ECOSYSTEM ECOLOGY - ORIGINAL PAPER

Nitrogen fixation in mixed *Hylocomium splendens* moss communities

O. Zackrisson · T. H. DeLuca · F. Gentili · A. Sellstedt ·
A. Jäderlund

Abstract The pleurocarpus feather moss, *Hylocomium splendens*, is one of two co-dominant moss species in boreal forest ecosystems and one of the most common mosses on earth, yet little is known regarding its capacity to host cyanobacterial associates and thus contribute total ecosystem N. In these studies, we evaluated the N-fixation potential of the *H. splendens*–cyanobacteria association and contrasted the N-fixation activity with that of the putative N-fixing moss–cyanobacteria association of *Pleurozium schreberi*. Studies were conducted to: quantify N-fixation in *H. splendens* and *P. schreberi* in sites ranging from southern to northern Fennoscandia; assess N and P availability as drivers of N-fixation rates; contrast season-long N-fixation rates for both mosses; and characterize the cyanobacteria that colonize shoots of *H. splendens*. Nitrogen-fixation rates were generally low at southern latitudes and higher at northern latitudes (64–69°N) potentially related to anthropogenic N deposition across this gradient. Nitrogen fixation in *H. splendens* appeared to be less sensitive to N deposition than *P. schreberi*. The season-long assessment of N-fixation rates at a mixed feather moss site in northern Sweden showed that *H. splendens* fixed a substantial quantity of N, but about 50% less total N compared to the contribution from *P. schreberi*. In total, both species provided 1.6 kg fixed N ha^{-1} year^{-1}. Interestingly, *H. splendens* demonstrated somewhat higher N-fixation rates at high fertility sites compared to *P. schreberi*. *Nostoc* spp. and *Stigonema* spp. were the primary cyanobacteria found to colonize *H. splendens* and *P. schreberi*. These results suggest that *H. splendens* with associated *Nostoc* or *Stigonema* communities contributes a significant quantity of N to boreal forest ecosystems, but the contribution is subordinate to that of *P. schreberi* at northern latitudes. Epiphytic cyanobacteria are likely a key factor determining the co-dominant presence of these two feather mosses across the boreal biome.

Keywords Feather moss · Nitrogen · Boreal ecosystems · *Pleurozium schreberi*

Communicated by Hakan Wallander.

O. Zackrisson · T. H. DeLuca · A. Jäderlund
Department of Forest Ecology and Management,
Swedish University of Agricultural Sciences,
Umeå 901 83, Sweden

T. H. DeLuca (✉)
Ecology and Economics Research Department,
The Wilderness Society, 503 West Mendenhall,
Bozeman, MT 59715, USA
e-mail: tom_deluca@tws.org

F. Gentili · A. Sellstedt
Umeå Plant Science Center,
Department of Plant Physiology,
Umeå University, 901 87 Umeå, Sweden

Introduction

The feather mosses *Hylocomium splendens* (Hedw.) B.S.G. and *Pleurozium schreberi* (Bird.) Mitt. are ubiquitous in boreal regions and are some of the most widespread plants on earth (Persson and Viereck 1983; Schofield 1992; Kuc 1997; DeLuca et al. 2002). They often dominate the ground vegetation in boreal forests but are also common in temperate and arctic ecosystems (Nyholm 1965; Walter and Breckle 1989; Ochyra and Brednarek-Ochyra 2002; Mäkipää and Heikinen 2003). These two feather mosses can form dense monospecific carpets on the forest floor with the ground cover reaching 80% or more in mature tree stands.

They comprise a smaller part of the total ecosystem biomass but their annual growth can easily exceed that of trees in many northern boreal forests (Weetman 1969; Oechel and Van Cleve 1986; Bisbee et al. 2001; Bond-Lamberty and Gower 2007). The factors behind this unique ability to monopolize resources and dominate over other species on the forest floor is not well understood (Zackrisson et al. 1997; Nilsson and Wardle 2005). Classical explanations include their unusual ability to compete for and acquire nutrients exclusively from precipitation and throughfall, and their limited susceptibility to natural parasites or predators (Chapin et al. 1987; Brown and Bates 1990; Turetsky 2003). *Hylocomium splendens* tends to dominate in mesic and more productive sites while *P. schreberi* is found across a broader array of ecological conditions (Påhlsson 1994). The ground cover of *H. splendens* typically increases with successional age in pristine mesic boreal forest stands (Payette 1992; Engelmark 1999) and it is also commonly found in mature stages of managed coniferous forests (Makipää and Heikkinen 2003; Hart and Chen 2008).

The importance of *H. splendens* in nutrient cycling of boreal forests was first brought to attention by Tamm (1953, 1964). He demonstrated that the annual input of nutrients in precipitation and throughfall could account for the annual accumulation of all major nutrients in *H. splendens* with the exception of N. Several more recent studies have confirmed this paradox in *H. splendens* (Binkley and Graham 1981; Brown and Bates 1990; Longton 1992). The commonly insufficient availability of inorganic (and organic) N in boreal forests has remained an enigma. However, increased anthropogenic N deposition over recent centuries may have drastically altered the primary N resources for feather mosses (Huttunen et al. 1981; Lovett and Lindberg 1993; Forsum et al. 2006) and thereby also changed the possibilities to study natural availability and transfer of N between cyanobacteria and mosses in polluted areas. Until recently, the suspicion that biological N-fixation could account for the missing N-resources in boreal forests (DeLuca et al. 2002) had mainly remained unexplored. Previous efforts to examine N-fixation patterns in boreal forests likely underestimated the N-fixation potential in moss and lichen communities by being performed in high summer season when actually very little N-fixation takes place, in contrast to early spring and late autumn when N-fixation is at its peak in northern latitudes (DeLuca et al. 2002). Alternatively, many N-fixation studies have been performed in regions with raised levels of anthropogenic N deposition and other pollutants that can effectively mask or totally exclude N-fixation in cyanobacterial associations with mosses (Zackrisson et al. 2004).

It is well established that N-fixation both influences and is influenced by ecosystem processes (Rastetter et al. 2001; Vitousek et al. 2002; Gruber and Galloway 2008). Vegetation

dynamics can be profoundly influenced by the activity of N-fixing organisms in serial plant communities (Chapin et al. 1991). It has often been suggested that N-fixation has the most substantial effects in early primary succession (Cleveland et al. 1999). However, recent studies indicate that this may not be the case in northern boreal ecosystems, where N-fixation is apparently maximized in late secondary succession (Zackrisson et al. 2004). The functional aspects of this late successional N-fixation and its importance for the large net N accumulations found in growing humus layers associated with late seral plant communities is, however, far from being well understood (Zackrisson et al. 1998; Nilsson and Wardle 2005; Wardle and Zackrisson 2005).

The recovery of organic N resources that are lost to combustion during severe wildfire events are likely the result of N accumulation through biological N-fixation (Smithwick et al. 2005). Our understanding of the origin of boreal N (Binkley et al. 2000) and its dynamics as related to C and P cycling is unsatisfactory (Wardle et al. 2003a, b; Lagerström et al. 2007), especially given the size of boreal forest biome and its important role in global cycles (Bonan and Shugart 1989; Walter and Breckle 1989; Nilsson and Wardle 2005).

Recently, it has been shown that cyanobacteria present on the leaves of *P. schreberi* fix substantial amounts of N in pristine and previously unpolluted boreal forests (DeLuca et al. 2002; Zackrisson et al. 2004). This type of epiphytic cyanobacterial association has previously received limited attention (Usher et al. 2007; Adams and Duggan 2008). Up to three distinct genera of cyanobacteria have been identified in association with *P. schreberi* (Gentili et al. 2005). The ecological similarities that exist between the two major boreal feather mosses led us to investigate whether *H. splendens* accounts for a significant proportion of total biological N-fixation in boreal forests where this moss is a major component of the moss bottom layer.

The purpose of the work reported was to: (1) quantify N-fixation patterns in *H. splendens* and compare them with that in *P. schreberi* across a variety of sites ranging from southern to northern Fennoscandia; (2) assess N and P availability (by experimental applications) as drivers of N-fixation rates; (3) describe N-fixation in a fertility gradient typical of northern boreal forests; (4) assess season-long N-fixation rates in a natural forest reserve; and (5) isolate and characterize the cyanobacteria that colonize shoots of *H. splendens*.

Materials and methods

Regional study of N-fixation

Studies were conducted to evaluate the N-fixation potential of *H. splendens* communities at 40 independent sites in Fennoscandia (most sites in Sweden, but also a few in Nor-

way and Finland) between latitudes 59–69°N and longitudes 12–23°E during the 5-year period 2003–2007. All forest sites were late successional (secondary successions estimated to be older than 130 years) and dominated by coniferous trees, *Picea abies* L. (Karst.) or *Pinus sylvestris* L. Sites were pristine and uninfluenced by modern forestry practises and situated more than 300 m from the nearest main road to reduce effects of traffic emission (e.g., Zechmeister et al. 2005; DeLuca, unpublished). Most sites were chosen within existing nature reserves and national parks. The ground vegetation was dominated by ericaceous dwarf shrubs (*Vaccinium* sp., *Empetrum* sp., and *Calluna vulgaris* (L.) Hull) and dense carpets of feather mosses. Sites with a mixture of *H. splendens* and *P. schreberi* were used for sampling. Of the sites located near the timberline, samples were taken within the low alpine zone where feather mosses grow in plant communities with dwarf shrubs and grasses. Soils at the vast majority of sites are classified as Haplocryods. Most sampling was performed in early spring or late fall when N-fixation could expect to be high and in a manner such that latitude should not have been confounded by date. In each of the 40 sites, 12 samples of 10 moss shoots of *H. splendens* and *P. schreberi* were randomly collected within a 2-ha area. The 10 moss shoots were placed into a 20-ml glass culture tube and analyzed for N-fixation using a 24-h incubation and acetylene reduction assay as described below.

Nitrogen and phosphorus fertilization experiments

The influence of N and P fertilization rates was studied in a late successional site (Reivo) with mixed tree layer of Norway spruce and Scots pine. This site has been described in detail elsewhere (DeLuca et al. 2007). Ground vegetation was dominated by a field layer of ericaceous dwarf shrubs and a bottom layer of *H. splendens*. Three rates of N (0, 5, and 10 kg N ha^{-1}) applied as NH_4NO_3 and two rates of P (0 and 5 kg P ha^{-1}) applied as NaH_2PO_4 were applied in factorial combination to seven replicate blocks of 1-m^2 plots of *H. splendens* creating a total of 42 plots. Fertilizer treatments were applied in four increments during the growing season. Nitrogen-fixation rates were measured just before treatment and then at 2, 8, 52, and 104 weeks after treatment by placing 10 moss shoots in a 20-ml tube and using a 24-h incubation and acetylene reduction assay as described below.

Nitrogen-fixation in a fertility gradient

Three forest reserves, each with a distinct local fertiliy gradient, were used to study N-fixation differences between *H. splendens* and *P. schreberi*. These forest reserves, Vargisån, Piteälven, and Kryddgrovan, are all situated in northern Sweden between latitudes 65–66°N and at alti-

tudes between 290 and 360 m above sea level (for further details on soil nutrients, etc. within gradients, see Wardle et al. 1998). Mean annual temperature and precipitation are 1°C and 570 mm, respectively. Three fertility levels were identified in each reserve. Sites used for sampling within each reserve were situated less than 300 m from each other to reduce local climatic differences. The low level productivity sites had a sparse tree layer dominated by Scots pine and ground vegetation mainly composed of reindeer lichens such as *Cladina rangiferina* (L.) Nyl. and *C. stellaris* (Opiz) Brodo. Feather moss carpets, composed primarily of *P. schreberi*, were mostly found in distinct patches mixed into the lichen mats. This vegetation is characteristic of oligotrophic sites of low nutrient availability (Hägglund and Lundmark 1977). The soils of the low fertility stands are Typic Haplocryods with a 2-cm Oe horizon. The middle level productivity sites had a mixed tree layer of Scots pine and Norway spruce and the ground vegetation were dominated by a dense layer of ericaceous dwarf shrubs (*Vaccinium myrtillus* L., *V. vitis-idaea* L and *Empetrum hermaphroditum* Hagerup) and thick carpets of feather mosses. The soils in the middle fertility level are also Typic Haplocryods formed in alluvial sediments with a 5-cm Oe horizon. The high level productivity site was dominated by Norway spruce and herbaceous species such as *Geranium sylvaticum* L., *Gymnocarpium dryopteris* (L.) Newm., and *Melica nutans* L. characteristic of highly productivity, fertile areas (Hägglund and Lundmark 1977). The two feather mosses were mostly found on decaying wood (downed logs and stumps) or near stem bases of conifers while other mosses such as *Rhytidiadelphus triquetrus* (Hedw.) Warnst., *Brachythecium* spp., and *Mnium* spp were growing directly on the ground among herbs and grasses. Soils of the high fertility sites are Oxyaquic or Aquic Haplocryods with a 10-cm Oa horizon underlain by a 5-cm A horizon. All sites represented stand successional ages older then 120 years (estimated by tree ring counts).

Sampling of *H. splendens* and *P. schreberi* for N-fixation analysis within the fertility gradient was performed in September 2004 when N-fixation rates were high. Four samples of 30 shoots of each species were randomly taken within each of the three fertility level stands at each of the three sites. In total, 36 samples, each composed of 30 shoots, were analyzed for each species. This represents approximately 8.4 cm^2 for *P. schreberi* and 29.4 cm^2 for *H. splendens*. All samples were placed into a 60-ml culture tube and analyzed for N-fixation using a 24-h field incubation and acetylene reduction assay as described below.

Season-long assessment of N-fixation rates

We also conducted a season-long in situ field assessment of N-fixation rates at the Reivo forest reserve (65°47''N,

19°06″E, 460 m above sea level), northern Sweden. The studied forest is in a late successional stage after fire perturbation about 350 years ago. The tree layer (average basal area 22 m^2) is dominated by a mixture of Norway spruce and Scots pine, and the understory vegetation is formed by ericaceous dwarf shrubs such as *Vaccinium myrtillus*, *V. vitis-ideae*, and *Empetrum hermaphroditum*. *Hylocomium splendens* and *P. schreberi* form a dense carpet in the forest floor with a minor cover of other moss species (e.g., *Dicranum fuscecens* Sm., *Ptilium crista-castrensis* (Hedw.) DeNot., and *Barbilophozia lycopodioides* (Wallr.) Loeske). At each sampling, 10 shoots of *H. splendens* and *P. schreberi* were randomly collected from 12 separate 1 × 1 m field plots with monospecific dominance of the respective moss species. The 10 moss shoots were placed in 20-ml glass culture tubes and analyzed for N-fixation using a 24-h incubation and acetylene reduction assay as described below. Samples were collected and field incubated on a total of 33 occasions from August 2004 to October 2006.

To determine the amount of N fixed per unit area, 12 replicate samples of mosses of both species were plugged out using a 45-mm-diameter PVC tube, and dry weight and number of shoots per m^2 calculated for each of the 12 sample points. *Pleurozium schreberi* and *H. splendens* were found to posses an average area of 3.58 shoots cm^{-2} (±0.18 SE) and 0.98 shoots cm^{-2} (±0.08 SE), respectively. Percent ground cover of field and bottom layer vegetation was also estimated at the species level in 12 replicated large plots (11.3 m diameter) according to Hägglund and Lundmark (1977). The ratio between shoot numbers and area were also used for calculating N-fixation in the latitudinal study.

Acetylene reduction assay

Nitrogen-fixation rates were estimated using a calibrated acetylene reduction technique (Schöllhorn and Burris 1967). Ten moss shoots were removed from each plot and placed into a 20-ml glass culture tube. In the fertility gradient experiment, 30 moss shoots were placed in a 60-ml culture tube. In all cases, 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 with the stopper down. Tubes were allowed to incubate for 24 h at a natural forest site near the laboratory in Arvidsjaur, after which they were returned to the laboratory and analyzed for ethylene production. One ml of headspace was removed by use of a gas sampling syringe and analyzed for ethylene by injecting the sample into a gas chromatograph equipped with a Porapak T column (1 m) and a flame ionization detector (SRI Instruments, Torrence, California, USA). Data were reported as acetylene reduced in μmol m^{-2} day^{-1}.

The acetylene reduction assay was calibrated by incubation of moss shoots in tubes with acetylene followed by a parallel incubation with $^{15}N_2$. Details on the calibration process are described in DeLuca et al. (2007).

There are potential problems comparing mosses of different sizes when placed in test tubes. The ratio of moss shoot volume to assay tube volume was similar for the 30 shoots in the 60-ml tubes and 10 shoots in the 20-ml tubes; however, shoot mass in the tubes differs between *H. splendens* and *P. schreberi*. To determine whether the larger size and thus higher density of *H. splendens* shoots in tubes might result in reduced efficiency of the acetylene reduction assay, we conducted a comparison of acetylene reduction rates in tubes with variable moss volume. Fresh samples of both *P. schreberi* and *H. splendens* were collected from areas of known high fixation activity. Twelve replicate samples of 10 or 30 moss shoots of each species were placed into 60-ml polyethylene tubes and were fitted with a septum and the acetylene reduction assay was performed as described above. The tubes containing 30 shoots were found to have approximately three times (2.75 for *H. splendens* and 3.0 for *P. schreberi*) greater acetylene reduction activity than that in tubes containing 10 shoots suggesting that differences in shoot mass between *P. schreberi* and *H. splendens* do not appear to represent a confounding factor in the acetylene reduction assay.

A short-term study was also performed to evaluate if N-fixation in *H. splendens*, as measured using the acetylene reduction method, follows a linear pattern when experimentally performed in glass tubes for a period of 24 h or more. Six tubes with 10 moss shoots of *H. splendens* in each were placed in a climate chamber with day/night cycle of 17 h/ 7 h and a temperature cycle of 22°C/15°C. The tubes were kept in the climate chamber for 30 h and acetylene reduction was measured at eight different occasions.

Identification and characterization of cyanobacteria

Shoots of *H. splendens* for identification of cyanobacteria were collected in spring 2005 from plots used for the season long assessment of N-fixation at Reivo. Individual pieces of moss shoots were placed on agar plates prepared with BG11-N media. The plates were incubated in a growth cabinet at 21°C with a light regime of 16 h at 60 μmol m^{-2} s^{-1} and 8 h of darkness. As soon as a single colony was observed on the plate surface, the colony was transferred using a sterile plastic loop to a fresh BG11-N agar plate. The cells were then harvested by centrifugation and DNA was extracted as described in Gentili et al. (2005).

The DNA obtained was amplified in the tRNA-Leu(UAA) intron by use of a nested PCR. The outer primers were: 5′ggaattcGGGGRTRTGGYGRAAT3′ and 5′tcccGGGGRYRGRGGGACTT3′. The inner primers were: 5′agaattcGGTAGACGCWRCGGACTT3′ and 5′acccggg

TWTACARTCRACGGATTTT3′ as described in Gentili et al. (2005). The obtained result was visualized by use of an agarose gel run in electrophoresis. The reaction was purified with the Viogene Kit "PCR-M Clean up system" according to the manufacturer's description. Then, samples were prepared for sequencing with a mixture of 300 fmol DNA and 15 pmol of the primer in a total volume of 15 μl dH$_2$O, and sequencing was performed in an ABI Sequencer. The obtained sequences were analyzed by comparison with the sequences in the NCBI database: http://www.ncbi.nlm.nih.gov/blast/Blast.cgi.

Statistical analysis

Descriptive statistical analyses were applied to all data sets. Data from the north–south gradient were simply presented using mean and standard error with no evaluation of mean separation between sites. Nitrogen-fixation rates south of 64°N were compared to those north of 64°N and N-fixation in *P. schreberi* was compared to that in *H. splendens* at this latitudinal distinction using a students *t* test. A two-way Analysis of variance (ANOVA) was used to contrast N-fixation rates across the two moss species at three fertility gradient conditions at three study sites in northern Sweden. A two-way ANOVA was used to contrast N-fixation rates in the comparison of three rates of N fertilizer and two rates of P fertilizer at a study site in northern Sweden. Since there was no N fertilizer × P fertilizer interaction, a one-way ANOVA was used to contrast treatments and the Fischer's least significant difference post hoc test was used to determine mean separation in N-fixation rates with the different N and P fertilizer treatments. Fisher's test was also used to determine mean separation in N-fixation rates within moss species at the three fertility conditions and three sites. Regression analyses were used to analyze the influence of latitude on N-fixation rate with location serving as the independent variable. All data were analyzed for conformation to the assumptions of parametric statistics. Any data not conforming to these assumptions were transformed prior to analysis or analyzed using non-parametric analyses. All data were analyzed using SPSS.

Results

Nitrogen-fixation rates, as measured by acetylene reduction, in both *P. schreberi* and *H. splendens* were clearly influenced by latitudinal position (Fig. 1). There is a marked transition in N-fixation rates in cyanobacterial associated with *P. schreberi* and with *H. spendens* at approximately 64°N. Nitrogen-fixation rates were significantly greater ($P < 0.001$) in the northern regions as compared to southern regions when data are grouped as south of 64°N
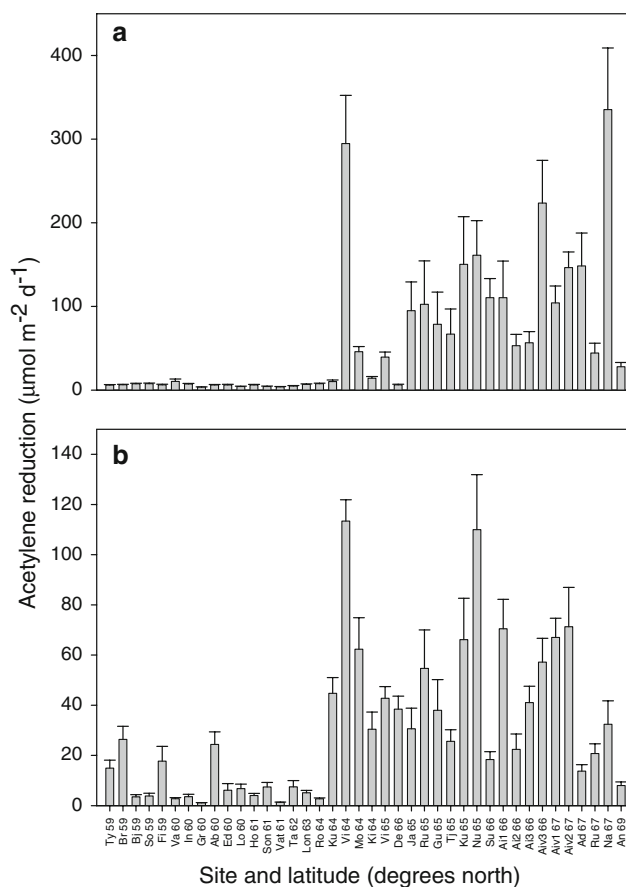

Fig. 1 Nitrogen-fixation rates as measured by acetylene reduction in **a** *P. schreberi* and **b** *H. splendens* at 40 individual sites along a latitudinal gradient (59–69°N) in Fennoscandia. *Error bars* represent 1 standard error ($n = 12$). Note differences in scale between the two mosses

and north of 64°N. At southern latitudes, there was no significant difference in N-fixation rates between *H. splendens* and *P. schreberi* whereas *P. schreberi* fixed significantly more N ($P < 0.01$) than *H. splendens* at latitudes north of 64°N.

Nitrogen fertilizer applications of 10 kg N ha^{-1} to the moss layer reduced N-fixation rates in *H. splendens* (Fig. 2) while the rate of 5 kg N ha^{-1} had no effect on N-fixation rates. Phosphorus addition alone to the moss layer had no effect on N-fixation nor was there a significant N × P interaction. This pattern was repeated at all four sampling periods over a 2 year period with no treatment by time interaction, thus Fig. 2 represents an average across all four sample dates.

Nitrogen-fixation rates were found to be directly influenced by site conditions at three fertility gradient sites in northern Sweden (Fig. 3). Data were averaged across all three sites as there was no significant site by fertility level interaction. Each of these gradients consisted of three vegetative indicators of site fertility conditions: (1) low fertility, lichen heath; (2) medium fertility, ericaceous shrub; and (3)
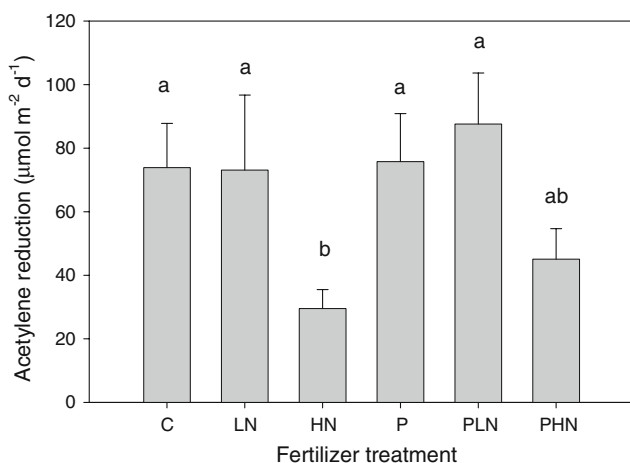
Fig. 2 Nitrogen-fixation rates as measured by acetylene reduction in *H. splendens* as influenced by N and P fertilizer treatments (*C* control, *LN* 5 kg N ha^{-1}, *HN* 10 kg N ha^{-1}, *P* 5 kg P ha^{-1}) averaged across four sample dates over a 2-year period. Differences ($P < 0.05$) in N-fixation between the fertility gradient sites (within species) are indicated by different *letters* as determined using Fisher's LSD multiple range test. *Error bars* represent 1 standard error ($n = 7$)
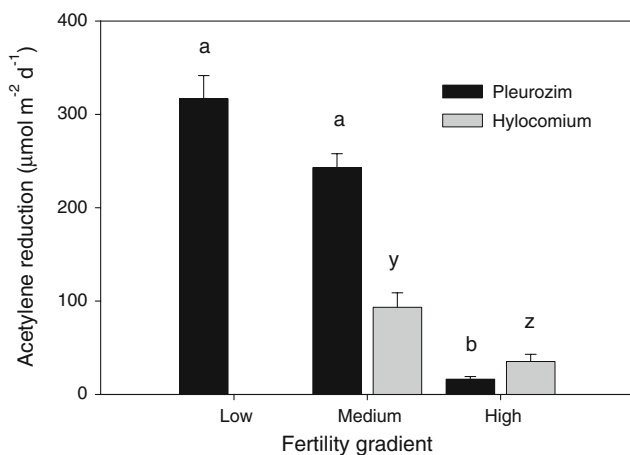

Fig. 3 Nitrogen-fixation rates as measured by acetylene reduction in *P. schreberi* and *H. splendens* as influenced by site conditions, lichen heath, ericaceous, and herb averaged across three separate fertility gradients in northern Sweden with four subplots per gradient at each site. Differences ($P < 0.05$) in N-fixation as influenced by fertility gradient (within species) are indicated by different *letters* as determined using Fisher's LSD multiple range test. *Error bars* represent 1 standard error ($n = 3$) for data averaged across the three separate sites

Fig. 4 Nitrogen-fixation rates as measured by acetylene reduction in *P. schreberi* (*closed circles*) and *H. splendens* (*open circles*) as influenced by date, measured from August 2004 through October 2006 at the Reivo Forest reserve in northern Sweden. *Error bars* represent 1 standard error ($n = 12$)

high fertility, herbaceous vegetation (see "Materials and methods"). There was a significant effect of fertility condition on N-fixation in both mosses ($P < 0.001$) and a significant effect of moss type on N-fixation rates. *Pleurozium schreberi* had higher N-fixation rates in the medium and low fertility site than in the high fertility herb site (Fig. 3). *Hylocomium splendens* was completely absent from the low fertility heath site and had significantly higher rates of
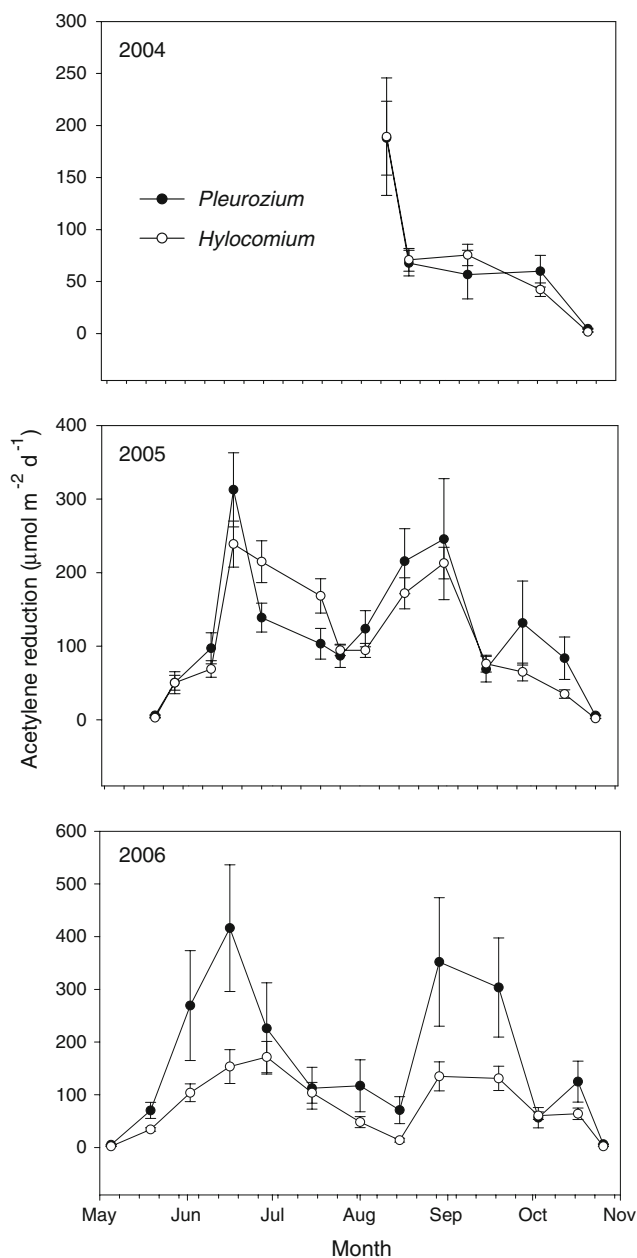
N-fixation in the high fertility herb site compared to the medium fertility ericaceous sites.

Nitrogen-fixation rates in both *P. schreberi* and *H. splendens* followed a distinct trend with season when measured from August 2004 through October 2006 (Fig. 4). Nitrogen-fixation increased in spring to a peak in June, followed by a sharp decrease in July and a subsequent peak in September. Activity was measured into late October once

snow cover was established. *Pleurozium schreberi* and *H. splendens* had a ground cover of 44 and 36%, respectively at the Reivo site where the season long assessment of N-fixation was performed. Moss shoot density was 3.6 and 1.0 shoot cm^{-2} for *P. schreberi* and *H. splendens,* respectively. The shoot density and ground cover data were used to scale up the acetylene reduction values to ecosystem N-fixation rates (as described in "Materials and methods"). The total feather moss N-fixation was estimated at 1.4 kg ha^{-1} year^{-1} for 2005 and 1.8 ha^{-1} year^{-1} for 2006. In 2005 and 2006, *P. schreberi* contributed 0.8 and 1.3 kg ha^{-1} year^{-1}, respectively, and *H. splendens* contributed 0.6 and 0.5 kg ha^{-1} year^{-1}, respectively.

There was no diurnal variation in N-fixation rates as measured as acetylene reduction (data not shown). Interestingly, the N-fixation rates steadily rose during the period of measurement from the morning, when the experiment started, and continued this trend through the night, reflecting N-fixation during periods of darkness.

By use of PCR amplification, we found that the cyanobacteria on the leaves of *H. splendens* is a *Nostoc* sp., *Peltigera pruinosa* cyanobiont (AF509435.1; Rikkinen et al. 2002). The sequence is TCAAAACTCCTAACTCATA ACTGTTCGGAAGGTGCAGAGACCCGA-GGGGAGC TACCCTAACGTTAAGTCGAGGGTAAAGGGAGAGT CCAATTCTCAAAATCTGAGCAAGCTATTGTCATCA GGTAGCAGTGAAAACTGCGGGAGAATGAAAATCC GTTGACCGTAA. In addition, species of *Stigonema* were found and identified by use of microscopy, but it was not possible to isolate this organism.

In contrast, the sequence for Nostoc sp. *Placopsis parellina* cyanobiont found on *P. schreberi* is: CTCTCAGACTC AGGGAAACCTAAATCTGGTGACAGACATGGCAAT CCTGAGCCAAGCCCAAAAATTTTAGATTTGCGATT TTAGATTTGGGATTAATTTTCAATCCAAAATCCAA AATCCAAAACCCAAAATTGAGGGAAGGTGCAGAG ACCCGACGGGAGCTACCCTAACGTTAAGTCGAGG GTAAAGGGAGAGTCCAATTCTTAAAACCTGAACT GGCTATAGCTATCAGGTAGCAGTGAAAACTGCGGA AGAATGAAAATCCGTCGATTGTA.

There is a 92% similarity between the two sequences (ncbi blast alignment, http://www.ncbi.nlm.nih.gov/BLAST/ bl2seq).

Calibration of the acetylene reduction method using ^{15}N$_2$ gas demonstrated a ratio between ethylene formed and N$_2$ reduced of 3.3 for *H. splendens* which is just slightly higher than the ratio of 3.0 reported for *P. schreberi* (DeLuca et al. 2002, 2007). However, since the N fixing cyanobacteria associated with *H. splendens* are similar to that reported for *P. schreberi* (Gentili et al. 2005), we used the ratio of 3.0 for both feather moss species when converting acetylene reduction rates to N-fixation per unit area.

Discussion

Hylocomium splendens was found to be highly similar to *P. schreberi* in terms of serving as a host to cyanobacteria associates throughout northern Fennoscandia. This relationship appears to be specific to these two feather mosses along with *Ptillium crista-castrensis* which is also found to harbor cyanobacterial associates (see also Solheim et al. 2004; Houle et al. 2006), but little or no N-fixation was observed in any other typical forest mosses in northern Sweden (Zackrisson, unpublished data) excluding *Sphagnum*. Together, *P. schreberi* and *H. splendens* often make up 80–90% of the moss cover in boreal forests making these two the most important species with regard to N accumulation in boreal ecosystems (data from the Swedish Forest Survey).

Nitrogen-fixation rates were found to be higher in cyanobacteria associated with *P. schreberi* as compared to those in *H. splendens* (Fig 1). Although *P. schreberi* accounts for greater ground cover at northerly latitudes, the differences in N-fixation along the latitudinal gradient do not include relative percentage cover, rather they are based on total acetylene reduction per m^2 assuming 100% cover within that area. The relative increase in total N-fixation with increasing latitude is likely a function of the diminishing N deposition along this same gradient (Phil-Karlsson et al. 2003), as increased N deposition has been found to yield decreased N-fixation rates in feather mosses (DeLuca et al. 2008). Although it is clear that spatial and temporal variation influenced results along the north–south gradient, variation in N-fixation in both species of moss was greatly explained by increasing latitude with N-fixation rates increasing exponentially with latitude ($y = 3E - 10e^{4E4-06x}$, $R^2 = 0.63$, $P < 0.001$).

The results from the N fertilization experiment at Reivo support the notion that increasing N deposition decreases N-fixation rates (Fig 2). In a separate study, low rates of N fertilization were found to greatly reduce N-fixation rates associated with *P. schreberi* (Zackrisson et al. 2004; DeLuca et al. 2007). Interestingly, N-fixation rates in *H. splendens* were found to be somewhat less sensitive to low dosages of N fertilizer compared to *P. schreberi* which may partially explain why *H. splendens* is capable of fixing N at southern latitudes with higher atmospheric N depositions where *P. schreberi* seemed to be inactive. Phosphorus applications did not significantly ($P > 0.05$) alter N-fixation rates when applied with N, nor did P alone significantly increase N-fixation in either moss species. This differs somewhat from earlier findings which suggested that P fertilization increased N-fixation in shoots of *P. schreberi* (Zackrisson et al. 2004), implying some level of P limitation in this species.

Moss samples collected from three natural fertility gradients in northern Sweden demonstrated a close link between

natural resource availability and N-fixation rates in both mosses. Data presented for this gradient do not take into account total coverage of the mosses at these sites, although it is reported on a square meter basis. Nitrogen-fixation in *P. schreberi* was highest in the low fertility plots and the lowest in the high fertility stands (Fig. 3). The understory of the low fertility stands at each site was dominated by reindeer lichens and *P. schreberi*, but more or less lacked the presence of *H. splendens* making it impossible to test N-fixation in *H. splendens*. The mid-fertility stand was dominated by dwarf shrubs (*Vaccinium* spp. and *E. hemaphroditum*) and demonstrated intermediate N-fixation rates in both moss species. The high fertility stands were dominated by various herbaceous species including *Geranium sylvaticum* L., *Gymnocarpium dryopteris* (L.) Newm., and *Melica nutans* L. The presence of feather mosses in these stands was greatly limited, to just that on decaying logs and stumps. The low amounts of N fixed at that herb site came almost exclusively from cyanobacteria associated with *H. splendens* as the contribution from *P. schreberi* was minimal.

The mechanism for the low N-fixation rates in mosses in the high fertility stand is not completely clear. These high fertility stands have much greater levels of N turnover than lower fertility stands (Högberg et al. 2006) and are potentially more P limited (Giesler et al. 2002). Early successional stands that exhibit higher levels of N availability have been found to have lower levels of N-fixation within feather mosses which may be attributed to greater throughfall N deposition (Zackrisson et al. 2004; DeLuca et al. 2008). Stands that had higher rates of N deposition were also observed to have extremely low rates of cyanobacterial colonization while stands with low N deposition had high rates of colonization (DeLuca et al. 2007). All three fertility gradient sites examined in this study exhibited a similar pattern wherein the high fertility herb site exhibited the significantly greater N content in throughfall and wet deposition compared to the medium and low fertility stands (data not shown) and, accordingly, the high fertility sites had the lowest levels of N-fixation in mosses.

Seasonal changes in N-fixation rates were similar in *H. splendens* and *P. schreberi* (Fig. 4). Both species exhibited a distinct drop in N-fixation rate in mid-summer, a pattern that has been repeatedly observed in *P. schreberi* (DeLuca et al. 2002; Zackrisson et al. 2004). This drop to near nondetectable levels of activity could be a function of drying mid-season conditions (cf. Chapin et al. 1991; Solheim et al. 2002) or it could be a function of photo-inhibition of the feather mosses (cf. Murray et al. 1993) as both of these conditions are greatest under the 24-h light conditions that prevail in late June and most of July. The lower N-fixation rates reported for *H. splendens* compared to *P. schreberi* is both a function of lower fixation rates per moss shoot and a lower density of moss shoots per unit area. The total ground

cover is also somewhat lower (36% for *H. splendens* compared to 44% for *P. schreberi*) at the study site. It appears that *P. schreberi* is the primary source of N via cyanobacterial N-fixation in northern boreal ecosystems where the two species co-dominate (see also Fig. 1). The mean total N-fixation of 1.6 kg ha^{-1} year^{-1} for the period 2005 and 2006 is somewhat lower than we have previously reported for late successional forests exclusively dominated by *P. schreberi* in this region (DeLuca et al. 2007). Site conditions which favor *H. splendens* and allow a co-dominance of the two feather mosses appear to reduce the total ecosystem N-fixation compared to conditions that favor pure *P. schreberi* carpets at similar late successional stages. We assume that higher N turnover in sites with a mixture of the two mosses may be a factor that down-regulates N-fixation in *P. schreberi* (DeLuca et al. 2008). Since cyanobacteria associated with *H. splendens* only fix about half that in *P. shreberi*, it proportionally adds to the lower levels of N-fixation found in carpets of pure *P. schreberi*.

The N contribution from the cyanobacterial-feather moss association in forests south of latitude 64°N is greatly restricted (on average about 0.1 kg ha^{-1} year^{-1} calculated from the assumption of 75% ground cover and co-dominance of the two feather mosses and a total season of 200 days of N-fixation). This pattern is likely best explained by the much higher atmospheric deposition of N found at lower latitudes (Phil-Karlsson et al. 2003; Fisher et al. 2007) that appears to reduce moss N-fixation (Zackrisson et al. 2004; DeLuca et al. 2008). The mean N contribution from feather moss N-fixation north of latitude 64°N was ten-fold higher (on average about 1.1 kg ha^{-1} year^{-1} for the sites studied) and may represent somewhat more natural levels of moss N-fixation that existed before the industrial era started. However, *P. schreberi* appears to be extremely sensitive to low doses of increased N deposition (DeLuca et al. 2008) and may therefore have faced reduced N-fixation rates at many northern sites (especially sites near settlements, roads and industrial sites) long before the anthropogenic air pollution effects was first noticed some 50 years ago. The air pollution effects on *H. splendens* may have been less dramatic over time, but is still of significance for existing patterns of N-fixation found along the north–south gradient.

Interestingly, there was a difference in efficiency in N-fixation between the cyanobionts on *P. schreberi* and *H. splendens*, with the latter being less efficient. This is most likely due to the fact that the *Nostoc* cyanobiont inhabiting the two mosses are different, which is also verified with the molecular analysis of the cyanobiont showing 95% similarity to *Nostoc* sp. *Placopsis parellina* cyanobiont for *P. schreberi* (Gentili et al. 2005) and 97% similarity to *Nostoc* sp. *Peltigera pruinosa* for *H. splendens*. The fact that both *H. splendens* and *P. schreberi* from the same site harbor

different *Nostoc* cyanobionts strengthens the notion that plant associations with cyanobacteria are highly selective. Selection (cost-benefit driven) in resource rich and resource poor habitats of these two feather mosses is likely a significant evolutionary driver of those differences we observed between the moss cyanobionts. Feather mosses and cyanobacteria are both extremely old organisms and may have coexisted for over 400 million years (Wellman et al. 2003; Usher et al. 2007). Evolution during this period may have led to a variety of associations between cyanobacteria and feather mosses as an adaptation strategy to allow colonization in varied geologic strata and climatic conditions worldwide. The epiphytic cyanobacterial associations found on feather mosses includes several genera (at least *Calotrix* sp., *Stigonema* sp., and *Nostoc* sp.) each with unique ecological characteristics such as temperature optima (DeLuca et al. 2002; Solheim et al. 2004; Gentili et al. 2005; Houle et al. 2006; DeLuca et al. 2007). It is tempting to speculate that the global success of these two feather mosses is at least partly dependent upon the ability to form functional associations with a range of cyanobacteria. Diversity and specificity of epiphytic cyanobacteria is, however, poorly understood (Adams and Duggan 2008), making it difficult to ascribe such cyanobiont characteristics to the global proliferation and function of the two feather mosses.

It seems reasonable to assume that the "*Hylocomium* N availability paradox" (N in precipitation and throughfall cannot account for the N used for growth) originally described by Tamm (1953, 1964) may partly be explained by the N input from cyanobacteria living in association with the moss. The amount of N fixed by cyanobacteria may be comparable to that delivered with precipitation (ca. 2 kg N ha^{-1} year^{-1}) in northern Fennoscandia where levels of atmospheric N are low (Lagerström et al. 2007). Unfortunately, it is difficult to determine the portion of the N budget in mosses that is accounted for by cyanobacteria. Further studies are required to quantify the amount of fixed N that is acquired and recycled by feather mosses and how that is influenced by other N sources available for moss growth (cf. Ayres et al. 2006; Startsev and Lieffers 2006; Salemää et al. 2008; Mankovska and Oszlanyi 2008). Experiments that include the N-budgets of these two feather mosses must also take into account the potential N input of epiphytic cyanobacterial associations. Importantly, however, the transfer and availability of fixed N to other boreal biota and soils remains poorly understood and requires further attention.

Conclusion

The two feather mosses, *H. splendens* and *P. schreberi*, fix substancial amounts of nitrogen in association with cyanobacteria in northern boreal forests. While *P. schreberi* has much higher (approximately double) N-fixation capacity at northern latitudes, both species fix N at similarly low levels at southern latitudes potentially as a result of the higher levels of anthropogenic N deposition in this region. The ecological niche separation for the two mosses found along natural fertility gradients is also reflected in their respective N-fixation rates in association with cyanobacteria. Nitrogen-fixation is highest in communities dominated by *P. scheberi* at poor sites while *H. splendens* communities appear to fix more N in more fertile locations. The cyanobacteria–*P. schreberi* association seems to be more negatively influenced by the input of exogenous or ecosystem recycled N compared to *H. splendens*.

The extremely broad global distribution of both these feather mosses is a unique characteristic. It is likely that the epiphytic association with cyanobacteria is one of the key factors contributing to the ability of these species to successfully occupy or dominate such a broad array of habitats where N available for plant growth is limited. Feather moss communities, in arctic, boreal, and temperate biomes, may together represent some of the greatest single N-fixing capacity (at least prior to anthropogenic down-regulation) of all terrestrial plant communities, not by an extremely high nitrogen fixation capacity per unit area but through the enormous large global distribution. *Pleurozium schreberi* alone may have the largest total N-fixing capacity of all naturally distributed species on earth.

References

Adams DG, Duggan PS (2008) Cyanobacteria–bryophyte symbioses. J Exp Bot 59:1047–1058

Ayres E, van der Wal R, Sommerkorn M, Bardgett RD (2006) Direct uptake of soil nitrogen by mosses. Biol Lett 2:286–288

Binkley D, Graham RL (1981) Biomass, production and nutrient cycling of mosses in an old-growth Douglas-fir forest. Ecology 62:1387–1399

Binkley D, Son Y, Valentine DW (2000) Do forest receive occult inputs of nitrogen? Ecosystems 3:321–331

Bisbee KE, Gower ST, Norman JM, Nordheim EV (2001) Environmental controls on ground cover species composition and productivity in a boreal black spruce forest. Oecologia 129:261–270

Bonan GB, Shugart HH (1989) Environmental factors and ecological processes in boreal forests. Annu Rev Ecol Syst 20:1–28

Bond-Lamberty B, Gower ST (2007) Estimation of stand level leaf area for boreal bryophytes. Oecologia 151:584–592

Brown DH, Bates JW (1990) Bryophytes and nutrient cycling. Bot J Linn Soc 104:129–147

Chapin FS, Oechel WC, Van Cleve K, Lawrence W (1987) The role of mosses in the phosphorus cycling of an Alaskan black spruce forest. Oecologia 74:310–315

Chapin DM, Bliss LC, Bledsoe LJ (1991) Environmental regulation of Nitrogen-fixation in a high arctic lowland ecosystem. Can J Bot 69:2744–2755

Chapin FS, Walker LR, Fastie CL, Sharman LC (1994) Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. Ecol Monogr 64:149–175

Cleveland CC, Townsend AR, Schimel DS, Fisher H, Howarth RW, Hedin LO, Perakis SS, Latty EF, Von Fischer JC, Elseroad A, Wasson MF (1999) Global patterns of terrestrial biological nitrogen (N_2) fixation. Global Biogeochem Cycles 13:623–645

DeLuca TH, Zackrisson O, Nilsson M-C, Sellstedt A (2002) Quantifying nitrogen-fixation in feather moss carpets of boreal forests. Nature 419:917–920

DeLuca TH, Zackrisson O, Gentili F, Sellstedt A, Nilsson M-C (2007) Ecosystem controls on nitrogen-fixation in boreal feather moss communities. Oecologia 152:121–130

DeLuca TH, Zackrisson O, Gundale MJ, Nilsson M-C (2008) Ecosystem feedbacks and nitrogen-fixation in boreal forests. Science 320:1181–1181

Engelmark O (1999) Boreal forest disturbance. In: Walker RL (ed) Ecosystems of disturbed ground. Elsevier, Burlington, pp 161–186

Fisher R, Mues V, Ulrich E, Becher G, Lorenz M (2007) Monitoring of atmospheric deposition in European forests and an overview on its implication on forest condition. Appl Geochem 22:1129–1139

Forsum Å, Dahlman T, Näsholm T, Nordin A (2006) Nitrogen utilization by *Hylocomium splendens* in a boreal forest fertilization experiment. Funct Ecol 20:421–426

Gentili F, Nilsson MC, Zackrisson O, DeLuca TH, Sellstedt A (2005) Physiological and molecular diversity of feather moss associative N_2 fixing cyanobacteria. J Exp Bot 56:3121–3127

Giesler R, Högberg P, Peterssen T (2002) Phosphorus limitation in boreal forests: effects of aluminum and iron accumulation in the humus layer. Ecosystems 5:300–314

Gruber N, Galloway JN (2008) An earth-system perspective of the global nitrogen cycle. Nature 451:293–296

Hägglund B, Lundmark JE (1977) Site index estimation by means of site properties. Stud For Suec 138:1–34

Hart SA, Chen HYH (2008) Fire, logging and understory abundance, diversity and composition in boreal forest. Ecol Monogr 78:123–140

Högberg MN, Myrold DD, Giesler R, Högberg P (2006) Contrasting patterns of soil N-cycling in model ecosystems of Fennoscandian boreal forests. Oecologia 147:96–107

Houle D, Gauthier SB, Paquet S, Planas D, Warren A (2006) Identification of two genera of nitrogen fixing cyanobacteria growing on three feathermoss species in boreal forests of Quebec, Canada. Can J Bot 84:1025–1029

Huttunen S, Karhu M, Kallio S (1981) The effect of air pollution on transplanted mosses. Silva Fenn 15:495–504

Kuc M (1997) The northernmost extension of the moss *Pleurozium schreberi* (Brid.) Mitt. in the Canadian high arctic. Can Field Nat 111:630–633

Lagerström A, Nilsson M-C, Zackrisson O, Wardle D (2007) Ecosystem input of nitrogen through biological fixation in feather mosses during ecosystem retrogression. Funct Ecol 21:1027–1033

Longton RE (1992) Role of bryophytes and lichens in terrestrial ecosystems. In: Bates JW, Farmer AM (eds) Bryophytes and lichens in an changing environment. Clarendon, Oxford, pp 32–76

Lovett GM, Lindberg ST (1993) Atmospheric deposition and canopy interactions of nitrogen in forests. Can J For Res 23:1603–1616

Makipää R, Heikkinen J (2003) Large-scale changes in abundance of terricolous bryophytes and macrolichens in Finland. J Veg Sci 14:497–508

Mankovska B, Oszlanyi J (2008) Mosses and foliage of forest tree species as biomonitors of nitrogen pollution. Int J Environ Stud 65:377–387

Murray MJ, Tenhunen JD, Nowak RS (1993) Photoinhibition as a control on photosynthesis and production of sphagnum mosses. Oecologia 96:200–207

Nilsson M-C, Wardle D (2005) Understory vegetation as a forest ecosystem driver: evidence from the northern Sweden boreal forest. Front Ecol Environ 3:421–428

Nyholm E (1965) Illustrated moss flora of Fennoscandia. II. Musci. Gleerup, Lund

Ochyra R, Brednarek-Ochyra H (2002) Pleurozium schreberi recorded for tropical Africa and a review of its world distribution. Cryptogam Bryol 23:355–360

Oechel WC, Van Cleve K (1986) The role of bryophytes in nutrient cycling in the taiga. In: Van Cleve K, Chapin FS, Flanagan PW, Viereck L, Dyrness LA (eds) Forest ecosystems in the Alaskan taiga. Springer, New York, pp 121–137

Påhlsson L (1994) Vegetationstyper i Norden. Nordiska Ministerrådet, Köpenhamn

Payette S (1992) Fire as a controlling process in the North American boreal forest. In: Shugart HH (ed) A system analysis of the global boreal forest. Cambridge University Press, Cambridge, pp 144–169

Persson H, Viereck LA (1983) Collections and discussions of some bryophytes from Alaska. Lindbergia 9:5–20

Phil-Karlsson G, Blomgren H, Pettersson K, Svensson A, Sjöberg K (2003) Nationell miljöövervakning av luft- och nederbördskemi 2002. IVL Report U 852. Swedish Environmental Institute, Göteborg, Sweden

Rastetter EB, Vitousek PM, Field C, Shaver GR, Herbert D, Agren GI (2001) Resource optimization and symbiotic nitrogen-fixation. Ecosystems 4:369–388

Rikkinen J, Oksanen I, Lothander K (2002) Lichen guilds share related cyanobacterial symbionts. Science 297:357

Salemää M, Mäkipää R, Oksanen J (2008) Differences in growth response of three bryophyte species to nitrogen. Environ Pollut 152:82–91

Schofield WB (1992) Bryophyte distribution patterns. In: Bates JW, Farmer AM (eds) Bryophytes and lichens in a changing environment. Clarendon Press, Oxford, pp 103–130

Schöllhorn R, Burris RH (1967) Acetylene as a competitive inhibitor of Nitrogen-fixation. Proc Natl Acad Sci USA 58:213–218

Smithwick EAH, Turner M, Mack MC, Chapin FSIII (2005) Post fire soil N cycling in northern conifer forests affected by severe, stand replacing wildfires. Ecosystems 8:163–181

Solheim B, Johanson U, Callaghan TV, Lee JA, Gwynn-Jones D, Björn LO (2002) The Nitrogen-fixation potential of arctic cryptogram species is influenced by enhanced UV-B radiation. Oecologia 133:90–93

Solheim B, Wiggen H, Roberg S, Spaink HP (2004) Associations between arctic cyanobacteria and mosses. Symbiosis 37:169–187

Startsev NA, Lieffers VJ (2006) Dynamics of mineral nitrogen released from feathermosses after dehydration or handling stress. Bryologist 109:551–559

Tamm CO (1953) Growth, yield and nutrition in carpets of a forest moss (*Hylocomium splendens*). Medd Statens Skogsforskningsinst (Swed) 42:1–140

Tamm CO (1964) Growth of *Hylocomium splendens* in relation to tree canopy. Bryologist 64:423–426

Turetsky MR (2003) The role of bryophytes in carbon and nitrogen cycling. Bryologist 106:395–409

Usher KM, Bergman B, Raven JA (2007) Exploring cyanobacterial mutualism. Annu Rev Ecol Evol Syst 38:255–273

Vitousek PM, Cassman K, Cleveland C, Crews T, Field CB, Grimm NB, Howarth RW, Marino R, Martinelli L, Rastetter EB, Sprent JI (2002) Towards an ecological understanding of biological Nitrogen-fixation. Biogeochemistry 57(58):1–45

Walter H, Breckle SW (1989) Ecological systems of the geobiosphere. Springer, Berlin

Wardle DA, Zackrisson O (2005) Effects of species and functional group loss on island ecosystem properties. Nature 435:806–810

Wardle DA, Zackrisson O, Nilsson MC (1998) The charcoal effect in boreal forests: mechanisms and ecological consequences. Oecologia 115:419–426

Wardle DA, Hörnberg G, Zackrisson O, Coomes DA (2003a) Long-term effects of wildfire on ecosystem properties across an island area gradient. Science 300:972–975

Wardle DA, Nilsson M-C, Zackrisson O, Gallet C (2003b) Determinants of litter mixing effects in a Swedish boreal forest. Soil Biol Biochem 35:827–835

Weetman GF (1969) The relationship between feather moss growth and nutrient content under upland black spruce. Proceedings of 3rd International Peat Congress. Quebec, Canada, pp 366–370

Wellman CH, Osterloff PL, Mohiuddin U (2003) Fragments of the earliest land plants. Nature 425:282–284

Zackrisson O, Nilsson M-C, Dahlberg A, Jäderlund A (1997) Interference mechanisms in conifer-Ericaceae-feathermoss communities. Oikos 78:209–220

Zackrisson O, Dahlberg A, Norberg G, Nilsson M-C, Jäderlund A (1998) Experiments on the effects of water availability and exclusion of fungal hyphae on nutrient uptake and establishment of *Pinus sylvestris* seedlings in carpets of the moss *Pleurozium schreberi*. Ecoscience 5:77–85

Zackrisson O, DeLuca TH, Nilsson M-C, Sellstedt A, Berglund L (2004) Nitrogen-fixation increases with successional age in boreal forests. Ecology 85:3327–3334

Zechmeister HG, Hohenwallner D, Riss A, Hanus-Illar A (2005) Estimation of element deposition derived from road traffic sources by using mosses. Environ Pollut 138:238–249