Nitrogen fixation by cyanobacteria associated to feathermosses
- A comparison between Scots pine and Norway spruce stands

Foto: Felicia Dahlgren Lidman

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Kvävefixering av cyanobakterier associerade till hus- och väggmossa. - En jämförelse mellan tall och granbestånd

Felicia Dahlgren Lidman

Keywords / Nyckelord:
Boreal skog, Hylocomium splendens, pleurozium schreberi, Pinus sylvestris, Picea abies, acetylenreduktions analys / Boreal forest, Hylocomium splendens, Pleurozium schreberi, Pinus sylvestris, Picea abies, acetylene reduction assay
I denna rapport redovisas ett examensarbete utfört vid Institutionen för skogens ekologi och skötsel, Skogsvetenskapliga fakulteten, SLU. Arbetet har handletts och granskats av handledaren, och godkänts av examinator. För rapportens slutliga innehåll är dock författaren ensam ansvarig.

This report presents an MSc/BSc 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 limitations of available nutrients are strong in the boreal forest, especially of nitrogen (N). Due to slow mineralization rate of organic soil nitrogen, caused by low temperatures and acidic soils. Few organisms can fixate atmospheric nitrogen. Among those who have the ability there are some that are common in the boreal forest. Cyanobacteria associated to feather mosses are one example. This study is focused on finding if there is a difference in N2 fixation rate by cyanobacteria associated to feather mosses between forest stands of different tree species. The two tree species that are compared are Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) H. Karst). In a study by Nilsson *et al.* (2012) the Scots pine stands produced much more volume compared to the Norway spruce. The hypothesis of this study is that N2 fixation by cyanobacteria associated to feather mosses, would enhance N input in particularly Scots pine stands. Thereby contribute to the larger volume production of these stands compared to the Norway spruce stands. The study is carried out on some of the plots used by Nilsson *et al.* (2012), along with some other similar ones. Samples of the feather moss species *Hylocomium splendens* (Hedwig) and *Pleurozium schreberi* (Bridal) was collected from 14 sites on 6 locations, half of which was Scots pine stands and half Norway spruce stands. An acetylene reduction assay was used in combination with a moss biomass inventory, to estimate N2 fixation rates. Results showed that the moss biomass of the two species *Hylocomium splendens* and *Pleurozium schreberi* was significantly larger in the Norway spruce stands than in the Scots pine stands. There was also a tendency that estimated N2 fixation rate per unit moss mass was higher in Norway spruce than Scots pine stands. The difference between the stands in estimated N2 fixation rate per hectare and year was not significant. In conclusion the study indicates that the N2 fixation in the moss layer does not have notable effect on the volume growth of the trees in this study.

**Keywords:** boreal forest, *Pinus sylvestris*, *Picea abies*, acetylene reduction assay.
**Sammanfattning**

I den boreala skogen är tillgången på näringsämnen, speciellt av kväve, begränsande. Detta beror på den långsamma nedbrytningen av organiskt material orsakad av låga temperaturer och jordar med lågt pH. Få organismer kan fixera kväve från luften. Ett exempel på en av de organismer som har denna förmåga är cyanobakterier associerade till boreala bladmossor, vilka är vanligt förekommande på grund av låg pH. Fruktbakterier kan fixera kväve från luften.

**Tillämpning**


**Métoden**

Studien använde acetyleen-reduktions analys i kombination med en mossbiomassainventering i de olika bestånden, för att skatta kvävefixeringen per hektar. Resultaten visade att moss-biomassan för två arterna *Hylocomium splendens* (Hedwig) och *Pleurozium schreberi* (Bridal) samlades in från 14 bestånd på 6 olika lokaler. Hälften av bestånden var tallbestånd och hälften granbestånd. Acetylen-reduktions analys användes i kombination med en mossbiomassainventering i de olika bestånden, för att skatta kvävefixeringen per hektar. Resultaten visade att moss-biomassan för de två arterna *H. splendens* och *P. schreberi* var signifikant större i gran- än i tallbestånden. Det fanns även en tendens till att kvävefixeringshastigheten per gram mossor var högre i gran- än i tallbestånden, men skillnaden var inte signifikant. Kvävefixering associerad till mossor verkade alltså inte ha någon betydelse för den observerade högre trädtillväxten i tall- än i granbestånd.

**Nyckelord:** boreal skog, kväve fixering, *Pinus sylvestris, Picea abies, acetyl- len reduktions analys.*
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1 Introduction

1.1 The boreal forest

The boreal forest is the biome that occupies the zone between 50° and 65° N, which includes most of Sweden (Ahti et al., 1968). It is the largest biome and holds one-third of earth’s forested land. The climate is characterized by low average annual temperature and low precipitation (Cain et al., 2014). Because of the last glaciation, the plant diversity is quite low in the boreal forest compared to other biomes (Townsend et al., 2008). Conifers are dominating the canopy of the boreal forest (Cain et al., 2014). Norway spruce (Picea abies (L.) H. Karst) and Scots pine (Pinus sylvestris L.) are the dominating tree species in Sweden. Ericaceous dwarf shrubs, bryophytes and lichen dominate the ground vegetation. Two bryophytes that are very common are the feather mosses Pleurozium schreberi (Bridal) and Hylocomium splendens (Hedwig), which in 2008-2016 was estimated to cover 19.5 and 12.9% respectively of the ground layer in Sweden’s productive forests (SLU, 2017).

1.2 The role of nitrogen in the boreal forest

The boreal forest stores more organic matter than any other terrestrial biome (Anderson, 1991) and it occupies 11% of the earth’s terrestrial surface (Bonan & Shugart, 1989). The limitations of available nutrients, especially nitrogen (N) are strong in many boreal forest ecosystems (Grime, 1979). This has been considered to be caused by slow mineralization rate of organic soil N (Tamm, 1991; Vitousek & Howarth, 1991). The slow rate of mineralization is caused by low temperatures in combination with acidic soils (Jarvis & Linder, 2000; Swift et al., 1979). The fact that the anthropogenic N deposition in boreal forests often are low (Gundale et al., 2011), is another reason to why N is more limiting here than in other biomes. Even tough N is dominating as atmospheric element, very few life forms can access it due to its none-reactive nature (N2) (Schelsinger, 1997). The microorganisms with the ability to fixate atmospheric N2 are called diazotrophs (Weil & Brady, 2008). Some of the few fungi and plants that are known to form symbiosis with diazotrophs in boreal forests are e.g. Alnus and cyanolichens (Henskens et al., 2012; Mitchell &
Furthermore, feather mosses *H. splendens* and *P. schreberi* hosts large populations of N\textsubscript{2} fixing cyanobacteria (Gundale *et al.*, 2012; Zackrisson *et al.*, 2009; Lagerström *et al.*, 2007; DeLuca *et al.*, 2002). N\textsubscript{2} fixing cyanobacteria associated to feather mosses is today believed to be a major source of N input to the boreal forest ecosystem (Markham, 2009; DeLuca *et al.*, 2002).

### 1.3 Nitrogen fixation by cyanobacteria

On the feather mosses *H. splendens* and *P. schreberi* in the boreal forest, several epiphytic cyanobacteria have been found, for example strains of *Nostoc sp.*, *Cylindrospermum sp.*, *Stigonema sp.* and *Calothrix sp.* (Bay, 2013; Ininbergs *et al.*, 2011; Zackrisson *et al.*, 2009; Houle *et al.*, 2006; Gentili *et al.*, 2005; DeLuca *et al.*, 2002). N\textsubscript{2} fixation rates of cyanobacteria associated to feather mosses are controlled by several factors. The fixation rate can be reduced or inhibited by external N supply (Sorensen *et al.*, 2012), such as anthropogenic N deposition (Gundale *et al.*, 2011; DeLuca *et al.*, 2008). It has also been shown that biomass of bacteria capable of biological N\textsubscript{2} fixation often is reduced by external N supply (Demoling *et al.*, 2008; Wallenstein *et al.*, 2006). The abundance and growth rate of cyanobacteria is shown to be light dependent, due to the N\textsubscript{2} fixations energetically expensive process (Staal *et al.*, 2001; Gerard *et al.*, 1990). Another factor of importance when it comes to biological N\textsubscript{2} fixation rates is temperature, since that the nitrogenase enzyme is temperature dependent when it comes to optimal activity, which also is species-specific (Gundale *et al.*, 2012; Gentili *et al.*, 2005; Zielke *et al.*, 2002; Kashyap *et al.*, 1991).

### 1.4 Scots pine and Norway spruce in the Swedish forest

Sweden’s total standing timber volume is estimated to 3445 m\textsuperscript{3}sk, the Norway spruce and Scots pine holds approximately 40% of it each (SLU, 2017). The Scots pine is a pioneer species and the spruce is a late successional species (Engelmark & Hytteborn, 1999). The Scots pine is in general growing faster than Norway spruce in northern Sweden (Bergh *et al.*, 2010; Heiskanen & Mäkitalo, 2002; Björkman, 1953) and the Norway spruce is in general growing faster than Scots pine in southern Sweden (Ekö *et al.*, 2008). Due to that the availability of nutrients are related to temperature (Björkman, 1953). Increased temperatures can indirectly affect the availability of nutrients, by increasing mineralization and decomposition of organic matter (Jarvis & Linder, 2000).

The study (Nilsson *et al.*, 2012) compared the stem volume production of the two tree species with the hypothesis that the yields of Norway spruce and Scots pine are similar on sites carrying medium fertility. This hypothesis were in line with the
results of Ekö et al. (2008), were both species was stated to have equal volume production in the north of Sweden. The plots used by Nilsson et al. (2012) consisted out of pairs of Norway spruce and Scots pine stands that was sown or planted next to each other at relatively the same point in time. Their replicates were spread out over several locations from middle to northern Sweden. The layout of their replicates is what makes their trial quite special compared to other studies. However, stem wood production of Norway spruce was only 29.4% of the Scots pine and did not support their hypothesis. Norway spruce was expected to give higher yields compared to Scots pine on rich sites in the same study, this was not the case, contradicting previous studies. Over all this means that some of Nilsson et al. (2012) results isn’t corresponding to the common perception that Norway spruce is more suited for fertile sites then Scots pine (Albrektson et al., 2012). This deviant result is what caused the idea of this study to occur.

1.5 Nitrogen fixation and volume growth

This study is to be done on some of the stands used by (Nilsson et al., 2012), with a focus on biological N\textsubscript{2} fixation by cyanobacteria associated to feather mosses. The hypothesis that has grown out of the facts presented above is that the N\textsubscript{2} fixation might be greater in Scots pine stands then in Norway spruce stands. The higher N\textsubscript{2} fixation would then lead to a higher concentration of N in the humus layer, which over time would decompose and be of favour to the trees volume growth. This would then perhaps explain partly why the Scots pine is producing more volume than the Norway spruce in the study by Nilsson et al. (2012).

1.6 Aim

The aim of this study is to estimate and compare between Scots pine and Norway spruce forest stands: 1) the N\textsubscript{2} fixation rate of cyanobacteria associated to feather mosses Pleurozium schreberi and Hylocomium splendens, and 2) moss-biomass for the two species of moss.
2 Material and Methods

2.1 Plots

The 14 plots used in this study are part of a long-term experiment of forest management called Tiréns plots, managed by the Swedish University of Agricultural Sciences (SLU). The 14 plots are spread over the county of Jämtland and the county of Västerbotten, in the northern parts of Sweden. Seven out of the 14 plots are Scots pine \((\textit{Pinus sylvestris})\) stands and seven are Norway spruce \((\textit{Picea abies})\) stands. The plots are spread over six locations between 63.14° and 64.18° N. There is one spruce stand and one pine stand on each location, except for Flakaträsk which has double replicates (Figure 1, Table 1). All the Scots pine stands have produced larger volumes than the Norway spruce stands (Table 1).
Figure 1. Map over Sweden with the six locations of the 14 study plots.

Table 1. Descriptions over the 14 plots and their locations.

<table>
<thead>
<tr>
<th>Site name</th>
<th>Tree species</th>
<th>Establishment Method and year</th>
<th>Stand volume b m³ ha⁻¹ and (year for latest inventory)</th>
<th>Centrum coordinates wgs84</th>
</tr>
</thead>
<tbody>
<tr>
<td>1028 Kulbäcksliden</td>
<td>Scots pine</td>
<td>Pl 1950</td>
<td>421.5 (2008)</td>
<td>64.178727, 19.606340</td>
</tr>
<tr>
<td>1061 Övra</td>
<td>Scots pine</td>
<td>Ds 1943</td>
<td>485.6 (2017)</td>
<td>63.977328, 16.740322</td>
</tr>
<tr>
<td>1062 Björkheden</td>
<td>Scots pine</td>
<td>Pl 1948</td>
<td>495.9 (2017)</td>
<td>63.425684, 16.061748</td>
</tr>
<tr>
<td>2458 A Flakaträsk</td>
<td>Scots pine</td>
<td>Pl 1966</td>
<td>400.2 (2015)</td>
<td>64.270760, 18.497102</td>
</tr>
<tr>
<td>2458 E Flakaträsk</td>
<td>Scots pine</td>
<td>Pl 1966</td>
<td>362.3 (2015)</td>
<td>64.270760, 18.497102</td>
</tr>
<tr>
<td>1017 Fillsta</td>
<td>Norway spruce</td>
<td>Pl 1952</td>
<td>135.1 (2011)</td>
<td>63.142899, 14.545037</td>
</tr>
<tr>
<td>1029 Kulbäcksliden</td>
<td>Norway spruce</td>
<td>Pl 1949</td>
<td>63.8 (2008)</td>
<td>64.179375, 19.605235</td>
</tr>
<tr>
<td>1037 Häsjön</td>
<td>Norway spruce</td>
<td>Pl 1949</td>
<td>107.3 (2009)</td>
<td>63.472234, 16.223675</td>
</tr>
<tr>
<td>1061 Övra</td>
<td>Norway spruce</td>
<td>Ds 1943</td>
<td>123.5 (2017)</td>
<td>63.977328, 16.740322</td>
</tr>
<tr>
<td>1062 Björkheden</td>
<td>Norway spruce</td>
<td>Pl 1952</td>
<td>165.4 (2017)</td>
<td>63.425684, 16.061748</td>
</tr>
<tr>
<td>2458 A Flakaträsk</td>
<td>Norway spruce</td>
<td>Pl 1966</td>
<td>129.5 (2015)</td>
<td>64.270760, 18.497102</td>
</tr>
<tr>
<td>2458 E Flakaträsk</td>
<td>Norway spruce</td>
<td>Pl 1966</td>
<td>162.7 (2015)</td>
<td>64.270760, 18.497102</td>
</tr>
</tbody>
</table>

* Pl = Planting, Ds = direct seeding.

2.2 Moss samples for acetylene reduction assay

At each of the 14 plots (Figure 1, Table 1), five moss samples were randomly collected from each of the two species of moss, *H. splendens* and *P. schreberi* resulting in a total of 140 moss samples. Each sample consisted of five *H. splendens* shoots or ten *P. schreberi* shoots that were placed in glass vials. Only the upper 2.5 cm was collected of each shoot, following DeLuca *et al.* (2002). All the vials were thereafter stored in a Styrofoam tray in the shade during the sampling on each plot. The samples were placed in a cooler and transported to the laboratory in Umeå at SLU. All samples were collected between the 14th and 15th of September 2017. The moss samples were stored in a climate chamber in the lab which had the approximate temperature of 16 °C, 75% humidity and 80% light intensity. All samples were stored for three to four days in the climate chamber, before the acetylene reduction assay. The moss samples were incubated 24h before they were run through the gas chromatograph (*Clarus 500 Gas Chromatograph, with autosampler Turbomatrix Headspace Sampler*). At incubation the vials were sealed, 2 ml of air was removed and 2 ml of acetylene gas was injected in each vial. Two vials without moss were injected in parallel with the moss samples and used as controls. The gas chromatograph quantified the amount of acetylene converted to ethylene by each sample during the incubation. The ratio of three moles ethylene reduced per mole N₂ fixated was used to calculate the amount N₂ fixated by the cyanobacteria, following (Lagerström *et al.*, 2007; Zackrisson *et al.*, 2004; DeLuca *et al.*, 2002). The samples were then dried for 48h at 70 °C and weighed so g N fixated kg⁻¹ moss d⁻¹ could be determined.

2.3 Collection of moss biomass data

The moss biomass data was collected approximately three weeks after the samples for the acetylene reduction assay were collected on the 14 plots (Figure 2). On each of the 14 plots two 25m long transects were laid out diagonally. On each of the two transects, circular sample plots were placed with the interval of two meters, starting two meters from each site corner. From each sample plot all aboveground living moss biomass was collected and stored in plastic zip-lock bags, including the moss that was still recognizable as moss but brown in the colour.
The samples were sorted by species, dried for 48h at 70 °C and weighed. The results from the moss biomass inventory were used in combination with the results from the acetylene reduction assay to upscale the N fixation rate to kg N ha\(^{-1}\) yr\(^{-1}\). A growth period of 200 days was used, following DeLuca \textit{et al.} (2002).

\textit{Figure 2.} Pictures of two of the plots showing:
a) Plot 2458A Flakaträsk a Norway spruce stand,
b) Plot 2458A Flakaträsk a Scots pine stand.
Red pole for proportion. Photo: Felicia Lidman.
2.4 Statistics

To test the significance of the difference in moss-cyanobacteria N₂ fixation rate between the Scots pine and Norway spruce forest stands, a statistical analysis in the form of a paired t-test was performed. The variables included in the statistical analysis were average moss biomass per hectare and the yearly N₂ fixation per hectare, for both moss species *H. splendens* and *P. schreberi*. As well as average N₂ fixation rate per unit moss mass and day, for each of the two moss species individually. All statistical analyses were done in Minitab 17.
3 Results

There was a significant difference in moss biomass between the stands of the two tree species (Table 2). Moss biomass was approximately four times higher in the Norway spruce stands than in the Scots pine stands (Figure 3). In the Scots pine stands the average biomass for both moss species was $342.6 \text{ kg hectare}^{-1}$ while in the Norway spruce stands it was $1408.3 \text{ kg hectare}^{-1}$ (Figure 3). There was no significant difference in average N$_2$ fixation rate by cyanobacteria associated to the feather mosses *H. splendens* and *P. schreberi* separately per unit moss mass and day ($\mu$g N g$^{-1}$ moss day$^{-1}$), between Norway spruce and Scots pine stands (Figure 3), (Table 2). For *H. splendens* the average daily N$_2$ fixation rate per unit moss mass was estimated to $0.78 \mu$g N g$^{-1}$ moss day$^{-1}$ in the Scots pine stands and $3.13 \mu$g N g$^{-1}$ moss day$^{-1}$ in the Norway spruce stands (Figure 3). For *P. schreberi* the average daily N$_2$ fixation rate per unit moss mass was estimated to $0.03 \mu$g N g$^{-1}$ moss day$^{-1}$ in the Scots pine stands and $0.23 \mu$g N g$^{-1}$ moss day$^{-1}$ in the Norway spruce stands (Figure 3). There was no significant difference in yearly N$_2$ fixation per hectare for both moss species *H. splendens* and *P. schreberi* combined between Scots pine and Norway spruce stands (Table 2). The average yearly N$_2$ fixation per hectare for both mosses was estimated to $0.04 \text{ kg N hectare}^{-1} \text{ year}^{-1}$ in the Scots pine stands and $0.432 \text{ kg N hectare}^{-1} \text{ year}^{-1}$ in the Norway spruce stands (Figure 3).

There is a large variation in average yearly N$_2$ fixation per hectare between plots, dominating tree species and moss species (Figure 4). There is large variation within the same location as well, for example the plots 2458A and 2458E Flakasträsk for Scots pine and Norway spruce are all in the same location (Figure 4). The large variations between plots and within the same moss and tree species (Figure 4) is causing the large standard errors (Figure 3).
Figure 3. Mean of: a) moss biomass per hectare (kg dry mass hectare\(^{-1}\)), b) N\(_2\) fixation rate per gram moss and day (µg N g\(^{-1}\) moss day\(^{-1}\)) and c) N\(_2\) fixation rate per hectare and year (kg N hectare\(^{-1}\) year\(^{-1}\)) for the moss species *Hylocomium splendens* and *Pleurozium schreberi*, in stands dominated either by Norway spruce or Scots pine. Error bars show ±1 SE.
Figure 4. Average N₂ fixation rate by cyanobacteria per hectare and year (kg N hectare⁻¹ year⁻¹), for moss species *Hylocomium splendens* and *Pleurozium schreberi* for each plot, in stands dominated either by a) Norway spruce or b) Scots pine.
Table 2. The results of paired t-tests for moss biomass (kg dry mass hectare$^{-1}$) and $\text{N}_2$ fixation per hectare and year (kg $\text{N}$ hectare$^{-1}$ year$^{-1}$) for the moss species *Hylocomium splendens* and *Pleurozium schreberi* combined. Results of paired t-tests for $\text{N}_2$ fixation rate per unit moss mass and day ($\mu$g $\text{N}$ g$^{-1}$ moss day$^{-1}$) for each of the mosses *Hylocomium splendens* and *Pleurozium schreberi* separately. All four variables are compared between Scots pine and Norway spruce stands. n=7

<table>
<thead>
<tr>
<th>Dominating tree species</th>
<th>Moss biomass (kg dry mass hectare$^{-1}$)</th>
<th>$\text{N}_2$ fixation (kg $\text{N}$ hectare$^{-1}$ year$^{-1}$)</th>
<th>$\text{N}_2$ fixation ($\mu$g $\text{N}$ g$^{-1}$ moss day$^{-1}$) for <em>P. schreberi</em></th>
<th>$\text{N}_2$ fixation ($\mu$g $\text{N}$ g$^{-1}$ moss day$^{-1}$) for <em>H. splendens</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>-18.78</td>
<td>-1.95</td>
<td>-1.57</td>
<td>-1.51</td>
</tr>
<tr>
<td>P</td>
<td>0.000</td>
<td>0.099</td>
<td>0.167</td>
<td>0.181</td>
</tr>
</tbody>
</table>
4 Discussion

4.1 The difference in moss biomass and $\text{N}_2$ fixation rate between Scots pine and Norway spruce stands

This study states that average moss biomass per hectare was significantly higher in the Norway spruce stands than in the Scots pine stands. Also, the average $\text{N}_2$ fixation rate per unit moss mass and day associated to each of the mosses $H. \text{splendens}$ and $P. \text{schreberi}$, tended to be higher in the Norway spruce stands, but the difference between the stand types was not significant. The same result was found for average $\text{N}_2$ fixation rate per hectare and year. However, the number of replicates in this study was rather low and the sampling took place at only one occasion during the snow free season. $\text{N}_2$ fixation rate by cyanobacteria associated to feather mosses is varying throughout the growth season (DeLuca et al., 2007; DeLuca et al., 2002). This is resulting in that repeated measurements during a longer period might have strengthen the results of this study.

The results of the acetylene reduction assay in this study showed $\text{N}_2$ fixation rates varying between 0 and 1.6 kg N ha$^{-1}$ yr$^{-1}$ depending on moss species, location and tree species. This is in line with the results of similar studies, in which $\text{N}_2$ fixation rates associated to mosses varies from 1.5 to 2 kg N ha$^{-1}$ yr$^{-1}$ (Lagerström et al., 2007; Zackrisson et al., 2004; DeLuca et al., 2002; Cleveland et al., 1999), depending on the successional stage of the forest. That $\text{N}_2$ fixation associated to $H. \text{splendens}$ was larger than that of $P. \text{schreberi}$ for almost all plots is also consistent with the results of previous studies (Lagerström et al., 2007; Houle et al., 2006). Houle et al. (2006) found a larger abundance of $\text{N}_2$ fixation capable cyanobacteria on $H. \text{splendens}$ compared to $P. \text{schreberi}$, explaining the difference between the species in $\text{N}_2$ fixation.
4.2 Factors affecting N\textsubscript{2} fixation rates

There are several factors known to affect the N\textsubscript{2} fixation rate of cyanobacteria associated to feather mosses, like for example moisture, as the rate increases with increasing moisture (Jackson et al., 2011; Stewart et al., 2011; Gundale et al., 2009). Latitude is another factor that affects the N\textsubscript{2} fixation rate of cyanobacteria associated to feather mosses significantly, due to the larger N deposition in southern than northern Sweden (Zackrisson et al., 2009; DeLuca et al., 2008). Increasing N availability correlates with decreasing N\textsubscript{2} fixation of cyanobacteria associated to feather mosses (Sorensen et al., 2012; DeLuca et al., 2008; Zackrisson et al., 2004). In addition different species of cyanobacteria have different temperature optima for N\textsubscript{2} fixation (Gentili et al., 2005). Light is affecting the N\textsubscript{2} fixation rate of cyanobacteria (Gundale et al., 2012; Zielke et al., 2002), especially when interacting with temperature for cyanobacteria associated to feather mosses. Light increases N\textsubscript{2} fixation at low temperatures and can have damaging effects at high temperatures (Gundale et al., 2012). Several of these factors can be excluded to have any effect in this study, since the climatic conditions after collection and during incubation was the same for all samples. The relevant factors here are those that potentially differ between Scots pine and Norway spruce stands, and are thereby contributing to a difference in N\textsubscript{2} fixation rate between the two stand types. N availability seems to be a possible non-climatic variable explaining the variation in N\textsubscript{2} fixation rate between the stands of the two tree species in this study.

4.3 Stand turnover and availability of N - a decisive factor?

There is no other study focusing on the difference in N\textsubscript{2} fixation rate by cyanobacteria associated to feather mosses between Scots pine and Norway spruce stands. However, there are studies that have similarities in the layout to this study, for example DeLuca et al. (2007) and Zackrisson et al. (2004), which have relevant results. DeLuca et al. (2007) and Zackrisson et al. (2004) both used the same natural regenerated stands for their studies. Zackrisson et al. (2004) stated that the N\textsubscript{2} fixation rate by cyanobacteria associated to feather mosses increases with increasing time since last disturbance, in this case fire. DeLuca et al. (2007) transplanted feather mosses from early successional stands (42-102 years since fire) to late successional stands (245-356 years since last fire) and vice versa. The mosses from early successional stands transplanted to late successional stands increased their cyanobacteria associated N\textsubscript{2} fixation rate. The mosses transferred from late successional to early successional stands decreased their N\textsubscript{2} fixation rate (DeLuca et al., 2007).
Before fire the stands were mixed Scots pine and Norway spruce stands, after fire the young stands were dominated by Scots pine with a secondary occurrence of Norway spruce (Zackrisson et al., 2004). It is relevant to assume that as time goes the stands will get more dominated by Norway spruce, due to Norway spruce being a secondary species and Scots pine being a primary species (Engelmark & Hytteborn, 1999). This could be further implying that the dominating tree species may have an effect on N\textsubscript{2} fixation rate of cyanobacteria associated to feather mosses and thereby showing a similar pattern as to the results of this study. In this study N\textsubscript{2} fixation rates by cyanobacteria associated to feather mosses were higher in Norway spruce stands then in Scots pine stands. The same thing applies for the results of the moss coverage in the study by Zackrisson et al. (2004). Moss coverage is increasing logarithmically with time since last disturbance in the study by Zackrisson et al. (2004) and in this study the moss-coverage was significantly higher in the Norway spruce than in the Scots pine stands, despite that there was no difference in age. Hence my results suggest that tree species is a more important factor than time since disturbance to explain differences in moss-cover between forest stands.

DeLuca et al. (2007) also looked at available inorganic N and P in the forest floor on the same sites. The early successional stands had high N availability and the late successional stands had low N availability (DeLuca et al., 2007). N availability has been shown to affect the N\textsubscript{2} fixation rate by cyanobacteria associated to feather mosses (Sorensen et al., 2012; Zackrisson et al., 2009; DeLuca et al., 2008). Which in this case could imply that it is the available inorganic N and perhaps not the dominant tree species that has an effect on N\textsubscript{2} fixation rates in the plots used by DeLuca et al. (2007) and Zackrisson et al. (2004). On the other hand, a possible explanation to the decreasing N availability and increasing N\textsubscript{2} fixation with time since last disturbance could be that the tree species in fact has an effect on the N availability and thereby also on the N\textsubscript{2} fixation rate.

4.4 The effect of the tree species

In summary my results could be interpreted as there could be more available N in the Scots pine stands than the Norway spruce stands. The N\textsubscript{2} fixation is perhaps a reaction to the N turnover rate more than a contributing factor to the volume growth of the trees. The higher the N turnover rate, the larger volume growth of the trees and the smaller the N\textsubscript{2} fixation by the cyanobacteria. One possible explanation for this is the differences in canopy structure between the tree species and particularly the potential difference in climate underneath the canopy, which could affect the turnover rate. This potential climatic difference underneath the canopy could then
perhaps also be one of the potential explanations to the difference in moss abundance between the stands of the two tree species, among species competition and other explanations. Further studies on this possible connection between the N\textsubscript{2} fixation rate of cyanobacteria and dominating tree species is needed to fully understand its possible significance and specific effects.

4.5 Conclusions

I found that the mosses *H. splendens* and *P. schreberi* were significantly more abundant in Norway spruce stands than in Scots pine stands, although the trees were established at the same time (on average 60-70 years ago) in the two types of stands. In contrast to my hypothesis, there was no significant difference in N\textsubscript{2} fixation rate by cyanobacteria associated to the mosses between the stands of the two tree species. The significantly faster growth of Scots pine than of Norway spruce was thus not correlated to larger input of N into the former stands by moss associated N\textsubscript{2} fixation. Instead I suggest that the faster growth of Scots pine may indicate that the supply of plant available N is higher in these stands, due to higher N turnover rates, which may hamper moss associated N\textsubscript{2} fixation.
References


Acknowledgements

First of all, I would like to thank my supervisor Annika Nordin and assisting supervisor Michael Gundale for their help and advice. I’m sending a great thanks to Kelley Gundale for her much-appreciated help and assistance with the gas chromatograph and lab work. I would like to thank Hilda Edlund and Jonas Dahlgren for their advice on statistics. Another great thanks go to Tinkara Bizjak for her assistance in the field, and for all her patience during the weeks we sorted mosses. Thank you, Johannes Larson, for commenting on my use of words. Last but not least I would like to thank my father Andreas Lidman for accompanying me out in the field during one intense day of driving and moss gathering, as well as for his ingenuity when it comes to field equipment.

Tack!

Felicia Lidman,
January 2018.
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