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Stimulated growth of *Betula pubescens* and *Molinia caerulea* on ombrotrophic bogs: role of high levels of atmospheric nitrogen deposition

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Summary

1 In order to test whether the observed invasion of ombrotrophic bogs in the Netherlands by *Molinia caerulea* and *Betula pubescens* is the result of long-term high nitrogen (N) loads, we conducted a 3-year fertilization experiment with *Sphagnum fallax* turfs. Six different N treatments were applied ranging from 0 (control) to 4 g N m^{-2} $year^{-1}$.
2 During the experimental period, ammonium concentrations in the peat moisture remained very low due to high uptake rates by *Sphagnum*. Tissue N concentrations in *S. fallax* showed a linear response to the experimental N addition. Excess N was accumulated as N-rich free amino acids such as arginine, asparagine and glutamine, especially at N addition rates of 0.25 g m^{-2} $year^{-1}$ or higher, indicating N-saturation.
3 Despite the high tissue N : P ratio (above 35), above-ground biomass production by *Molinia* was still stimulated at N addition rates of 4 g m^{-2} $year^{-1}$, and foliar nutrient concentrations were unaffected compared to the control. In contrast to *Molinia*, *Betula* was unable to increase its above-ground biomass. Foliar N concentrations in *Betula* were significantly higher at N addition rates of 4 g m^{-2} $year^{-1}$ and excess N was stored in foliar arginine, making up 27% of the total N concentration. Evapotranspiration was increased at higher N addition rates due to stimulated total above-ground biomass production of the vegetation.
4 N addition at the actual Dutch deposition rate of 4 g m^{-2} $year^{-1}$ stimulated the growth of *Molinia* in this experiment, providing evidence that the observed dominance of *Molinia* on ombrotrophic bogs in the Netherlands is caused by high N deposition levels. Based on the observed changes in biomass production and tissue nutrient concentrations, we assume that a long-term deposition of 0.5 g N m^{-2} $year^{-1}$, or higher, leads to undesirable changes in species composition and increased risk of desiccation.

Key-words: *Betula pubescens*, *Molinia caerulea*, nitrogen deposition, ombrotrophic bog, *Sphagnum fallax*

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Introduction

Ombrotrophic bogs are traditionally regarded as nitrogen (N)-limited. In areas with increased N deposition levels, however, productivity of *Sphagnum* may change from being N-limited to phosphorus (P)-limited (Aerts *et al.* 1992). In non-forest ecosystems in central and western Europe, present N deposition rates can amount to 2–6 g N m^{-2} $year^{-1}$ (Bobbink & Heil 1993). Ombrotrophic bogs are probably among the systems most sensitive to N enrichment and the empirical critical N load for ombrotrophic bogs has been estimated as 0.5–1 g N m^{-2} $year^{-1}$ (Bobbink & Roelofs 1995). Increased atmospheric N inputs can have important effects on the vegetation composition in various (semi)natural ecosystems (Bobbink *et al.* 1998; Bobbink & Lamers 2002). In *Calluna vulgaris* dominated heathlands, high N deposition levels have been found to allow invasion by species like *Molinia caerulea* (e.g. Heil & Bruggink 1987; Aerts & Berendse 1988).

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In ombrotrophic bogs, invasion of certain species of grass (e.g. *Molinia caerulea*) and trees (*Betula pubescens*) has been observed, together with a decline of ombrotrophic species (Barkman 1992; Aaby 1994; Hogg *et al*. 1995; Risager 1998). However, several authors have ascribed these changes to increased mineralization as a result of desiccation of the peat (e.g. Aerts & Ludwig 1997) rather than to increased levels of N deposition.

Although an effect of increased N availability on the growth of *Sphagnum* has been observed in other experiments (e.g. Ferguson & Lee 1983; Aerts *et al*. 1992), findings have not been consistent across the various studies. The actual background deposition has a significant effect on the response of *Sphagnum* to increased availability of N (Aerts *et al*. 1992; Gunnarsson & Rydin 2000). At relatively low atmospheric input (< 1 g N m^{-2} $year^{-1}$), *Sphagnum* has been found to respond to increased N deposition levels by increased growth, indicating N-limitation (Malmer 1990). At higher N loads (1–2 g N m^{-2} $year^{-1}$), N no longer limits growth but the *Sphagnum* layer does not reach its maximum organic N content (Pitcairn *et al*. 1995; Lamers *et al*. 2000; Berendse *et al*. 2001). Above 2 g N m^{-2} $year^{-1}$, the *Sphagnum* layer reaches its maximum N content and *Sphagnum* growth is affected (Lamers *et al*. 2000; Gunnarsson & Rydin 2000). In this situation, N leaches from the *Sphagnum* layer to the roots of vascular plants (Lee & Woodin 1988; Aerts *et al*. 1992; Lamers *et al*. 2000).

Various N addition experiments have also found changes in the species composition of the *Sphagnum* layer (e.g. Press *et al*. 1986; Lütke Twenhöven 1992; Risager 1998). Under N-limiting conditions, complete N immobilization by the *Sphagnum* layer causes vascular plants to depend on N mobilized by mineralization processes in the underlying peat (Malmer 1993; Malmer *et al*. 1994). Increased availability of nutrients in the rhizosphere leads to an increased cover of vascular plants and a reduction in *Sphagnum* growth due to shading (Hayward & Clymo 1983; Heijmans *et al*. 2001; Berendse *et al*. 2001). Hogg *et al*. (1995) found that cutting back *Molinia* reduced the competition for light and stimulated the growth of *Sphagnum*. Thus, increased N deposition levels also causes changes in the competition between *Sphagnum* and vascular plants.

As increased growth of *Molinia* and *Betula* is also observed on floating rafts that are permanently wet (personal observations), the invasion of *Molinia* and *Betula* in Dutch ombrotrophic bogs could very well be the result of increased N deposition levels, although experimental evidence is limited. Therefore, the effects of N on the growth of *B. pubescens* and *M. caerulea* in *Sphagnum fallax* turfs were determined in a 3-year laboratory experiment in which N fertilization was applied under permanently wet conditions. In the Netherlands, *Sphagnum fallax* is one of the most dominant *Sphagnum* species, probably because it is a better competitor for N than the other species (Lee & Woodin 1988; Lütke Twenhöven 1992; Risager 1998). Six different N addition rates were used, ranging from 0 to 4 g m^{-2} $year^{-1}$. It was hypothesized that high atmospheric N loads would lead to high N concentrations in the peat moisture, stimulating the growth of *Betula* and *Molinia*.

Materials and methods

EXPERIMENTAL SET-UP

Turfs were collected from an ombrotrophic floating raft (4 ha) in the 'De Hamert' nature reserve in the Netherlands (51°32′N, 6°10′E). The upper 10 cm were used in the experiment and the vegetation consisted mainly of *Sphagnum fallax* (klinggr.) Klinggr. (synonymous with *Sphagnum recurvum* P. Beauv. Var. *mucronatum* (Russ.) Warnst.; 95–100% cover) along with some *Vaccinium oxycoccus* L. and *Drosera rotundifolia* L. The turfs (n = 24) were cut and were placed in glass containers (24 × 24 × 32 cm) on the same day. All containers were placed in a temperature-regulated water bath in a climate control room with a light intensity of 100 μmol m^{-2} s^{-1} at the vegetation level (Fig. 1). Summer and winter were simulated by gradually increasing or decreasing the temperature and photoperiod (between 15 °C, 16 hours and 3 °C, 8 hours). The length of the winter period differed slightly between the various years due to technical problems. This variation, however, stayed within the natural range. Concentrations of atmospheric CO_2 at the vegetation level were ambient (approx. 370 μmol CO_2 mol^{-1}). Three soil moisture samplers (Rhizon SMS – 10 cm; Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) were placed in each turf (at depths of 0–10 cm) to allow the chemical composition of the peat moisture to be analysed.

Six treatments were applied, differing in N concentrations and leading to N loads of 0, 0.25, 0.5, 1, 2 and 4 g N m^{-2} $year^{-1}$ (0–0.29 mol N m^{-2} $year^{-1}$). N was added as ammonium (65%) and nitrate (35%) using NH_4NO_3 and NH_4Cl based on the actual ratios in the Netherlands (situation 1994; Lamers 1995). The background deposition level of N in the climate control room was negligible (< 0.05 g m^{-2} $year^{-1}$, data not shown). Artificial rainwater was sprayed directly on the turfs three times a week, at a rate equivalent to a rainfall of 750 mm (the mean annual rainfall in the Netherlands). Besides the various N concentrations, the solution contained 5 mg L^{-1} sea salt ('Marine mix + Bio-elements', Wiegandt GmbH, FRG), 30 μmol L^{-1} KCl, 10 μmol L^{-1} $CaCl_2$, 10 μmol L^{-1} Fe-EDTA, 10 μmol L^{-1} KH_2PO_4, 0.7 μmol L^{-1} $ZnSO_4$, 0.8 μmol L^{-1} $MnCl_2$, 0.2 μmol L^{-1} $CuSO_4$, 0.8 μmol L^{-1} H_3BO_3 and 0.008 μmol L^{-1} $(NH_4)_6Mo_7O_{24}$. The surplus water was removed via an overflow system in order to keep the water level at 4 cm below the capitula (maximum fluctuation 5 mm). Each treatment consisted of four replicates, randomly distributed over the water bath. After a pre-treatment period of 4 months (rainwater without N), the concentration of ammonium in the

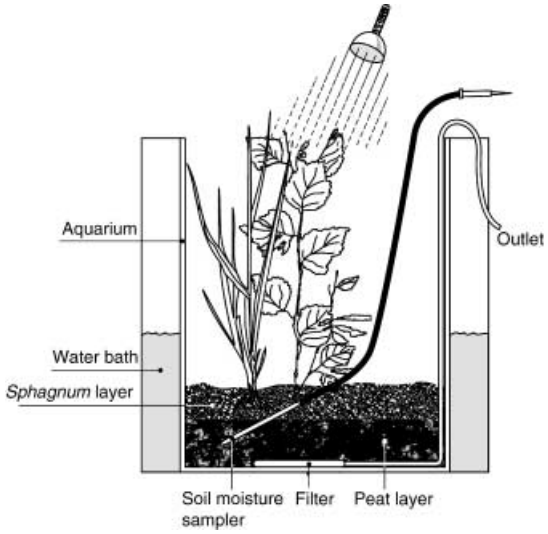

Fig. 1 Experimental set-up for one turf including introduced *Betula pubescens* and *Molinia caerulea*.

peat moisture had dropped from 50 to 60 μmol L^{-1} to < 10 μmol L^{-1} (data not shown). Six saplings of *Betula pubescens* Ehrh. (collected in a nearby heathland; 4.25 ± 0.59 cm high; total fresh weight 6.14 ± 0.91 g) and five vegetative shoots of *Molinia caerulea* (L.) Moench (collected at 'De Hamert'; total fresh weight 3.10 ± 0.27 g) were then planted in each container. Growth of *Betula* and *Molinia* was measured non-destructively every 3 months. Growth of *M. caerulea* was determined by counting the number of living shoots and that of *Betula* by measuring the length and number of leaves. The total N concentrations of the capitula (upper 2 cm) and stems (2–4 cm) of *S. fallax* were determined twice a year. After 3 years of N addition, the total biomass of *Betula* and *Molinia* plants was determined.

SAMPLING

Peat moisture was collected by connecting vacuum infusion flasks (30 mL) to each sampler. The three subsamples were pooled and pH and carbon dioxide concentration were measured. After the addition of citric acid to a final concentration of 0.6 mmol L^{-1} to prevent metal precipitation, water samples were stored (for a maximum of 6 weeks) in iodated polyethylene bottles (100 mL) at –20 °C until further analysis. Above-ground and below-ground biomass of *Betula* and *Molinia* was carefully removed and sorted into leaves, stems, flowers, roots and litter. Samples of *Sphagnum fallax* were prepared by dividing the upper 4 cm into two parts (capitulum and stem). Nutrients, leaf pigments and free amino acids were analysed in green leaves of *Betula* and *Molinia*, and in capitula and stems of *Sphagnum*. Subsamples of the peat from each turf were taken to determine the potential carbon mineralization rate.

CHEMICAL ANALYSIS

pH was determined using a combination glass electrode with an Ag/AgCl internal reference (Orion Research, Beverly, USA). CO_2 concentrations were measured using an infrared carbon analyser (model PIR-2000, Horiba Instruments, Irvine, USA). Leaf pigment concentrations were determined in frozen and ground fresh tissue shaken for 24 hours (4 °C) with 96% ethanol. Leaf pigment concentrations in the supernatant fraction were measured spectrophotometrically according to Wellburn & Lichtenthaler (1984). To analyse nutrient concentrations in plant tissue and peat, dried samples (48 hours at 70 °C) were ground in liquid nitrogen. Samples were digested in sealed Teflon vessels in a Milestone microwave oven (type mls 1200 Mega, Sorisole, Italy) adding nitric acid and hydrogen peroxide. After dilution, the digestates were kept at 4 °C until analysis. Nitrogen and carbon concentrations were measured in dried samples with a CNS analyser (type NA1500; Carlo Erba Instruments, Milan, Italy).

CO_2 and CH_4 production rates were measured by incubating 200 g of fresh peat in 500 mL infusion flasks, sealed with an airtight rubber stopper. Incubations were carried out in duplicate for each turf. After filling, the flasks were repeatedly vacuumed and flushed with oxygen-free nitrogen gas to remove all CO_2 and CH_4 from the peat and the headspace. The flasks were kept in the dark at 20 °C, and CO_2 and CH_4 concentrations were measured weekly over a period of 4 weeks. CO_2 and CH_4 production rates were calculated by linear regression of the measurements, and expressed on a dry weight basis.

Ortho-phosphate concentrations were determined colorimetrically with a Technicon AA II system, using ammonium molybdate (Henriksen 1965). Nitrate and ammonium were measured colorimetrically with a Traacs 800+ auto-analyser, using hydrazine sulphate (Technicon 1969) and salicylate (Grasshoff & Johannsen 1977), respectively. Potassium was measured by flame photometry (FLM3 Flame Photometer, Radiometer, Copenhagen, Denmark). Phosphorus was determined by inductively-coupled plasma emission spectrophotometry (Spectro Analytical Instruments, type Spectroflame, Kleve, Germany).

Free amino acids were extracted according to Van Dijk & Roelofs (1988). They were quantified by measuring fluorescence after precolumn derivation with 9-Fluorenylmethyl-Chloroformate (FMOC-Cl) and measured using HPLC (with a Star 9050 variable wavelength UV-VIS and Star 9070 fluorescence detector; Varian Liquid Chromatography, Palo Alto, USA) with norleucine as the internal standard. Twenty amino acids were detected (alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tyrosine and valine) and all were expressed on a dry weight basis.

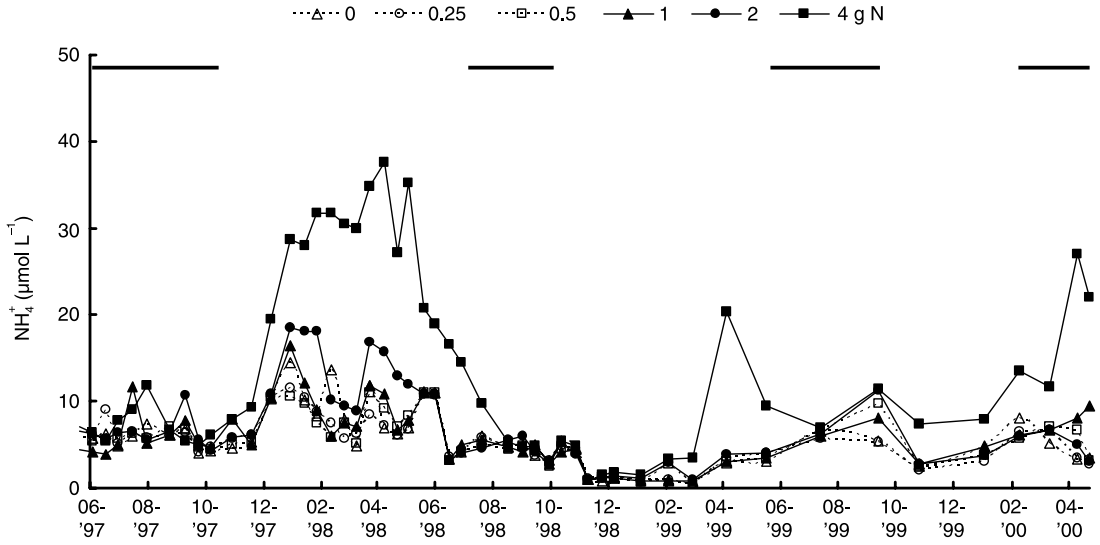

Fig. 2 Peat moisture ammonium concentrations between June 1997 and May 2000 at different experimental N addition rates (n = 4). Summer periods (15 °C and photoperiod of 16 hours) are indicated by horizontal lines.

STATISTICAL ANALYSIS

Prior to statistical analysis, data were log-transformed to make the variance less dependent on the means and to fit a normal distribution. All statistical analyses were carried out using the SPSS for Windows software package (version 10.0.7; SPSS Inc., Chicago, USA). Differences between treatments were tested with a one-way ANOVA at the 0.05 confidence limit. Tukey's student range tests were used to identify differences between treatments. Differences in peat moisture concentrations during the experiment were tested with the GLM (General Linear Model) procedure for repeated measures. Linear regression was used to determine significant relationships between N addition rates and N concentrations in *S. fallax*, and between above-ground biomass of *Molinia* and *Betula* and evapotranspiration rate. For clarity of presentation, the means and standard errors (SEs) presented in the figures represent the non-transformed data.

Results

WATER CHEMISTRY

The ammonium concentrations in the peat moisture showed a seasonal pattern and were significantly influenced by time ($P < 0.001$; Fig. 2). N addition significantly raised the concentration of ammonium in the peat moisture ($P < 0.01$). During the first growing season (6 months) the concentrations of ammonium in the peat moisture remained very low (Fig. 2). Lowering the temperature led to a strong increase in peat moisture ammonium concentration at the highest N treatment (4 g m^{-2} $year^{-1}$) during the first winter period. From the start of the second growing season, the ammonium concentrations gradually dropped again to concentrations comparable with those in the other treatments. During the second and third winter periods, no clear ammonium peak was measured. At the end of the experiment, peat moisture ammonium concentrations at the highest N addition rate were significantly elevated compared to those in the treatments with N addition rates of 1 g m^{-2} $year^{-1}$ or less (Fig. 2; $P < 0.05$). Nitrate concentrations were low throughout the experiment (≤ 6 μmol L^{-1}; Table 1).

Peat moisture pH fluctuated between 3.0 and 4.0 during the experimental period, and from spring 1999 onwards, pH was lower at higher N addition rates ($P < 0.001$). During the third growing season, the addition of 4 g N m^{-2} $year^{-1}$ led to pH 3.4, vs. 3.9 for the control (Table 1). The average phosphate concentration in the peat moisture remained low during the entire experiment: 0–0.5 μmol L^{-1} (Table 1). However, one of the 4 g N m^{-2} $year^{-1}$ replicates contained very high phosphate concentrations at the start of the experiment. The concentration dropped from 129 μmol L^{-1} at the start of the experiment to 0.5 μmol L^{-1} at the end. Potassium (K) concentrations at the start of the experiment ranged from 55 to 75 μmol L^{-1}. During the pre-treatment period, K concentrations dropped below 20 μmol L^{-1} and stabilized around 10 μmol L^{-1}. At the end of the experiment, K concentrations in the peat moisture increased slightly, with the highest concentrations found in the control treatment (30 μmol L^{-1}; Table 1).

SPHAGNUM LAYER

At the start of the experiment (field measurement, winter 1996–97), the N concentrations in the capitula and stems of *S. fallax* were 1214 and 1115 μmol N g^{-1} dry wt., respectively (Fig. 3). During the 4 months of the pre-treatment period (no N addition) the N concentrations

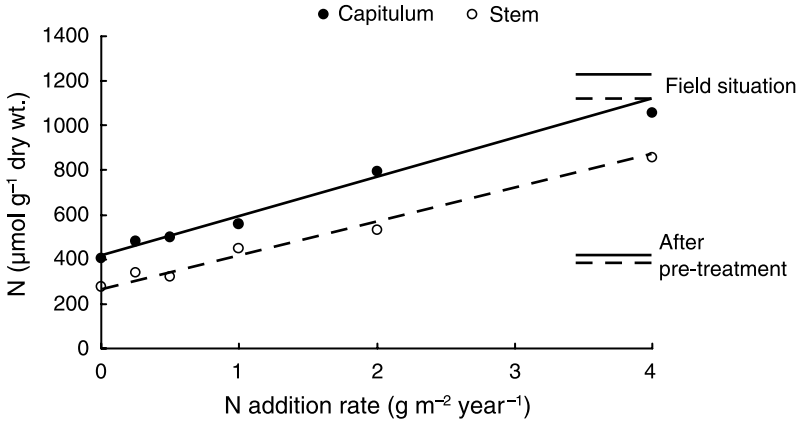

Fig. 3 N concentrations in capitula and stems of *Sphagnum fallax* after 3 years at different rates of experimental N addition (linear regression: $R^2_{capitul} = 0.988$ and $R^2_{stem} = 0.985$). Solid and dashed horizontal lines indicate N concentrations in capitula and stems, respectively, measured in the field and after the pre-treatment period.

Table 1 Peat moisture pH and carbon dioxide, nitrate, phosphate and potassium concentrations (µmol L^{-1}) during the third growing season (March until May 2000) at different experimental N addition rates (means ± 1 SE; $n = 12$). Different letters indicate significant differences ($P < 0.05$) between N treatments (one-way ANOVA)

N addition rate g m^{-2} $year^{-1}$	pH	CO_2 µmol L^{-1}	NO_3^- µmol L^{-1}	PO_4^{3-} µmol L^{-1}	K^+ µmol L^{-1}
0	3.91 ± 0.04^{a}	61 ± 6	2.2 ± 0.9^{a}	0.38 ± 0.18	30 ± 5^{a}
0.25	3.83 ± 0.03^{ab}	49 ± 8	1.7 ± 0.3^{ab}	0.19 ± 0.04	17 ± 5^{ab}
0.5	3.81 ± 0.04^{ab}	56 ± 6	5.6 ± 1.2^{ab}	0.40 ± 0.14	15 ± 6^{ab}
1	3.73 ± 0.04^{bc}	59 ± 7	4.5 ± 0.8^{ab}	0.54 ± 0.16	23 ± 5^{ab}
2	3.61 ± 0.04^{c}	49 ± 5	3.2 ± 0.6^{ab}	0.48 ± 0.18	7 ± 2^{b}
4	3.44 ± 0.04^{d}	64 ± 8	6.1 ± 1.5^{b}	0.43 ± 0.10	9 ± 2^{b}

Table 2 Concentrations (µmol g^{-1} dry wt.) of phosphorus and potassium, and C : N and N : P ratios (g g^{-1}) in capitula and stems of *Sphagnum fallax* subjected to different experimental N addition rates (means ± 1 SE; $n = 4$). Different letters indicate significant differences ($P < 0.05$) between N treatments (one-way ANOVA)

	N addition rate (g m^{-2} $year^{-1}$)	P µmol g^{-1} dry wt.	K µmol g^{-1} dry wt.	C : N ratio g g^{-1}	N : P ratio g g^{-1}
Capitula	0	25.9 ± 1.1^{a}	179 ± 10^{a}	78 ± 5^{a}	7 ± 0^{a}
	0.25	22.2 ± 0.7^{ab}	151 ± 5^{ab}	66 ± 5^{ab}	10 ± 1^{b}
	0.5	21.8 ± 0.9^{ab}	144 ± 6^{ab}	63 ± 4^{ab}	10 ± 0^{b}
	1	21.9 ± 0.9^{ab}	168 ± 6^{a}	56 ± 2^{b}	12 ± 1^{b}
	2	20.2 ± 1.0^{b}	133 ± 13^{ab}	40 ± 3^{c}	18 ± 1^{c}
	4	22.6 ± 2.2^{ab}	115 ± 18^{b}	30 ± 2^{c}	22 ± 1^{c}
Stems	0	15.3 ± 1.1	201 ± 14^{a}	114 ± 10^{a}	8 ± 1^{a}
	0.25	13.0 ± 0.6	175 ± 9^{ab}	92 ± 9^{ab}	12 ± 1^{ab}
	0.5	13.6 ± 1.4	156 ± 11^{abc}	98 ± 8^{ab}	11 ± 1^{ab}
	1	14.0 ± 0.9	162 ± 11^{abc}	69 ± 4^{bc}	15 ± 1^{bc}
	2	12.3 ± 1.9	128 ± 13^{bc}	61 ± 6^{c}	20 ± 1^{cd}
	4	14.5 ± 2.7	115 ± 11^{c}	36 ± 2^{d}	29 ± 4^{d}

in the capitula and stems of *S. fallax* dropped to 409 and 365 µmol g^{-1} dry wt., respectively, at the start of the N addition (Fig. 3). As a result of higher N addition rates, the N concentrations in *S. fallax* were significantly higher after 3 years ($P < 0.001$; Fig. 3). Concentrations were consistently slightly higher in capitula than in stems. The relationship between N addition rates and the tissue N concentrations in capitula and stems of *S. fallax* was linear. Capitulum N concentrations in *S. fallax* receiving no N were comparable with those measured directly after the pre-treatment period, whereas stem concentrations in *S. fallax*, receiving 0.5 g N m^{-2} $year^{-1}$ or less, decreased (Fig. 3). Compared to the N concentrations measured at the field location, both capitulum and stem concentrations were lower for all N treatments.

P concentrations in the capitulum tissue of *Sphagnum* were significantly lower than those in the controls at an addition of 2 g N m^{-2} $year^{-1}$ ($P < 0.05$; Table 2). For an addition rate of 4 g N m^{-2} $year^{-1}$ the effect was

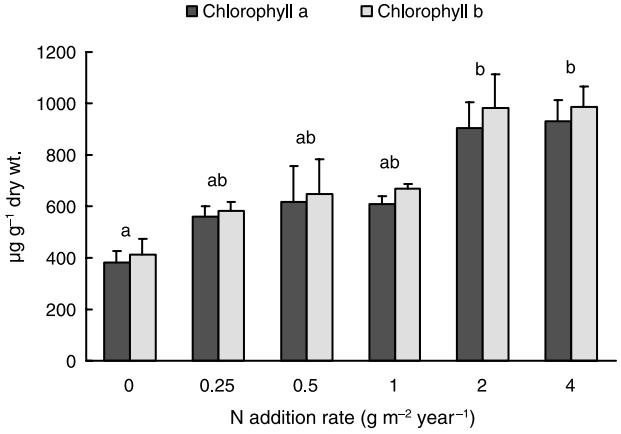

Fig. 4 Concentrations of chlorophyll a and b in capitula of *Sphagnum fallax* after 3 years at different rates of experimental N addition (means + 1 SE; $n = 4$). Different letters indicate significant differences ($P < 0.05$) between N treatments (one-way ANOVA).

not significant due to high variance. Stem tissue concentrations in different treatments were all comparable, though lower than capitulum concentrations. K concentrations in capitula and stems were significantly lower at higher N addition rates ($P < 0.05$; Table 2). Due to increasing N concentrations and slightly decreasing carbon concentrations (data not shown), C : N ratios in capitula and stems were significantly lower at higher N loads ($P < 0.001$; Table 2). C : N ratios in stem tissue were higher than those in capitulum tissue. N : P ratios in capitula and stems were significantly increased at higher N addition rates ($P < 0.001$; Table 2). N : P ratios at N addition rates of 2 and 4 g m^{-2} $year^{-1}$ were over 16, suggesting P limitation (Koerselman & Meuleman 1996).

The concentrations of the leaf pigments chlorophyll a and b in the capitula of *S. fallax* increased as a result of N addition ($P < 0.001$; Fig. 4). The capitulum concentrations of the free amino acids arginine, asparagine, glutamine and glutamic acid increased significantly with higher N addition rates (Table 3, $P < 0.001$, $P < 0.001$, $P < 0.01$ and $P < 0.01$, respectively). Compared to the control (no N addition) arginine and asparagine concentrations were significantly higher at N loads of 0.25 g m^{-2} $year^{-1}$. Free amino acids started to accumulate even after only 1 year of N addition (Tomassen *et al.* 2000). The fraction of N stored in amino acids significantly increased at a N load of 1 g m^{-2} $year^{-1}$ or higher (Table 4). After 3 years of addition at 4 g N m^{-2} $year^{-1}$, approximately 18% of the total N concentration was stored in N-rich free amino acids.

BIOMASS AND NUTRIENT CONCENTRATIONS IN *MOLINIA*

N addition significantly increased the above-ground biomass of *Molinia* ($P < 0.05$; Fig. 5). Individual above-ground biomass of *Molinia* increased by more than three fold, from 0.2 to 0.7 g dry wt., with increasing N addition rates. Root to shoot ratio of *Molinia* varied between 1.6 and 2.0 and did not differ significantly between treatments. Litter production after 3 years was significantly higher upon addition of 4 g N m^{-2} $year^{-1}$ compared to the other N treatments ($P < 0.01$; data not shown). N addition stimulated inflorescence production ($P < 0.05$). The total mean number of inflorescences per aquarium at the end of the experiment varied from 0 (0.25 g N m^{-2} $year^{-1}$) to 3.7 (4 g N m^{-2} $year^{-1}$). The concentration of N in the green leaves of *Molinia* ranged between 900 and 1230 µmol g^{-1} dry wt. However, there were no significant differences (Table 5), nor for the concentrations of P and K in the leaves (Table 5). N : P ratios in *Molinia* leaves ranged between 33 and 44 (no significant differences), suggesting growth limitation by P (Koerselman & Meuleman 1996). The concentrations of all free amino acids measured were very low (Tables 3 and 4) and made up only a small fraction of the total N concentration.

BIOMASS AND NUTRIENT CONCENTRATIONS IN *BETULA*

Above-ground biomass of *Betula* was approximately 0.5 g dry wt. at a load of 2 g N m^{-2} $year^{-1}$ or lower (Fig. 5). Although addition of 4 g N m^{-2} $year^{-1}$ almost doubled the biomass of *Betula* to 1.0 g dry wt., no significant effects could be detected on the above-ground biomass of *Betula*. Root to shoot ratio in *Betula* varied between 0.8 and 1.1 and was not affected by N addition. Litter production was significantly stimulated after 3 years of N addition ($P < 0.01$; data not shown).

Foliar N concentration in *Betula* increased from 560 to 730 µmol g^{-1} dry wt. at additions of 2 g N m^{-2} $year^{-1}$ and lower, to 1400 µmol g^{-1} dry wt. at the highest N treatment (Table 5, $P < 0.01$). The rather constant N concentration at lower N addition rates may indicate that *Betula* growth was limited by N. The concentrations of P and K in the leaves showed no significant differences (Table 5). N : P ratios in leaf tissue of *Betula* at loads of 0.25 g N m^{-2} $year^{-1}$ and higher were above 16, suggesting growth limitation by P (Koerselman & Meuleman 1996). However, only at an addition of

Table 3 Concentrations (μmol g^{-1} dry wt.) of arginine (ARG), asparagine (ASN), glutamine (GLN), aspartic acid (ASP), glutamic acid (GLU) and serine (SER) in *Sphagnum fallax*, *Betula pubescens* and *Molinia caerulea* subjected to different experimental N addition rates (means ± 1 SE; $n = 4$). Different letters indicate significant differences ($P < 0.05$) between N treatments (one-way ANOVA)

		N addition rate (g N m^{-2} $year^{-1}$)					
		0	0.25	0.5	1	2	4
ARG	*Sphagnum*	0.4 ± 0.1^{a}	1.7 ± 0.4^{b}	1.4 ± 0.4^{b}	2.0 ± 0.3^{b}	11.5 ± 2.2^{c}	28.4 ± 2.1^{c}
	Betula	1.8 ± 1.1^{ab}	0.5 ± 0.2^{ab}	0.1 ± 0.0^{a}	0.5 ± 0.3^{ab}	4.5 ± 1.9^{b}	93.3 ± 24.3^{c}
	Molinia	3.7 ± 3.4	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.0
ASN	*Sphagnum*	1.3 ± 0.4^{a}	3.6 ± 0.4^{b}	3.8 ± 0.5^{b}	7.4 ± 2.4^{b}	22.6 ± 4.7^{c}	28.3 ± 4.9^{c}
	Betula	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.7 ± 0.3
	Molinia	2.9 ± 2.2	0.3 ± 0.2	0.6 ± 0.4	0.5 ± 0.4	0.2 ± 0.1	3.4 ± 1.7
GLN	*Sphagnum*	0.8 ± 0.1^{a}	1.1 ± 0.3^{ab}	0.6 ± 0.6^{abc}	0.9 ± 0.4^{ab}	1.7 ± 0.6^{bc}	1.7 ± 1.0^{c}
	Betula	0.2 ± 0.1	0.2 ± 0.1	0.6 ± 0.2	0.2 ± 0.0	0.3 ± 0.1	0.6 ± 0.1
	Molinia	0.4 ± 0.2^{a}	1.1 ± 0.1^{ab}	1.1 ± 0.3^{ab}	1.7 ± 0.3^{b}	1.6 ± 0.2^{b}	0.8 ± 0.1^{ab}
ASP	*Sphagnum*	1.5 ± 0.5	2.1 ± 0.4	2.4 ± 1.5	5.0 ± 0.9	3.0 ± 0.6	3.6 ± 1.6
	Betula	0.3 ± 0.1^{a}	1.0 ± 0.2^{ab}	2.5 ± 1.5^{b}	0.5 ± 0.2^{a}	0.6 ± 0.3^{ab}	0.5 ± 0.1^{a}
	Molinia	2.4 ± 0.6^{ab}	3.2 ± 0.5^{ab}	3.0 ± 0.6^{ab}	4.1 ± 0.9^{b}	3.7 ± 0.1^{b}	1.2 ± 0.1^{a}
GLU	*Sphagnum*	2.8 ± 0.4^{a}	3.4 ± 0.1^{ab}	4.7 ± 0.6^{ab}	5.3 ± 0.2^{b}	5.6 ± 0.9^{b}	6.1 ± 0.8^{b}
	Betula	1.1 ± 0.1	2.0 ± 0.3	2.9 ± 0.1	1.4 ± 0.1	1.7 ± 0.2	1.6 ± 0.4
	Molinia	4.1 ± 0.9	3.2 ± 0.4	3.4 ± 0.2	3.0 ± 0.4	4.1 ± 0.4	3.6 ± 0.5
SER	*Sphagnum*	1.5 ± 0.1	1.6 ± 0.4	1.1 ± 0.6	0.6 ± 0.4	1.3 ± 0.5	0.6 ± 0.3
	Betula	0.3 ± 0.1	0.5 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
	Molinia	1.5 ± 0.7^{ab}	0.4 ± 0.2^{a}	0.7 ± 0.1^{ab}	1.2 ± 0.1^{ab}	1.0 ± 0.1^{ab}	1.3 ± 0.1^{b}

Table 4 Concentrations of amino acid N, and fractions of amino acid N of total tissue N in *Sphagnum fallax*, *Betula pubescens* and *Molinia caerulea* subjected to 3 years of different experimental N addition rates (means ± 1 SE; $n = 4$). Different letters indicate significant differences ($P < 0.05$) between N treatments (one-way ANOVA)

	N addition rate g m^{-2} $year^{-1}$	Amino acid N μmol g^{-1} dry wt.	Fraction amino acid N %
Sphagnum	0	11 ± 2^{a}	2.8 ± 0.5^{a}
	0.25	23 ± 3^{b}	4.9 ± 0.1^{ab}
	0.5	23 ± 1^{b}	4.6 ± 0.3^{ab}
	1	35 ± 6^{b}	6.3 ± 0.7^{b}
	2	104 ± 18^{c}	13.6 ± 2.6^{c}
	4	184 ± 5^{d}	17.5 ± 1.1^{c}
Betula	0	10 ± 5^{a}	1.5 ± 0.6^{a}
	0.25	6 ± 1^{a}	1.0 ± 0.2^{a}
	0.5	4 ± 3^{a}	1.0 ± 0.0^{a}
	1	4 ± 2^{a}	0.9 ± 0.3^{a}
	2	21 ± 8^{a}	2.7 ± 0.7^{a}
	4	378 ± 97^{b}	26.9 ± 6.5^{b}
Molinia	0	29 ± 19	3.7 ± 2.6
	0.25	10 ± 1	1.1 ± 0.1
	0.5	11 ± 1	1.0 ± 0.2
	1	13 ± 2	1.4 ± 0.2
	2	13 ± 1	1.2 ± 0.1
	4	16 ± 4	1.3 ± 0.4

4 g N m^{-2} $year^{-1}$ was N accumulated as arginine (Table 3, $P < 0.001$). The concentrations of the other amino acids measured remained very low. Addition of 4 g N m^{-2} $year^{-1}$ resulted in the storage of 378 μmol g^{-1} N in amino acids, which was 27% of the total N concentration (Table 4).

EVAPOTRANSPIRATION

Evapotranspiration was positively correlated with the above-ground biomass of *Molinia* and *Betula* (Fig. 6). As indicated, the total above-ground biomass of both species was stimulated by N addition.

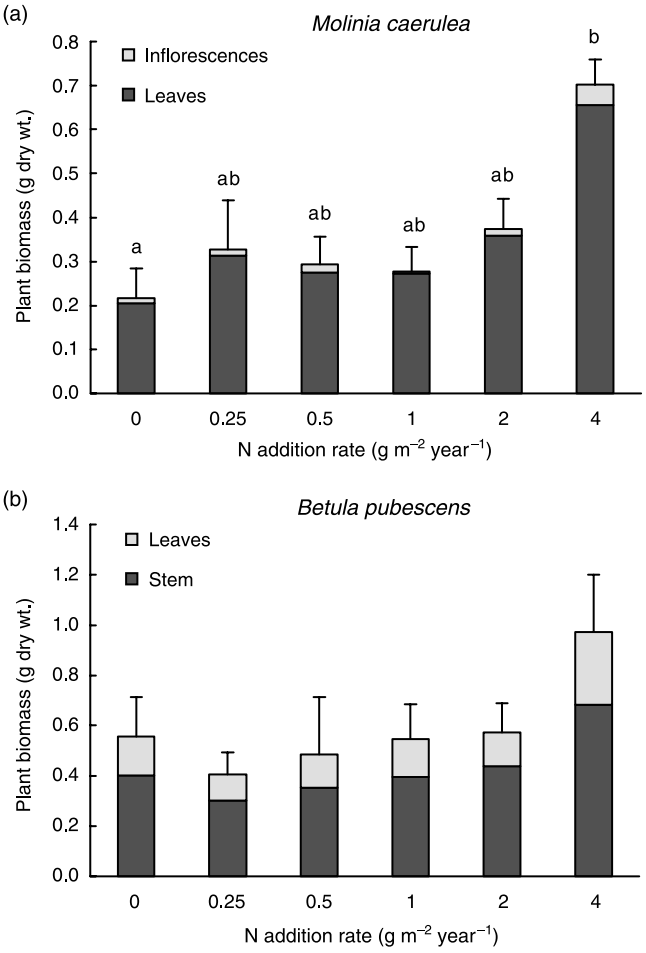

Fig. 5 Individual above-ground biomass of *Molinia caerulea* (a) and *Betula pubescens* (b) after 3 years at different rates of experimental N addition (means + 1 SE; $n = 4$). Different letters indicate significant differences ($P < 0.05$) between N treatments (one-way ANOVA).

Table 5 Foliar concentrations (μmol g^{-1} dry wt.) of nitrogen, phosphorus and potassium, and N : P ratios (g g^{-1}) in *Betula pubescens* and *Molinia caerulea* subjected to different experimental N addition rates (means ± 1 SE; $n = 4$). Different letters indicate significant differences ($P < 0.05$) between N treatments (one-way ANOVA)

	N addition rate (g m^{-2} $year^{-1}$)	N μmol g^{-1} dry wt.	P μmol g^{-1} dry wt.	K μmol g^{-1} dry wt.	N : P ratio g g^{-1}
Betula	0	608 ± 48[a]	25.3 ± 5.9	228 ± 16	14 ± 5[a]
	0.25	594 ± 59[a]	16.1 ± 3.0	193 ± 30	18 ± 2[ab]
	0.5	676 ± 96[a]	19.0 ± 4.7	209 ± 27	18 ± 3[ab]
	1	561 ± 62[a]	16.8 ± 4.0	205 ± 30	16 ± 2[ab]
	2	733 ± 101[a]	13.4 ± 1.1	232 ± 14	25 ± 3[ab]
	4	1403 ± 80[b]	23.0 ± 5.9	241 ± 24	32 ± 6[b]
Molinia	0	904 ± 99	11.4 ± 1.5	391 ± 38	38 ± 6
	0.25	928 ± 59	11.2 ± 0.3	325 ± 33	38 ± 3
	0.5	1059 ± 79	14.9 ± 3.8	372 ± 12	38 ± 9
	1	930 ± 31	12.4 ± 2.2	400 ± 28	38 ± 7
	2	1094 ± 30	11.6 ± 1.0	394 ± 19	44 ± 5
	4	1233 ± 108	26.4 ± 12.3	297 ± 74	33 ± 10

Discussion

EXPERIMENTAL DESIGN

We investigated the possible effects of elevated N deposition levels on the growth of *Betula* and *Molinia* in *Sphagnum fallax* turfs in a laboratory experiment that enabled us to eliminate the high background level of 4 g N m^{-2} $year^{-1}$ in the field. In contrast to most earlier fertilization experiments, in which N was added only six times a year, N was added three times a week in the present study. A low application frequency leads to an

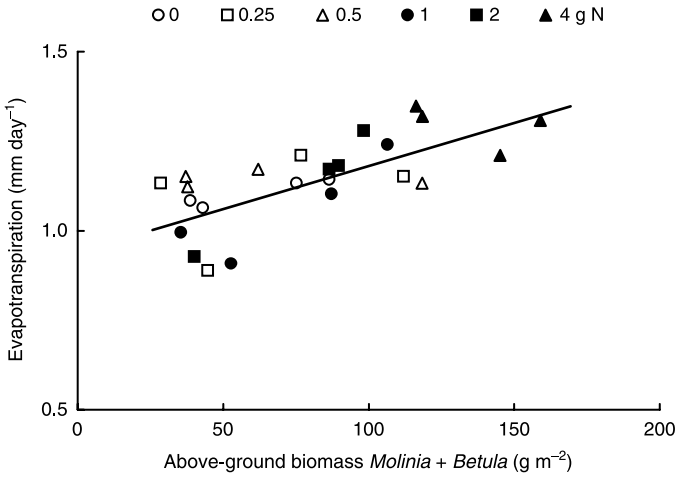

Fig. 6 Relationship between above-ground biomass of *Betula pubescens* plus *Molinia caerulea* (g m^{-2}) and evapotranspiration (mm day^{-1}) during the final growing season of the experiment. Different N treatments are indicated by different symbols (linear regression: $R^2 = 0.485$; $P < 0.001$).

imbalance between the supply and demand of nutrients in the vegetation. It is not only the net rate, but also the regime of N deposition which influences its long-term effects. Many fertilization experiments have been conducted over short periods and several of these studies mention the discrepancy between short-term (≤ 1–2 years) and long-term (≥ 3–4 years) responses (e.g. Rochefort *et al*. 1990; Gunnarsson & Rydin 2000; Aerts *et al*. 2001). This is why we conducted a 3-year experiment.

RHIZOSPHERE CHEMISTRY

Addition of 2 g N m^{-2} $year^{-1}$ or less had hardly any effect on the concentrations of free ammonium in the peat moisture of *S. fallax* turfs (Fig. 2). During the first winter, an increase in free ammonium was only observed at an addition rate of 4 g N m^{-2} $year^{-1}$. At the other addition rates, the N added was completely taken up by the vegetation (especially *S. fallax*). Jauhiainen, Wallén & Malmer (1998) also found high N uptake rates in *Sphagnum fallax*. *Sphagnum* species lack cuticles and, their leaves being only one cell layer thick, they are able to efficiently capture and utilize the atmospheric supply, thus making it unavailable for the roots of vascular plants (Woodin & Lee 1987; Lee & Woodin 1988; Malmer *et al*. 1994). In this situation, with low N availability in the rhizosphere, hardly any N was available for vascular plants like *Betula* and *Molinia*.

In the second and third years, there was still no major increase in N concentrations in the peat moisture, as the further development of the vegetation took up all added N. Only the addition of 4 g N m^{-2} $year^{-1}$ led to increased ammonium concentrations during the final months of the experiment (Fig. 2). However, these concentrations were still considerably lower than those measured in the surface layer of the peat at the site of origin (50 µmol L^{-1}), where a strong increase in abundance of *Molinia* and *Betula* has been observed in recent decades (personal observations).

Peat moisture pH at the end of the experiment was significantly lower at higher N addition rates due to cation exchange by *S. fallax*. Uptake of ammonium by *Sphagnum* is compensated for by excretion of protons, resulting in lower peat moisture pH (Clymo 1987). Carbon dioxide concentrations were very low compared to those measured in the top layer of peat in other Dutch peat bogs (500–1500 µmol L^{-1}; Tomassen & Smolders unpublished data). Smolders *et al*. (2001) found that *S. magellanicum* growing in a terrestrial situation not only depends on atmospheric CO_2 but also on high CO_2 concentrations in the peat moisture. Therefore, the growth of *S. fallax* in this experiment may have been limited by carbon, especially at the higher N addition rates.

Despite relatively high phosphate concentrations in the artificial rainwater, peat moisture phosphate concentrations were below 0.5 µmol L^{-1} (Table 1) due to high *Sphagnum* uptake rates, and probably limited optimal *Betula* and *Molinia* growth.

NUTRIENT SUPPLY AND *SPHAGNUM*

The high uptake of N by *S. fallax* led to a change in colour due to increased chlorophyll a and b concentrations (Fig. 4). *Sphagnum* which received 0.5 g N m^{-2} $year^{-1}$ or more contained elevated concentrations of chlorophyll a + b compared to those measured in *S. fallax* from Northern Italy (1.2 µmol g^{-1} dry wt.; Gerdol *et al*. 1996). It has been found for *Sphagnum cuspidatum* that mosses from a high-N site contained higher chlorophyll concentrations than those from a low-N site (Baxter *et al*. 1992). An increase in tissue chlorophyll concentrations can be the result of decreased growth dilution when P becomes limiting (Marschner 1986). In addition, if growth of *Sphagnum* is limited by CO_2, increased production of chlorophyll can enhance CO_2 fixation (Rice 1994; Smolders *et al*. 2001).

After 3 years of N addition, only the N concentrations in the capitula of *S. fallax* in the 4 g N m^{-2} $year^{-1}$ treatment were in the same range of those measured at

the field location (Fig. 3). This was to be expected, as the *S. fallax* turfs were collected from a site with a long-term total deposition of approx. 3.5 g N m^{-2} $year^{-1}$. At lower N addition rates, the added N was insufficient to maintain constant tissue N concentrations, due to dilution by growth. The low capitulum N concentration of 400 µmol g^{-1} dry wt. in *S. fallax* receiving no N appears to be the lower limit, since N concentrations after 3 years remained at the range of those measured directly after the pre-treatment. This is consistent with data from Malmer (1990) showing 410 µmol g^{-1} dry wt. as the lowest N concentration measured in the apical part of *Sphagnum*.

N concentrations in *S. fallax* showed a strong linear correlation with the amount of N added (Fig. 3). Increased N concentrations due to elevated N deposition rates have also been found for other *Sphagnum* species, including *S. fuscum*, *S. magellanicum*, *S. palustre*, *S. angustifolium* and *S. papillosum* (Pitcairn *et al.* 1995; Williams & Silcock 1997; Jauhiainen, Vasander & Silvola 1998). *Sphagnum* species can therefore be used as biological indicators to estimate N deposition levels based on their tissue N concentrations (e.g. Risager 1998; Gunnarsson & Rydin 2000; Lamers *et al.* 2000). A maximum N concentration (Lamers *et al.* 2000; Berendse *et al.* 2001) was not reached in our experiment. Van der Heijden *et al.* (2000) propose a capitulum N concentration of 15 mg g^{-1} dry wt. (= 1071 µmol g^{-1} dry wt.) as an indication of N pollution stress in *S. fallax*. In our experiment, the N concentrations in the capitula of *Sphagnum* receiving 4 g N m^{-2} $year^{-1}$ equalled this critical value.

Based on the N : P ratio, *Sphagnum* growth appeared to be limited by N at loads of 1 g N m^{-2} $year^{-1}$ and lower (Table 2; Koerselman & Meuleman 1996). Higher N loads resulted in P limitation (N : P ratio > 16). To prevent ammonium toxicity, many plants respond by synthesizing specific amino acids and amines, particularly those with a low C : N ratio (Marschner 1986). The concentrations of free amino acids in *S. fallax* strongly increased above an addition rate of 0.5–1 g N m^{-2} $year^{-1}$, corresponding to P-limiting conditions according to the N : P ratio (Tables 2 and 3). Arginine (C : N ratio 1.5) and asparagine (C : N ratio 2.0) concentrations in particular were elevated, due to nutrient imbalance in *Sphagnum* at increased ammonium availability. Several other studies have mentioned the production of free amino acids including arginine, asparagine and glutamine for different *Sphagnum* species at high N loads (Thönes & Rudolph 1983; Baxter *et al.* 1992; Nordin & Gunnarsson 2000; Smolders *et al.* 2001; Limpens & Berendse in press). The present experiment allows the conclusion that the concentrations of N-rich free amino acids, which are produced as a detoxification mechanism, can be used as a good indication of future N saturation. Based on the concentrations of N-rich amino acids, we propose that N loads above 0.25–0.5 g m^{-2} $year^{-1}$ lead to N saturation.

NUTRIENT SUPPLY AND GROWTH OF *MOLINIA* AND *BETULA*

It will be obvious from the above that the amount of N available for *Betula* and *Molinia* was strongly limited by the high N uptake rate by *Sphagnum fallax*. In peat-forming systems with a *Sphagnum* layer, vascular plants do not have direct access to N, P and K supplied from the atmosphere but rely almost entirely on their release from organic matter during mineralization (Malmer 1993). However, leaves may absorb nutrients through the cuticle, thereby providing a net source of nutrients when concentrations in rainwater are high (Marschner 1986).

N addition had no effect on the above-ground biomass production by *Molinia* in the first experimental year, probably due to low N availability because of immobilization by *Sphagnum*. After 1 year, N effects became more obvious and addition of 4 g N m^{-2} $year^{-1}$ had a significant effect on above-ground biomass production by *Molinia* compared to the control treatment (Fig. 5). Various experiments in other ecotypes have also shown stimulation of the growth of *M. caerulea* by N (e.g. Roelofs 1986; Heil & Bruggink 1987; Aerts & Berendse 1988). In addition, N addition had a significant stimulating effect on the production of inflorescences by *Molinia.* In a few of the turfs receiving high N loads, *Molinia* expanded by producing seedlings.

Growth and architecture of the *Betula* saplings (above-ground biomass, length, number of branches and leaves, and leave surface) was not significantly influenced by N addition within 3 years (Fig. 5). High N addition rates did, however, lead to higher litter production, which may have negative effects on nutrient cycling and *Sphagnum* growth by shading (Heijmans *et al.* 2001). In the long-term, however, growth of *Betula* in our study might significantly be stimulated. If the uptake of nutrients by *Sphagnum* is hampered by shading, and if nutrient mobilization from litter is stimulated, nutrient availability will increase for *Betula*.

NUTRITIONAL STATUS OF *MOLINIA* AND *BETULA*

Increased N availability had no effect on foliar N concentrations in *Molinia*, indicating that all N was used for biomass production and N was limiting growth. However, the N : P ratio was above 33 for all treatments (Table 5), suggesting that *Molinia* was strongly limited by P (Koerselman & Meuleman 1996). Güsewell *et al.* (1998) proposed that the N : P ratio could be a appropriate tool to predict short-term effects of nutrient enrichment at the level of individual species. The observed growth response at high N : P ratio supports the idea that *Molinia* is a species adapted to low P availability, as has also been found in earlier studies (Kirkham 2001). High N deposition levels have changed a substantial proportion of *Calluna*-dominated

uplands in England and Wales from N-limited ecosystems into P-limited ones, favouring species like *Molinia* that are better adapted to P limitation (Kirkham 2001). Despite high N : P ratios, the growth of *Molinia* in our experiment was still limited by N. This is in agreement with Thornton (1991), who found an absence of growth response to P supply at low N availability, indicating growth limitation of *Molinia* by N. Based on our results, therefore the N : P ratio is not a suitable tool for detecting the absence of N limitation in *Molinia*. The N : P tool developed by Koerselman & Meuleman (1996) was based on results obtained at the vegetation level and not for individual plant species.

Based on the N : P ratio, the growth of *Betula* was limited by P above a load of 0.25 g N m^{-2} $year^{-1}$ (Table 5; see also Koerselman & Meuleman 1996). Optimum nutrition for *Betula pendula* growth are known to be achieved at N : P ratios between 10 and 12, although a higher relative P requirement has been observed under nutritional stress conditions (Ericsson & Ingestad 1988). Foliar nutrient concentrations in our experiment were relatively low (Table 5). At P concentrations below 65 μmol g^{-1} dry wt., growth of *Betula* is limited by P, and normal concentrations have been reported to be 65–130 μmol g^{-1} dry wt. (Hytönen & Kaunisto 1999). Fertilization experiments on drained mires in northern Finland showed a positive effect of NPK and PK fertilization on the growth of *Betula pubescens* (Penttila & Moilanen 1997), and no effect of N fertilization. In contrast to *Molinia*, the *Betula* saplings in our experiment were not able to use the added N at high loads, due to P shortage. Peat moisture P concentrations in the rhizosphere of Dutch bogs are relatively high (0.5–2.5 μmol o-PO_4 L^{-1}) compared to those measured in Ireland and Norway (< 0.5 μmol o-PO_4 L^{-1}) (Tomassen & Smolders unpublished data). The high P concentrations in Dutch bogs probably enable expansion of *Betula* at high levels of N deposition.

Just like *Sphagnum*, trees can respond to N saturation by detoxifying the excess ammonium to N-rich free amino acids, especially arginine (e.g. Van Dijk & Roelofs 1988). In our experiment, *Betula* accumulated foliar arginine upon addition of 4 g N m^{-2} $year^{-1}$, but unlike what we found in *S. fallax*, none of the other N-rich free amino acids were formed (Table 3). The fraction of amino acid N was 27% of the total N concentration, indicating a strong nutrient imbalance in the *Betula* saplings (Table 4). Näsholm & McDonald (1990) studied *Betula pendula* seedlings and found higher concentrations of total amino acid N in root and shoot at greater N supply. In their study, higher concentrations of amino acid N were mainly due to high concentrations of citrulline, glutamine, γ-aminobutyric acid and arginine, but amino acid N made up only 3–4% of the total foliar N concentration.

Uptake of nutrients by trees is greatly influenced by symbiosis with mycorrhizal fungi (Smith & Read 1997). The *Betula* saplings in our experiment grown under wet conditions were associated with ectomycorrhizal fungi (J. Baar personal communications). Among the ectomycorrhizal species, *Laccaria* sp. was identified by the use of PCR-based molecular techniques (e.g. Baar *et al*. 1999). Ectomycorrhizal fungi were also observed on *Alnus glutinosa* trees in waterlogged peaty soils (Baar *et al*. 2000; 2002). Baar *et al*. (2000) discussed the functional role of ectomycorrhizal fungi under wet conditions. In peaty soils, the N : P ratios of the soil water are usually high, resulting in P limitation. Therefore, Baar *et al*. (2000) suggested that trees growing in waterlogged peaty soils are dependent on mycorrhizal symbionts for their P uptake. In our study, however, the growth of the *Betula* saplings was limited by P despite the fact that ectomycorrhizal symbionts were present. Activity of the ectomycorrhizal symbionts was presumably inhibited by the acidic conditions, particularly at high N addition rates.

INTERACTIONS BETWEEN *SPHAGNUM* AND VASCULAR PLANTS

Sphagnum growth can be inhibited by high N availability (e.g. Jauhiainen, Vasander & Silvola 1998). As N deposition levels increases, the growth of *Sphagnum* may decrease, and with it its function as a 'sink' for atmospheric elements (Lee & Woodin 1988). The resulting enhanced availability of N in the rhizosphere stimulates the growth of higher plants. *Sphagnum* peat produced under high N loads probably has a lower C : N ratio and is therefore more easily decomposed by bacteria (Aerts & Chapin 2000). However, mineralization rates of peat from the present experiment showed no relationship with the N addition rates (data not shown). Despite the effect of N on the tissue N concentration in *Sphagnum*, no differences in peat N concentrations were found between the various treatments. The added N was completely reabsorbed from dying *Sphagnum* parts, leading to similar mineralization rates and no extra N source for vascular plants. Several other studies have found that P had a stronger effect on the mineralization than N (Hogg *et al*. 1994; Aerts & Chapin 2000; Aerts *et al*. 2001).

Increased growth of vascular plants in bog systems at higher N deposition rates has been observed in various studies (e.g. Heijmans *et al*. 2001; Berendse *et al*. 2001; Limpens *et al*. personal communication). Such plants include the shallowly rooted species *Vaccinium oxycoccus* (e.g. Lütke Twenhöven 1992; Heijmans *et al*. 2001), *Andromeda polyfolia* and *Eriophorum vaginatum* (Redbo-Torstensson 1994). Previous studies have suggested that shading by vascular plants may reduce *Sphagnum* growth (Hayward & Clymo 1983; Heijmans *et al*. 2001). In the present experiment, an increase in the above-ground biomass of vascular plants, especially *Vaccinium oxycoccus*, was observed at the expense of *Sphagnum* growth (data not shown).

Increased total cover by *Betula* and *Molinia* had a stimulating effect on the evapotranspiration (Fig. 6).

Takagi *et al*. (1999) found similar increased evapotranspiration rates due to the invasion of vascular plants. The stimulated growth of vascular plants in bog vegetation also increases the vegetation structure and thereby the interception of water and input of dry deposition (e.g. Heil *et al*. 1988; Tomassen & Roelofs in press). The concomitant desiccation and N eutrophication hampers the growth of *Sphagnum* species and may ultimately enhance decomposition processes and stimulate the growth of N-dependent vascular plants. In the Netherlands, the dominance of *Betula* and *Molinia* is a common feature in the bog relicts, probably resulting from prolonged high N deposition levels combined with relatively high P availability.

VEGETATION CHANGES IN N-POLLUTED BOGS

The empirical critical load for ombrotrophic bogs has been estimated to be around 0.5–1.0 g m^{-2} $year^{-1}$ (Bobbink & Roelofs 1995). Risager (1998) proposed a critical load of 0.7 g m^{-2} $year^{-1}$ based on experiments and literature study and Gunnarsson & Rydin (2000) suggested that the critical load of N had to be below their lowest treatment rate of 1 g m^{-2} $year^{-1}$. Based on ecotoxicological parameters indicating N excess for *S. fallax* in the present experiment we propose that this threshold must be around 0.5 g m^{-2} $year^{-1}$. High N deposition levels do indeed appear to be responsible for the observed rapid vegetation changes in ombrotrophic bogs. Our experiment shows that even after 3 years of N addition at levels present in the Netherlands in recent decades, *Molinia* growth and dispersion was significantly stimulated. *Molinia* shows a rapid response to increased N availability, as it is able to grow under P-limited conditions. *Betula* is only able to expand if enough P is available.

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