New Phytologist

Research

N deposition affects N availability in interstitial water, growth of *Sphagnum* and invasion of vascular plants in bog vegetation

Juul Limpens, Frank Berendse and Herman Klees

Nature Conservation and Plant Ecology Group, Wageningen University, Bornsesteeg 69, 6708 PD Wageningen, the Netherlands

Author for correspondence:
Juul Limpens
Tel: +31-317-483173
Fax: +31-317-484845
Email: Juul.Limpens@WUR.nl

Received: *28 August 2002*
Accepted: *18 October 2002*

Summary

• We studied the effects of N deposition on shrub–moss competition and the establishment and growth of invasive *Betula pubescens* and *Molinia caerulea* in intact bog vegetation removed from a site subject to 40 kg N ha^{-1} yr^{-1}.

• Mesocosms with and without introduced *Betula* seedlings and *Molinia* sprouts were kept under a roof and received an equivalent of 0, 40 and 80 kg N ha^{-1} yr^{-1} for two growing seasons.

• N concentration in both interstitial water and *Sphagnum* decreased when N input ceased and increased when N input was doubled. *Molinia* biomass was positively related to the inorganic N concentration in the interstitial water. Adding N increased production of *Molinia* and prolonged survival of *Betula* seedlings in the first year. *Sphagnum* height increment showed a hump-shaped relationship with light interception by vascular plants.

• N deposition encouraged vascular plants to grow by enhancing N availability in the rhizosphere. Water table level and the availability of P were found to be important in explaining species-specific responses to N deposition. The underlying mechanisms and the reversibility of N effects are discussed.

Key words: *Betula*, deposition, global change, *Molinia*, nitrogen, *Sphagnum*, raised bogs, water chemistry.

© *New Phytologist* (2003) **157**: 339–347

Introduction

Many ecosystems, including bogs, have evolved under low nitrogen (N) inputs and as a result, both species composition and ecosystem processes are geared to these nutrient-poor conditions (van Breemen, 1995; Chapin *et al.*, 1997). When the availability of N increases, for example by an increase in N deposition, the competitive balance between species may alter and previously subordinate species may become invasive (Heil & Diemont, 1983; Bobbink & Willems, 1987; Lee, 1998; Turkington *et al.*, 1998; Nordin *et al.*, 2002). For undrained bogs in north-western Europe, where critical deposition loads have been exceeded (Bobbink & Roelofs, 1995; Risager, 1998), this is illustrated by the establishment and subsequent expansion of more nutrient demanding species such as *Betula* sp. and *Molinia caerulea* (L.) Moench., and the increase in overall vascular plant cover since the 1970s (Aaby, 1994; Hogg *et al.*, 1995). Apart from affecting species composition, the shift from a *Sphagnum*-dominated to a more vascular plant-dominated vegetation also impacts on the rate of carbon sequestration in these systems; dead *Sphagnum* decomposes more slowly than the litter of vascular plants (Coulson & Butterfield, 1978; Bartsch & Moore, 1985; Hobbie, 1996).

To improve our understanding of how, and when, we can expect increasing N deposition to affect *Sphagnum*-dominated peatlands, Lamers *et al.* (2000) and Berendse *et al.* (2001) have proposed a three-phased mechanism. They argue that *Sphagnum* growth is limited by N when deposition is low. Consequently, the moss takes up all available N and uses it for growth and maintenance (phase one). When deposition increases to intermediate levels, N no longer limits growth, but still all or almost all of the deposited N is taken up, and excess N is stored for future use (phase two). When N deposition increases further, the living *Sphagnum* layer becomes saturated with N and can no longer retain all the deposited N.

The N filter fails, and N becomes available for vascular plants who respond by increasing in cover. In addition, the enhanced N availability may also facilitate the invasion of more N-demanding species, such as *Betula* and *Molinia*. Subsequently, the increase in vascular plant cover reduces light availability at the moss-surface, ultimately suppressing growth of *Sphagnum* and thus carbon sequestration (phase three). The authors disagree about the point at which *Sphagnum* becomes saturated, however. Lamers *et al.* (2000) argue that at a deposition of *c.* 18 kg N ha^{-1} yr^{-1}, a threshold concentration of 12–13 mg N g^{-1} is reached, above which N concentration in *Sphagnum* no longer increases and the interstitial water becomes enriched. Results of Berendse *et al.* (2001) indicate a maximum N concentration of 20 mg N g^{-1} for *Sphagnum* in the upper 3 cm.

In this study we pursued two objectives. The first was to test the described conceptual N deposition model for *Sphagnum*-dominated peatlands proposed by Lamers *et al.* (2000) and Berendse *et al.* (2001); the second was to test the effect of N on establishment and growth of *Molinia* and *Betula*. We removed peat cores with intact vegetation from a Dutch bog with high N deposition, and subjected them to *in situ* N inputs (40 kg N ha^{-1} yr^{-1}), no N inputs (0 kg N ha^{-1} yr^{-1}), and doubled inputs (80 kg N ha^{-1} yr^{-1}). Additionally, the nutrient treatments were crossed with introduction of *Betula* and *Molinia*. We hypothesised that first: N availability in interstitial water would be a function of N deposition: a decrease of deposition would result in lower concentrations, whereas an increase of deposition would lead to higher concentrations of N in relation to field conditions. Second: vascular plants, *Betula* and *Molinia* in particular, would profit from the increased N availability in the high deposition treatment and decline at low deposition. Third: expansion of vascular plants would result in increased shading of *Sphagnum* and thus decreased *Sphagnum* growth.

Materials and Methods

Plant material

In early March 1999, 45 peat cores (diameter 34 cm, 30–35 cm deep) were cut from a raised bog vegetation in a former heath pool situated in the State Forest of Dwingeloo (52°49′-N, 6°25′-E). The vegetation was dominated by *Sphagnum magellanicum* Brid. and had a sparse cover (5–15%) of *Rhynchospora alba* L., *Vaccinium oxycoccus* L., *Erica tetralix* L., *Drosera rotundifolia* L., *Eriophorum angustifolium* Honck., and *Calluna vulgaris* L. Some of the cores were also found to contain *Sphagnum papillosum* Lind.

Seeds of *Betula pubescens* Ehrh. were collected in a Dutch bog in November 1998 and stored at 4°C. At the end of February the seeds were scattered on nutrient-poor sand and kept in a heated glasshouse until they had a minimum of two leaves and were about 15 mm tall. For *Molinia caerulea* L., vegetative buds were collected from the control treatment of another experiment in which *Molinia* had been grown on nutrient-poor sand. The buds from a single individual were used. They were grown in the same glasshouse as the *Betula* seedlings until they had developed into a sprout with 4–5 leaves and were *c.* 10 cm tall.

In early June 1999, both *Betula* and *Molinia* were carefully rinsed with demineralised water and numbers of leaves were measured. Five seedlings or clones were used per container. Until the end of June, dead seedlings and sprouts were replaced.

Experimental set-up

The 45 peat cores were placed into plastic containers, which were kept in an open glasshouse with a transparent roof (light transmission of 80%, LICOR probe) and walls of coarse shade mesh, thus allowing for some air movement. We will hitherto refer to the whole of container and vegetation as mesocosm. The treatments were randomly assigned to the mesocosms, which were placed in two sunken concrete basins and arranged in five replicated blocks. The basins were filled with water in order to keep the temperature within the peat columns as close to natural as possible. Water exchange between basin and mesocosms was impossible. There were nine treatments, which consisted of a factorial combination of three deposition levels (0, 40 and 80 kg N ha^{-1} yr^{-1}) and three 'seedling' treatments (no seedlings, *Betula* seedlings, *Molinia* sprouts). After an acclimatisation period of three months, the fertilisation treatments were started in early June The experiment lasted for 18 months and was harvested in the second week of October 2000.

The water level in the mesocosms was adjusted to 5 cm below the capitula twice a week, using an artificial rainwater solution without N (Garrels & Christ, 1965). During the growing season, nitrogen as NH_4NO_3, dissolved in 1 l demineralised water was added once every 2 wk. Both rainwater and N solution were applied with a watering can.

Physical measurements

In each container two Rhizon Soil Moisture Samplers (Eijkelkamp, Agrisearch Equipment, Giesbeek, The Netherlands) with a porous length of 5 cm were inserted to depths of 0–5 and 10–15 cm. Approximately once every 3 months, soil moisture was sampled through vacuumed syringes, and analysed. We were careful to wait at least 1 wk between nutrient addition and water sampling. Soil moisture samples were analysed colorimetrically, using a continuous flow analyser (SKALAR SAN plus system, the Netherlands) for NH_4^+, NO_3^- and PO_4^{3-}.

In the summer of 2000, maximum light interception by the vascular plants in the mesocosms was calculated by comparing simultaneous PAR measurements inside and outside the canopy. Light was measured with two small light probes

above the *Sphagnum* capitula (digital multimeter Mx190). As the distribution of the vascular plants in some mesocosms was rather clumped, we measured in the densest patch.

Measurements on *Sphagnum* and vascular plants

Height increment of *Sphagnum* was measured twice a year using two metal rods that could be fastened to the container edge. A bar fitted between the rods provided a stable horizontal benchmark above the vegetation. At five marked points, we measured the distance between the bar and the *Sphagnum* surface with a ruler. At final harvest, *Sphagnum* sods of 5×35 cm and 20 cm deep were cut from 30 randomly selected mesocosms, and stored at 1°C. We decided on a random selection, because the presence of seedlings did not affect *Sphagnum* height increment. *Sphagnum* was removed from the sods and after the capitula had been counted, the individuals were separated into one capitulum (0–1 cm) and two stem fractions (1–2 and 2–3 cm). The *Sphagnum* was oven dried at 70°C for 48 h before d. wt was determined. Production (g m^{-2} yr^{-1}) in the final year was calculated as:

$$P = (L * B_s) + (\triangle B_c/1.5)$$

with L standing for height increment in 2000; B_s, bulk density of the 1–3 cm stem fraction; $\triangle B_c/1.5$ difference in capitulum bulk density between the 0 or 80 kg N treatments and the 40 kg N treatment, developed over 1.5 growing seasons. We hereby assumed that capitulum bulk density in the 40 kg N treatment did not change in the course of the experiment, because this treatment was supposed to reflect ambient field deposition in the Netherlands.

To monitor the biomass response of *Molinia* during the experimental period, we counted the number of leaves per individual; *Molinia* shoot biomass and number of leaves turned out to be well related ($r^2 = 0.72$, $P = 0.001$; linear regression). For *Betula* we counted the number of seedlings per container at regular time intervals in the first year. At the beginning of October 2000, the above-ground vegetation in all 45 mesocosms was cut flush with the top of the *Sphagnum* and sorted into species. The samples were dried at 70°C for at least 48 h before d. wt was determined.

Vegetation samples were pulverised using a ball mill and digested with sulphuric acid, salicylic acid, hydrogen peroxide and selenium. N and P were analysed colorimetrically. For *Sphagnum* the C and N concentrations were also measured, using an elemental analyser (Fisions Instruments, EA 1108, Milan, Italy). Data on C and N were corrected for water and ash content.

Statistical analysis

Data were tested for normality and equality of variance and, when necessary, were $\log_e$-transformed prior to analysis. When no block effect was detected, which was usually the case, block was omitted from the analysis to gain enough degrees of freedom to perform a posthoc test. All analyses were conducted using the SPSS statistical package for Windows (10.0).

Data on water chemistry were analysed for each depth separately with a repeated measures ANOVA (RM-ANOVA), with N treatment and presence of *Molinia* as fixed factors. Data from the mesocosms with introduced *Betula* seedlings were pooled with data from the mesocosms without seedlings; presence of *Betula* had no significant effect on water chemistry. To discern when interstitial water had been affected by *Molinia* and N, an additional two-way ANOVA was performed for each sampling date separately, with the same factors as above, but block included as a random factor. The effect of N on the increase in number of *Molinia* leaves was tested with a RM-ANOVA, with N treatment as a fixed factor. To test the N effect on the cumulative *Sphagnum* height increment, we also used a RM-ANOVA, but included block as a random factor. In the latter case, as well as for the following analyses on organic nutrient concentrations and vascular plant biomass, data on the seedling treatments were pooled, as neither presence nor type of seedling had a significant effect on the dependant variables. Nutrient concentrations in moss tissue were tested for each fraction separately (capitulum and stem fractions) with a one-way ANOVA, with N treatment as fixed factor, and for all fractions together with a two-way ANOVA, with N treatment and plant fraction as fixed factors. Both tissue nutrient concentration and biomass of vascular plants were tested with an ANOVA, with N treatment as a fixed factor. For vascular plant biomass, we initially included cumulative height increment of *Sphagnum* as a covariable, to correct for overgrown stems and leaves (Svensson, 1995). As this covariable had no significant effects, we omitted it from the design to be able to perform a posthoc test.

Differences between the three N treatments were analysed using a Tukey posthoc test. Spearman's rho was used for correlation analysis.

Results

Water chemistry

The N treatments affected inorganic N concentration in the interstitial water at both 0–5 and 10–15 cm depth (N effect: $P = 0.001$, $n = 15$, RM-ANOVA), bringing about a gradient from low concentrations in the 0 kg N treatment to high concentrations in the 80 kg N treatment (Fig. 1a,b). The differences between the N treatments had become significant at the end of the 1999 growing season (N effect: $P = 0.05$, $n = 15$ two-way ANOVA). These differences were sustained throughout the following year, although there was a temporary drop at the end of July, probably because the vegetation was growing rapidly at that time. In all N treatments, ammonium was the dominant source of inorganic

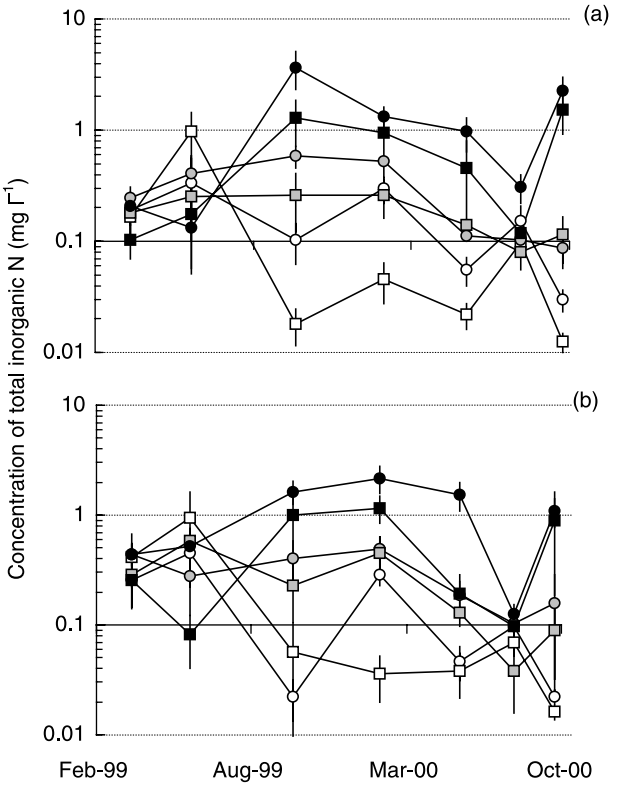

Fig. 1 The effect of N deposition on the inorganic N concentration in interstitial water (mean ± 1 SE, $n = 5$) at (a) 0–5 cm and (b) 10–15 cm depth. Circles, without *Molinia*; squares, with *Molinia*; open symbols, 0 kg N ha^{-1} yr^{-1}; Closed grey symbols, 40 kg N ha^{-1} yr^{-1}; Closed black symbols, 80 kg N ha^{-1} yr^{-1}. Application of nutrients started early June 1999.

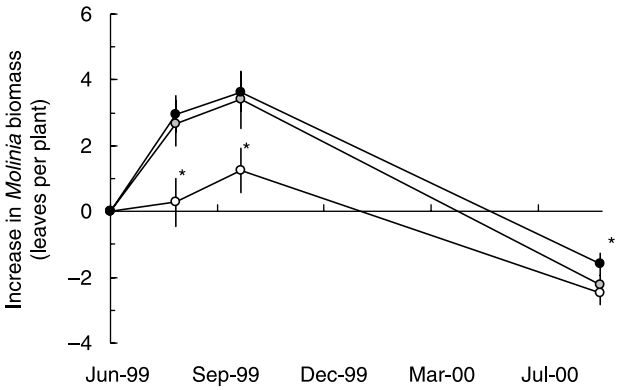

Fig. 2 The effect of N deposition on the increase in number of green leaves of *Molinia caerulea* (mean ± 1 SE, $n = 5$). Open circles, 0 kg N ha^{-1} yr^{-1}; closed grey, 40 kg N ha^{-1} yr^{-1}; closed black, 80 kg N ha^{-1} yr^{-1}; *, $P = 0.05$ (RM ANOVA).

N. P concentrations hardly ever reached the detection limit of 0.01 mg l^{-1} (data not shown).

The presence of *Molinia* (Fig. 1a,b) resulted in a significantly lower N concentration in the interstitial water at both depths (*Molinia* effect: $P = 0.05$, $n_{+Molinia} = 15$, $n_{-Molinia} = 30$, RM-ANOVA). This effect was most consistent in the highest N treatment at 10–15 cm depth.

Vascular plants

Soon after the first *Betula* seedlings had been inserted, it became evident that they could not compete with the fast-growing *Sphagnum*. By November 1999, all seedlings had been overgrown. The time it took to overgrow the seedlings was influenced by adding N (Table 1), however. In the 0 kg N treatment it took about 2 months for all the seedlings to die, in the 40 and 80 kg N treatments, they survived 1 month longer. *Molinia* fared better. Growth, as expressed by the increase in number of leaves, strongly depended on year (Fig. 2). In 1999, growth was retarded in the 0 kg N treatment, but did not differ between the 40 kg N and 80 kg N treatment. In 2000, *Molinia* declined in all treatments, although it performed best in the high N treatment. This sudden decline was probably responsible for the absence of a treatment effect on shoot biomass at harvest time (Table 2). An indirect N effect through water chemistry could be detected: the biomass correlated positively with the average inorganic N concentration of the interstitial water in the rhizosphere of *Molinia* in the previous year (Fig. 3). At the same time as growth of *Molinia* stagnated, a rapid expansion of *Rhynchospora alba* could be observed in the mesocosms that received additional N, resulting in a significantly higher biomass in the 80 kg N treatment (Table 2). The same response, albeit weaker, was found for *Vaccinium oxycoccus*. Other vascular plants, such as *Erica*, were only present in low densities and showed no clear biomass response to the N treatments.

	0 kg N	40 kg N	80 kg N	Treatment effect
No. of times *Betula* seedlings in a container were overgrown and replaced before June 26	1.2A ± 0.1	0.5B ± 0.1	0.7AB ± 0.1	*
No. of days it took to overgrow all the Betula seedlings in a container after June 26	59A ± 5	98B ± 5	85AB ± 5	*

Table 1 Effect of N deposition on survival of *Betula* seedlings (mean ± 1 SE, $n = 5$). Different letters indicate significant differences between N treatments, *$P = 0.05$ (one-way ANOVA)

Table 2 The effect of N deposition on shoot biomass of vascular plants (mean ± 1 SE). Different letters indicate significant differences between N treatments, ns = $P > 0.05$; * = $P = 0.05$, ***$P = 0.001$ (one-way ANOVA). Data on the seedling treatments were pooled

		n	0 kg N	40 kg N	80 kg N	Treatment effect
Drosera	g m^{-2}	15	1.8 ± 0.3	2.8 ± 0.4	2.7 ± 0.4	ns
Erica	g m^{-2}	15	11.6 ± 3.4	12.0 ± 3.7	16.7 ± 4.3	ns
Eriophorum	g m^{-2}	15	14.4 ± 3.8	25.0 ± 7.1	14.1 ± 3.6	ns
Molinia	g m^{-2}	5	12.0 ± 3.3	9.0 ± 0.7	11.2 ± 1.4	ns
Rhynchospora	g m^{-2}	15	136.0^{a} ± 13.3	182.5^{a} ± 18.6	290.6^{b} ± 31.8	***
Vaccinium	g m^{-2}	15	9.0^{a} ± 0.9	8.3^{a} ± 1.0	11.6^{b} ± 0.9	*

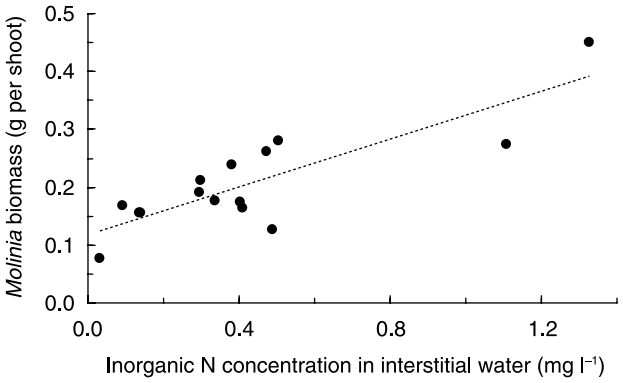

Fig. 3 The relationship between *Molinia* shoot biomass in 2000 and the average inorganic N concentration in interstitial water at 10–15 cm depth of three sampling dates in the growing season of the previous year. Correlation: $P = 0.01$, $r^2 = 0.44$ (Spearman's rho).

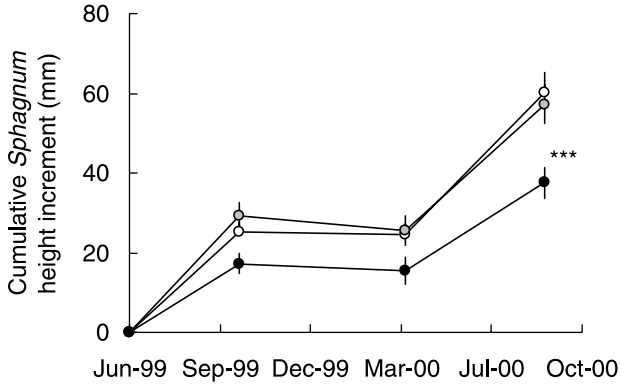

Fig. 4 The effect of N deposition on the cumulative height increment of *Sphagnum magellanicum* (mean ± 1 SE, $n = 15$). Open circles, 0 kg N ha^{-1} yr^{-1}; closed grey, 40 kg N ha^{-1} yr^{-1}; closed black, 80 kg N ha^{-1} yr^{-1}. ***, $P = 0.001$ (RM-ANOVA).

Total vascular plant biomass had increased in the 80 kg N treatment ($P = 0.001$, $n = 15$; one-way ANOVA) and was positively correlated with interception of light ($r^2 = 0.22$, $P = 0.05$; data not shown).

In all species, except the deep-rooting *Eriophorum angustifolium*, N tissue concentration increased with N supply (Table 3). Due to increasing N concentrations and slightly decreasing P concentrations, a significant N effect was found on the N : P ratio in the shoots of all species except *Eriophorum*, with the highest values for the 80 kg N treatment. Values below 16 were found for *Drosera* and *Rhynchospora* in the 0 kg N treatments and for some *Rhynchospora* in the 40 kg N treatment, suggesting N limitation or NP colimitation (Koerselman & Meuleman, 1996). *Erica* and *Molinia* showed remarkably high N : P ratios, exceeding 30 and 45, respectively.

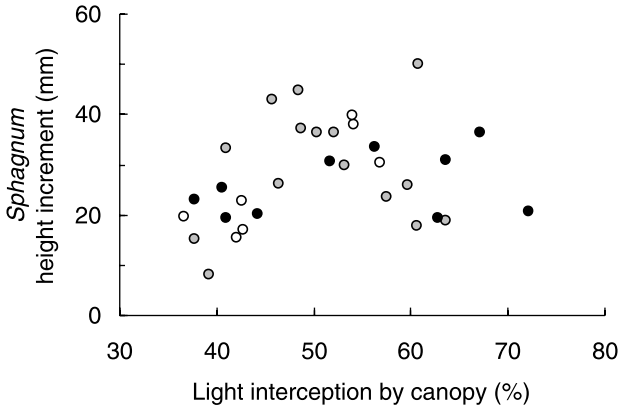

Fig. 5 The relationship between shading and height increment of *Sphagnum* in 2000. Open circles, 0 kg N ha^{-1} yr^{-1}; closed grey, 40 kg N ha^{-1} yr^{-1}; closed black, 80 kg N ha^{-1} yr^{-1}. Quadratic regression: $r^2 = 0.31$, $P = 0.005$. Mesocosms with unevenly clumped distribution of vascular plants have been omitted.

Sphagnum

The cumulative height increment of *Sphagnum* over the experimental period was depressed by adding N in surplus of field background deposition ($P = 0.01$, $n = 15$, RM-ANOVA) and amounted to 6 cm for the 0 and 40 kg N treatments and 4 cm for the 80 kg N treatment (Fig. 4). The N effect on *Sphagnum* production was less clear, due to considerable variability in capitulum and stem d. wt (data not shown), but it was still significant ($P = 0.05$, $n = 10$, one-way ANOVA). The height increment of *Sphagnum* showed a quadratic relationship with light interception by the canopy (Fig. 5). An interception of 53% was the threshold above

Table 3 The effect of N deposition on the N and P concentrations and the N/P ratio of vascular plant shoots (mean ± 1 SE). Different letters indicate significant differences between N treatments, ns = $P > 0.05$, $^{**}P = 0.01$, $^{***}P = 0.001$ (one-way ANOVA). Data on the seedling treatments were pooled

	n	0 kg N	40 kg N	80 kg N	Treatment effect
Drosera					
N mg g^{-1}	15	$14.44^{a} \pm 0.62$	$18.64^{b} \pm 0.98$	$23.08^{c} \pm 0.92$	***
P mg g^{-1}	15	1.25 ± 0.09	1.23 ± 0.05	1.11 ± 0.05	ns
N/P ratio	15	$12^{a} \pm 1$	$15^{b} \pm 1$	$21^{c} \pm 1$	***
Erica					
N mg g^{-1}	15	$7.47^{a} \pm 0.50$	$9.78^{b} \pm 0.44$	$13.51^{c} \pm 0.52$	***
P mg g^{-1}	15	0.27 ± 0.03	0.28 ± 0.02	0.21 ± 0.01	ns
N/P ratio	15	$31^{a} \pm 3$	$37^{a} \pm 3$	$66^{b} \pm 5$	***
Eriophorum					
N mg g^{-1}	15	9.13 ± 0.50	9.34 ± 0.23	9.98 ± 0.27	ns
P mg g^{-1}	15	0.18 ± 0.02	0.18 ± 0.01	0.18 ± 0.01	ns
N/P ratio	15	28 ± 5	25 ± 2	25 ± 1	ns
Molinia					
N mg g^{-1}	5	$8.04^{a} \pm 0.24$	$10.50^{ab} \pm 0.74$	$12.99^{b} \pm 1.14$	**
P mg g^{-1}	5	0.17 ± 0.01	0.16 ± 0.01	0.15 ± 0.02	ns
N/P ratio	5	$45^{a} \pm 6$	$64^{ab} \pm 5$	$95^{b} \pm 3$	**
Rhynchospora					
N mg g^{-1}	15	$8.20^{a} \pm 0.24$	$9.68^{b} \pm 0.38$	$10.18^{b} \pm 0.37$	***
P mg g^{-1}	15	$0.56^{a} \pm 0.03$	$0.51^{a} \pm 0.02$	$0.41^{b} \pm 0.03$	**
N/P ratio	15	$15^{a} \pm 1$	$19^{a} \pm 1$	$27^{b} \pm 2$	***
Vaccinium					
N mg g^{-1}	15	$9.40^{a} \pm 0.32$	$10.38^{a} \pm 0.29$	$12.62^{b} \pm 0.41$	***
P mg g^{-1}	15	0.50 ± 0.03	0.43 ± 0.02	0.49 ± 0.04	ns
N/P ratio	15	$19^{a} \pm 1$	$24^{b} \pm 1$	$27^{b} \pm 1$	***

Table 4 Effect of N deposition on the N and P concentrations and the C/N and N/P ratios of *Sphagnum* (mean ± 1 SE, $n = 10$). Different letters indicate significant differences between N treatments, ns = $P > 0.05$, $^{***}P = 0.001$ (one-way ANOVA). Data on seedling treatments were pooled

	0 kg N	40 kg N	80 kg N	Treatment effect
Capitulum				
N (mg g^{-1})	$9.12^{a} \pm 0.15$	$13.19^{b} \pm 0.41$	$16.33^{c} \pm 0.49$	***
P (mg g^{-1})	0.41 ± 0.02	0.43 ± 0.02	0.45 ± 0.04	ns
C/N	48 ± 1^{a}	$34^{b} \pm 1$	$28^{c} \pm 1$	***
N/P	23 ± 1^{a}	$31^{b} \pm 1$	$38^{c} \pm 3$	***
1–2 cm stem				
N (mg g^{-1})	$8.32^{a} \pm 0.22$	$13.03^{b} \pm 0.16$	$17.86^{c} \pm 0.60$	***
P (mg g^{-1})	0.28 ± 0.02	0.26 ± 0.01	0.31 ± 0.03	ns
C/N	$56^{a} \pm 1$	$34^{b} \pm 1$	$28^{b} \pm 3$	***
N/P	$31^{a} \pm 1$	$50^{b} \pm 2$	$62^{b} \pm 6$	***
2–3 cm stem				
N (mg g^{-1})	$7.85^{a} \pm 0.30$	$12.82^{b} \pm 0.28$	$17.75^{c} \pm 0.67$	***
P (mg g^{-1})	0.23 ± 0.02	0.25 ± 0.01	0.27 ± 0.02	ns
C/N	$58^{a} \pm 2$	$36^{b} \pm 1$	$26^{c} \pm 1$	***
N/P	$35^{a} \pm 2$	$52^{b} \pm 3$	$70^{c} \pm 6$	***

which height increment decreased; production remained unaffected.

N concentration in *Sphagnum* differed between all three N treatments (Table 4), resulting in distinctive C : N ratios. Values for the stem fraction ranged from 57 in the 0 kg N treatment to 26 in the 80 kg N treatment. The distribution of N over stem and capitulum was also affected. In the 0 kg N treatment, the capitulum C : N ratio was significantly lower than the C : N ratio of the stem fractions; this difference disappeared in the 40 and 80 kg N treatments.

There was no correlation between the inorganic N concentration in the interstitial water at 0–5 cm depth and the N

tissue concentrations at harvest time, suggesting that the amount of N associated with the water in the hyaline cells was negligible.

The P concentration in *Sphagnum* was not influenced by adding N (Table 4) and was higher in the capitulum fraction than in the stem fractions (fraction effect: $P = 0.001$, $n = 30$, two-way ANOVA). The mean N : P ratios in the capitulum ranged from 23 in the 0 kg N treatment to 38 in the 80 kg N treatment, suggesting limitation by P (Koerselman & Meuleman, 1996).

Discussion

Model

In general, our results are in agreement with the conceptual model describing the influence of N deposition on *Sphagnum* dominated vegetation proposed by Lamers *et al.* (2000) and Berendse *et al.* (2001). As was hypothesised, the concentration of inorganic N in the interstitial water decreased and increased in accordance with the changes in N deposition (Fig. 1a,b). Adding N did encourage vascular plants to grow, although an increase in the deposition above field background values, benefited only few vascular plant species (Table 2). *Rhynchospora* and *Vaccinium* increased their biomass when N deposition increased from 40 to 80 kg N ha^{-1} yr^{-1}. Other species accumulated the excess N in their tissue (Table 3). This observation, coupled with the high N : P ratio in most vascular plant species and *Sphagnum* (Tables 3 and 4), suggests that those species that did not expand their biomass after adding N were limited by P (Verhoeven & Schmitz, 1991; Koerselman & Meuleman, 1996).

Both Lamers *et al.* (2000) and Berendse *et al.* (2001) suggest that in intact vegetation, the depression of *Sphagnum* by elevated N deposition is a result of increased shading by vascular plants. In our study, the observed growth reduction of *Sphagnum* in the 80 kg N treatment (Fig. 4) cannot be wholly explained by increased shading (Fig. 5): only less than half the data points indicating a combination of heavy shading and short height increment coincide with the 80 kg N treatment (Fig. 5). An alternative explanation, which may have confounded the effect of shading, is a direct toxic effect of N on *Sphagnum* at a high N supply (Limpens & Berendse, 2003; Press *et al.*, 1986; Gunnarsson & Rydin, 2000). The hump-shaped relationship we found between shading and height increment (Fig. 5) does point to an additional effect of shading, however. It seems that the observed response to shading below 53% was due to elongation of *Sphagnum* as a result of diminished light availability. Above 53% shading however, length increment decreased albeit the etiolation process taking place. This result suggests a decreased production, as has been observed by Hayward & Clymo, 1983).

There are some further discrepancies between our study and the conceptual model proposed by Lamers *et al.* (2000). In our study (Table 4), as well as in other similar studies (Ferguson *et al.*, 1984; Press *et al.*, 1986; Aerts *et al.*, 1992; Pitcairn *et al.*, 1995; Williams *et al.*, 1999; Nordin & Gunnarsson, 2000; Berendse *et al.*, 2001; Heijmans *et al.*, 2001), tissue N concentration of *Sphagnum* showed a linear increase with N deposition rather than a logarithmic one, and subsequently reached higher values than the proposed maximum N tissue concentration of 12–13 mg Ng^{-1}. For *S. magellanicum* the maximum value recorded was 24.3 (± 0.06) mg Ng^{-1} in the 0–3 cm fraction (Heijmans *et al.*, 2001). This value was reached in a fertilisation treatment in which the total deposition load was *c.* 100 kg N ha^{-1} yr^{-1}. When these concentrations are taken into account, a maximum organic N concentration of *c.* 20 mg N g^{-1} for *S. magellanicum*, as proposed by Berendse *et al.* (2001), seems more likely. Of course, one can argue that the cited studies in which nutrient concentration exceeded 12–13 mg N g^{-1} refer to relatively short-term fertilisation experiments, which lasted 1–3 yr. Experimentally fertilised systems undergo forced rapid change, whereas peat bogs have been subject to at least 10 yr of rather constant, or slowly changing, deposition loads. Lamers *et al.* (2000) only used values for *Sphagnum* derived from these latter 'natural' conditions. Nevertheless, there is no reason to assume that *Sphagnum* would behave so differently under experimentally fertilised conditions.

Another anomaly is that, according to the theory as proposed by Lamers *et al.* (2000), N concentration in the rhizosphere should only begin to increase strongly when the N tissue concentration in *Sphagnum* has peaked. Our results indicate that the process must be more gradual. While still accumulating N in its tissue, *S. magellanicum* is not capable of retaining all atmospherically derived N, as indicated by the increasing inorganic N concentration in the interstitial water (Fig. 1a,b) and the increased concentration of N in vascular plants (Table 3). It seems more likely that, as tissue N concentration of *Sphagnum* increases, its capability to absorb N declines, as shown by Woodin & Lee (1987). The rate, at which *Sphagnum* and subsequently the rhizosphere become loaded with N, is likely to be determined by *Sphagnum* production. In turn, the latter may be influenced by factors such as P availability, degree of shading, water table 1evel and the occurrence of extreme climatic conditions (Hayward & Clymo, 1983; Malmer, 1988; Aerts *et al.*, 1992, 2001; Takagi *et al.*, 1999; Heijmans *et al.*, 2001). When modelling the impact of N deposition on *Sphagnum* dominated peatlands, we must take this interaction between the abiotic and biotic environment into account.

The above implies that we cannot separate phases two and three of the proposed mechanism on account of the maximum N concentration in *S. magellanicum*: the *Sphagnum* filter fails before *Sphagnum* reaches its maximum inorganic N content. As the transition between phase two and three is not well defined, phase two only seems to serve a theoretical purpose, maybe indicating a time lag between exceeding phase one (N

no longer limits *Sphagnum* growth) and the ensuing N effects on the vegetation due to positive feed-back through litter quality (Berendse *et al.*, 1989, van Breemen, 1995). On account of this, we expect N-induced changes in the species composition of *Sphagnum* dominated peatlands at the end of phase one, shortly after the N concentration of *Sphagnum* in the upper 3 cm surpasses 8–9 mg N g^{-1} (Lamers *et al.*, 2000).

Invasive species

We anticipated that growth of *Betula* and *Molinia* would improve with an increase in N deposition and decline when deposition approached zero. Although the growth of both species was much less than expected, their response to N was in accordance with our hypothesis (Table 1, Figs 2 and 3). Nevertheless, it seems that the expansion of these species in bogs cannot be solely explained by high atmospheric N deposition. The level of the water table in combination with the availability of other nutrients than N, probably codetermines the success of these species in undrained bogs.

For *Betula* the circumstances were obviously too severe for establishment; the combination of a high water table and substantial *Sphagnum* growth successfully prevented survival of *Betula* seedlings. A less vigorous *Sphagnum* growth seems a prerequisite to a successful establishment.

Molinia also suffered indirectly from the high water table by becoming vulnerable to competition with *Rhynchospora*. In the first year *Molinia* profited from adding N (Fig. 2) and took up a significant part of the N available in the interstitial water (Fig. 1b). At the beginning of the following year, the amount of N taken up in the previous year was used to form new leaves (Thornton & Millard, 1993). Between May and June 2000, the presence of *Molinia* no longer suppressed N availability in the interstitial water (Fig. 1b). This period coincided with the rapid expansion of *Rhynchospora* in the mesocosms treated with extra N. At the end of the growing season, no effect of N treatment was found on biomass of *Molinia*, but adding N did increase *Rhynchospora* biomass (Table 2). The extremely high N : P ratio of *Molinia* (Table 3) indicates limitation by P (Verhoeven & Schmitz, 1991; Koerselman & Meuleman, 1996). On this account it seems likely that during the second growing season, insufficient P was available to sustain further growth of both *Molinia* and *Rhynchospora* at the same time. Although *Rhynchospora* is not characterised as a successful competitor (Ohlson & Malmer, 1990), under the conditions in our experiment it was apparently able to outcompete *Molinia*. That a low P availability, at least in natural wet ecosystems, can curb expansion of *Molinia*, has also been shown by Aerts & Berendse (1988). In their fertilisation experiment, conducted in wet heath, they only found a significant increase in cover for *Molinia* when P had been added.

From the above, we infer that extensive expansion of *Molinia* in intact bog vegetation is likely to occur only when P availability is also enhanced.

Reversibility

Raised bog vegetation seems able to recover its nutrient poor state after the source of enrichment is taken away. The latter is illustrated by the decrease in inorganic N concentration of interstitial water (Fig. 1a,b) and the N tissue concentrations of both vascular plants and *Sphagnum* (Tables 3 and 4) in the 0 kg N treatment. In addition, the poor performance of *Betula* and *Molinia* in this treatment (Table 1, Fig. 2) shows that when N availability decreases, survival of invasive species becomes challenged. These findings, combined with the vigorous *Sphagnum* growth (6 cm in 2 yr, Fig. 4), suggest that in time vascular plant establishment and growth would slowly decrease and again be in line with the re-created extreme nutrient-poor environment. These observations are supported by the work of Maksimova & Yudina (1999), who mention a similar reversibility of fertilisation effects. They observed that the vegetation had almost completely recovered 18 yr after a 3-yr application of high doses of mineral fertiliser (30 kg N and 60 kg *P* ha^{-1} yr^{-1}). These findings imply that prospects for the conservation of bogs are good, if critical loads are met in the near future.

Acknowledgements

This research was supported by the Ministry of Agriculture, Nature Management and Fisheries of the Netherlands. We are indebted to Monique Heijmans for comments on the manuscript and Jan van Walsem and Frans Möller for chemical analysis and practical assistance.

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