

# Nitrogen concentration and $\delta^{15}\text{N}$ signature of ombrotrophic *Sphagnum* mosses at different N deposition levels in Europe

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## Abstract

Alteration of the global nitrogen (N) cycle because of human-enhanced N fixation is a major concern particularly for those ecosystems that are nutrient poor by nature. Because *Sphagnum*-dominated mires are exclusively fed by wet and dry atmospheric deposition, they are assumed to be very sensitive to increased atmospheric N input. We assessed the consequences of increased atmospheric N deposition on total N concentration, N retention ability, and  $\delta^{15}\text{N}$  isotopic signature of *Sphagnum* plants collected in 16 ombrotrophic mires across 11 European countries. The mires spanned a gradient of atmospheric N deposition from about 0.1 up to about  $2\text{ g m}^{-2}\text{ yr}^{-1}$ . Mean N concentration in *Sphagnum* capitula was about  $6\text{ mg g}^{-1}$  in less polluted mires and about  $13\text{ mg g}^{-1}$  in highly N-polluted mires. The relative difference in N concentration between capitulum and stem decreased with increasing atmospheric N deposition, suggesting a possible metabolic mechanism that reduces excessive N accumulation in the capitulum. *Sphagnum* plants showed lower rates of N absorption under increasing atmospheric N deposition, indicating N saturation in *Sphagnum* tissues. The latter probably is related to a shift from N-limited conditions to limitation by other nutrients. The capacity of the *Sphagnum* layer to filter atmospheric N deposition decreased exponentially along the depositional gradient resulting in enrichment of the mire pore water with inorganic N forms (i.e.,  $\text{NO}_3^- + \text{NH}_4^+$ ). *Sphagnum* plants had  $\delta^{15}\text{N}$  signatures ranging from about  $-8\%$  to about  $-3\%$ . The isotopic signatures were rather related to the ratio of reduced to oxidized N forms in atmospheric deposition than to total amount of atmospheric N deposition, indicating that  $\delta^{15}\text{N}$  signature of *Sphagnum* plants can be used as an integrated measure of  $\delta^{15}\text{N}$  signature of atmospheric precipitation. Indeed, mires located in areas characterized by greater emissions of  $\text{NH}_3$  (i.e., mainly affected by agricultural activities) had *Sphagnum* plants with a lower  $\delta^{15}\text{N}$  signature compared with mires located in areas dominated by  $\text{NO}_x$  emissions (i.e., mainly affected by industrial activities).

**Keywords:** eutrophication, global change, inorganic nitrogen, internal nitrogen relocation, isotope, nitrogen retention, nitrogen saturation, peatlands, pore water chemistry

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## Introduction

Nitrogen (N) is a fundamental element for all biota. Because most organisms are not able to use the inert  $\text{N}_2$ , N frequently is a limiting nutrient (Vitousek & Howarth, 1991). In the present industrial world, human activities are strongly increasing the fixation of biologically reactive N through energy production, fertilizer production, and legume cultivation. Nowadays, the amount of N converted by human activities into biologically active forms is approximately equal to the amount of N naturally fixed on continents (Galloway *et al.*, 1995). The most abundant reactive N compounds in the atmosphere are represented by oxidized forms (i.e.,  $\text{N}_2\text{O}$  and  $\text{NO}_x$ ) and reduced forms (i.e.,  $\text{NH}_x$ ). Both forms, with the exception of  $\text{N}_2\text{O}$ , can be deposited onto terrestrial and aquatic ecosystems as dry and wet deposition. The main anthropogenic sources for  $\text{NO}_x$  emissions are transport, industry, and energy production (estimated to contribute up to 70% of total  $\text{NO}_x$  emissions). Additional sources include soil emission, particularly under high N inputs (Levy *et al.*, 1999). Emission sources of  $\text{NH}_x$  are primarily related to agricultural activities such as animal husbandry, as well as application and production of fertilizers (Asman *et al.*, 1998).

The increased emissions and depositions of reactive N are responsible for many environmental concerns dealing with, for example, increased tropospheric ozone, acidification, global climate change, and eutrophication (Galloway *et al.*, 2003). The latter, in particular, has been proved to cause a loss in plant biodiversity by favouring a few strong competitors (Sala *et al.*, 2000). This threat is particularly high for ecosystems such as ombrotrophic mires (or bogs) whose nutrient balance depends totally on atmospheric input (Bobbink *et al.*, 1998).

Ombrotrophic mires are peat-accumulating ecosystems, hydrologically insulated from local minerotrophic groundwater and hence fed only by atmospheric deposition. A genus of plants typical of ombrogenous mires is *Sphagnum* (Van Breemen, 1995). *Sphagnum* mosses are characterized by indefinite apical elongation in the so-called capitulum (i.e., the apical portion characterized by a dense aggregation of branches). The lower portion of *Sphagnum* plants gradually dies and this, under anaerobic conditions, favours peat accumulation that insulates the mire surface from minerotrophic groundwater.

The bog surface appears as a layer of tightly connected *Sphagnum* capitula that efficiently intercept nutrients coming from the atmosphere or from the canopy of the few vascular plants adapted to thrive in such nutrient-poor ecosystems. Accordingly, the reten-

tion ability of *Sphagnum* mosses has been demonstrated to be very high, particularly under low levels of atmospheric N availability (Williams *et al.*, 1999; Aldous, 2002). Like all bryophytes, *Sphagnum* plants accumulate nutrients through direct influx of atmospheric inputs into living cells, making these mosses extremely sensitive to present and past atmospheric conditions (e.g., Shotyk *et al.*, 1998; Bragazza *et al.*, 2003). In addition, recent findings obtained from mesocosm fertilization experiments (see Limpens & Berendse, 2003) suggest a reduced relocation of N towards the capitulum under high N availability associated with an increase of N content in the underlying stem segment.

Recent models on the relationship between increased atmospheric N inputs and N accumulation by *Sphagnum* plants have suggested a three-phase mechanism (Lamers *et al.*, 2000; Berendse *et al.*, 2001). The authors reason that at very low atmospheric deposition, N is completely and rapidly absorbed by the *Sphagnum* layer (phase 1), but under increasing input *Sphagnum* plants become N saturated (phase 3). During this phase the *Sphagnum* layer is assumed to lose its filtering ability, leading to increased N availability to vascular plants that may expand to the detriment of *Sphagnum*. As such, the degree in which *Sphagnum* is able to retain N, and thus, withhold it from the more competitive vascular plants under increasing N inputs determines the resilience of the bog ecosystem to N deposition.

The  $\delta^{15}\text{N}$  value of a plant is the result of three major mechanisms: (a) assimilatory fractionation during N compounds uptake; (b) mycorrhizal association; and (c) preferential use of isotopically distinct N sources (Evans, 2001). In vascular plants, N fractionation during root uptake of  $\text{NO}_3^-$  was demonstrated to be absent or very low, whereas  $^{15}\text{N}$  discrimination can take place during  $\text{NH}_4^+$  uptake at high external ammonium concentrations (Evans *et al.*, 1996; Yoneyama *et al.*, 2001). Data on N fractionation by mosses during uptake are not available but, in the light of the direct influx of nutrients to the living moss cells, we assume that no significant fractionation occurs during N absorption by *Sphagnum* plants. Accordingly, the different  $\delta^{15}\text{N}$  signature of reactive N forms in the atmosphere (see, e.g., Heaton, 1986) would make isotopic composition of ombrotrophic *Sphagnum* mosses a reliable monitor of N emission sources. Indeed, in contrast to root uptake of vascular plants, the initial signature of atmospheric N compounds should not significantly change as the deposited N is promptly absorbed by the *Sphagnum* plants without undergoing changes mediated by soil processes (Högberg, 1997; Hobbie *et al.*, 2000; Evans, 2001). The possibility to differentiate between N emission sources based on *Sphagnum* tissue  $\delta^{15}\text{N}$

signature represents an easy and low-cost way to assess the dominant N form in atmospheric deposition; which N form dominates, determines, to a large extent, the effect of N deposition on *Sphagnum* N metabolism (e.g., Jauhiainen *et al.*, 1998), and greenhouse gas emission from bogs (e.g., Aerts & de Caluwe, 1999; Saarnio & Silvola, 1999).

In the light of the claimed alteration of the biogeochemical N cycle in bogs under increased atmospheric N inputs, this paper aims at addressing four questions using *Sphagnum* plants as target organisms: (1) How does N tissue concentration in *Sphagnum* plants change in relation to the amount of atmospheric N deposition? (2) Is the difference between N concentration in the actively growing capitulum and that in the underlying stem in *Sphagnum* plant affected by increased atmospheric N supply? (3) Is there any sign of N saturation in the tissues of European *Sphagnum* under high N supply? If so, what are the effects of a failing filtering capacity of the moss layer on mire pore water chemistry? (4) Can  $\delta^{15}\text{N}$  isotopic signature of *Sphagnum* plants be used to identify atmospheric N sources?

The above questions are addressed using field data across a gradient of N deposition in Europe. In this way, the effects of increased atmospheric N deposition on ombrotrophic mires will be assessed under chronic N input avoiding possible toxic effects associated with abrupt N supply as happens in short-term fertilization experiments. In addition, sampling plants across 11 European countries permitted to take into account the role of different climatic conditions, particularly for what concerns the N isotopic signature of *Sphagnum* tissues.

## Materials and methods

### Sampling protocol

Plant sampling was carried out at 16 mires distributed in 11 European countries during the period 2001–2003. At each mire three to six plots were set up in hummocks (i.e., relatively drier microhabitats) and, whenever possible, three to six plots were set up in lawns (i.e., relatively wetter microhabitats). All plots were located in ombrotrophic sectors of the mire. The sampling was performed in areas with a dense cover of healthy *Sphagnum* plants and a very low, if any, cover of vascular plants. The hummocks had *Sphagnum fuscum* (Schimp.) Klinggr. and/or *S. capillifolium* (Ehrh.) Hedw. as dominant species in the moss layer. In contrast, lawns mostly had *S. magellanicum* Brid. as dominant species with the exception of two mires where the dominant species was *S. papillosum* Lindb. At each of the sampling plots all *Sphagnum* plants were picked from a 10 cm × 10 cm quadrat.

In a subset of eight mires, three pore water samples were collected close to the *Sphagnum* sampling plots. Free-standing pockets of water were selected and sampled with a syringe. The water was immediately filtered in the field through a 0.45 µm Whatman filter (Whatman Inc., Clifton, NJ, USA).

### Precipitation chemistry

Mean atmospheric depositions of  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N were determined based on data obtained, whenever possible, during the 3 years preceding the sampling, primarily from national organizations responsible for bulk deposition monitoring (Table 1). Even though dry deposition can account for a great portion of total deposition in highly polluted areas, we decided to use bulk deposition values for the following reasons: (1) the existence of an extensive network of bulk deposition monitoring stations in many European countries permitted us to select a monitoring station as close as possible to the study mires; (2) because of the uncertainty of dry deposition models (Erismann *et al.*, 1998b), the bulk deposition measured with permanently open funnels can partly compensate for the amount of total dry deposition (Cape & Leith, 2002); and (3) bulk deposition measurements are based on the same sampling method (i.e., open funnels) making large-scale comparisons practicable and reliable.

### Chemical analyses

After collection, all *Sphagnum* plants were cut into two parts: the apical portion characterized by a dense aggregation of branches (the capitulum) and the next 2 cm long portion (the stem). The density and the weight of capitula at each sampling plot were determined after air drying.

Before performing chemical analyses, *Sphagnum* capitula and stem portions were ground in a titanium mill through a 0.2 mm screen to ensure homogeneity of the sample. Subsamples of powdered material were oven-dried for 48 h at 70 °C to convert air-dry weight as well as element concentrations to standard oven-dry conditions.

Total N concentration and N isotopic signature of *Sphagnum* samples were determined with an elemental analyser (EA 1110, Carlo Erba, Milan, Italy) connected online with an isotope ratio mass spectrometer (delta S, Finnigan MAT, Bremen, Germany). Standard reference material (NIST Citrus leaves 1572, National Bureau of Standards) was analysed along with *Sphagnum* samples to ensure accuracy within 5% of known total N concentration.

N isotopic abundance is reported as  $\delta^{15}\text{N}$  and calculated as follows:

$$\delta(\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000,$$

**Table 1** List of the mires investigated with identification codes, geographical position (latitude and longitude), climatic data, average atmospheric nitrogen (N) bulk deposition, and microhabitats selected for *Sphagnum* sampling

Mire, country and identification code	Geographical coordinates	Mean annual precipitation (mm)	Mean annual temperature ( $^{\circ}\text{C}$ )	N bulk deposition ( $\text{g m}^{-2}\text{yr}^{-1}$ )	Sampled microhabitats
Malmmyran – Norway (N1)	63°41'N 8°28'E	1200	+ 7.0	0.17	Hummock
Djupvik – Norway (N2)	69°45'N 20°20'E	760	+ 3.0	0.08	Hummock
Salmisuo – Finland (FIN)	62°47'N 30°56'E	600	+ 2.0	0.20	Hummock
Klockamyran – Sweden (S1)	63°18'N 12°29'E	857	+ 1.1	0.14	Hummock
Ryggmossen – Sweden (S2)	60°3'N 17°20'E	554	+ 5.6	0.38	Hummock; lawn
Laxford Bridge – Great Britain (UK1)	58°22'N 5°00'W	1430	+ 8.0	0.22	Hummock; lawn
Ringinglow Bog – Great Britain (UK2)	53°23'N 1°29'W	981	+ 9.5	0.90	Lawn
Ballinahocun – Ireland (IRE)	53°19'N 7°58'W	820	+ 10.0	0.40	Hummock
Store Vildmose – Denmark (DK)	57°10'N 9°47'E	853	+ 8.3	0.68	Lawn
Reigersplas – the erlands (NL)	52°50'N 6°27'E	780	+ 8.6	2.0	Hummock; lawn
Čihadla – Czech Republic (CZ1)	49°59'N 13°49'E	1476	+ 4.4	1.9	Hummock; lawn
Mrtvý luh – Czech Republic (CZ2)	48°52'N 13°53'E	700	+ 5.1	0.49	Hummock; lawn
Etang de la Gruère – Switzerland (CH)	47°14'N 7°03'E	1200	+ 6.5	0.58	Hummock; lawn
Šijec – Slovenia (SLO)	46°20'N 13°59'E	1580	+ 3.0	1.11	Hummock; lawn
Coltrondo – Italy (I1)	46°30'N 12°45'E	1227	+ 2.2	0.45	Hummock; lawn
Wölfl Moor – Italy (I2)	46°26'N 11°24'E	808	+ 6.3	0.82	Hummock; lawn

Precipitation chemistry sources were: NILU-Norsk Institutt for Luftforskning; Finnish Meteorological Institute; IVL-Svenska Miljöinstitutet; NETCEN – AEA Technology Env.; Aherne & Farrell (2002); Ellermann *et al.* (2002); Rijksinstituut voor Volksgezondheid en milieu; Český hydrometeorologický ústav; OFEFP/NABEL; Slovenian Forestry Institute; Regione Veneto-ARPAV; Tait & Thaler (2000).

where  $R$  is the  $^{15}\text{N}/^{14}\text{N}$  ratio and the standard is atmospheric  $\text{N}_2$ . The analytical precision was in general better than  $\pm 0.2\text{‰}$  for the reference  $\text{KNO}_3$ .

Pore water samples were analysed colorimetrically, using a continuous flow analyser (FLOWSYS, Systea, Italy) for  $\text{Cl}^-$ ,  $\text{NO}_3^-$ -N, and  $\text{NH}_4^+$ -N.

#### Calculation and statistical analyses

Because no significant differences in N content and  $\delta^{15}\text{N}$  signature of *Sphagnum* plants between hummocks and lawns were found, all statistical analyses were performed averaging the hummock and lawn data when both the microhabitats were sampled in the same bog.

The N retention coefficient of *Sphagnum* capitula was calculated as the ratio between capitulum N concentration per square meter and atmospheric N deposition.

The relative difference in N concentration between the capitulum and the stem was calculated as follows:

$$[(N_{\text{capitulum}} - N_{\text{stem}})/N_{\text{capitulum}}] \times 100.$$

Concentration of inorganic N forms ( $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N) in pore water was corrected for evaporation by dividing N concentration for the ratio  $\text{Cl}^-_{\text{pore water}}/\text{Cl}^-_{\text{rain}}$ , assuming that chlorine has a conservative behaviour in mire pore water (Appelo & Postma, 1994).

Multiple regression related N isotopic signatures to selected environmental variables. In particular, mean annual temperature and total annual rainfall were selected as representative of climatic conditions, whereas N bulk deposition and the ratio  $\text{NH}_4^+:\text{NO}_3^-$  in bulk deposition were considered representative of atmospheric deposition chemistry.

All statistical analyses were performed using Statistica for Windows v. 6.0 (StatSoft Italia srl 2001).

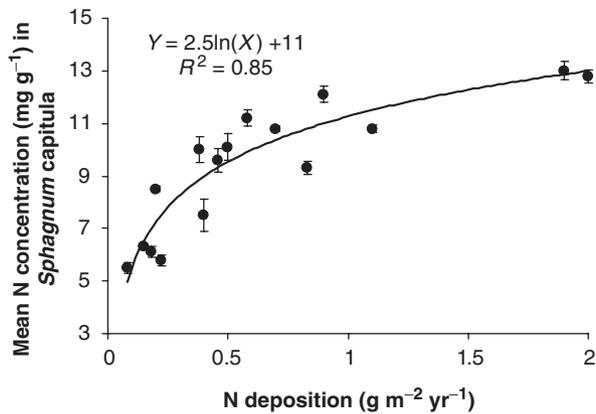
## Results

### Atmospheric N input over the study mires

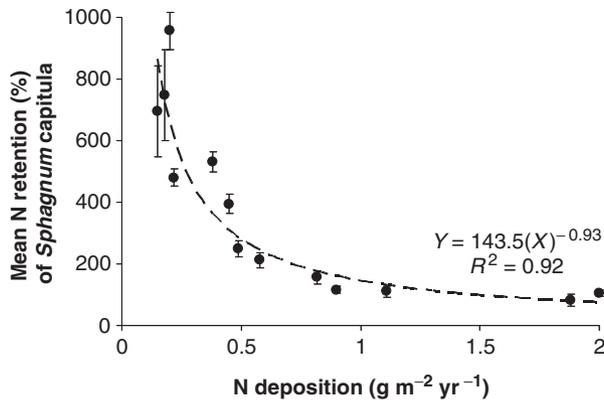
Geographic location of the mires investigated, and climatic and precipitation chemistry data are summarized in Table 1. Atmospheric N inputs ranged from about 0.1 to about  $2\text{ g m}^{-2}\text{ yr}^{-1}$ . Less N-polluted sites were located in Fennoscandia and in northern Scotland, whereas the most N-polluted sites were in the Czech Republic and the Netherlands.

### N concentration, N retention and relative N concentration in *Sphagnum* plants

Mean N concentration in *Sphagnum* capitula from less polluted mires was about  $6\text{ mg g}^{-1}$  compared with a



**Fig. 1** Trend of mean ( $\pm$  SE) nitrogen (N) concentration in *Sphagnum* capitula along the gradient of atmospheric N deposition.

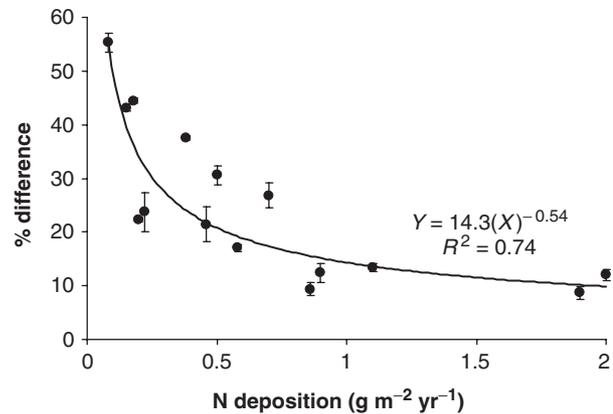


**Fig. 2** Mean ( $\pm$  SE) N retention by *Sphagnum* capitula along the gradient in atmospheric N deposition.

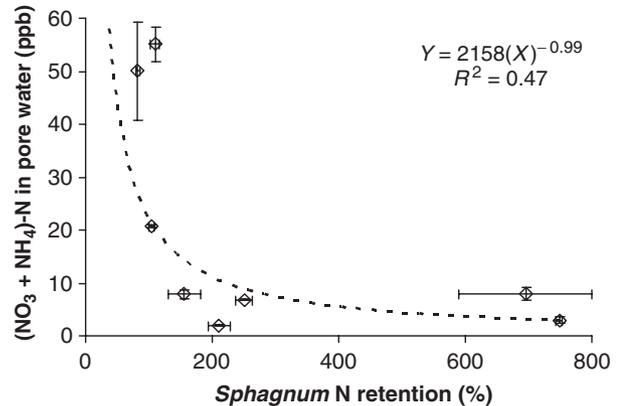
mean N concentration of about  $13 \text{ mg g}^{-1}$  under highest atmospheric N depositions (Fig. 1). The increase of N concentration in *Sphagnum* capitula along the atmospheric N deposition gradient was much faster at low than at high atmospheric N input (Fig. 1).

Retention of atmospheric N by *Sphagnum* capitula decreased exponentially along the gradient of atmospheric deposition with highest retention rates at less polluted mires (Fig. 2). *Sphagnum* plants collected in bogs subject to highest N deposition (i.e., the Netherlands and the Czech Republic) showed a retention ability up to fivefold lower than plants collected in less polluted mires (Fig. 2).

The relative difference in N concentration between capitulum and stem decreased exponentially along the gradient of atmospheric N deposition. In particular, *Sphagnum* plants in highly polluted areas showed a relative difference in N concentration about fivefold



**Fig. 3** Relative mean ( $\pm$  SE) difference in N concentration between capitulum and stem of *Sphagnum* plants along the gradient atmospheric N deposition.



**Fig. 4** Relationship between mean ( $\pm$  SE) N retention by the *Sphagnum* layer and mean ( $\pm$  SE) inorganic N in mire pore water. Dissolved inorganic N concentration in mire pore water was corrected for the effect of evaporation (see text for details).

lower than *Sphagnum* plants collected under low atmospheric N input (Fig. 3).

#### *Inorganic N concentrations in mire pore water*

Decreasing retention of N in the *Sphagnum* layer was accompanied by an exponential increase in the concentration of inorganic N in mire pore water (Fig. 4). Total corrected concentration of inorganic N in pore water showed mean values  $<10$  ppb in less polluted mires and mean values of about 50 ppb in mires subject to the highest atmospheric N deposition.

#### $\delta^{15}\text{N}$ isotopic signature of *Sphagnum* plants

*Sphagnum* plants showed a range of mean  $\delta^{15}\text{N}$  from about  $-8.0\text{‰}$  to about  $-3.5\text{‰}$  over the study

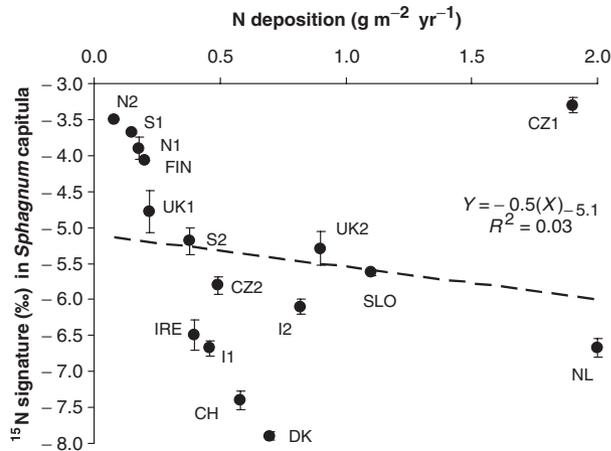


Fig. 5 Mean  $\delta^{15}\text{N}$  isotopic signature ( $\pm$  SE) of *Sphagnum* capitula along the gradient of atmospheric N deposition. The identification codes of mires follow Table 1.

Table 2 Summary of multiple linear regression between  $\delta^{15}\text{N}$  in *Sphagnum* capitula and selected environmental variables

Variables in the model	Slope	P-value
	( $n = 25$ ) $R^2 = 0.41$ , $F = 3.3$ , $P < 0.05$	
Intercept	-3.3	0.01
N bulk deposition	0.9	0.13
$\text{NH}_4^+ : \text{NO}_3^-$ in bulk deposition	-2.2	0.01
Mean annual temperature	-0.02	0.87
Total annual precipitation	$-4 \times 10^{-4}$	0.63

$n$  is the number of samples.

mires (Fig. 5). The relationship between  $\delta^{15}\text{N}$  isotopic signature and atmospheric N deposition did not show any significant trend (Fig. 5), indicating that bulk atmospheric N input did not affect the N isotopic signature of *Sphagnum* plants (Table 2). Instead, a significant contribution to N isotopic signature of *Sphagnum* plants was found for the  $\text{NH}_4^+ : \text{NO}_3^-$  ratio in atmospheric deposition (Table 2). In particular, based on literature data reporting the ratio of reduced ( $\text{NH}_x$ ) and oxidized ( $\text{NO}_x$ ) N compounds in dry and wet deposition, a significant correlation was found between the  $\text{NH}_x : \text{NO}_x$  ratio in dry deposition and  $\delta^{15}\text{N}$  signatures in *Sphagnum* plants ( $r = -0.87$ ,  $P = 0.023$ ,  $n = 6$ ), whereas the correlation was not significant with the  $\text{NH}_x : \text{NO}_x$  ratio in wet deposition ( $r = -0.40$ ,  $P = 0.44$ ,  $n = 6$ ) (Fig. 6).

Climatic conditions as expressed by mean annual temperature and annual precipitation did not signifi-

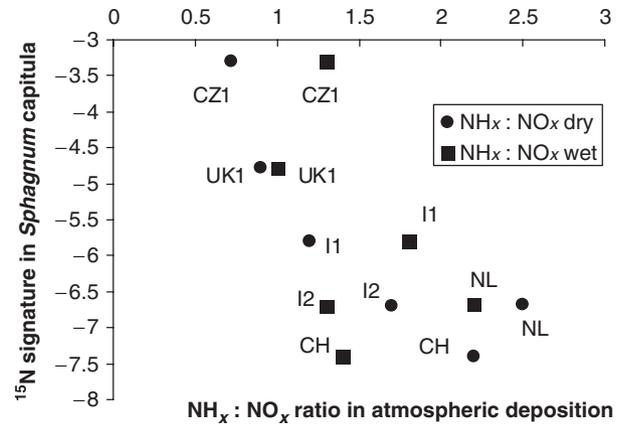


Fig. 6 Pattern of variation of isotopic signature in *Sphagnum* capitula measured at CZ1, UK1, I1, I2, NL, and CH in relation to the ratio of reduced ( $\text{NH}_x$ ) and oxidized ( $\text{NO}_x$ ) nitrogen compounds in dry and wet deposition obtained by different authors for areas representative of the selected study mires. Literature data are from: Zapletal (1998) for CZ1; Metcalfe *et al.* (2001) for UK1; Puxbaum & Gregori (1998) for I1; Balestrini *et al.* (2000) for I2; Erisman *et al.* (1998a) for NL; Rihm & Kurz (2001) for CH.

cantly affect the  $\delta^{15}\text{N}$  isotopic signature of *Sphagnum* plants over the study area (Table 2).

## Discussion

### Pattern of variation of N concentration in *Sphagnum* plants

In our study, the *Sphagnum* capitula showed higher rates of N absorption under low N atmospheric input, with a gradual decrease of N absorption as atmospheric N input increased. Rapid N absorption at low atmospheric inputs is related to the limiting role played by N in relatively unpolluted mires (Aerts *et al.*, 1992; Soares & Pearson, 1997; Bragazza *et al.*, 2004), whereas the saturating trend in the capitulum N concentration at high atmospheric input probably is a consequence of nutritional constraints caused by other nutrients, namely P and K (Pearson & Stewart, 1993; Bragazza *et al.*, 2004).

However, the Dutch and Czech (CZ1) *Sphagnum* plants, subject to the highest atmospheric N input, are expected to absorb further N supplies even if nutritional conditions are no longer N-limited. Indeed, N fertilization experiments carried out in the Netherlands with artificial N supply up to 10-fold greater than the amount in bulk deposition reported total N concentration in *Sphagnum* capitula up to about  $20 \text{ mg g}^{-1}$ , well above the value of  $13 \text{ mg g}^{-1}$  observed in the present survey (see, e.g., Van der Heijden *et al.*, 2000; Berendse

*et al.*, 2001; Heijmans *et al.*, 2001; Limpens & Berendse, 2003). This indicates an effective metabolic adaptation of *Sphagnum* plants to a broad range of external N supplies, even beyond the present levels of natural bulk deposition observed in polluted regions (Baxter *et al.*, 1992; Limpens & Berendse, 2003).

#### *N relocation in Sphagnum plants*

High input of N compounds can be detrimental to mosses (Soares & Pearson, 1997). However, *Sphagnum* plants have been shown to cope with increased N availability through a set of metabolic adaptations such as reduced nitrate reductase activity (Woodin & Lee, 1987; Soares & Pearson, 1997), reduced N uptake (Jauhiainen *et al.*, 1998), and conversion of excess N into amino acids (Nordin & Gunnarsson, 2000; Limpens & Berendse, 2003). A further metabolic adaptation emerging from our study is an increase in the relative N concentration in the stem compared with the capitulum. Although, the capitulum represents the most actively growing part of *Sphagnum* plants, characterized by a high content of nutrients, in particular N, P, and K, as well as amino acids and soluble proteins (van der Heijden *et al.*, 2000), our findings suggest that under enhanced N availability more N is stored in the stem probably as a means to avoid toxic effects of high N concentration in the capitulum. This conclusion is in accordance with the results obtained by Limpens & Berendse (2003) reporting higher free amino acid concentrations in the stem than in the capitulum at enhanced N input.

#### *The N filtering ability of the moss layer*

To thrive in nutrient-deficient habitats *Sphagnum* mosses rely on an efficient capitulum absorption of atmospheric N input (Williams *et al.*, 1999; Aldous, 2002; Heijmans *et al.*, 2002). In contrast to the model proposed by Lamers *et al.* (2000) and Berendse *et al.* (2001), our data showed that N retention in the *Sphagnum* layer began to decline even at low levels of N deposition, well before N saturation in *Sphagnum* tissues had been reached. This failure in efficiently filtering the N deposition by the moss layer was accompanied by an increase of inorganic N concentration in pore water. Increased N availability in pore water favours vascular plant growth (Berendse *et al.*, 2001; Heijmans *et al.*, 2002; Limpens *et al.*, 2003a), and can also increase parasitic attacks in *Sphagnum* plants (Limpens *et al.*, 2003b). Hence, it seems that the disappearance of *Sphagnum* plants under increasing atmospheric N input in Europe (e.g., Hogg *et al.*, 1995) is not exclusively attributable to direct toxic effects of

increased N supply, at least not at the present levels of atmospheric N deposition but, instead, to the alteration of competitive equilibria triggered by the improved N availability.

#### *$\delta^{15}\text{N}$ in Sphagnum plants and the source of N input*

To our knowledge, there are few data available on  $\delta^{15}\text{N}$  signature in *Sphagnum* plants under field conditions (e.g., Kohzu *et al.*, 2003), and no data on *Sphagnum* isotopic signature in relation to atmospheric N deposition. *Sphagnum* plants from hummocks and lawns were depleted in  $^{15}\text{N}$  relative to atmospheric  $\text{N}_2$ . However, the variation of  $^{15}\text{N}$  content in *Sphagnum* plants was not simply related to the total amount of atmospheric N deposition. Indeed, the  $\delta^{15}\text{N}$  signature seems primarily related to the relative ratio of reduced N forms to oxidized N forms in atmospheric deposition, particularly in dry deposition. Even though historical reconstruction of  $\delta^{15}\text{N}$  variation based on herbarium plant specimens (e.g., Peñuelas & Filella, 2001), and ice cores (e.g., Freyer *et al.*, 1996) reported a general depletion of  $^{15}\text{N}$  with increasing N deposition, our findings suggest that the type of N prevailing at a given locality, as a consequence of human activities, significantly influences the  $\delta^{15}\text{N}$  signature of the mosses (Pearson *et al.*, 2000). In particular, the much lower  $\delta^{15}\text{N}$  value in the Netherlands, Denmark, Switzerland, and Italy (I2) appeared to reflect a major role of local ammonia emissions from livestock during animal husbandry, manure storage and spreading (Asman *et al.*, 1998), in accordance with the very negative  $\delta^{15}\text{N}$  signature detected for reduced N forms in wet ( $\text{NH}_4^+$ ) and, particularly, dry ( $\text{NH}_3$ ) atmospheric precipitation (Heaton, 1987; Freyer, 1991; Garten, 1992; Buzek *et al.*, 1998; Erskine *et al.*, 1998; Russell *et al.*, 1998; Gao, 2002; Xiao & Liu, 2002).

On the other hand, relatively higher  $\delta^{15}\text{N}$  signature for the Czech mire (CZ1) located in the so-called 'black triangle', one of the most polluted and acidified areas in Europe, seems to be related to the higher contribution of  $\text{NO}_x$  forms emitted by industrial activities, primarily fossil fuel burning. A similar pattern of relatively higher  $^{15}\text{N}$  content was observed in pine needles (Jung *et al.*, 1997; Amman *et al.*, 1999) and acrocarpous mosses (Pearson *et al.*, 2000) collected in industrial areas and was related to the enrichment in  $^{15}\text{N}$  of  $\text{NO}_x$  forms in dry and wet deposition (Freyer, 1991; Garten, 1992; Buzek *et al.*, 1998; Russell *et al.*, 1998; Yeatman *et al.*, 2001; Gao, 2002; Xiao & Liu, 2002). This pattern is consistent with the significant relationship between the ratio of reduced N forms to oxidized N forms in wet and dry deposition obtained by recent literature data and the  $\delta^{15}\text{N}$  of *Sphagnum* plants measured in this

study. This trend points out the role of precipitation quality with particular respect to the dominant type of N forms in dry deposition.

The  $\delta^{15}\text{N}$  values in *Sphagnum* plants growing in Norway, Scotland, Finland, and Sweden could be taken as representative of the  $^{15}\text{N}$  signature under relatively pristine conditions of atmospheric N deposition.

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