Ecophysiological adjustment of two *Sphagnum* species in response to anthropogenic nitrogen deposition

Magdalena M. Wiedermann¹, Urban Gunnarsson², Lars Ericson¹ and Annika Nordin³

¹Department of Ecology and Environmental Science, Umeå University, SE-901 87 Umeå, Sweden; ²Evolutionary Biology Centre, Department of Plant Ecology, Uppsala University, Villavägen 14, SE-752 36 Uppsala, Sweden; ³Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden

**Summary**

• Here, it was investigated whether *Sphagnum* species have adjusted their nitrogen (N) uptake in response to the anthropogenic N deposition that has drastically altered N-limited ecosystems, including peatlands, worldwide.

• A lawn species, *Sphagnum balticum*, and a hummock species, *Sphagnum fuscum*, were collected from three peatlands along a gradient of N deposition (2, 8 and 12 kg N ha⁻¹ yr⁻¹). The mosses were subjected to solutions containing a mixture of four N forms. In each solution one of these N forms was labeled with ¹⁵N (namely ¹⁵NH₄⁺, ¹⁵NO₃⁻ and the amino acids [¹⁵N]alanine (Ala) and [¹⁵N]glutamic acid (Glu)).

• It was found that for both species most of the N taken up was from NH₄⁺, followed by Ala, Glu, and very small amounts from NO₃⁻. At the highest N deposition site N uptake was reduced, but this did not prevent N accumulation as free amino acids in the *Sphagnum* tissues.

• The reduced N uptake may have been genetically selected for under the relatively short period with elevated N exposure from anthropogenic sources, or may have been the result of plasticity in the *Sphagnum* physiological response. The negligible *Sphagnum* NO₃⁻ uptake may make any NO₃⁻ deposited readily available to co-occurring vascular plants.

**Introduction**

Since the industrial revolution, increasing anthropogenic nitrogen (N) pollution, mainly originating from agriculture and fossil fuel combustion, has given rise to high levels of atmospheric N deposition over natural ecosystems across vast areas of Europe and North America (e.g. Lövblad & Erisman, 1992; Asman *et al*., 1998; Galloway, 2001; Holland *et al*., 2005). This has had a drastic effect on species composition and the functioning of former N-limited aquatic (Bergström & Jansson, 2006) and terrestrial (Bobbink *et al*., 1998; Nordin *et al*., 2005) ecosystems.

One ecosystem that is particularly vulnerable to an increased atmospheric N load is oligotrophic peatland, which responds with a shift from *Sphagnum*-dominated to vascular plant-dominated vegetation (e.g. Gunnarsson *et al*., 2002; Wiedermann *et al*., 2007). This is because the dominant group of organisms, the *Sphagnum* mosses, has several morphological and physiological features that are uniquely adapted to nutrient-limited conditions (cf. Van Breemen, 1995). The specific leaf area of *Sphagnum* mosses normally exceeds those of feather mosses, and can be close to 500 cm² g⁻¹ dry weight (DW) (Bond-Lamberty & Gower, 2007). *Sphagna* have lots of small unistratose leaf-like structures, which allow effective nutrient absorption over the entire moss surface (Clymo & Hayward, 1982). A high cation-exchange capacity (Clymo, 1963; Woodin & Lee, 1987a; Li & Vitt, 1997), coupled with a high nutrient use efficiency, through translocation of nutrients from senescing leaves (Aldous, 2002a; Bridgham, 2002), enables *Sphagna* to monopolize the limited nutrient supply (Lamers *et al*., 2000; Turetsky, 2003). This suggests that in areas of high N pollution these features, which facilitate persistence under extremely nutrient-constrained conditions, will ultimately result in elevated N accumulation beyond the demands of *Sphagnum* for growth and maintenance.

However, in recent years it has been increasingly emphasized that anthropogenic environmental change may cause directional selection, and that evolutionary processes cannot be ignored in studies of ecological processes (Carroll *et al*., 2007; Strauss *et al*., 2008). Over a period of about a century, genetic
adaptations in response to different fertilization regimes have been documented for several plant species (Snaydon & Davies, 1972; Silvertown et al., 2006). One possible adaptation of Sphagnum to increased N supply is an adjustment in N uptake; this could be the result of either physiological responses induced by high internal N concentrations in the Sphagnum tissue or directional selection.

For higher plants, N uptake is strictly regulated on the basis of whole plant demand, so that N uptake rates decrease as plant internal N concentrations increase (for reviews see, e.g., Crawford & Glass, 1998; Miller et al., 2008). For Sphagnum mosses, it has been suggested that regulation of N uptake is less strict (Jauhiainen et al., 1998). Although not explicitly addressing N uptake rates, it has been demonstrated repeatedly that Sphagna subjected to a high N supply accumulate elevated amounts of N (Nordin & Gunnarsson, 2000; Van der Heijden et al., 2000; Limpens & Berendse, 2003), show reduced growth rates (Press et al., 1986; Gunnarsson & Rydin, 2000), and decline in abundance (Bubier et al., 2007; Wiedermann et al., 2007). However, the conclusions of many of these studies were constrained, either by their short duration (from 1 to 3 yr, which may exclude long-term adjustments) or by the very high N doses used, which may lead to immediate physiological damage.

A few studies of Sphagna from areas in north-western Europe, where the moss has been subjected to increased N supply via atmospheric deposition, have indicated that long-term ecophysiological adjustment to high N supply may occur. The first two of these were British studies of Sphagnum cuspidatum. In one, Press et al. (1986) conducted a short-term transplantation experiment, which showed that S. cuspidatum specimens originating from a high N deposition area took up less inorganic N than those originating from a low N deposition area. Later, Baxter et al. (1992) found that, when S. cuspidatum was exposed to high N supply in a culture solution, specimens from a site with low atmospheric N deposition showed reduced growth, while growth was stimulated in specimens originating from a high N deposition site. Similarly, Limpens & Berendse (2003) observed that, in a glasshouse experiment, growth of Sphagnum magellanicum was maintained despite a high N supply rate, as long as this rate did not exceed N input rates at the site of origin. They suggested that decreased N uptake may be a long-term adaptation in mosses subjected to high N supply (Limpens & Berendse, 2003), and argued that support for their hypothesis was also provided, at least indirectly, by a comparison of results from two independent studies on Sphagnum N uptake rates performed in Germany (a high N deposition area; Twenhöven, 1992) and in Sweden (a low N deposition area; Jauhiainen et al., 1998). In these two studies, lower N uptake rates were reported in the high compared with the low deposition area (Limpens & Berendse, 2003). Direct experimental support for decreased N uptake as an ecophysiological adjustment by Sphagnum to high loads of atmospheric N deposition, however, is still lacking.

In this study we wanted to examine whether two Sphagnum species, Sphagnum balticum, a lawn species, and Sphagnum fuscum, a hummock species, reduced their N uptake in response to high loads of atmospheric N deposition. For this purpose we made use of a natural gradient of anthropogenic N deposition across Sweden, ranging from 2 to 12 kg N ha\(^{-1}\) yr\(^{-1}\). We sampled Sphagnum specimens from three peatlands and exposed them to experimental solutions containing organic (alanine (Ala) and glutamic acid (Glu)) and inorganic (NH\(_4\)\(^+\) and NO\(_3\)\(^-\)) N sources. The amino acids Ala and Glu were chosen because they represent the major amino acids found in precipitation (Fonselius, 1954; Gorzelska et al., 1992).

We investigated whether Sphagna adjust to long-term high N input by means of reduced N uptake and, if so, how species differ in this respect.

**Materials and Methods**

**The N deposition gradient**

For this study we chose three mires in Sweden representing a gradient of decreasing inorganic (NH\(_4\)\(^+\) and NO\(_3\)\(^-\)) N deposition. The study sites (Fig. 1, Table 1) were exposed to different levels of N deposition; from highest to lowest: Öresjömossen, a slightly raised ombrotrophic bog; Åkultmyrern, an eccentric ombrotrophic bog; and Degerö Stormyr, an aapa mire with topogenous nutrient-poor fens and ombrotrophic hummocks. Peat water pH was recorded as 3.9 at all three locations. The annual wet N deposition for the three sites amounted to 12, 8, and 2 kg ha\(^{-1}\) yr\(^{-1}\), respectively. In the following, the sites are referred to as the HD (high N deposition), MD (mid N deposition), and LD (low N deposition) sites, respectively (Table 1).

**Field sampling**

We selected two species: Sphagnum balticum (Russ.) C.E.O. Jensen, a lawn species, and Sphagnum fuscum (Schimp.) H. Klinggr., a hummock species (Rydin et al., 1999). Sampling took place in June 2006. Five moss cores (0.2 m x 0.15 m deep) were collected for each of the two Sphagnum species from each site. For both species all plots had a 100% cover of Sphagna. However, an increasing vascular plant cover was recorded at the two sites with enhanced N supply. Within each locality, the sampling points were spaced at least 20 m apart. The cores were transported to the laboratory in plastic trays and kept moist using peat water from their original site.

**Experimental set-up in the laboratory and chemical analyses**

In Sphagna, metabolic activity and nutrient uptake are highest in the upper part of the plant, the capitulum (Malmer et al., 1994; Aldous, 2002b). Therefore, in the laboratory we...
cut off capitula and separated them from the stems. The capitula were shaken for 30 min in 500 ml of 0.5 mM CaCl₂ solution to rinse them and to guarantee cell membrane integrity (cf. Kielland, 1997). They were gently dried with paper towels, and then immersed in the experimental mixed solutions containing four N forms ( , , Ala and Glu). In each solution, one of the four N forms was labeled with 15N (98 atom%), namely 15 , 15 , [15N]alanine (Ala) and [ 15N]glutamic acid (Glu). We also prepared two solutions that differed in N concentration (10 and 100 µM). (The 10 µM solution was only used for S. balticum.) These concentrations were chosen to mimic actual values during rainfall events at the LD and HD sites, respectively (IVL, Svenska Miljöinstitutet AB (http://www.ivl.se/miljo/projekt/ ned_net/)). To avoid significant N depletion of the solutions during the course of the experiment, excessive volumes (100 ml for the 100 µM N solution and 500 ml for the 10 µM N solution) of the solutions were used. Sphagnum N uptake rates were assumed to be similar to those observed by Kielland (1997). To maintain cell membrane integrity during the experiment all solutions also contained 0.5 mM CaCl₂ (Epstein, 1961; Kielland, 1997). In accordance with the natural pH conditions recorded in peat water samples from the three peatlands, the pH in the solutions was adjusted to 3.9 using concentrated HCl.

We used five cores from each site (n = 5). From each core we used six capitula (from each species) as a pooled sample, which were put into one of the four uptake solutions. Hence, we used a total of 24 capitula from each species and core. The uptake solutions were shaken during the 2-h experimental period to ensure aeration of the solution and to avoid local depletion zones. Light conditions in the laboratory were kept constant (c. 500 lux). After 2 h, the mosses were taken out of the solution, gently dried with paper towels, and then placed in a 1 M KCl solution (also containing 0.5 mM CaCl₂) and shaken for c. 2 min. The highly concentrated salt solution was intended to remove N not taken up into the cytosol, but attached to apoplastic surfaces of the moss tissues. Thereafter the mosses were dried again with paper towels and immediately frozen. The amount of 15N (atom%) in the dried (60 °C for 24 h) and ground moss material was determined using isotope ratio mass spectrometry (IRMS). The atom% 15N values calculated from the mass spectrophotometric data were corrected for the natural abundance of 15N. Total N and soluble amino acid contents were analyzed in additional capitulum samples, which were not exposed to the uptake solution but were collected at the same time as the capitula used in the uptake experiment. They were also immediately frozen and the frozen plant material was subsequently ground for 30 s using a ball-mill; the vials and balls were cooled with liquid N to prevent thawing of the specimens. After drying (at 60°C for 24 h), the total N content of the ground plant material was analysed using a Carlo Erba model 1108 high-temperature combustion elemental analyzer (Thermo Electron, Milan, Italy). Amino acids were extracted from the ground, frozen plant material with 10 mM HCl and analyzed on a high-performance liquid chromatograph capable of detecting nanomolar concentrations of 18 standard amino acids (Nordin & Gunnarsson, 2000).

Statistical analyses

For the statistical analyses, we used R.2.2.1 (Vienna, Austria; ISBN 3-900051-07-0; URL http://www.R-project.org.). Data met with the requirements for ANOVA (data were...
examined for normality and homogeneity of variance). First, ANCOVAs were used to test the effect of different factors (site, species, and soluble amino acid N (NAA) and total N (N\text{tot}) tissue concentrations, respectively) on total \(^{15}\)N uptake by the two species. Based on results of the ANCOVAs we used ANOVAs to examine the N uptake of the two species from each of the N sources (\(\text{NH}_4^+\), Alfa, Glu and \(\text{NO}_3^-\)) and total \(^{15}\)N uptake from the three uptake solutions. For between-site comparisons we used treatment contrasts, which compare each treatment level (in our case each of the two sites with enhanced N deposition; MD and HD) separately with the control (here the LD site). Total tissue N and soluble amino acid N concentrations along the N deposition gradient were tested with linear models.

**Results**

Along the atmospheric N deposition gradient we observed a clear effect of increased N supply on the *Sphagnum* species investigated. For both *S. balticum* and *S. fuscum* the total tissue N and soluble amino acid N concentrations increased with increased N deposition (Table 2).

The N uptake experiment demonstrated that *S. balticum* and *S. fuscum* took up considerable amounts of N, in all forms, from the mixed solutions (Table 2, Fig. 2). The N form with the highest uptake in both species was \(\text{NH}_4^+\), followed by the amino acids Alfa and Glu, with intermediate uptake, while only very small amounts of \(\text{NO}_3^-\) were taken up (Fig. 2). For both species, the total N uptake from the 100 µM N solution was lower for specimens from the HD site than for specimens from the MD and LD sites (Table 2). However, interspecific differences were found with respect to the preferred form of N. For *S. fuscum*, \(\text{NH}_4^+\) and amino acid uptake was lower in specimens from the HD site than in specimens from the MD and LD sites (Fig. 2). For *S. balticum*, \(\text{NO}_3^-\) and amino acid uptake was lower at the HD site (Fig. 2).

For \(\text{NH}_4^+\) uptake by *S. balticum* a different pattern was apparent for the two concentrations of nutrient solution. In

**Table 1** List of investigated peatlands with abbreviations, geographic position (latitude and longitude), altitude, climatic data, and averaged nitrogen (N) wet and dry deposition

<table>
<thead>
<tr>
<th>Sites (abbreviation)</th>
<th>Geographical coordinates</th>
<th>Altitude (m asl)</th>
<th>Mean annual precipitation (mm)</th>
<th>Mean annual temperature (°C)</th>
<th>N wet deposition (kg ha(^{-1}) yr(^{-1}))</th>
<th>N dry deposition (kg ha(^{-1}) yr(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degerö Stormyr (LD)</td>
<td>64°11′N/19°33′E</td>
<td>270</td>
<td>523</td>
<td>1.2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Åkghultmyren (MD)</td>
<td>57°06′N/14°32′E</td>
<td>225</td>
<td>939</td>
<td>6.0</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Öresjömossen (HD)</td>
<td>57°03′N/12°51′E</td>
<td>150</td>
<td>1095</td>
<td>6.4</td>
<td>12</td>
<td>4</td>
</tr>
</tbody>
</table>

Meteorological data are from the Swedish Meteorological and Hydrological Institute (SMHI) (Alexandersson et al., 1991). Nitrogen wet deposition data (\(\text{NO}_3^-\) and \(\text{NH}_4^+\) contributing equally) for Åkghultmyren and Öresjömossen were obtained from the nearby IVL measuring stations Aneboda and Boa Berg, respectively (IVL Svenska Miljöinstitut AB: http://www.ivl.se/miljo/projekt/ned_net/) and averaged for the period 1995–2003. N wet deposition at Degerö Stormyr was measured at the nearby field station (Granberg et al., 2001). Nitrogen dry deposition data (\(\text{NO}_3^-\) and \(\text{NH}_4^+\)) for all sites are from SMHI (Persson et al., 2004). For dry deposition, \(\text{NO}_3^-\) contributed 80% of the total deposition at the low N deposition (LD) site, 60% at the mid N deposition (MD) site and 50% at the high N deposition (HD) site.

**Table 2** Total tissue nitrogen (N) and total amino acid N (N\text{AA}) for *Sphagnum balticum* and *Sphagnum fuscum*, and total amount of \(^{15}\)N uptake from the 100 µM N solution for *S. balticum* and *S. fuscum*, and from the 10 µM N solution for *S. balticum*, in samples originating from the three peatland sites with different levels of N deposition from low (LD) through mid (MD) to high (HD) (cf. Table 1)

<table>
<thead>
<tr>
<th>Species</th>
<th>Sites</th>
<th>Total tissue N (mg g(^{-1}) DW)</th>
<th>Total N\text{AA} (mg g(^{-1}) DW)</th>
<th>(^{15})N uptake 10 µM (µmol g(^{-1}) DW)</th>
<th>(^{15})N uptake 100 µM (µmol g(^{-1}) DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sphagnum balticum</em></td>
<td>LD</td>
<td>5.5 \pm 0.2</td>
<td>0.1 \pm 0.0</td>
<td>24.6 \pm 1.7</td>
<td>48.6 \pm 1.6</td>
</tr>
<tr>
<td></td>
<td>MD</td>
<td>9.4 \pm 0.2</td>
<td>0.6 \pm 0.0</td>
<td>33.9 \pm 1.6**</td>
<td>50.0 \pm 2.3</td>
</tr>
<tr>
<td></td>
<td>HD</td>
<td>10.9 \pm 0.5</td>
<td>1.0 \pm 0.1</td>
<td>27.8 \pm 1.8</td>
<td>42.5 \pm 1.9**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(F_{(1,13)} = 122.2)</td>
<td>(F_{(1,13)} = 152.6)</td>
<td>(F_{(2,12)} = 7.8)</td>
<td>(F_{(2,12)} = 4.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
<td>(P = 0.007)</td>
<td>(P = 0.044)</td>
</tr>
<tr>
<td><em>Sphagnum fuscum</em></td>
<td>LD</td>
<td>0.1 \pm 0.0</td>
<td>7.1 \pm 0.4</td>
<td>50.1 \pm 2.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MD</td>
<td>0.3 \pm 0.1</td>
<td>8.9 \pm 0.6</td>
<td>48.7 \pm 1.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HD</td>
<td>0.7 \pm 0.1</td>
<td>11.1 \pm 0.4</td>
<td>38.6 \pm 2.5**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(F_{(1,13)} = 32.4)</td>
<td>(F_{(1,13)} = 26.8)</td>
<td>(F_{(2,12)} = 7.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
<td>(P = 0.008)</td>
<td></td>
</tr>
</tbody>
</table>

For total tissue N and N\text{AA} linear regression models were applied and for \(^{15}\)N uptake ANOVAs were used. To test for differences between sites, treatment contrasts were used (each of the two sites with enhanced N deposition, MD and HD, were tested against the LD site). Statistical differences between sites (columns and species) are indicated by \(* * P < 0.01; n = 5\).
the 10 µM N solution, NH$_4^+$ uptake was higher in specimens from the MD and HD sites than in those from the LD site (Fig. 2a). In the 100 µM N solution, S. balticum from all three sites took up similar amounts of NH$_4^+$ (Fig. 2b). However, amino acid and NO$_3^-$ uptake was similarly reduced in S. balticum from the HD site irrespective of the concentration of the uptake solution (Fig. 2a,b). A comparison of N uptake between the two solutions showed that, despite there being a tenfold difference in concentration, NO$_3^-$ uptake was rather similar, and amino acid and NH$_4^+$ uptake was less than twice as high from the stronger solution (Fig. 2a,b).

ANCOVAs testing the effect of different factors (site, species, and N$_{AA}$ and N$_{tot}$ tissue concentrations) on total $^{15}$N uptake by the two species, S. balticum and S. fuscum, revealed that sampling site (representing the three levels of N deposition) was the only factor influencing total $^{15}$N uptake (Table 3, Fig. 3). Thus, no difference in $^{15}$N uptake was found between the two species, and furthermore neither N$_{AA}$ nor N$_{tot}$ tissue concentration influenced $^{15}$N uptake (Table 3, Fig. 3).

**Discussion**

**Site effects**

Our study demonstrates that N uptake by the two Sphagnum species studied varies according to the prevailing atmospheric N deposition. However, for both species, reduced N uptake was only observed at the HD site. We found that for the hummock species S. fuscum and the lawn species S. balticum, amino acid uptake was lower in specimens from the HD site than in those from the MD and LD sites. Our data further show that the two species differed with regard to inorganic N uptake reduction. Sphagnum fuscum took up less NH$_4^+$ and S. balticum less NO$_3^-$ at the HD site. Hence, our data suggest that Sphagna subjected to long-term N enrichment have the capacity to adjust to the new environmental conditions through decreased N uptake.

Results based on gradient studies should, however, be interpreted with great care (e.g. Rustad, 2006). In our case the studied north–south gradient is a gradient not only of increasing N deposition but also of increasing temperature and precipitation (see Table 1). However, for the current study performed in a laboratory under strictly controlled conditions, the Sphagnum specimens from three peatlands along the north–south N deposition gradient were collected during peak growing season at a time when none of the sites was affected by drought. Hence, we believe that long-term N deposition along the gradient is the main factor influencing Sphagnum N uptake. In support of this conclusion, it has previously been demonstrated that N deposition effects on N-sensitive species supersede other factors varying along with the N deposition gradient in Sweden (Strengbom et al., 2003).

In higher plants, N uptake through the roots is down-regulated on the basis of whole plant demands, as N metabolites (e.g. glutamine) accumulate in the root cells (Imsande & Touraine, 1994; Rawat et al., 1999; Vidmar et al., 2000). As N uptake in Sphagnum occurs solely through the shoot tissue, N concentrations in the shoot tissue should provide an appropriate measure of N uptake regulation. Both Sphagna in our study show a linear increase in internal N$_{AA}$ tissue concentrations in response to the increased N supply along the N deposition gradient. This response did not, however, result in a gradual decrease in N uptake along the gradient for either of the

![Fig. 2](https://example.com/image2.png)

Fig. 2 $^{15}$N uptake from the different nitrogen (N) sources (NH$_4^+$, alanine (Ala), glutamic acid (Glu), and NO$_3^-$) by two Sphagnum species, Sphagnum balticum and Sphagnum fuscum, originating from a low N deposition site (LD, dark gray bars), a mid N deposition site (MD, gray bars), and a high N deposition site (HD, white bars) for: (a) S. balticum in a 10 µM N solution; (b) S. balticum in a 100 µM N solution; and (c) S. fuscum in a 100 µM N solution. Bars represent means ± SE (n = 5). Statistical differences for between-site comparisons are indicated by: ***, P < 0.001; **, P < 0.01; *, P < 0.05. DW, dry weight.
investigated species. Instead, reduced N uptake was found only at the HD site. Our results reveal that the site effect, that is the effect of local N deposition, overrides the importance of internal N tissue concentration (Table 3, Fig. 3).

One explanation for this nongradual reduction in uptake may be that the HD site is located in the part of south-western Sweden where there has been not only the highest deposition of anthropogenic air pollutants, including N (Fig. 1; Hole & Engart, 2008), but deposition over the longest period (Brännvall et al., 2001; Bindler et al., 2002). Plant adaptations within a period of about a century have been documented in relation both to different fertilization regimes (Naydon & Davies, 1972; Silvertown et al., 2006) and to mine deposits with high concentrations of heavy metals (Jain & Bradshaw, 1966; Antonovics et al., 1971). This suggests that contemporary evolution should not be disregarded in studies aiming to address ecosystem responses to anthropogenic change, in particular in those parts of the world where biota were drastically affected by high deposition of N.

### Table 3
Results of the ANCOVAs testing the effect of different factors (site, species, total amino acid N (N\textsubscript{AA}) and total tissue N (N\textsubscript{tot})) on total \textsuperscript{15}N uptake for the two species *Sphagnum balticum* and *Sphagnum fuscum* (for values of total \textsuperscript{15}N uptake see Table 2)

<table>
<thead>
<tr>
<th>Response variable</th>
<th>F\textsubscript{(4,25)}</th>
<th>R\textsuperscript{2}</th>
<th>Model significance</th>
<th>Source</th>
<th>df</th>
<th>F-value</th>
<th>P (\textgreater F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textsuperscript{15}N uptake</td>
<td>6.4</td>
<td>0.51</td>
<td>P = 0.001</td>
<td>Site</td>
<td>2</td>
<td>11.7586</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Species</td>
<td>1</td>
<td>0.4899</td>
<td>P = 0.490</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N\textsubscript{AA}</td>
<td>1</td>
<td>1.6469</td>
<td>P = 0.211</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Site</td>
<td>2</td>
<td>11.6061</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Species</td>
<td>1</td>
<td>0.4835</td>
<td>P = 0.490</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N\textsubscript{tot}</td>
<td>1</td>
<td>1.3015</td>
<td>P = 0.265</td>
</tr>
</tbody>
</table>

\textsuperscript{15}N uptake was used as a continuous response variable, N deposition along the gradient as a categorical variable, *Sphagnum* species (*S. balticum* and *S. fuscum*) as a categorical variable, and *Sphagnum* tissue chemistry, represented by N\textsubscript{AA} and N\textsubscript{tot} separately, as continuous explanatory variables.
by the industrial revolution. Thus one possible interpretation of our data is that the result indicates an adaptive response at the HD site, but not at the MD site; this could be the result of stronger directed selection acting over a longer period at the HD site. However, the findings of the current and earlier studies (Press et al., 1986; Baxter et al., 1992; Limpens & Berendse, 2003) that have reported reduced N uptake cannot exclude the possibility that the change in N uptake with enhanced atmospheric N deposition is purely a physiological response induced by a high internal N concentration, exceeding a threshold value.

N forms
Uptake of amino acid and NO\textsubscript{3}\textsuperscript{−} by S. balticum did not differ between the two solutions with different N concentrations (10 and 100 µM). Regardless of N concentration in the solution, Ala, Glu and NO\textsubscript{3}\textsuperscript{−} uptake was reduced in specimens from the HD site. For S. balticum, NH\textsubscript{4}\textsuperscript{+} uptake was not reduced in specimens from the HD site, irrespective of the solution concentration. Unlike amino acid and NO\textsubscript{3}\textsuperscript{−} uptake, the pattern of NH\textsubscript{4}\textsuperscript{+} uptake from the 10 µM N solution diverged (Fig. 2a) from that of the 100 µM N solution (Fig. 2b): we found a higher NH\textsubscript{4}\textsuperscript{+} uptake in plants from the MD and HD sites compared with those from the LD site. To our knowledge, the mechanisms for NH\textsubscript{4}\textsuperscript{+} uptake by Sphagna are unknown (cf. Glime, 2007). Assuming that shoot N uptake in Sphagna is similar to root N uptake in vascular plants, there appear to be two possible explanations for the different patterns in NH\textsubscript{4}\textsuperscript{+} uptake between the two experimental solutions (and between the two species). However, we admit that this assumption can be questioned because in vascular plants NH\textsubscript{4}\textsuperscript{+} uptake differs substantially between the metabolically regulated root uptake and the more unregulated shoot uptake.

The first possibility is that long-term high N deposition has the potential to induce potassium (K\textsuperscript{+}) deficiency in Sphagna (Bragazza & Limpens, 2004; Carfrae et al., 2007; Gerdol et al., 2007). For higher plants, NH\textsubscript{4}\textsuperscript{+} uptake may occur as a side effect of K\textsuperscript{+} uptake, as some K\textsuperscript{+} channels also can be employed for NH\textsubscript{4}\textsuperscript{+} uptake (Maathuis, 2007). It has been suggested that up-regulated K\textsuperscript{+} uptake, compensating for the induced K\textsuperscript{+} deficiency, could enhance NH\textsubscript{4}\textsuperscript{+} uptake (Britto & Kronzucker, 2002). However, although this would explain the uptake pattern from the 10 µM N solution (Fig. 2a), it does not explain the similar values of NH\textsubscript{4}\textsuperscript{+} uptake from the 100 µM N solution by S. balticum from all three sites (Fig. 2b), nor the reduced NH\textsubscript{4}\textsuperscript{+} uptake recorded for S. fuscum from the HD site (Fig. 2c). Furthermore, such an explanation for NH\textsubscript{4}\textsuperscript{+} uptake by S. fuscum from the HD site would contradict the hypothesis of Gerdol et al. (2007) that, in high N deposition areas, K\textsuperscript{+} deficiency is more pronounced in hummock species than in lawn species, in our case S. fuscum and S. balticum, respectively.

A second possible explanation is based on the potential for NH\textsubscript{4}\textsuperscript{+} influx and efflux through plant tissue, as discussed by Britto & Kronzucker (2002). With higher internal tissue concentrations, NH\textsubscript{4}\textsuperscript{+} efflux should be higher, thereby causing increased absolute values of 15NH\textsubscript{4}\textsuperscript{+} influx. To explain the observed pattern of 15NH\textsubscript{4}\textsuperscript{+} uptake from the 10 µM N solution, internal NH\textsubscript{4}\textsuperscript{+} concentrations would have to have exceeded toxic values over a long period (Britto & Kronzucker, 2002). Even in that case, this hypothesis fails to explain the patterns of NH\textsubscript{4}\textsuperscript{+} uptake from the 100 µM N solution (Fig. 2b,c). Thus, we are unable to explain the differences in response patterns for NH\textsubscript{4}\textsuperscript{+} uptake between either the two experimental solutions or the two species.

Ecological implications
Our study clearly shows that amino acids constitute a source of N for the investigated Sphagnum species, thus concurring with earlier studies (Simola, 1975; Kielland, 1997; Krab et al., 2008). However, the availability of amino acid N in ombrotrophic peatlands is unknown, although we can assume some airborne deposition. In addition to inorganic N, rainwater contains small amounts of soluble amino acids (Fonselius, 1954; Neff et al., 2002; Cornell et al., 2003). Also, during certain periods of the year, particularly spring/early summer, pollen dispersal may constitute a significant additional N supply. The ability of Sphagna to sequester amino acids may help the genus to monopolize the limited nutrients and might, therefore, significantly affect N cycling in peatlands. In areas of anthropogenic N pollution, Bragazza & Limpens (2004) reported indications of an enhanced amino acid supply: they found elevated levels of dissolved organic N (DON) in the peat water. Although not much is known about amino acid availability in peatlands under high N deposition regimes, reduced amino acid uptake by the Sphagnum carpet – as shown in this study – might contribute to high peat water DON (Bragazza & Limpens, 2004).

In Sweden, NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{−} contribute in approximately equal amounts to anthropogenic N pollution (IVL, Svenska Miljöinstitutet AB (http://www.ivl.se/miljo/projekt/ned_net/)). Our study shows that N uptake by both Sphagnum species at all three sites was dominated by NH\textsubscript{4}\textsuperscript{+}, whilst only small amounts of NO\textsubscript{3}\textsuperscript{−} were taken up, despite the well-known fact that Sphagna have the potential to utilize NO\textsubscript{3}\textsuperscript{−} as an N source. For example, Woodin et al. (1985) and Woodin & Lee (1987b) reported nitrate reductase activity in Sphagna. Enzymatic activity of nitrate reductase is induced following NO\textsubscript{3}\textsuperscript{−} exposure. The 2-h experimental period used in the current study was not sufficient to induce maximal enzyme activity, but c. 70% of the maximal activity has been reported for S. capillifolium following an exposure period of 2 h (Woodin et al., 1985). However, the pronounced higher NH\textsubscript{4}\textsuperscript{+} than NO\textsubscript{3}\textsuperscript{−} uptake accords with earlier studies (Twenhöven, 1992; Kielland, 1997; Jauhiainen et al., 1998) and has direct implications for direct and indirect effects of N deposition upon a Sphagnum carpet. Firstly, the biggest direct threat to N-sensitive
peat-forming Sphagnum species seems to be anthropogenic NH$_4^+$ pollution. Secondly, the low capacity of Sphagnum to take up NO$_3^-$ suggests that, under circumstances of high external NO$_3^-$ input, there are likely to be indirect effects on the Sphagnum cover through a drastic shift of the ecosystem N cycle: increased amounts of NO$_3^-$ will be available for uptake by vascular plants. Nitrate is readily taken up by vascular plants, and, for example, the graminoid Erhöphorum vaginatum, a characteristic species of oligotrophic peatlands, has been shown to have a high capacity for utilizing NO$_3^-$ as a source of N (Nordin et al., 2004). An increased abundance of this graminoid has, indeed, been observed in response to NH$_4$NO$_3$ enrichment of peatlands (e.g. Wiedermann et al., 2007). Thus, even though NO$_3^-$ seems to be hardly taken up by Sphagnum species (cf. also Twenhöven, 1992; Jauhiainen et al., 1998), high levels of NO$_3^-$ input into ombrotrophic peatlands still constitute a potential indirect threat through enhanced shading effects as a result of increased vascular plant cover over the Sphagnum carpet (e.g. Heijmans et al., 2002; Tomassen et al., 2004).

Moreover, in the current study we found species-specific differences in the inorganic N uptake patterns as S. fuscum reduced its NH$_4^+$ uptake, while S. balticum reduced its NO$_3^-$ uptake, at the HD site. In addition, the two species show different tissue amino acid accumulation rates along the N deposition gradient, the accumulation rates being more pronounced for S. balticum than for S. fuscum (Table 3). Several other studies (e.g. Jauhiainen et al., 1998, Van der Heijden et al., 2000; Limpens et al., 2004) confirm species-specific responses to enhanced N supply by different Sphagna. For example, decreased abundance of S. balticum was observed at N doses of 15 kg ha$^{-1}$ yr$^{-1}$ (Wiedermann et al., 2007), while biomass production of S. fuscum was positively influenced by N doses as high as 14 kg ha$^{-1}$ yr$^{-1}$ (Gunnarsson & Rydin, 2000; Vitt et al., 2003). Our demonstration of reduced NH$_4^+$ uptake by S. fuscum at the HD site, as well as the lower amino acid accumulation rate along the N deposition gradient in S. fuscum than in S. balticum, appears to support the idea that S. balticum is more sensitive than S. fuscum to high N supply.

Conclusion

This gradient study included sites that have been exposed to enhanced N deposition, at comparatively moderate doses, for a long time. This allowed us to examine the potential long-term adjustment of Sphagnum to anthropogenic N pollution. Despite reduced N uptake by both species collected from the site with the highest N deposition, we found that the uptake regulation was not strict enough to prevent accumulation of excess N in the form of free amino acids in either of the studied species. For both species the majority of the N taken up was from NH$_4^+$, followed by the amino acids Ala and Glu, while only small amounts of NO$_3^-$ were taken up.

Acknowledgements

We gratefully acknowledge financial support from Stiftelsen Anna och Gunnar Vidfelt’s fond (to MMW) and from the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS) to LE and AN, respectively.

References


© The Authors (2008).
Journal compilation © New Phytologist (2008)


Li YH, Vitt DH. 1997.


About New Phytologist

- New Phytologist is owned by a non-profit-making charitable trust dedicated to the promotion of plant science, facilitating projects from symposia to open access for our Tansley reviews. Complete information is available at www.newphytologist.org.

- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication ‘as-ready’ via Early View – our average submission to decision time is just 29 days. Online-only colour is free, and essential print colour costs will be met if necessary. We also provide 25 offprints as well as a PDF for each article.

- For online summaries and ToC alerts, go to the website and click on ‘Journal online’. You can take out a personal subscription to the journal for a fraction of the institutional price. Rates start at £139 in Europe/$259 in the USA & Canada for the online edition (click on ‘Subscribe’ at the website).

- If you have any questions, do get in touch with Central Office (newphytol@lancaster.ac.uk; tel +44 1524 594691) or, for a local contact in North America, the US Office (newphytol@ornl.gov; tel +1 865 576 5261).