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

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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 ^{15}N (namely $^{15}NH_4^+$, $^{15}NO_3^-$ and the amino acids [^{15}N]alanine (Ala) and [^{15}N]glutamic acid (Glu)).
- It was found that for both species most of the N taken up was from NH_4^+, followed by Ala, Glu, and very small amounts from NO_3^-. 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* NO_3^- uptake may make any NO_3^- 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 cm^2 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, for example, 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; Åkhultmyren, 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 (0.2×0.2 m, and 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

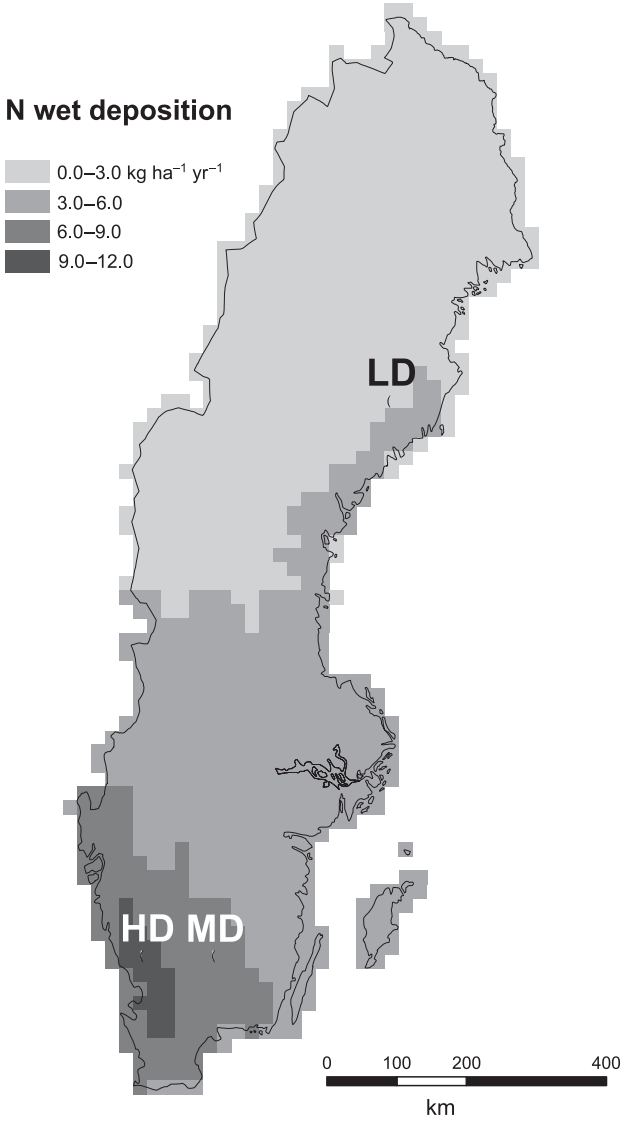

Fig. 1 Map of Sweden with the location of the studied peatlands: a low nitrogen (N) deposition site (LD), a mid N deposition site (MD), and a high N deposition site (HD). The map also shows N ($NO_x + NH_x$) wet deposition (kg ha^{-1} yr^{-1}) across the country. Data were measured and modeled by the Swedish Meteorological and Hydrological Institute (SMHI) and represent averaged values over a 10-yr period (1995–2005).

cut off capitula and separated them from the stems. The capitula were shaken for 30 min in 500 ml of 0.5 mM $CaCl_2$ 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 (NH_4^+, NO_3^-, Ala and Glu). In each solution, one of the four N forms was labeled with ^{15}N (98 atom%), namely $^{15}NH_4^+$, $^{15}NO_3^-$, [^{15}N]alanine (Ala) and [^{15}N]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 $CaCl_2$ (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 $CaCl_2$) 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 ^{15}N (atom%) in the dried (60°C for 24 h) and ground moss material was determined using isotope ratio mass spectrometry (IRMS). The atom% ^{15}N values calculated from the mass spectrophotometric data were corrected for the natural abundance of ^{15}N. 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 (N_{AA}) and total N (N_{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 (NH_4^+, Ala, Glu and 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 NH_4^+, followed by the amino acids Ala and Glu, with intermediate uptake, while only very small amounts of 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*, 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*, NO_3^- and amino acid uptake was lower at the HD site (Fig. 2).

For 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

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

Meteorological data are from the Swedish Meteorological and Hydrological Institute (SMHI) (Alexandersson *et al*., 1991). Nitrogen wet deposition data (NO_3^- and NH_4^+ contributing equally) for Åkhultmyren and Öresjömossen were obtained from the nearby IVL measuring stations Aneboda and Boa Berg, respectively (IVL Svenska Miljöinstitutet 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 (NO_3^- and NH_4^+) for all sites are from SMHI (Persson *et al*., 2004). For dry deposition, 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_{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)

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

For total tissue N and N_{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 $^{**}P < 0.01$; $n = 5$.
DW, dry weight.

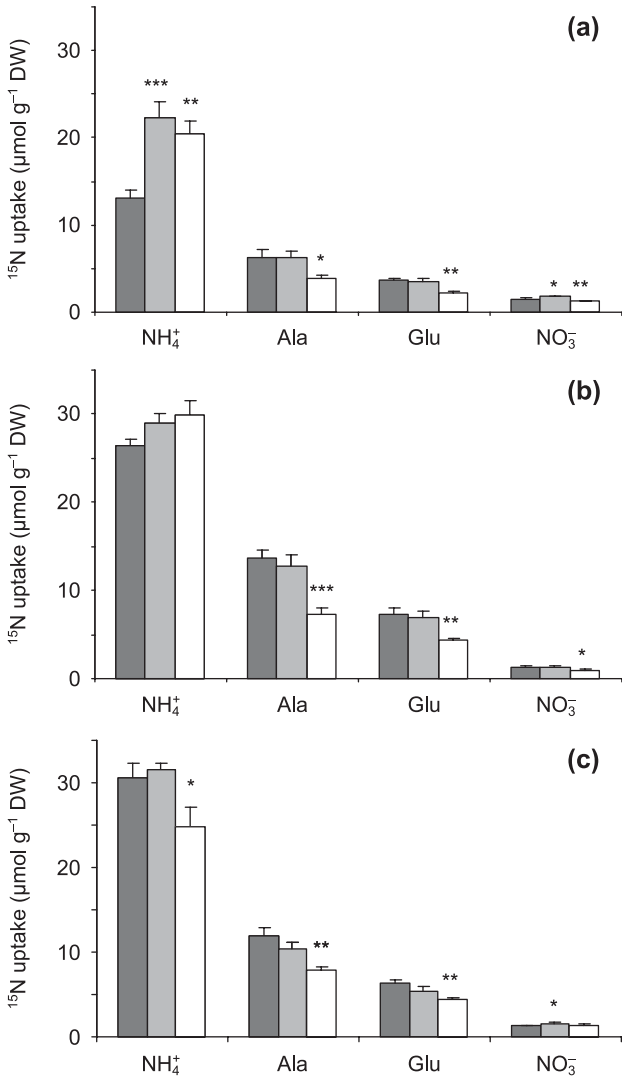

Fig. 2 ^{15}N uptake from the different nitrogen (N) sources (NH_4^+, alanine (Ala), glutamic acid (Glu), and NO_3^-) by two *Sphagnum* species, *Sphagnum balticum* and *Sphagnum fuscum*, originating from a low N deposition site (LD, dark gray bars), a mid N deposition site (MD, gray bars), and a high 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.

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

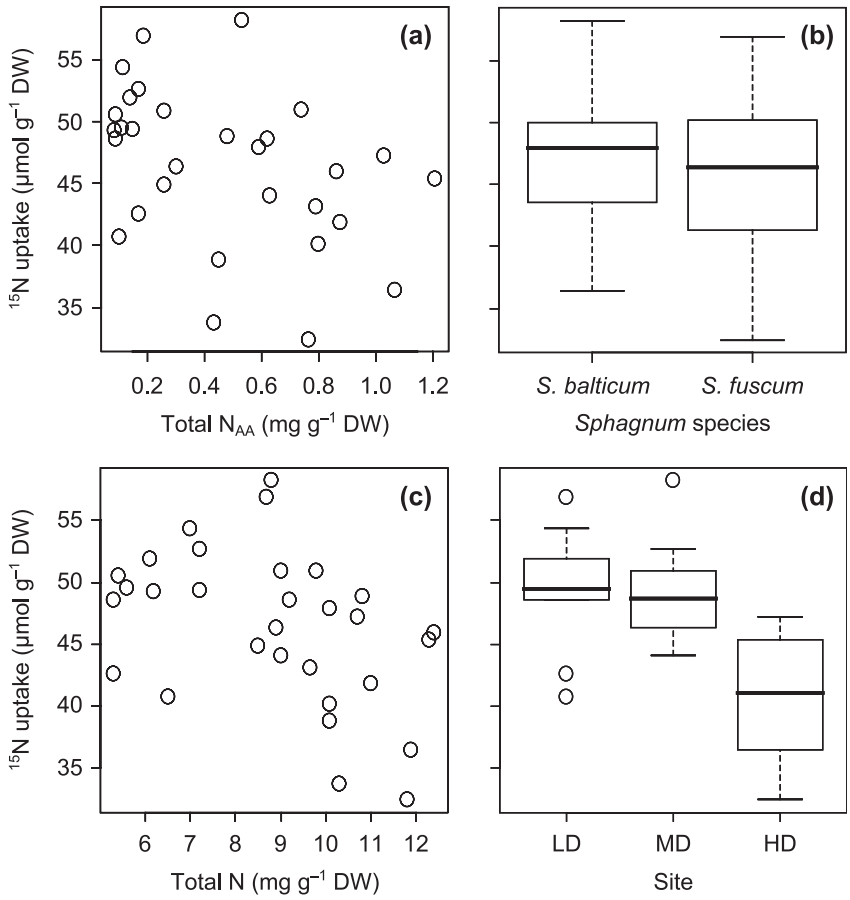

Fig. 3 Total ^{15}N uptake by the two *Sphagnum* species, *Sphagnum balticum* and *Sphagnum fuscum*, from the 100 μM uptake solutions after 2 h of exposure in relation to: (a) total soluble amino acid N tissue content (N_{AA}); (b) the two *Sphagnum* species; (c) total tissue N content; and (d) sites differing in N wet deposition (low, LD, 2 kg ha^{-1} yr^{-1}; mid, MD, 8 kg ha^{-1} yr^{-1}; and high N deposition site, HD, 12 kg ha^{-1} yr^{-1}); (for statistics see Table 3). DW, dry weight.

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

Response variable	$F_{(4,25)}$	R^2	Model significance	Source	df	*F*-value	*P* (> *F*)
^{15}N uptake	6.4	0.51	$P = 0.001$	Site	2	11.7586	$P < 0.001$
				Species	1	0.4899	$P = 0.490$
				N_{AA}	1	1.6469	$P = 0.211$
^{15}N uptake	6.2	0.50	$P = 0.001$	Site	2	11.6061	$P < 0.001$
				Species	1	0.4835	$P = 0.490$
				N_{tot}	1	1.3015	$P = 0.265$

Total ^{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_{AA} and N_{tot} separately, as continuous explanatory variables.

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 (Snaydon & 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 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_3^- 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_3^- uptake was reduced in specimens from the HD site. For *S. balticum*, NH_4^+ uptake was not reduced in specimens from the HD site, irrespective of the solution concentration. Unlike amino acid and NO_3^- uptake, the pattern of NH_4^+ 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_4^+ uptake in plants from the MD and HD sites compared with those from the LD site. To our knowledge, the mechanisms for NH_4^+ 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_4^+ 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_4^+ 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^+) deficiency in Sphagna (Bragazza & Limpens, 2004; Carfrae *et al.*, 2007; Gerdol *et al.*, 2007). For higher plants, NH_4^+ uptake may occur as a side effect of K^+ uptake, as some K^+ channels also can be employed for NH_4^+ uptake (Maathuis, 2007). It has been suggested that up-regulated K^+ uptake, compensating for the induced K^+ deficiency, could enhance NH_4^+ 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_4^+ uptake from the 100 μM N solution by *S. balticum* from all three sites (Fig. 2b), nor the reduced NH_4^+ uptake recorded for *S. fuscum* from the HD site (Fig. 2c). Furthermore, such an explanation for NH_4^+ uptake by *S. fuscum* from the HD site would contradict the hypothesis of Gerdol *et al.* (2007) that, in high N deposition areas, K^+ 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_4^+ influx and efflux through plant tissue, as discussed by Britto & Kronzucker (2002). With higher internal tissue concentrations, NH_4^+ efflux should be higher, thereby causing increased absolute values of $^{15}NH_4^+$ influx. To explain the observed pattern of $^{15}NH_4^+$ uptake from the 10 μM N solution, internal NH_4^+ 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_4^+ uptake from the 100 μM N solution (Fig. 2b,c). Thus, we are unable to explain the differences in response patterns for NH_4^+ 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_4^+ and NO_3^- 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_4^+, whilst only small amounts of NO_3^- were taken up, despite the well-known fact that Sphagna have the potential to utilize NO_3^- 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_3^- 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_4^+ than NO_3^- 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 *Eriophorum 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_4NO_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.

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