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Ecosystem controls on nitrogen fixation in boreal feather moss communities

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Abstract N fixation in feather moss carpets is maximized in late secondary successional boreal forests; however, there is limited understanding of the ecosystem factors that drive cyanobacterial N fixation in feather mosses with successional stage. We conducted a reciprocal transplant experiment to assess factors in both early and late succession that control N fixation in feather moss carpets dominated by *Pleurozium schreberi*. In 2003, intact microplots of moss carpets (30 cm × 30 cm × 10–20 cm deep) were excavated from three early secondary successional (41–101 years since last fire) forest sites and either replanted within the same stand or transplanted into one of three late successional (241–356 years since last fire) forest sites and the transverse was done for late successional layers of moss. Moss plots were monitored for changes in N-fixation rates by acetylene reduction (June 2003–September 2005) and changes in the presence of cyanobacteria on moss shoots by microscopy (2004). Forest nutrient status was measured using ionic resin capsules buried in the humus layer. Late successional forests exhibit high rates of N fixation and consistently high numbers of cyanobacteria on moss shoots, but low levels of available N. Conversely, early successional forests have higher N availability and have low rates of N fixation and limited presence of cyanobacteria on moss shoots. Transplantation of moss carpets resulted in a significant shift in presence and activity of cyanobacteria 1 year after initiation of the experiment responding to N fertility differences in early versus late successional forests.

Keywords *Pleurozium schreberi* · Nitrogen fixation · Reciprocal transplant

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Introduction

N fixation in feather moss carpets (DeLuca et al. 2002a) is one of the primary sources of N in pristine northern boreal forests (Cleveland et al. 1999; DeLuca et al. 2002a); however, there is currently limited understanding of the factors that drive N-fixation rates in these ecosystems. N is a limiting nutrient in most forest ecosystems (Vitousek and Howarth 1991) and certainly limiting in northern European boreal forests (Tamm 1991). N-fixation rates in boreal feather moss communities contribute approximately 2 kg N ha^{-1} $year^{-1}$ in mature forest ecosystems (DeLuca et al. 2002a); however, N-fixation rates are low in forests recently exposed to fire (Zackrisson et al. 2004). This increase in N fixation with time since disturbance stage contrasts with the accepted notion that N-fixation rates decline with primary succession (Rastetter et al. 2001). Sites exposed to severe ground fires can result in the complete elimination (Engelmark 1999) of the ground

vegetation from the forest floor, a significant loss of total N capital on site (Smithwick et al. 2005), and a short term (0–100 year) increase in N availability and turnover rates (DeLuca et al. 2002b). These fire-exposed sites would likely have higher N turnover after fire (Smithwick et al. 2005) and thus might experience lower N-fixation demand than sites with low fire severity and low N turnover rates.

N fixation in feather moss carpets has been found to vary greatly both spatially and temporally (DeLuca et al. 2002a) as well as with stage of secondary succession in forest stands (Zackrisson et al. 2004). The cause of this variation in N-fixation rates with time since last fire is not completely clear. Although it appears that N deposition has an inhibitory effect on nitrogenase activity in mosses (Huttunen et al. 1981; Zackrisson et al. 2004); there has been no attempt to date to determine the influence of ecosystem properties on the presence of cyanobacteria in feather mosses. Early successional forests in northern Sweden tend to have greater availability of inorganic N (DeLuca et al. 2002b) and likely have greater N deposition via throughfall, which may ultimately reduce N-fixation rates (Zackrisson et al. 2004). Although light is often cited as a controlling mechanism in plant community dynamics in secondary succession (Kimmins 2003), light is not likely a major controlling factor of N fixation in feather moss carpets as late successional Scots pine stands of northern Sweden tend to be more open (greater light penetration to the forest floor) than mid successional stands (DeLuca et al. 2002b; Engelmark 1999). Importantly, cyanobacteria are thought to function as heterotrophs in many associations (Meeks 1998) and we have found no relationship between forest stand structure and N fixation in feather moss carpets (Zackrisson et al. 2004).

Currently there is only limited understanding of environmental and ecological variables that drive N fixation in cyanobacteria associated with boreal feather moss carpets. Experimental analysis of factors controlling N fixation in various associations and symbioses are often carried out under artificial conditions (laboratory, greenhouse or garden plot) due to the difficulties associated with the manipulation and measurement of N fixing organisms (Weaver and Danso 1994). The nature of feather moss carpets (mosses lack roots and the cyanobacteria are present on their leaves) provides the unique opportunity for field manipulation and measurement of N-fixation under natural conditions.

Herein we investigated ecosystem controls on N-fixation rates in feather moss carpets by using a reciprocal transplant approach allowing us to analyze the influence of biotic and abiotic factors (that differ with successional stage) on N fixation in northern Sweden. Rapid changes in N-fixation rates following transplantation would implicate immediate response factors such as an alteration of light or moisture conditions whereas more gradual changes would implicate slow response factors such as colonization of moss leaves by cyanobacteria or exposure to subtle differences in available N. A fertilization experiment was monitored to evaluate how N deposition (e.g., reintroduced as throughfall) influences N-fixation rates in feather moss carpets. Finally, ionic resin capsules were installed in the forest floor of early and late successional sites to simply assess the amount of bioavailable N in these respective stand types.

Materials and methods

Site descriptions

Studies were conducted at natural boreal forest preserves in northern Sweden on three early successional sites that exhibit low N-fixation rates and high N availability (Zackrisson et al. 2004) and three late successional sites that exhibit the opposite condition (see Table 1). Greater detail on each site and the means of determining site history is described by Zackrisson et al. (2004). All sites occur within the Northern Boreal zone of Sweden (65°35′–66°07′N; 17°15′–19°26′E). Each site comprises Scots pine (*Pinus sylvestris* L.) and Norway spruce [*Picea abies* L. (Karst.)]. The ground vegetation in mature stands is dominated by ericaceous dwarf shrubs and dense carpets of feather mosses, mainly *Pleurozium schreberi* (Bird) Mitt. All mineral soils developed in granitic glacial till or glacial sediment with a 2- to 7-cm-thick Oe/Oa horizon, 10- to 20-cm-thick E horizon and a 30- to 40-cm-thick Bs horizon. Soils are classified as either Typic Haplocryods or Entic Haplocryods. Chemical characteristics of soil organic horizons at the six sites are presented in Table 1 and vegetation cover composition is given in Table 2.

Reciprocal transplant experiment

Replicated reciprocal transplant experiments were carried out at a total of six field sites in dense moss carpets where *P. schreberi* accounted for 65% or more of ground cover in the three late secondary successional sites and 9–50% in the three early secondary successional sites (Table 2). Moss transplant plots were established at three early successional sites (Järvliden, Granliden, and Avaviken) that range from 42 to 102 years since last fire and three late successional sites

Table 1 Selected soil chemical properties and depth of the soil organic horizon (Oe/Oa) (*O depth*) at three early successional sites and three late successional sites in northern Sweden

Site	Time since fire (years)	O depth (cm)	Total N (%)	Total C (%)	pH 0.01 M $CaCl_2$	Extractable P (mg kg^{-1})
Järvliden	42	5.0	1.05	45.8	2.2	17.2
Granliden	79	6.0	1.25	44.4	2.4	16.5
Avaviken	102	5.0	0.74	31.6	2.5	27.7
Tjadnes	245	7.0	1.27	43.9	2.2	18.4
Kuottavare	281	4.3	1.39	44.9	2.5	38.1
Ruttjeheden	356	5.3	1.02	42.3	2.5	22.4

Table 2 Percent vegetation cover[a] of dominant understory vegetation types and total tree basal area (number *in parentheses* represents 1 SE, $n = 6$) at three early successional sites and three late successional sites in northern Sweden

	Empetrum hermaphroditum	*Vaccinium myrtillus*	*Vaccinium vitis-idaea*	*Deschampsia flexuosa*	*Pleurozium schreberi*	*Hylocomium splendens*	*Cladina* spp.	Tree basal area
Järvliden	4.8 (1.2)	15.8 (2.4)	20.0 (0.8)	5.8 (0)	9.2 (1.5)	1.0 (0)	8.3 (2.1)	15.3 (1.2)
Granliden	2.0 (0.6)	37.5 (2.8)	9.2 (2.0)	8.3 (0)	40.0 (2.2)	12.5 (2.1)	0.0 (0)	20 (2.0)
Avaviken	12.5 (1.7)	35 (1.3)	12.5 (2.5)	5.3 (0.6)	50.8 (2.4)	2.3 (0.6)	2.3 (0.6)	25.2 (1.9)
Tjadnes	24.2 (2.4)	31.7 (2.1)	7.5 (1.1)	1.2 (0.2)	75.8 (1.5)	3.0 (0.6)	1.0 (0)	20 (0.8)
Kuottavare	21.7 (2.1)	27.5 (2.1)	7.0 (1.4)	2.3 (0.3)	70.8 (2.0)	7.8 (1.9)	1.0 (0)	17 (0.8)
Ruttjeheden	24.2 (1.5)	23.3 (1.1)	7.5 (1.1)	1.0 (0)	65.8 (3.7)	3.0 (0.6)	7.3 (2.5)	21 (1.5)

[a] Note that shrubs may overlap mosses and lichen creating greater than 100% cover

(Tjadnes, Kuottavare, and Ruttjeheden) that range from 245 to 356 years since last fire. Twelve replicate plots measuring 30 cm × 30 cm were cut out of the moss carpet at each of the sites removing all of the live and dead moss tissue within the carpet to the surface of the soil humus layer. Within each late successional site, six of the plots were removed and repositioned in a space vacated by the removal of another plot within the same site. The remaining six plots were transferred to one of the early successional sites. The same procedure was performed for the other two late successional sites. Within each early successional site, six of the plots were removed and repositioned in a space vacated by the removal of another plot within the same site. The remaining six plots were transferred to one of the late successional sites. The same procedure was performed for the other two early successional sites. Plots were established as a randomized complete block experiment on 2 June 2003. N fixation was estimated by a calibrated acetylene reduction method (described below) and performed on all transplanted and repositioned plots immediately following transplantation and then after a total of 6 weeks, 14 weeks, 15 months and 26 months following transplantation.

Assessment of available N and P at all sites

To assess available inorganic N and P in the forest floor of early and late successional stands, we installed spherical nylon capsules containing 1 g of mixed anionic and cationic resin (Unibest, Bozeman, Mont.) in June 2003. Capsules were installed at the interface of forest floor and mineral soil by cutting an access channel by use of a stainless steel knife inserted at a 45° angle. Ionic resin capsules were allowed to incubate in situ for 3 months to assess the total available inorganic N and P integrated across a single growing season. Resin-sorbed PO_4^{3-}, NO_3^- and NH_4^+ were determined by shaking the resin capsules with three sequential aliquots of 10 m of 2 M KCl, decanting the aliquot in between samples to create a total extract volume of 30 ml (DeLuca et al. 2002b). Extracts were analyzed for ion concentrations as described below.

N fertilization experiment

The influence of N fertilization on N-fixation rates was studied at a single late successional forest site (Ruttjeheden, 355 years since fire), a location where N-fixation rates had been observed to be relatively high. Acetylene reduction rates were assessed over the first 2 years and reported in Zackrisson et al. (2004); here we report on a third year of acetylene reduction rates and the number of cyanobacterial cells on moss shoots for the first time. Twelve replicate blocks of three N application rates (0, 4.25 and 25.5 kg N ha^{-1}) were laid out on dense moss carpets (in which *P. schreberi* accounted for about 65% of ground cover) in a

Table 3 Resin-sorbed inorganic N (NH_4^+–N + NO_3^-–N) and resin-sorbed PO_4^{3-} collected during the summer of 2003 from beneath the moss bottom layer at the interface of the humus layer and mineral soil at three early successional and three late successional study sites in northern Sweden (number *in parentheses* represents 1 SE, $n = 8$)

Site	Chemical parameter			
	NO_3^-–N (μg g^{-1})	NH_4^+–N (μg g^{-1})	Inorganic N (μg g^{-1})	PO_4^{3-}–P (μg g^{-1})
Järvliden	1.68 (0.11)	7.53 (3.82)	9.22 (3.73)	2.54 (0.56)
Granliden	2.02 (0.16)	14.68 (5.82)	16.7 (5.53)	3.69 (0.42)
Avaviken	2.52 (0.14)	9.46 (2.24)	11.98 (2.16)	6.58 (3.29)
Tjadnes	6.33 (3.77)	6.92 (4.37)	13.25 (5.05)	2.84 (2.02)
Kuottavare	3.31 (0.39)	0.97 (0.65)	4.29 (0.84)	1.32 (0.17)
Ruttjeheden	3.43 (0.58)	7.58 (3.44)	9.94 (3.51)	1.59 (0.27)

randomized complete block experiment established in spring of 2002. N fertilizer was applied as a 0.001 M solution of NH_4NO_3 and was applied to 12 replicate 1-m × 1-m plots at a rate of 0.56 kg ha^{-1} $week^{-1}$ and 3.18 kg ha^{-1} $week^{-1}$ to create a total application of 4.25 and 25.5 kg N ha^{-1}, respectively, for a total of 8 weeks. A volume of water was applied to the control and the low N plots to create a total moisture application that was consistent across all plots. Acetylene reduction activity and total cyanobacterial presence of moss shoots were then measured, as described below, 3 years following the original fertilizer application during the spring of 2002.

N-fixation analysis

N-fixation rates were estimated using a calibrated acetylene reduction technique (Schöllhorn and Burris 1967). Moss samples were analyzed for acetylene reduction rates in 12 separate 1-m × 1-m plots at each of the 12 sites two separate growing seasons. Ten moss shoots were removed from each plot and placed into a 20-ml glass culture tubes (ten moss shoots represent approximately 2.8 cm^2 of total land surface). The tubes were fitted with a septum and 10% of the total headspace evacuated and replaced with acetylene and settled back into the moss layer. Tubes were allowed to incubate for 24 h at which time they were brought back to the laboratory and analyzed for total ethylene production. The *Pleurozium–Nostoc* symbiosis (DeLuca et al. 2002a) as well as *Pleurozium* symbiosis with other cyanobacteria has been found to reduce acetylene at a ratio of 3 mol of ethylene per mol N (DeLuca et al. 2002a; Zackrisson et al. 2004).

Identification and enumeration of cyanobacteria

The presence of cyanobacteria on moss leaves was only measured after significant differences in N-fixation rates were observed between transplanted and non-transplant plots (14 months after establishment of the experiment). Moss samples were collected from all transplanted and repositioned plots in August of 2004 and observed by fluorescence microscopy for the type and presence of cyanobacteria. From each of four randomly chosen plots per site, counts of cells were made on two randomly chosen moss shoots from the living portion of the moss tissue. Each shoot was placed in a clean glass Petri dish, 200 μl of sterile distilled water was pipetted onto the shoot, and the wet shoot was thoroughly scratched with a 27-gauge needle to detach the cyanobacteria. Once the entire shoot was scratched and removed from the Petri dish, 50 μl of water solution was transferred to a microscope glass slide cover with a 24-mm× 24-mm cover slip. An area of 1 cm^2 was marked on the back of the glass slide. The bacteria present in this area were identified according to Rippka et al. (1979) and the number of total cells, heterocysts and vegetative cells counted; then the number of total cells, heterocysts and vegetative cells were estimated per shoot.

Soil and resin analyses

Resin extracts were centrifuged at 4,000 *g* for 10 min and then analyzed for PO_4^{3-} using the molybdate–ascorbic acid method on an Autoanalyzer II (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 (Bran Luebbe, Chicago, Ill.). Humus samples were analyzed for pH in a 2:1 (0.01 M $CaCl_2$) suspension, total C and N by dry combustion analysis using a Fissions EA 1100 (Milan, Italy), and 0.01 M $CaCl_2$-extractable P (Kuo 1996).

Statistical analysis

Data from all six sites were analyzed using a one-way ANOVA. All data were tested to determine if they met the assumptions of ANOVA (homogeneity of variance and normality of distribution). In the case of

percent colonization results a log + 1 transformation was applied to allow for conformation to normality of distribution (as a result of numerous samples containing zeros). Data not conforming to the assumptions of ANOVA following transformation were analyzed using the non-parametric Kruskal–Wallis test across all sites and the Mann–Whitney *U*-test as a test for mean separation within sites. Simple Pearson correlations were performed on summarized data from the September 2004 sampling to assess the relationship between N-fixation rates and leaf colonization and between nutrient sorption to resins and colonization rates. It should be noted that all moss samples collected from Tjadnes in August 2004 were lost following acetylene reduction analysis and had to be resampled in May 2005 to conduct colonization analyses. All data were analyzed using SPSS 6.2 for Windows (SPSS, Chicago, Ill.).

Results

N-fixation rates in feather moss carpets were low on all plots from early secondary successional forests (Fig. 1) where N availability was relatively high and cyanobacterial colonization rates were low (Fig. 2). Transplantation of these moss carpets into late secondary successional forests where N availability was low resulted in a gradual increase in N-fixation rate (Fig. 1a). The opposite trend was observed when late successional plots were transferred to early successional forests resulting in a gradual decline in N-fixation rates with time (Fig. 1b). N-fixation rates did not follow a specific trend in the late and early successional control plots that remained in their respective sites throughout the study period, but late successional mosses consistently had high rates of N fixation and early successional mosses consistently had low N-fixation rates.

Significantly greater numbers of N-fixing cyanobacteria, (specifically *Stigonema* spp. and *Nostoc* spp.) were observed on moss plots transplanted from early successional to late successional sites compared to those plots that remained on early successional sites (Fig. 2a). Conversely, the number of cyanobacterial cells on late successional moss plots transplanted to the early successional sites was significantly lower than late successional moss plots that remained on site (Fig. 2b). Cyanobacterial cells estimated per moss shoot were also found to be correlated with N-fixation rates measured during the same 14-month sampling period (Fig. 3).

The total number of heterocysts per moss shoot was twice as high on late successional sites compared to early successional sites. The average number of heterocysts was 420 (SE = 150, $n = 4$) in late successional stands and 220 (SE = 150, $n = 4$) on early successional plots. The number of heterocysts decreased to 210 (SE = 100, $n = 4$) on the late successional moss transplanted into early successional sites. The number of heterocysts on early successional moss that was repositioned in the same early successional site did not change (210, SE = 30, $n = 4$).

N fertilization of moss plots resulted in a drastic reduction in N-fixation activity some 3 years after application of N fertilizer (data not shown). N fertilization of mosses at the high rate (25.5 = kg N ha^{-1}) resulted in no observable cyanobacteria on moss shoots and fertilization of mosses at the low N rate (4.25 = kg N ha^{-1}) resulted in the presence of cyanobacteria on moss leaves in only one of eight plots. The control plots that received no N fertilizer at this late successional site had high numbers of cyanobacteria cells (16,800 cyanobacteria cells $shoot^{-1}$, SE = 5,600, $n = 8$).

Concentrations of inorganic N (NH_4^+–N + NO_3^-–N) and P (PO_4^{3-}–P) sorbed to ionic resins at the interface of humus and mineral soil tended to be higher at the early successional sites (Table 3). Both NH_4^+ and P sorption to resins were significantly higher at the early successional sites compared to late successional sites ($P < 0.05$, based on a non-parametric Kruskal–Wallis test). There was no significant difference in total inorganic N concentrations when comparing early and late successional sites. N-fixation rates had a weak negative relationship to NH_4^+ sorption to resins (r^2 = –0.77, $P = 0.07$, $n = 6$). Colonization of moss leaves by cyanobacteria was not significantly correlated with P or NO_3^- sorption to resins.

Discussion

N-fixation rates in feather moss carpets of Swedish boreal forests are sensitive to ecosystem variables that differ between late and early secondary successional forests. Using a reciprocal transplant approach, our studies demonstrate that N-fixation rates gradually decline in feather mosses from late successional forests (that exhibit low available N) when transplanted into early successional, high available N, forests. Conversely, mosses transplanted from early successional stands into late successional stands demonstrated a gradual increase in N-fixation demonstrating that the factors controlling N fixation are not transferred with the moss plots.

N-fixation rates for these late successional sites have been estimated at about 2 kg N ha^{-1} as compared to

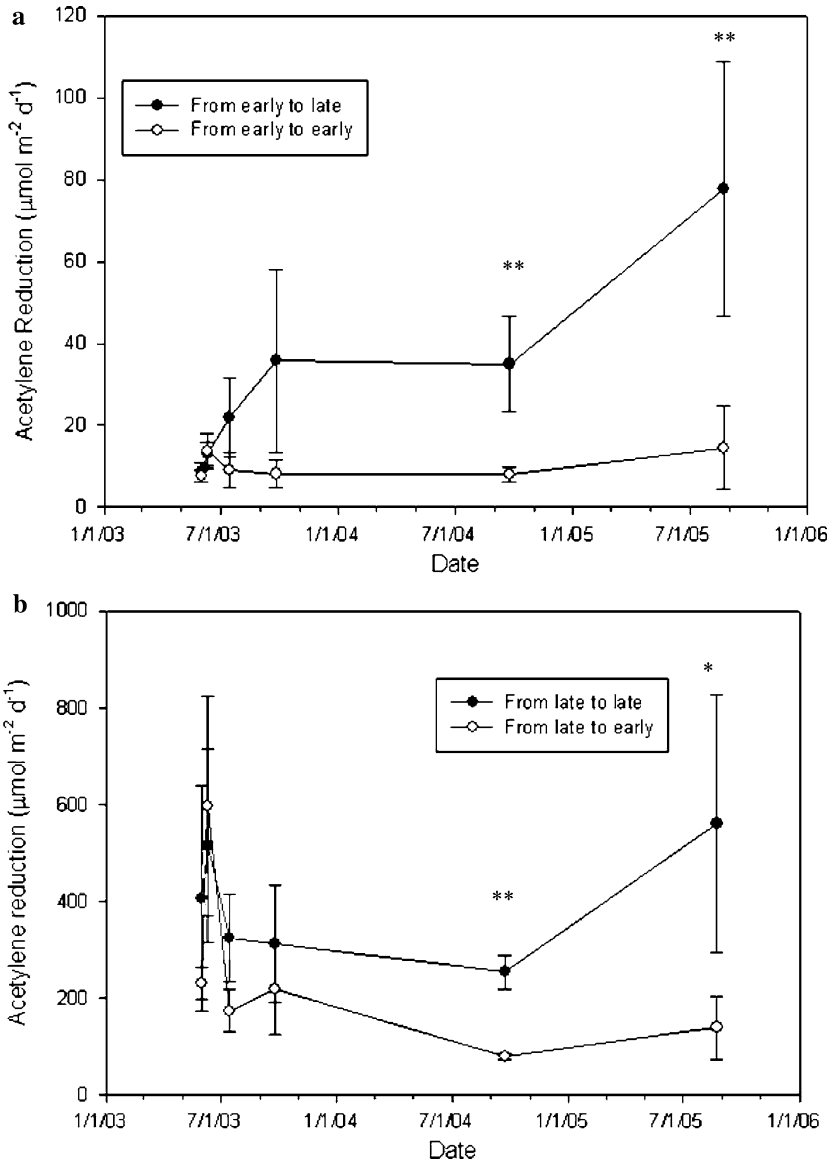

Fig. 1 **a** Change in N-fixation rates (as estimated by acetylene reduction) with time following removal and either repositioning of plots on the same early successional site (*open circles*) or transplantation of early successional feather moss plots to a late successional site (*closed circles*). *Each point* represents an average acetylene reduction value as a composite across three sites and 12 subsamples per site (*error bars* represent 1 SE, $*P < 0.05$ or $**P < 0.01$ demonstrate a significant difference in N fixation between transplanted and non-transplanted moss carpets, as determined using a Mann–Whitney *U*-test). **b** Change in N-fixation rates (as estimated by acetylene reduction) with time following removal and either repositioning of the plots on the same late successional site (*open circles*) or transplantation of late successional feather moss plots to an early successional site. *Each point* represents an average acetylene reduction value as a composite across three sites and 12 subsamples per site (*error bars* represent 1 SE, $*P < 0.05$ or $**P < 0.01$ demonstrate a significant difference in N fixation between transplanted and non-transplanted moss carpets, as determined using a Mann–Whitney *U*-test)

less than 0.5 kg N ha^{-1} for the early successional sites (Zackrisson et al. 2004). Average values for measurements made at these sites during the 2003–2005 seasons result in an estimate of 7 kg N ha^{-1} for the late successional sites and approximately 0.2 kg ha^{-1} for the early successional sites. However, these values are somewhat inflated as samples were only taken during periods of relatively high fixation rates; samples were not taken in early spring and late fall when fixation rates are low (Zackrisson et al. 2004) thereby inflating average annual fixation rates.

The mechanism for differences between late and early successional mosses is poorly understood. Low numbers of cyanobacteria were found on shoots of mosses from the early successional forests suggesting that colonization of regenerating moss shoots (after

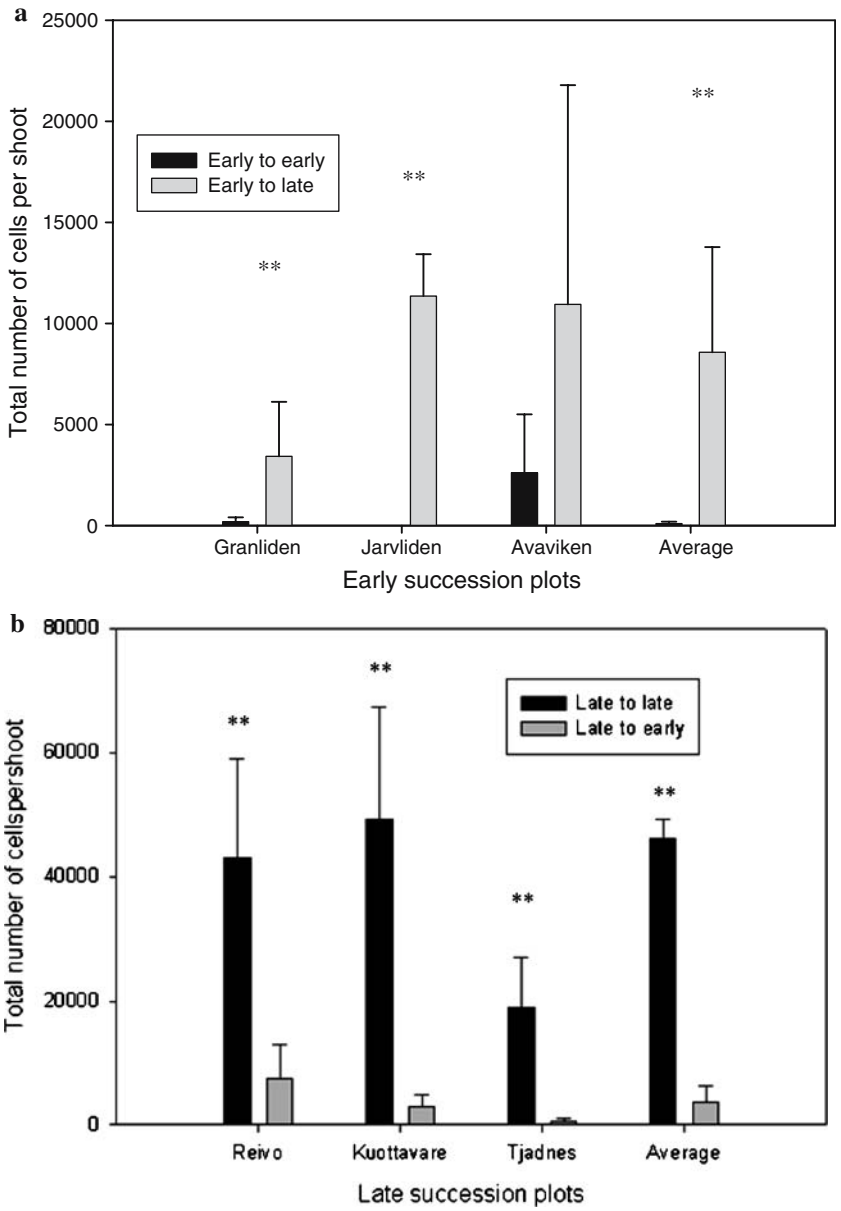

Fig. 2 **a** Number of cyanobacteria cells present on leaves of *Pleurozium schreberi* as influenced by removal and either repositioning of feather moss plots from an early successional site into a late successional forest site or transplantation of feather moss plots from an early successional site into the same site. *Values* represent the number of cyanobacteria per shoot of moss as determined by direct microscopy. *Each bar* represents an average from two shoots per plot and site (*error bars* represent 1 SE where $n = 4$, $^{*}P < 0.05$ or $^{**}P < 0.01$ demonstrate a significant difference in cells per moss shoot between transplanted and non-transplanted moss carpets, as determined using a Mann–Whitney *U*-test). **b** Number of cyanobacteria cells present on leaves of *P. schreberi* as influenced by removal and either transplantation of feather moss plots from an early successional site into a late successional forest site or repositioning of feather moss plots from an early successional site in the same site. *Values* represent the number of cyanobacteria per shoot of moss as determined by direct microscopy. *Each bar* represents an average from two shoots per plot and from four plots per site (*error bars* represent 1 SE where $n = 4$, $^{*}P < 0.05$ or $^{**}P < 0.01$ demonstrate a significant difference in cells per moss shoot between transplanted and non-transplanted moss carpets, as determined using a Mann–Whitney *U*-test)

fire) is either a slow process or perhaps colonization is denied or controlled by the moss during this stage of high N availability. The fact that mosses from late successional forests that were transplanted into early successional stands experienced a significant drop in the number of cyanobacteria on shoots favors the idea that an abiotic factor, such as nutrient availability or N deposition via canopy throughfall is a more important

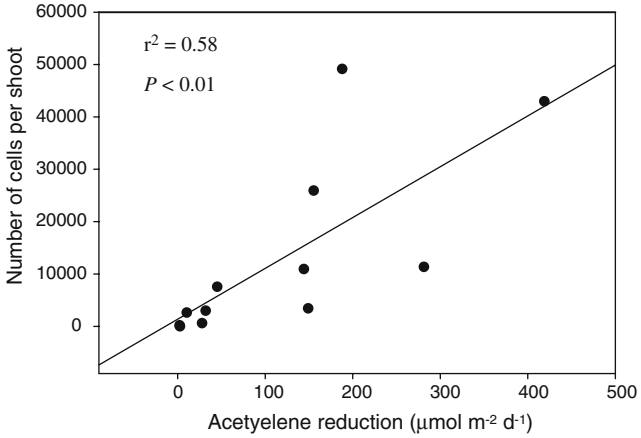

Fig. 3 Relationship between average acetylene reduction values for the early and late successional sites (n = 12) from September 2004 and total cyanobacterial cells estimated per moss shoot (r^2 = 0.58, uncorrected Pearson correlation, P = 0.01) for samples collected during the same sampling period. *Each data point* represents the mean of six replicate plots

factor influencing N-fixation rates as compared to speed of colonization. These results may also suggest that the moss exerts some level of control over the presence of cyanobacteria, because the number of cyanobacteria actually declines. If the moss did not control the presence of cyanobacteria, then one would expect the cyanobacteria to simply use available N rather than continuing to fix N_2 and therefore expect their numbers to remain constant (Meeks 1998).

N-fixation rates in moss carpets were almost not measurable in early succession. N-fixation rates at Järvliden (42 years since last fire) were below detection limits and were near detection limit at Granliden (79 years since last fire). Accordingly, Järvliden had no observed cyanobacteria on moss leaves and Granliden had the next lowest numbers of cyanobacteria of any of the other sites. Higher levels of N fixation and greater numbers of cyanobacteria were measured at Avaviken, which had not been exposed to fire for over 100 years prior to sampling. This difference in age and associated difference in N availability (DeLuca et al. 2002b) may account for the contrasting numbers of cyanobacteria on moss shoots at the three early successional sites.

The greatest presence of cyanobacteria was found at the late successional sites. The close correlation found between N-fixation rate and numbers of cyanobacteria in moss shoots 14 months after initiation of the experiment initially appears as strong evidence for the numbers of cyanobacteria on moss shoots driving N fixation (Fig. 3). However, the numbers of cyanobacterial cells per shoot in the early successional plots transplanted into late successional forests were similar to those in the late successional plots transplanted into early successional forests (Fig. 2a, b), yet N-fixation rates in these transplant plots were half those observed in the late successional moss plots transplanted into early successional forests.

The increase in N fixation in early successional mosses transplanted into late successional stands was not significant until 14 months following transplantation, which may relate to the gradual process of mechanical transfer (e.g., rain splash) of cyanobacterial cells from neighboring moss or to the growth of the cyanobacteria already present on moss leaves, but that only grow after the release of chemoattractants and initiation of the association or symbiosis (Rai et al. 2000). The presence of antimicrobial properties and leaf phenols (Turetsky 2003) may also function to inhibit leaf colonization under the more N-rich conditions found in early successional sites (DeLuca et al. 2002b) which would ultimately give way to chemoattractant production under the more N-poor conditions in the late successional sites.

The lack of an immediate change in N-fixation rates in the between-site transplanted plots greatly eliminates the possibility that temperature or light are driving differences between early and late successional stands. The late successional sites would have had greater light penetration as a function of the lower tree density (Zackrisson et al. 2004). Temperature optima are generally near 25 °C for most symbiotic cyanobacteria (Meeks 1998) and potentially somewhat lower for polar or subarctic ecosystems (Dickson 2000). However, recent studies demonstrated variable temperature optima for cyanobacteria associated with feather mosses in northern Sweden with some optima near 13°C depending on the genus of cyanobacteria that have colonized the moss (Gentili et al. 2005).

We found no consistent effect of successional stage on the genus of cyanobacteria that colonize *P. schreberi*. Both early and late successional stands were dominated by *Stigonema* spp. and secondarily by *Nostoc* spp. and early successional plots only occasionally by *Calothrix* spp.. Light conditions are likely influenced by successional stage (lower canopy density in late successional stands); however, here again, if light conditions were influencing N-fixation rates, then we would have likely seen an immediate increase in N-fixation rates following transplantation of early successional moss plots into late successional sites where basal areas are lower (Table 2) and light penetration is somewhat greater. The gradual response suggests that the mosses must be colonized by more cyanobacteria or that the mosses (like the host) need to adapt to the new environmental conditions (e.g., being depleted in

nutrients or recover from the relatively high N deposition in early successional sites), which may take several months or years.

N deposition is a potential inhibitor of N fixation in cyanobacteria (Kitoh and Shiomi 1991) and specifically in *P. schreberi* (Zackrisson et al. 2004). It is highly possible that the N available for plant uptake in early successional plots (DeLuca et al. 2002b) was high enough to inhibit N fixation. This is supported by the negative correlation between resin-sorbed NH_4^+ and N-fixation rates and by the significantly higher levels of resin-sorbed NH_4^+ at the early successional sites which may reflect a greater presence of available N in these early successional systems. It is therefore possible that transplantation of moss plots from late successional to early successional sites exposed the mosses to higher rates of N deposition thereby decreasing N-fixation rates while only modestly reducing the presence by cyanobacteria. N in throughfall is deposited at a rate of approximately 2.2 kg N ha^{-1} $year^{-1}$ (Kristensen et al. 2004). The concentration of N in throughfall (predominantly organic N and NH_4^+) was found to be correlated with increasing C:N of the organic horizon (Kristensen et al. 2004) where the C:N of plant tissue in boreal forests has been found to decrease with time since last fire (Wardle et al. 1997); these combined findings support the notion that N in throughfall would be greatest in sites most recently exposed to fire. In our fertilization experiment, colonization of moss shoots was greatly eliminated (few or no cyanobacteria observed on mosses in fertilized plots) by N additions of 4.5 kg N ha^{-1} (applied as NH_4NO_3) demonstrating that cyanobacterial presence on shoots of *P. schreberi* is highly sensitive to N additions.

N-fixation rates in feather moss carpets are clearly influenced by ecosystem factors that vary with successional stage (Zackrisson et al. 2004) and the number of cyanobacteria on moss shoots is definitely a major factor influencing this process. It is possible that the total numbers of cyanobacteria present at a given site are greatly reduced during fire events and are simply not present in the very early successional sites, thus requiring wind dissemination of cells from off site to initiate colonization. It is, however, more likely that feather mosses and their cyanobacterial associates survive in patches following fire events and that cyanobacteria and are slowly reintroduced to the mosses growing on site via abiotic (e.g., wind dissemination or rain splash) or biotic (e.g., hormogonia stage or inadvertent mechanical) transmission. The higher rates of N turnover in early successional forests (DeLuca et al. 2002b) might create a condition that inhibits colonization by cyanobacteria. A condition of high N in symbiotic plant–cyanobacteria relationships is known to eliminate the release of chemoattractants that initiate the horomogonia stage in symbiotic cyanobacteria (Rai et al. 2000). It is possible that this type of indirect effect of N controls long-term N-fixation rates in early successional stands; however, further work is required to determine whether such tight relationships exist between cyanobacteria and the feather moss host. It does appear that the high levels of bioavailable N in early successional ecosystems reduce both N-fixation rate and cyanobacterial colonization of moss shoots.

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