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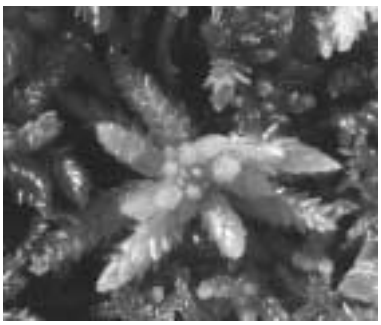

Quagmire, swampland, morass:
the slime kingdoms,
domains of the cold-blooded,
of mud pads and dirtied eggs.

But *bog*
meaning soft,
the fall of windless rain,
pupil of amber.

Is er een toekomst voor hoogveen vegetatie in Nederland?

Promotor: **P**rof. dr. F. Berendse
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Proefschrift

Ter verkrijging van de graad van doctor op gezag van de Recor Magnificus van Wageningen Universiteit, prof. dr. ir. L. Speelman.
In het openbaar te verdedigen op vrijdag 4 april 2003 des namiddags te vier uur in de Aula

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Poem: **S**eamus Heaney

Photographs front-page: ***S****phagnum papillosum*, *Vaccinium oxycoccus* and aerial overview of a part of Bargerveen

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Paranimfen: **H**ilde Tomassen & Gert-Jan van Duinen

Abstract

Limpens J. 2003. Prospects for *Sphagnum* bogs subject to high nitrogen deposition. Thesis at Wageningen University, the Netherlands, 143 pp.

S*phagnum* bogs harbour a wealth of rare vascular plant and bryophyte species, preserve an amazing pollen record and are long-term sinks for atmospheric carbon. Unfortunately, the relatively low production and decomposition rates, that make these bogs such important environments, also make them vulnerable to changes in atmospheric nitrogen (N) input. The research presented in this thesis had as its aim to investigate to what extent N deposition could affect bogs, and explore whether its influence could render initially successful restoration efforts futile.

We conducted a set of field and greenhouse experiments, aimed at delineating the effects of N and P on the interactions between *Sphagnum* and vascular plants, epiphytic algae, fungi and other *Sphagnum* species. In addition, we paid attention to the physiological effects of a high N supply on *Sphagnum*, and we studied the impact of an elevated N supply on litter quality and decomposition rate, to get an impression of its long-term effects on bog vegetation.

Our results unambiguously showed that a simulated increase in N deposition depressed *Sphagnum* growth. How this decreased vitality came about is not so straightforward, however. We can distinguish two types of negative N effects on *Sphagnum*. A direct toxic effect that seems to be linked to the N metabolism of *Sphagnum* and an indirect effect brought about by intensified interactions with other organisms. Additionally, our results showed that *Sphagnum* originating from sites with a high N deposition decomposed faster than *Sphagnum* from a site with intermediate N deposition. This combination of decreased *Sphagnum* production and increased decomposition nudges the carbon balance of these systems towards the negative, and thus challenges the survival of bogs.

A considerable part of the effects mentioned above depends on the amount of deposited N that *Sphagnum* can incorporate in its tissue and on the resulting tissue N concentration. As such, the impact of a high N supply is not so much determined by the level of N deposition *per se* than by the balance between the negative effects of N on the one hand and the supply of potentially growth-limiting factors such as water, P, CO_2, light and temperature on the other hand. Thus, it seems possible to circumvent an important part of the negative N effects by optimising the overall growing conditions of *Sphagnum*. However, we must realise that the resilience of the bog ecosystem and the range of conditions under which *Sphagnum* bogs can survive decrease with N deposition, and thus are limited.

Contents

Voorwoord

Vreemd om nu in het Nederlands te schrijven en nog vreemder om me niets van het wetenschappelijke keurslijf te hoeven aantrekken. Hier heb ik naar uitgekeken, maar nu het zover is weet ik niet goed waar en hoe te beginnen. In de afgelopen vijf jaar heb ik een groot aantal mensen ontmoet die in meer of mindere mate, elk op hun eigen manier, een stempel hebben gedrukt op dit boekje. Het zou helaas ondoenlijk zijn iedereen te noemen; hoewel het dan niet in tekst vastgesteld is, weet jullie verzekerd van mijn waardering.

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Emiel, je hebt je belofte meer dan waar gemaakt. Je vaak korte vragen, bedoeld om voor jezelf structuur in een door mij warrig uitgezette theorie te brengen, hebben me vaak op het goede spoor gebracht. Bedankt!

Juul

Front view of Clara House in 1999, county Offaly, Ireland.

Chapter 1

General introduction

Of man and bog

A question frequently put to me by Irish locals in the pub, was what brought me to Clara. More often as not my answer, that I was conducting research on the nearby bog, was met with a polite, but slightly pitying look. On my query whether they had ever visited the bog area themselves, some admitted of a stroll up and down the boardwalk or a short visit when they were kids. Most had never been there, but knew of Dutch people, mainly researchers, who had. The few, who were interested, were usually active in nature conservation. This dual attitude towards the bog further showed in the efforts of the village council to attract a nature education centre to Clara against a strong opposition of local farmers; the dissent even resulted in part of the best developed area of the bog being set to fire in the summer of 2000.

I'm sure that something similar could be told about other natural ecosystems in Europe, but I think it is especially true for bogs. The inaccessible and often dreary nature of these areas, has undoubtedly contributed to the uneasy relationship between man and bog, and fed the numerous tales of lost people and unnamed horrors (Lamers 2001, Vasander *et al.* 2000). Additionally, the stories of recent ancestors, toiling to extract peat for fuel or turn the wilderness into much needed arable land, must have left some residual resentment in the mind.

In contrast, the attention paid to bogs, and peatlands in general, by scientists and conservationists in the last decades is considerable, and has initiated numerous efforts to increase the general public's awareness of their intrinsic value. An apt example of the changing attitude towards bogs in Ireland can be found in the book *Celebrating Boglands* (Allen *et al.* 2002), about which the editor writes:

"*Reading … one is struck by the immensity of the journey, we as a nation have taken in just 20 years. We have come from a time and culture in which we looked on bogs from afar - wastelands to be dug, drained, burned, and planted, to a time and culture in which we have gotten off the fence and waded in for a close look".*

As a result, quite a few bogs have become conservation areas and a substantial amount of money is invested in the preservation, maintenance and restoration of these systems (e.g. Schouten 1994). The latter is certainly true for the Netherlands, where only a few thousand hectares (ha) of degenerate bog are left of the 250.000 that once covered a major part of the country (Pons 1992). At present, relatively undisturbed bog vegetation covers less than 100 ha, and is mainly restricted to former heath pools and patches in fen areas (Barkman 1992). Because restoration and preservation of these remains have proven more difficult than expected (Schouten and Le Gras 1999, Schouwenaars *et al.* 2002), bogs have been included in the general Dutch survival plan for woodland and nature (OBN[1]) in 1996. The research presented in this thesis was financed from this programme, and had as its aim to investigate to what extent nitrogen (N) deposition could affect bog vegetation, and might render initially successful restoration efforts futile.

The N cycle and N deposition

The human impact on the global N cycle has increased exponentially since the late 1950s, and has reached the point where more N is fixed annually by human driven than by natural processes (Vitousek *et al.* 1997, Smil 1999). In addition to fixing N, a considerable amount of N is mobilised from long-term storage pools through biomass burning and use of fossil fuels, deforestation, and draining of wetlands (Crutzen and Andrae 1990, Vitousek and Matson 1993, Galloway *et al.* 1995). As a result there is an increasing transfer from unavailable gaseous N_2 to biologically available (reactive) N in the oceans, biosphere and atmosphere (e.g. Vitousek 1994). It is likely that in this century the above process will continue, as projections indicate that in less developed regions of the world, Asia in particular, both the population size (e.g. Lutz *et al.* 2001) and the per capita resource use will continue to grow (e.g. Galloway and Cowling 2002).

The enrichment of the atmosphere, with oxidised (NO_x) and reduced N compounds (NH_x), has resulted in an averaged fourfold increase of pre-industrial deposition for temperate systems in the northern hemisphere (Holland *et al.* 1999). The main sources of NO_x are industry and traffic, whereas intensive animal husbandry and dairy farming are the chief sources of NH_x.

[1] OBN (Overlevingsplan Bos en Natuur) is a programme developed by the Dutch Ministry of Agriculture, Nature Management and Fisheries (LNV) to generate knowledge about key processes and threats, in order to improve restoration success of endangered ecosystems in the Netherlands (Bal *et al*. 2000).

The actual N load that an ecosystem is subject to, generally depends on the distance from local ammonia sources (Ivens 1990) and the canopy structure; the wider the leaf area and the rougher the deposition surface, the more atmospheric N is captured by the vegetation (Van Breemen *et al*. 1982, Heil *et al.* 1988, Matzner 1989). The interaction of NH_x with SO_2 may also affect the amounts of N deposited on a - wet - leaf surface. After deposition of both compounds, ammonium sulphate is formed, decreasing the pH of the water film covering the leaf surface. As a result, NH_3 dissolves more readily in the watery solution and cuticular uptake of N increases, thus facilitating further deposition of NH_3 (e.g. Adema *et al.* 1986, Flechard *et al.* 1999).

Due to a stricter legislation concerning emissions of SO_2 and NH_3, and the political reform in eastern Europe, the emissions of SO_2 and NH_3 in Europe have respectively declined from the 1960s and 1990s onwards (Thomas *et al.* 1988, Sutton *et al.* 2001). As a result, it is reasonable to assume that the aggravating effect of co-deposition of SO_2 and NH_3 has also diminished in this area, relieving the N pressure on ecosystems. Nevertheless, the input of atmospheric N in this region, the Netherlands in particular, is still among the highest in the world (Holland *et al.* 1999, Van Oene *et al.* 1999, EMEP-web site 2002) and likely to remain so in the foreseeable future.

Impacts of N on ecosystems

Although the human production of reactive N is a blessing for food production, the concomitant release of bio-available N in natural ecosystems is not so benign. As most temperate ecosystems have evolved under N limited conditions, and both species composition and ecosystem processes are geared to nutrient poor conditions, it is not remarkable that a steady increase in available N poses a serious threat to these low productive ecosystems (e.g. Van Breemen 1995a, Chapin *et al.* 1997). Numerous experimental studies have shown that in systems where N limits primary production, adding N alters the competitive balance between species, and previously subordinate species may become invasive. Ultimately, this may result in a shift from low productive and often diverse vegetation to high productive vegetation, consisting of a few nitrogen-responsive, often clonal, plant species (Heil and Diemont 1983, Tilman 1987, Bobbink 1997, Lee 1998, Turkington *et al.* 1998, Nordin *et al.* 2002).

The subsequent changes in competition between species and eventual species replacement have usually been attributed to interspecies differences in the ability to acquire and use different nutrient sources, potential growth rate and rates of nutrient loss (Berendse and Aerts 1987, Aerts and Berendse 1988, Berendse and Elberse 1990, Aerts and Chapin 2000). Not all the changes in the community assemblage can be wholly explained by interspecific competition, however. Changes in multi-trophic interactions, such as plant–insect (Brunsting and Heil 1985, Berdowski and Zeilinga 1987, Aerts *et al.* 1990), plant–fungus (Packer and Clay 2000, Strengbom *et al.* 2002) and

plant–algae (Wear *et al.* 1999, Fong *et al.* 2000) associations, may contribute to the observed shifts in species composition. N-induced changes in the nutrient concentration and palatability of the plants play a major role in the bi-trophic interactions mentioned above. These changes may involve an increase in the N tissue concentration and subsequent leaching of easily degradable N compounds (Ohlson *et al.* 1995, Flückiger and Braun 1998, Peveling *et al.* 2002) and possibly, a decrease in the concentration of secondary metabolites (e.g. Balsberg-Påhlsson 1992, Hättenschwiler and Vitousek 2000). On the long term, N-induced changes in the species composition and the litter quality may further aggravate the ecosystem effects of N deposition by speeding up N turnover within the system (Berendse *et al.* 1989, Van Vuuren *et al.* 1992, van Oene *et al.* 1999).

*S*phagnum as ecosystem engineer

Peatlands[1], cover *c*. 345 million hectares in the northern hemisphere between the 50°N and 70°N latitudes and make up around 25% of the global soil C pool (Gorham 1991, O'Neill 2000). A large proportion of these northern peatlands are dominated by peat mosses (*Sphagnum*, or veenmos) and as a consequence, a considerable amount of C is stored in their litter; it has even been claimed that *Sphagnum* alone, contains more carbon in its living and dead tissues than any other genus of plants, including trees (Clymo and Hayward 1982). Although the anoxic, cold, acidic and nutrient poor environment created by the unique properties of *Sphagnum* (e.g. Van Breemen *et al.* 1995b) enables but a low production, these same conditions and the refractory nature of *Sphagnum* litter (e.g. Verhoeven and Liefveld 1997), also guarantee an even lower rate of decomposition. As a result, *Sphagnum* peatlands sequester *c*. 12% of the human C emissions on a yearly basis, thus playing an important role in the global carbon cycle.

The key role of *Sphagnum* in maintaining the bog ecosystem also means that any disturbance that affects the dominance of this species ultimately impacts on the existence of the system itself. This is why a significant part of the research on the effects of N deposition on *Sphagnum* peatlands focuses on the effects of N on *Sphagnum*. However, as described under the previous caption, a considerable part of the ecosystem effects of N depend on the interactions between organisms, stressing the importance of field experiments where N effects can be studied on a vegetation as a whole, as is we set out to do in this thesis.

[1] In this thesis, peatlands have been defined as peat forming wetlands, in which an organic soil layer of at least 30 cm depth has developed (Gorham 1991). *Sphagnum* peatlands refer to those peatlands where *Sphagnum* dominates the bryophyte layer. When using *Sphagnum* bog, bog or raised bog, we mean *Sphagnum* peatlands that receive most of their nutrients from the atmosphere and, as such, are rainwater fed or ombrogenous (Wheeler and Proctor 2000).

Effects of N on *Sphagnum*

The effect of high N deposition on *Sphagnum* has received considerable attention in the last ten years, but most studies have only addressed the short-term effects of enhanced N availability on the performance of isolated *Sphagnum* under controlled conditions (Press *et al.* 1986, Rudolph and Voigt 1986, Baxter *et al.* 1992, Jauhiainen *et al.* 1994 and 1998a, Williams and Silcock 1997, Williams *et al.* 1999, Gunnarsson and Rydin 2000, Van der Heijden *et al.* 2000). These studies generally report a negative effect of high N deposition on *Sphagnum*, although the amount of N added before deleterious effects could be observed differed among *Sphagnum* species and sites where the mosses had been collected. Species adapted to more mesotrophic conditions, such as *S. fallax* or *S. angustifolium,* usually coped better with heavy N loads than those adapted to ombrotrophic habitats, such as *S. fuscum* (Jauhiainen *et al.* 1998a and 1998b; Gunnarsson and Rydin 2000). In the few studies on intact bog vegetation (Twenhöven 1992, Hogg *et al.* 1995, Heijmans *et al*. 2001, Berendse *et al.* 2001), N-stimulated vascular plant growth was observed in combination with depressed *Sphagnum* growth, suggesting that the *Sphagnum* suffered from shading (Clymo 1973, Hayward and Clymo 1983). In experiments in which vascular plants were excluded, differences in nutrient limitation, depending on background deposition at the study site, have proven to be important in explaining contradicting *Sphagnum* responses to N fertilisation. Thus, P limited *Sphagnum* from a site with intermediate N deposition (Aerts *et al.* 1992, Risager 1998), whereas N limited *Sphagnum* from a low deposition site (Aerts *et al.* 1992).

The research presented in this thesis gives experimental evidence for and elaborates on the proposed mechanisms (Lamers *et al.* 2000, Berendse *et al.* 2001) by which N affects intact bog vegetation. We paid special attention to the effects of N and P on the interactions between *Sphagnum* and vascular plants, epiphytic algae, fungi and other *Sphagnum* species, as well as to physiological effects of a high N supply on *Sphagnum*. In addition, we tried to get an impression of long term N effects on bog vegetation by studying the impact of an elevated N supply on litter quality and decomposition rate.

Outline of thesis

Our main questions and their associated hypotheses are listed in Table 1.1 and will be discussed at the end of this thesis.

In the coming chapter (2), we introduce our main field experiment in which we tried to elucidate which nutrient, N or P, limits growth of *Sphagnum* and vascular plants in five bogs in the Netherlands subject to high N deposition, and one bog in Ireland subject to intermediate deposition. In this chapter we also attempt to answer the question whether the expected negative N effects on *Sphagnum* can be attributed to N-induced changes in vascular plant cover, or must be a result of other factors instead.

In the next chapter (3), we present an experiment with mesocosms containing intact bog vegetation. In this experiment, we manipulated the level of N deposition to elucidate the relationships between N deposition, *Sphagnum* growth, N availability in the rhizosphere and vascular plant growth. In addition, we wanted to test whether the establishment of two invasive species in north-west European bogs, *Betula* sp. and *Molinia caerulea*, would be encouraged by an increase in N availability and impaired by a decrease in N availability.

In chapter 4, we focus on the mechanism behind the negative effects of N on *Sphagnum*. In this chapter we investigate how N affects the competitive balance between *Sphagnum* and species other than vascular plants, such as fungi and algae.

In the following chapter (5), we attempt to make out whether the accumulation of N-rich free amino acids may be held responsible for depressed *Sphagnum* growth subjected to increased N supply.

After having dealt with the effects of N on *Sphagnum* in general, we now (chapter 6) give attention to the consequences of nutrient enrichment on the competitive balance between *Sphagnum* species.

Chapter 7 is wholly devoted to the effects of *Sphagnum* litter quality on decomposition and N cycling and explores whether N deposition can initiate a positive feedback by influencing litter chemistry. As such, this chapter gives a glimpse of what the long-term ecosystem effects of a high N deposition may be.

We conclude with a short general discussion, aimed at integrating the results presented in the previous chapters. We also dwell briefly on the consequences of a high N deposition for the restoration of *Sphagnum* bogs.

Table 1.1 Main questions and hypotheses dealt with in this thesis

Question	Hypothesis	Chapters
How does a high N supply affect the vitality of *Sphagnum?*	N-induced depression of *Sphagnum* growth is a result of increased shading by vascular plants, and, possibly, of an increased metabolic effort to incorporate the excess N into N rich free amino acids.	2, 3, 4, 5
Does P limit *Sphagnum* at sites with an intermediate to high N deposition?	Adding P encourages *Sphagnum* growth at all intermediate[1] to high deposition sites.	2
Is the availability of inorganic N in the rhizosphere of bogs a function of N deposition?	When background deposition is high, the *Sphagnum* layer no longer retains all deposited N, and as a result, N becomes available in the rhizosphere. Therefore, a decrease in deposition will result in lower rhizosphere inorganic N concentrations, whereas an increase of deposition will lead to higher concentrations of inorganic N.	2, 3
Are vascular plants limited by N at sites with a high N deposition and can they profit from an enhanced N supply in their rhizosphere?	Vascular plants, *Betula* and *Molinia* in particular, are still limited by N at high deposition sites, and as a result, will profit from an increase in N availability in their rhizosphere, and decline when N availability decreases.	2, 3
Does a high N supply affect the competitive balance between *Sphagnum fallax* and hummock forming species, such as *S. magellanicum* and *S. papillosum*?	*S. fallax* can only expand at the expense of surrounding *Sphagnum* species when both N and P are available in sufficient quantities. As a consequence, expansion of this species at high N deposition sites is determined by P, and at low to intermediate deposition sites by N supply.	6
Does N-enrichment of *Sphagnum* litter affects its decomposability and does it speed up N turnover?	Both decomposition rate and N mineralisation will increase with the N:C quotient of the litter.	7

[1] Intermediate N deposition is defined as *c.* 10-25 kg N ha^{-1} yr^{-1}. High deposition is defined as 25 kg N ha^{-1} yr^{-1} and higher. The values refer to the total of wet and dry deposition.

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Impression of domed raised bog

Chapter 2

How P availability affects the impact of N deposition on *Sphagnum* and vascular plants in bogs

Juul Limpens, Frank Berendse and Herman Klees (SUBMITTED)

Abstract

To elucidate the impact of high nitrogen (N) deposition on intact bog vegetation, as affected by phosphorous (P) availability, we conducted a 4-year fertilisation experiment with N and P at one site with moderate, and five sites with high N deposition. N (40 kg ha^{-1} yr^{-1}) or P (3 kg ha^{-1} yr^{-1}) or a combination of both elements were applied to the vegetation during the growing season. Adding N increased the concentration of inorganic N in the rhizosphere at the moderate deposition site and at two of the three high deposition sites measured; adding P decreased the inorganic N concentration and increased the P concentration at two sites. N depressed *Sphagnum* height increment at four high deposition sites and reduced *Sphagnum* Net Primary Production (NPP) at two sites. In contrast, N stimulated vascular plant growth and litter production at three high deposition sites. P stimulated *Sphagnum* growth and *Sphagnum* NPP at two sites, and seemed to encourage growth at two others, including the moderate deposition site. Vascular plant growth remained largely unaffected, but was depressed at one high deposition site. Shading by vascular plants was of minor importance in explaining the negative N effects on *Sphagnum*. As P availability alleviates the negative impact N has on *Sphagnum* and increases its capacity to withhold N for other organisms, it should be taken into account when studying ecosystem responses to moderate to high N deposition.

Introduction

Northern peatlands sequester *c*. 12% of current human carbon (C) emissions per year (Clymo *et al.* 1998). The rate of C accumulation in these ecosystems is determined by low decomposition rates rather than by a high productivity (Clymo 1983). As *Sphagnum* litter decomposes at a slower rate than vascular plants (Coulson and Butterfield 1978, Bartsch and Moore 1985, Hobbie 1996), the carbon balance of *Sphagnum* bogs largely depends on the relative amount of *Sphagnum* litter and is thus vulnerable to changes in the competitive balance between *Sphagnum* and vascular plants.

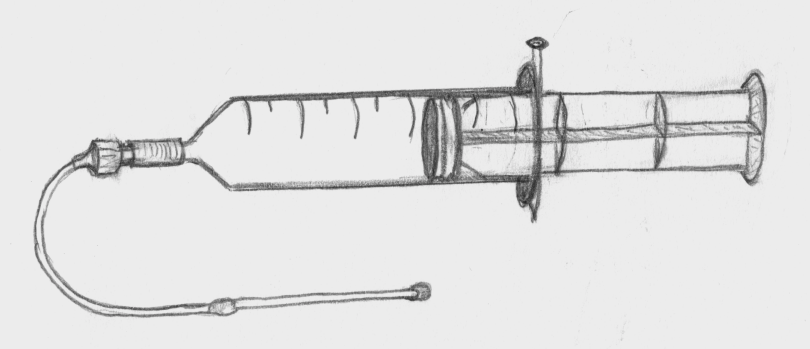
In undisturbed ombrotrophic peatlands, the balance between *Sphagnum* and vascular plants is maintained by their use of different sources of mineral nutrients. *Sphagnum* uses nutrients derived from atmospheric deposition and efficiently relocates nutrients from older tissue, whereas vascular plants depend more on nutrients released during decomposition of organic material (Malmer *et al.* 1994). The very presence of *Sphagnum* restricts the nutrient supply to vascular plants by intercepting deposited nutrients, and by slowing down decomposition through its recalcitrant litter (e.g. Van Breemen 1995). As long as the input of deposited nutrients, such as N, does not exceed the capacity of *Sphagnum* to retain them, nutrient availability to vascular plants is kept low. Once *Sphagnum* is no longer able to sufficiently restrict nutrient availability in the rhizosphere, the competitive balance may shift towards vascular plants that may ultimately outcompete *Sphagnum* (Malmer *et al.* 1994, Lamers *et al.* 2000, Berendse *et al.* 2001).

How N deposition affects *Sphagnum* bogs depends on what factors limit *Sphagnum* and vascular plant growth. Aerts *et al.* (1992) showed in a one-year fertilisation experiment, in which vascular plants were excluded, that N limited *Sphagnum* at a low deposition site in northern Sweden, whereas P limited *Sphagnum* at a site with moderate deposition in southern Sweden. When the experiment was continued for another three years, the effects on *Sphagnum* growth disappeared, presumably because water limited *Sphagnum* growth in the summer preceding the final harvest (Aerts *et al.* 2001). Other studies have shown that N limits vascular plant growth in bogs (Twenhöven 1992, Heijmans et al. 2001a). The above implies that when N deposition increases, nutrient limitation of the *Sphagnum* layer shifts from N to P (Aerts *et al.* 1992). Once N is no longer limiting *Sphagnum*, and the moss-layer becomes gradually saturated with N, it no longer retains all deposited N in its tissue (Woodin and Lee 1987, Lamers *et al.* 2000, chapter 3) and N becomes available for vascular plant growth.

In this study we pursued two objectives. The first was to investigate the impact of high N deposition on intact bog vegetation, as affected by P availability. Our second objective was to test whether the expected negative effect of N deposition on *Sphagnum* could be attributed to N-induced vascular plant growth (Lamers *et al.* 2000, Berendse *et al.* 2001, Heijmans *et al.* 2001a) or to toxic effects instead (e.g. Jauhiainen *et al.* 1994, Nordin and Gunnarsson

2000, Van der Heijden *et al.* 2000, chapter 5). We selected five sites with high N deposition in the Netherlands and one site in Ireland with a much lighter N load. The sites differed in whether the dominant *Sphagnum* species was *S. cuspidatum, S. fallax, S. papillosum* or *S. magellanicum*. N and P were subsequently applied to the vegetation for nearly four growing seasons.

We hypothesised that (I) P would encourage *Sphagnum* growth at all sites, whereas N would depress *Sphagnum* at our high deposition sites and have no effect at our moderate deposition site (II) N would limit vascular plant growth, whereas P would have no effect and (III) the negative N effect on *Sphagnum* would be mainly a result of increased shading by vascular plants.



Rhizon soil moisture sampler with syringe

Methods

Site descriptions

The experiment was carried out at five sites in the Netherlands and one site in Ireland, all of which are extremely poor fens or raised bogs. Although not all the sites had a continuous layer of *Sphagnum*, the individual plots were selected in areas with 100% *Sphagnum*. Two Dutch sites were situated in the Bargerveen reserve in the north-eastern part of the Netherlands (52°42'N, 7°03'E); three other sites were located in Dwingeloo State Forest. One moderate deposition site was chosen in Ireland to provide a reference for the Dutch sites of high deposition.

Bargerveen-Sp was chosen in an area dominated by *Sphagnum papillosum* (Lindb.), *Erica tetralix* (L.) and *Eriophorum angustifolium* (Honck.) Total vascular plant cover ranged from 25% to 50%.
Bargerveen-Sc comprised a floating peat layer 20-50 cm thick with *Sphagnum cuspidatum* (Hoffm.) as dominant *Sphagnum* species. The sparse vascular plant cover ranged from 5% to 10% and primarily consisted of *E. angustifolium*.
Reigersplas (52°50'N, 6°27'E) is a heath pool that has gradually become infilled with peat. It is surrounded on three sides by forest. Plots at this site were laid out on a floating peat layer (20-50 cm thick) dominated by *Sphagnum magellanicum* (Brid.) and *S. papillosum*. The herb cover ranged from 10% to 25% and mainly consisted of *E. tetralix*, *Vaccinium oxycoccus* L., *Rhynchospora alba* (L. Vahl.) and *M. caerulea*.
Harkeven (52°51'N, 6°25'E) is a depression in the landscape that is now filled with *c.* 1.75 m solid peat. It is screened from the surrounding arable fields by trees. *S. fallax* dominates the moss layer, whereas *Eriophorum vaginatum* (L.) dominates the herb layer that ranges in cover from 20% to 40%.
Rundeven (52°51'N, 6°23'E) is screened from the surrounding pastureland by trees. Half of this former heath pool has become infilled with *S. fallax* peat (20-70 cm thick), on which are scattered some hybrids of *Betula* . The herb layer in our plots was dominated by *V. oxycocus* and *Carex rostrata* (Stokes), ranging in cover from 5% to 20%.
Clara bog (53°20'N, 7°36'E) is a domed raised bog in the Midlands. We established our plots on peat rafts (20-50 cm thick) in erstwhile pools. *S. papillosum, S. magellanicum* and *Sphagnum rubellum* (Wils.) dominated the moss layer. *Calluna vulgaris* (L.), *E. tetralix*, *V. oxycoccus*, *Narthecium ossifragum* (L.), *E. angustifolium* and *Carex limosa* (L.), formed a sparse cover of 5% to 15%.

Experimental design

The experiment was set up at the Dutch and Irish sites in May and July 1998, respectively. At each site, 20 plots, measuring 1 x 1 m, were laid out in 5 replicated blocks. Treatments were randomly assigned to the plots, and consisted of a control treatment that received demineralised water only, an N treatment (40 kg N ha^{-1} yr^{-1}), a P treatment (3 kg P ha^{-1} yr^{-1}), and a combination of the latter two. The nutrients, NH_4NO_3 and $NaH_2PO_4.2H_2O$, were dissolved in 2 litres demineralised water for the Dutch sites and in sieved bog water (Table 2.1) for the Irish site. They were watered on, using a separate watering can for each treatment. The fertilisation treatments commenced on June 12th in the Dutch sites and on July 13th in the Irish site. Nutrients were added 6 times a year between March and September. We were careful to add the nutrients immediately prior to, during, or just after rainy weather. Because of the late start of the experiment, only half of the yearly doses were applied in 1998. The last treatments were given to all sites in July 2001. The Dutch sites were harvested in the second week of August 2001; the Irish site, two weeks later.

Measurements

Deposition and soil pore water

To establish the atmospheric nutrient input at our sites, we installed five rain collectors at each site. A collector consisted of a 2-litre polythene bottle with an attached funnel, 15 cm in diameter. This whole rested on a pole *c.* 0.75 m above the ground. Three taller poles were placed around each rain collector, and thread was wound between them to discourage birds from perching on the poles or the funnels. The bottles had been enveloped in tough white plastic to prevent penetration of light and thus growth of algae. At the Dutch sites, once every two weeks, the volume of the collected rainwater was measured, a sample was taken, and the bottles were cleaned on the spot with a brush and demineralised water. In Ireland, rain was collected every month in the growing season and once every two months in winter. To prevent growth of bacteria, some $HgCl_2$ was added to the bottles. Sampling and cleaning proceeded as above.

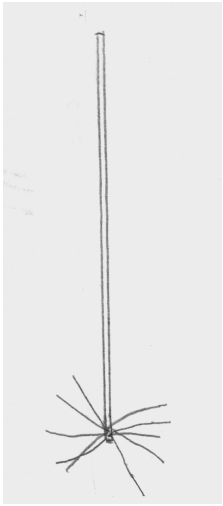
In addition to the rain collectors, five throughfall collectors (Heil and Van Dam 1986, Bobbink *et al.* 1992) were installed on a *Sphagnum* hummock beneath sparse *Erica* vegetation (cover *c*. 30%) in Reigersplas and Harkeven. The collectors consisted of a system with slanting half-open channels covered by polythene gauze that could be placed below the canopy. A polythene bottle, dug into in the peat, was connected to each collector. Once every two weeks, the bottles were emptied and cleaned and their contents measured and sampled. All water samples were amended with citric acid in the lab, and

stored at –20 °C till analysis. We sampled Dutch rainwater from April 1999 till April 2001 and Irish rainwater and throughfall from April 2000 till April 2001.

To establish whether the application of nutrients would result in enhanced nutrient availability in the rhizosphere, we analysed soil pore water during our experiment at 4 sites. In each plot in Bargerveen-Sc, Reigersplas, Rundeven and Clara bog, two Rhizon soil moisture samplers (Eijkelkamp Agrisearch Equipment, Giesbeek the Netherlands) with a porous length of 10 cm were inserted unto a depth of 10-15 cm. Soil moisture was sampled through vacuumed syringes. In 1998 and 1999, moisture was sampled once every four months. In 2000 and 2001 the sampling frequency was doubled. We were careful to wait at least three weeks between nutrient addition and water sampling. The samples were amended with citric acid in the lab, and stored at –20 °C till analysis. All samples were analysed colorimetrically, using a continuous flow analyser (SKALAR SAN plus system, the Netherlands) for NH_4^+, NO_3^- and PO_4^{3-}.

*S*phagnum growth and production

*S*phagnum growth, expressed as height increment, was measured using a variation of the cranked wire method (Clymo 1970). Four plastic rods per plot were inserted to a depth of *c*. 8 cm, and anchored by plastic broom bristles. When *Sphagnum* threatened to overgrow a rod, a new one was attached to the old one by fitting a piece of plastic tube over both rod ends. The length of the rod extending above the moss surface was measured twice a year, in March-April and September-November. The rods had a diameter of 1.5 mm and did not seem to interfere with the growth of the surrounding *Sphagna*.



Variation on the cranked wire. The bristles were anchored in the *Sphagnum.*

At the end of the experiment columns, with a diameter and depth of 10 cm, were cut around one of the plastic rods with a sharp knife. The number of columns cut per plot depended on the variation in the *Sphagnum* present. If the moss layer consisted of a homogeneous sward of one species identical in morphology, only one column was cut. If the condition, the morphology or the dominant species differed, up to four were cut to catch the variation. In the latter case means were calculated weighted by the covered area. Per day, only one site was harvested. The columns were stored at 1 °C for no longer than three weeks. *Sphagnum* was removed from the columns 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 hrs before dry mass was determined. The dried plant material was pulverised and the C concentration was measured using an elemental analyser (Fisions Instruments EA 1108, Milan Italy). Data on C were corrected

for water and ash content, both of which were determined from sub-samples after subsequent drying at 105 °C and igniting at 550 °C.

Net primary production (NPP: g C m^{-2} yr^{-1}) of *Sphagnum* in the final year was calculated as:

$$NPP = \%C_s * P \text{ where } P = (L * B_s) + ((B_c - B_{c\ control})/3.5)$$

$\%C_s$ refers to the percentage carbon measured in the 1-2 cm stem fraction, L is the height increment in cm in 2001, B_s the bulk density of the 1-2 cm stem fraction and B_c the capitulum (0-1 cm) bulk density. $B_{c\ control}$ is the bulk density of the capitula in the control treatment. We chose to divide by 3.5 because during the 3.5 years of the experiment, changes occurred in capitulum morphology, and thus in the bulk density. We assumed that during the experiment the capitulum bulk density in the control treatment did not change. As the %C of the capitulum did not differ from that in the 1-2 cm stem fraction, we did not separate between C sequestered in the stem and C sequestered in the capitulum, but multiplied production with the %C in the stem only.

Cover and vascular plant biomass

We used the point-intercept method (Jonasson 1988) to measure the cover of vascular plants and of *Sphagnum* in marked representative subplots. This entailed fixing a frame of 25 cm x 37.5 cm and with a 2.5 cm grid above this subplot. At 150 points, a stainless steel needle could be lowered to the moss surface. We recorded each species that was touched with the point of the needle; we did not distinguish between living or dead plant material. Cover was measured at the start of the experiment (June-July 1998) and each year at the end of the growing season thereafter (between August and early September).

Table 2.1 Precipitation (mm yr^{-1}) and deposition (kg ha^{-1} yr^{-1}) of inorganic nutrients per site (means + 1SE). Statistics refer to column-wise comparisons between sites. n = 5, * $P \leq 0.05$ (1-way ANOVA).

	mm	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	$P\text{-}PO_4^{3-}$
Clara bog				
Added bog water	12	0.02 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
Bulk deposition	1035 ± 7*	4.57 ± 0.62*	1.61 ± 0.03*	0.19 ± 0.10
Reigersplas				
Bulk deposition	988 ± 4	13.23 ± 0.87	5.03 ± 0.09	0.18 ± 0.02
Throughfall	677 ± 5	6.57 ± 0.30	2.67 ± 0.11	0.02 ± 0.00
Bargerveen				
Bulk deposition	863 ± 3	15.61 ± 1.55	5.73 ± 0.11	0.55 ± 0.31
Harkeven				
Bulk deposition	1001 ± 3	12.58 ± 0.28	4.97 ± 0.09	0.17 ± 0.02
Throughfall	679 ± 13	7.00 ± 0.30	2.78 ± 0.12	0.01 ± 0.00
Rundeven				
Bulk deposition	1003 ± 4	14.28 ± 1.87	5.08 ± 0.11	0.12 ± 0.01

At final harvest, the total aboveground vegetation in each subplot was cut flush with the top of the *Sphagnum*. The litter scattered on the moss surface was also collected. The harvested plants were sorted into species, and dead material, comprising both litter and standing dead, was kept separate. All plant material was dried at 70 ^{o}C for at least 48 hrs before dry mass was determined.

Data analyses

Data were tested for normality and equality of variance and, when necessary, were ln-transformed prior to analysis. All analyses were conducted with the SPSS statistical package for Windows (10.0).

As N and P concentrations in soil pore water varied considerably between the 17 sampling dates, all data on water chemistry were pooled and separately tested for an effect of N and P addition with a Kruskal-Wallis test. The effects of the fertilisation treatments and vascular plant cover on *Sphagnum* height increment were tested with an ANCOVA with N and P as fixed factors, block as a random factor and vascular plant cover and year as co-variables. The effects of N and P on *Sphagnum* NPP in 2001 were tested with a 2-way ANOVA, with N and P application as fixed factors and block as a random factor. Due to a high variability in vascular plant cover between plots, we could not use a repeated measures ANOVA to test treatment effects on vascular plant cover during our experiment (Potvin *et al.* 1990). We tested the treatment effects on the change in cover between 1998 and 2001, instead. For this, we used an ANCOVA with N and P application as fixed factors, block as a random factor and cumulative *Sphagnum* height increment during the experiment as a co-variable. The latter was to correct for parts of vascular plants that had been overgrown by the *Sphagnum* (Svensson 1995). To test whether the changes in vascular plant cover could be ascribed to changes in biomass or litter or both, we used an ANCOVA with block as a random factor and biomass and litter as co-variables.

Table 2.2 Inorganic N and P concentrations of soil pore water (mg l^{-1}) per treatment per site (means ± 1SE). x^2 values of separate Kruskal-Wallis tests for N and P are shown with their level of significance. + , – refer to direction effects. ns $P > 0.10$; (+, -) $P \leq 0.10$; ++,-- $P \leq 0.01$; +++,- - - $P \leq 0.001$ (n = 5*17 sampling rounds).

	C	N	P	NP	N effect	P effect
Clara Bog						
$N\text{-}NH_4^+$	0.08 ± 0.02	0.09 ± 0.02	0.06 ± 0.01	0.08 ± 0.02	ns	ns
$N\text{-}NO_3^-$	0.01 ± 0.01	0.06 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	7.650^{++}	ns
$P\text{-}PO_4^{3-}$	0.01 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	6.380^{-}	ns
Reigersplas						
$N\text{-}NH_4^+$	0.27 ± 0.03	0.45 ± 0.05	0.39 ± 0.04	0.44 ± 0.05	8.938^{++}	ns
$N\text{-}NO_3^-$	0.01 ± 0.00	0.09 ± 0.02	0.02 ± 0.00	0.03 ± 0.01	24.835^{+++}	ns
$P\text{-}PO_4^{3-}$	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	ns	$3.037^{(+)}$
Rundeven						
$N\text{-}NH_4^+$	0.24 ± 0.03	0.29 ± 0.05	0.36 ± 0.10	0.32 ± 0.06	ns	ns
$N\text{-}NO_3^-$	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	ns	ns
$P\text{-}PO_4^{3-}$	0.08 ± 0.02	0.07 ± 0.02	0.19 ± 0.02	0.10 ± 0.02	$3.664^{(-)}$	22.829^{+++}
Bargerveen-Sc						
$N\text{-}NH_4^+$	0.37 ± 0.08	2.41 ± 0.24	0.18 ± 0.04	0.85 ± 0.14	113.151^{+++}	30.481^{---}
$N\text{-}NO_3^-$	0.01 ± 0.00	0.03 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	ns	7.860^{--}
$P\text{-}PO_4^{3-}$	0.01 ± 0.01	0.00 ± 0.00	0.03 ± 0.01	0.02 ± 0.01	ns	14.873^{++}

Table 2.3 *F*-values for main effects influencing *Sphagnum* height increment, using ANCOVA. Levels of significance are indicated with + , – , * that refer to positive, negative or non-directional effects respectively. (+ ,*) $P \leq 0.10$; +,-,* $P \leq 0.05$; ** $P \leq 0.01$; - - - $P \leq 0.001$.

	Block	Year	Cover	N effect	P effect	N * P
Clara bog	0.916	0.611	5.101^{-}	0.000	$4.728^{(+)}$	0.583
Reigersplas	1.272	7.325^{**}	0.024	6.492^{-}	14.580^{+}	0.076
Bargerveen-Sp	4.476	$2.965^{(*)}$	2.774	25.986^{---}	$4.981^{(+)}$	0.551
Harkeven	0.308	1.029	0.006	1.011	1.989	0.257
Rundeven	150.368	8.834^{**}	0.375	4.229^{-}	3.022	0.012
Bargerveen-Sc	0.798	6.769^{*}	1.941	14.158^{-}	16.690^{+}	357.476

Results

Deposition and soil pore water

The total amounts of inorganic N and P deposited with bulk deposition, did not differ between the Dutch sites. Deposition of P at the Irish site was similar to those at the Dutch sites, but the amount of N compounds deposited was typically only a third. The inorganic nutrients added to the Irish plots via bog water (nutrients were dissolved in bog water) were very low and remained within the SE range of the bulk deposition (Table 2.1).

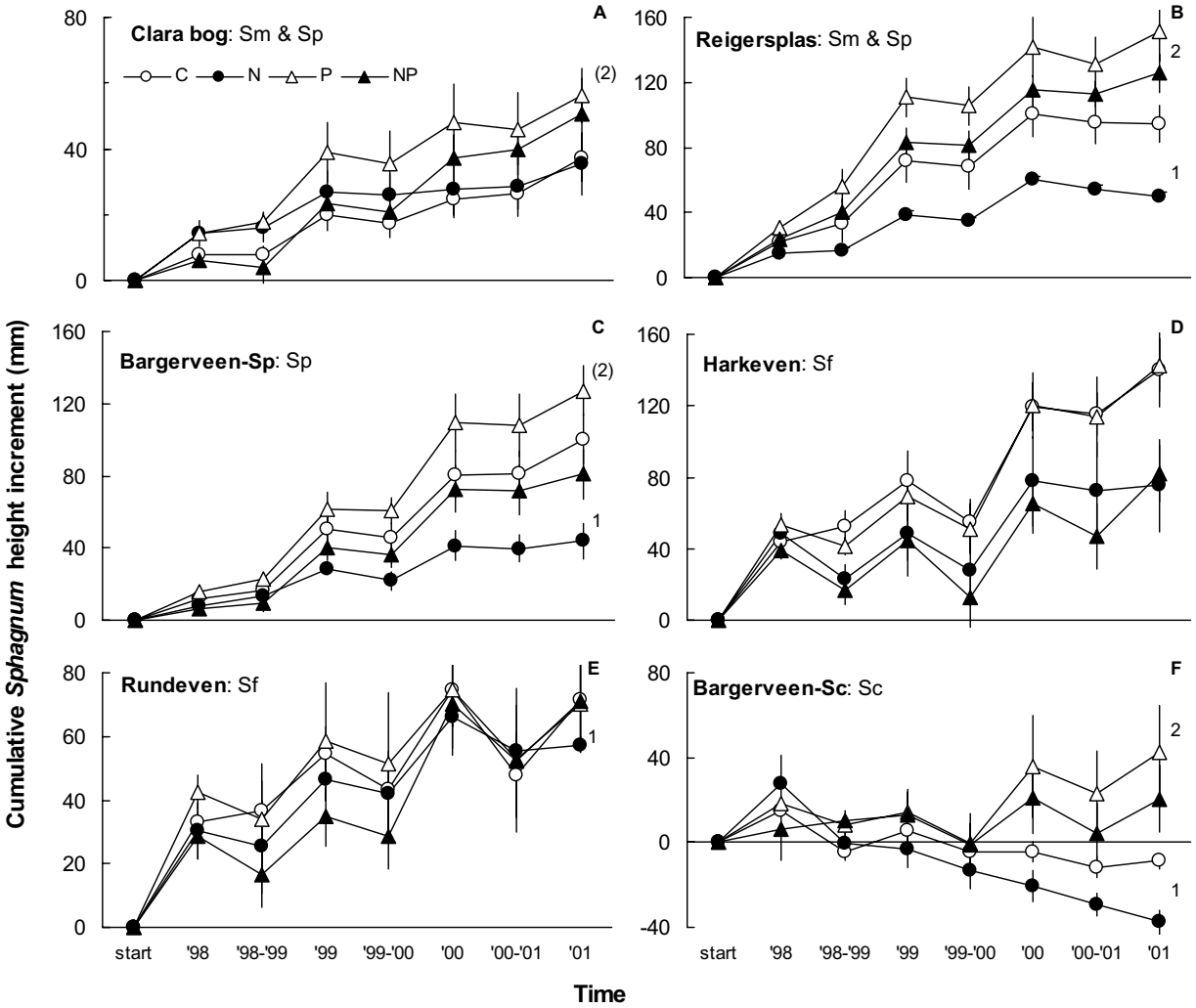


Figure 2.1 Cumulative height increment of *Sphagnum* (means ± 1SE) per summer and winter half year. Sm = *Sphagnum magellanicum*, Sp = *S. papillosum*, Sf = *S. fallax* and Sc = *S. cuspidatum*. 1 = significant N effect, 2 = significant P effect ($P \leq 0.05$). For statistics see Table 2.3.

The chemical composition of throughfall deposition (Table 2.1) differed from bulk deposition mainly in its N concentration; up to 40% of the N compounds were intercepted by the *Erica* canopy before they reached the *Sphagnum* surface. P-PO_4^{3-} could hardly be detected in the throughfall.

The Irish site had the lowest concentrations of N-NH_4^+ and P-PO_4^{3-} in the soil pore water (Table 2.2), whereas the N-NO_3^- concentration was similar to that at the Dutch sites. Rundeven was remarkably rich in inorganic P. The concentrations of N-NO_3^-, N-NH_4^+ and P-PO_4^{3-} were influenced by the fertilisation treatments. Adding N affected the N-NO_3^- concentration at three of the four sites, but the N-NH_4^+ concentration was elevated at one site only. Adding P seemed to temper this N effect, as the concentrations of inorganic N in plots treated with both N and P were lower than in plots treated with N alone (Table 2.2).

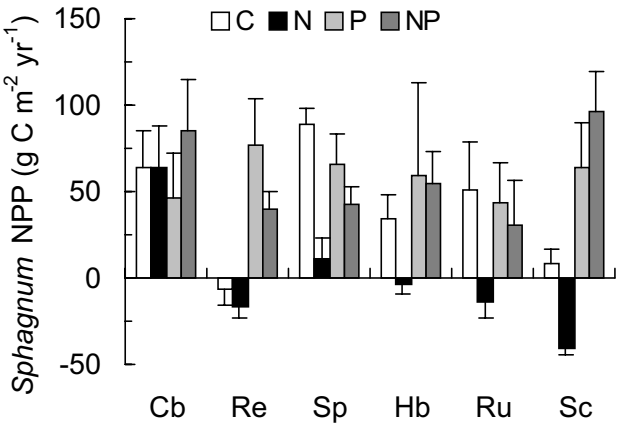


Figure 2.2 *Sphagnum* production per site in 2001, determined after 3.5 growing seasons of N or P treatments in 10 cm-diameter cores (means ± 1SE). **Cb** = Clara bog, **Re** = Reigersplas (N effect: $F = 5.499$, $P \leq 0.10$; P effect: $F = 9.935$, $P \leq 0.05$), **Sp** = Bargerveen-Sp (N effect: $F = 42.708$, $P \leq 0.001$), **Hb** = Harkeven, **Ru** = Rundeven (N effect: $F = 4.781$, $P \leq 0.10$), **Sc** = Bargerveen-Sc (P effect: $F = 23.330$, $P \leq 0.01$), N*P interaction: $F = 16.544$, $P \leq 0.05$). Only significant effects are shown (n = 10, 2-way ANOVA).

Sphagnum

Cumulative height increment varied strongly per site; the height increment of Dutch *S. papillosum* and *S. magellanicum* was more than twice that at Clara bog (Figure 2.1A-C). The height increment of *S. fallax* also varied considerably and ranged from 6 cm at Rundeven to 12 cm at Harkeven, and seemed to depend on some structural support by vascular plants (Figures 2.1D and E). The latter also applied to the height increment of *S. cuspidatum* at Bargerveen-Sc (Figure 2.1F). The negative 'increment' we observed at this site was mainly due to the *Sphagnum* dying as a result of the expansion of the fungus *Lyophyllum palustre* (Peck.) Singer, that first appeared in the summer of 1999 (chapter 4). In the graphs the difference in growth strategy between the species growing in hollows (*S. fallax* and *S. cuspidatum*) and the lawn-hummock species (*S. magellanicum* and *S. papillosum*) can clearly be seen. Each growing season, particularly in the wet summers of 1998 and 2000, the species of hollows showed an explosive growth in height followed by a relapse in the six months of winter, when the stems buckled and the *Sphagnum* layer compacted (Figures 2.1D-F). In contrast, the lawn-hummock

species had a more conservative growth in summer and less compaction in winter (Figures 2.1A-C). The seasonal fluctuation in *Sphagnum* height was most pronounced for *Sphagnum* treated with P, and resulted in a significant interaction between time and P addition at four sites.

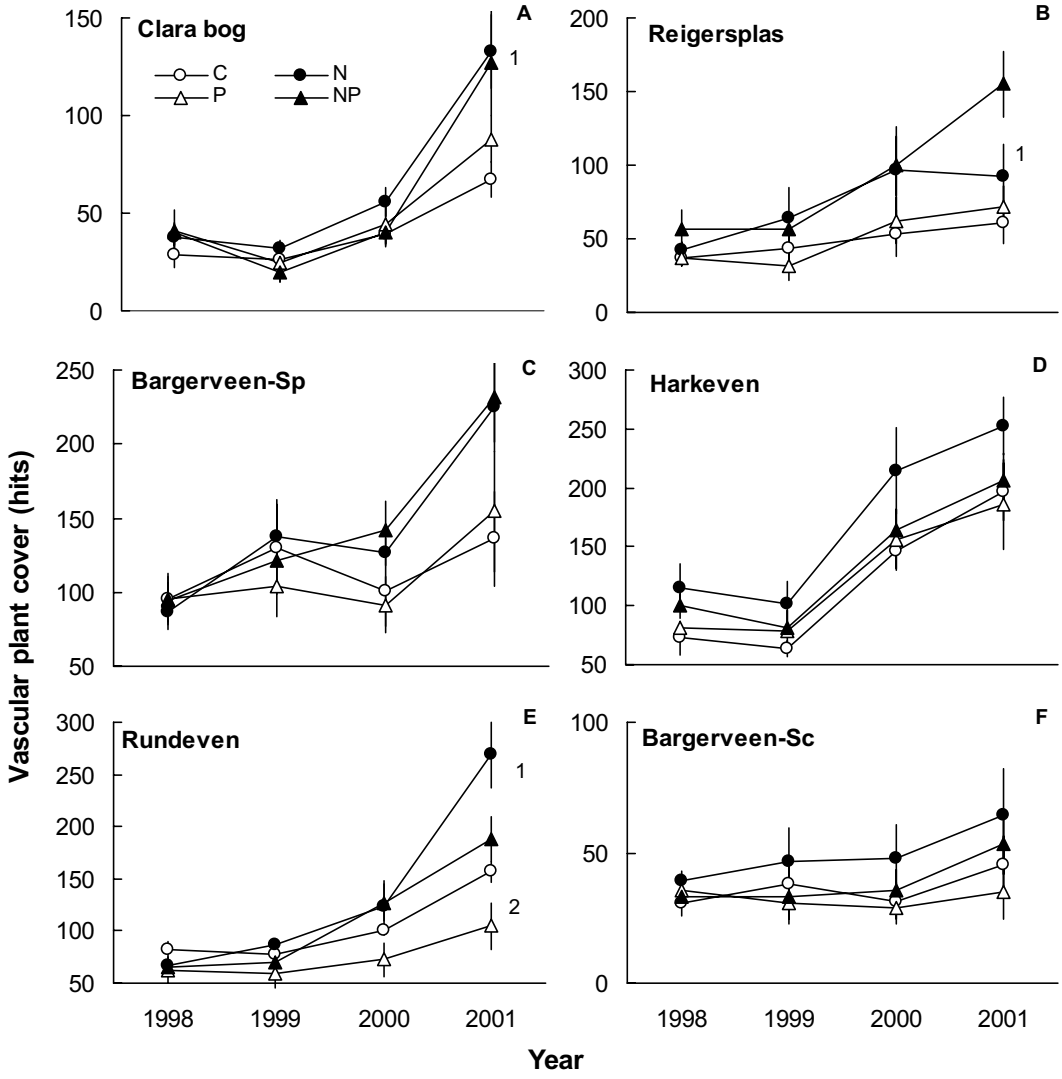


Figure 2.3 Vascular plant cover expressed as the number of hits (mean ± 1 SE), using the point-intercept method at peak standing crop. 1 = significant N effect, 2 = significant P effect ($P \leq 0.05$). For statistics see Table 2.4.

The effects of N and P on *Sphagnum* height increment were rather similar for all sites (Figure 2.1, Table 2.3). N depressed height increment at four high deposition sites (Figures 2.1B, C, E and F ,Table 2.3), but not at the intermediate deposition site (Figure 2.1A, Table 2.3). The negative N effect was most pronounced for all sites, save Rundeven, in the second year of our experiment. Adding P stimulated height increment at two high deposition sites (Figures 2.1B and F, Table 2.3) and seemed to encourage growth at two additional sites, including the intermediate deposition site (Figures 2.1A and C, Table 2.3). There was one site, Harkeven, where we failed to find a significant N or P effect on height increment (Table 2.3), despite the visible negative N effect on cumulative height increment over the experimental period (Figure 2.1D).

The fertilisation effects on *Sphagnum* height increment were mostly reflected in the NPP of *Sphagnum* (Figure 2.2), although high variability masked some of the effects detected previously. Adding N reduced *Sphagnum* NPP at half of the six sites, whereas adding P increased *Sphagnum* NPP at two sites. There was a significant interaction between N and P for one site, Bargerveen-Sc, where adding both elements yielded the highest *Sphagnum* NPP (Figure 2.2).

Vascular plants

During the experiment, vascular plant cover (Figure 2.3), including litter and standing dead, increased in the control treatments at four sites and showed a linear (Figures 2.3B and D) to quadratic (Figures 2.3A and E) relationship with time. Adding N stimulated the growth of vascular plants, resulting in a denser cover at three sites (Figures 2.3A, B and E, Table 2.4), with the biggest change occurring in the last year of the experiment. The change in cover was mainly explained by an increase in aboveground biomass; litter and standing dead were minor components (Table 2.5). Fertilisation with P depressed vascular plant cover at one site, Rundeven (Figure 2.3E, Table 2.4), and was mainly a result of a decrease in the abundance of *V. oxycoccus*.

Table 2.4 *F*-values for main effects testing change in vascular plant cover from 1998 to 2001, using ANCOVA. Moss refers to cumulative *Sphagnum* height increment. Levels of significance are indicated with + , – , * , that refer to positive, negative or non-directional effects respectively. +,* $P \leq 0.05$; ++,-- $P \leq 0.01$; +++ $P \leq 0.001$.

	Moss	Block	N effect	P effect	N * P
Clara bog	0.282	0.606	10.096 $^{++}$	0.121	0.440
Reigersplas	0.265	1.451	7.011 $^{+}$	0.423	1.369
Bargerveen-Sp	1.535	1.281	1.349	1.095	0.000
Harkeven	0.062	1.244	0.008	0.981	0.005
Rundeven	0.020	5.160 *	61.994 $^{+++}$	15.505 $^{--}$	0.576
Bargerveen-Sc	0.058	5.202 *	1.888	0.613	0.744

Shrub–moss competition

There was a shift from sparse vascular plant cover and large *Sphagnum* height increment in 1998 to dense cover and little height increment in 2001 (Figures 2.1 and 2.3), suggesting a negative relationship between vascular plant cover and *Sphagnum*. When tested, we found such a negative relationship for the Irish site only; the fertilisation treatments and experimental year explained most variation for the other sites (Table 2.3). Year had a significant effect on height increment for three sites and was almost significant for another site. When we omitted the fertilisation treatments from the model and performed an ANCOVA with block as a random factor and both year and vascular plant cover as co-variables, we found a significant relationship between vascular plant cover and *Sphagnum* height increment for one additional site (Bargerveen-Sp: F = 5.851, P = 0.018), whereas the relationship previously found for the Irish site had weakened (Clara bog: F = 3.478, P = 0.066). The effect of year, as shown in Table 2.3, was sustained at two sites only (Bargerveen-Sp: F = 4.026, P = 0.045, Reigersplas: F = 4.497, P = 0.037). As year and vascular plant cover are partly confounded, since cover increased with year, also in the control treatment (Figure 2.3), we tested the relationship between vascular plant cover and *Sphagnum* height increment for 2001 with two additional ANCOVA's. The first had N and P as fixed factors, block as a random factor and cover as the only co-variable. We chose for 2001, because vascular plant cover peaked in this year (Figure 2.3). In spite of cover at most sites being denser than at the Irish site, where we found an effect earlier, we failed to find a relationship between vascular plant cover and *Sphagnum*. The variation explained by cover for all sites was very small, the F-values being in the same range as shown in Table 2.3; the fertilisation treatments still explained most of the variation. When we omitted the fertilisation treatments from the above design in our second ANCOVA, the only significant effect that remained was a block effect for Rundeven. In turn, the cumulative height increment of *Sphagnum* did not affect vascular plant cover (Table 2.4) or biomass (data not shown).

	Block	Biomass	Litter
Clara bog	2.771 (*)	34.369 ***	4.783 *
Reigersplas	2.169	25.672 ***	1.300
Bargerveen-Sp	0.139	15.323 **	4.154 (*)
Harkeven	1.965	3.584	1.021
Rundeven	0.871	36.911 ***	0.612
Bargerveen-Sc	0.562	2.637	6.677 *

Table 2.5 *F*-values testing the contribution of biomass and litter to the observed change in vascular plant cover from 1998 to 2001, using ANCOVA. (*) P ≤ 0.10; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Discussion

Fertilisation effects

Our results show that P limits *Sphagnum* co-occurring with vascular plants at sites with moderate to high N deposition and can alleviate the negative impact of high N deposition on *Sphagnum* (Figure 2.1, Table 2.3). Nevertheless, the P effect we found was less pronounced than expected; two high deposition sites, Harkeven and Rundeven, did not respond to P at all (Figure 2.1D and E, Table 2.3). As the chemical composition of the precipitation at both sites did not differ from the other high deposition sites (Table 2.1), it seems reasonable to assume that there was some other source of P input, such as run-off, that reduced the P demand of *Sphagnum*. This observation is supported by the relatively high P concentrations measured in the pore water in Rundeven (Table 2.2). As *Sphagnum* can take up nutrients across its whole surface (Brown 1982) and is able to actively relocate P from stem parts to the growing point in the capitulum (Rydin and Clymo 1989) it may have profited from such an enrichment of the pore water. The question remains, however, what factor or factors limited *Sphagnum* growth at these sites instead. As *S. fallax* carpets have a relatively high evaporation rate (Overbeck and Happach 1957), it is possible that water may have limited *Sphagnum* growth at these sites (Thormann and Bayley 1997, Aerts *et al.* 2001).

As expected, both *Sphagnum* height increment and NPP were severely depressed by adding N (Figure 2.1, Table 2.3). The elevated inorganic N concentrations in the soil pore water (Table 2.2) show that the N supply in the plots receiving N was such that *Sphagnum* could no longer retain all deposited N. As a result, vascular plants profited from the high N supply and increased in cover (Figure 2.3, Table 2.4), which is in accordance with current theory (e.g. Lamers *et al.* 2000, Berendse *et al.* 2001). In this study we cannot distinguish between the N sources used by the vascular plants. It is likely, that they have profited both from the enhanced N supply in the rhizosphere, as has been shown in other studies (Heijmans *et al.* 2002, chapter 3), and from the N deposited on their leaves (i.e. Parker *et al.* 1983, Heil *et al.* 1988, Bobbink *et al.* 1992). The latter is suggested by the lower annual N input measured beneath the canopy (Table 2.1). Some indication for the importance of rhizosphere N for vascular plant growth in this study, can be found in the negative effect of P on vascular plant cover and biomass at Rundeven (Figure 2.3E, Table 2.4). As *Sphagnum* height increment was not affected by adding P at this site (Figure 2.1E, Table 2.3) and vascular plant growth was clearly limited by N (Figure 2.3E, Table 2.4), it seems reasonable to assume that vascular plant cover decreased because vascular plant growth could not keep up with the height increment of *Sphagnum*, due to increasingly severe N limitation.

Shrub–moss competition

In spite of the N-induced increase in vascular plant cover, our results indicate that the latter has not been the main factor responsible for the severe effect of N deposition on *Sphagnum* growth (Figure 2.1, Table 2.3). As such, our results seemingly contradict previous studies in which N-encouraged vascular plant growth has been held responsible for the observed negative effects on *Sphagnum* (Berendse *et al.* 2001, Heijmans *et al.* 2001a). Other negative effects of an experimentally enforced high N supply, such as toxic effects (e.g. Baxter *et al.* 1992, Nordin and Gunnarsson 2000, Van der Heijden *et al.* 2000, chapter 5), expansion of epiphytic algae, or increased infection by parasitic fungi (chapter 4), may have masked the relatively minor effect of cover, however. It is also possible that cover in our experiment was not dense enough to measure negative effects with the methods we used. Clymo and Hayward (1982) reported that *Sphagnum* height increment decreased when the moss was subjected to 50% shading under optimal water supply. This level of shading roughly corresponds with an estimated cover of *c*. 70% for vegetation dominated by *Erica* (Renske van Eekelen, unpublished). As there was only one site, Bargerveen-Sp, where cover became this dense, and for which we found tentative evidence of a negative relationship between vascular plant cover en *Sphagnum* height increment, the above explanation may apply. However, if we take into account, that we found a similar, but more consistent, negative relationship for the Irish site (Table 2.3), where vascular plant cover was at most 50% at the end of the experiment, it no longer seems this straightforward. On the one hand, *Sphagnum* may profit from the presence of vascular plants that give structural support (Malmer *et al.* 1994), reduce evaporation by providing shelter from wind and solar radiation (Heijmans *et al.* 2001b) and may prevent photo inhibition at low N availability (Murray *et al.* 1989 and 1993). On the other hand, vascular plants may depress *Sphagnum* by reducing light availability through shading and litter deposition (Hayward and Clymo 1983, Hogg *et al.* 1995, Berendse *et al.* 2001, Heijmans *et al.* 2001a, Ohlson *et al.* 2001) and by diminishing the availability of water. The latter may be achieved by intercepting precipitation by the canopy (Table 2.1), hampering capillary water rise in *Sphagnum* by a dense root matrix or by enhancing evaporation as a result of looser *Sphagnum* carpets (Malmer *et al.* 1994). When vascular plant cover is sparse, it is reasonable to assume that *Sphagnum* profits from the presence of vascular plants; when cover is dense the negative effects of vascular plants will start to dominate and *Sphagnum* growth will be depressed. It is likely, that between these extremes there exists a range in vascular plant covers that may or may not negatively affect *Sphagnum*, dependant on whether the remaining environmental factors, such as water and nutrient availability, are optimal or not. Such an interrelationship between environmental factors has been described by Hayward and Clymo (1983) who showed that the effect of shading on *Sphagnum* depended on water availability.

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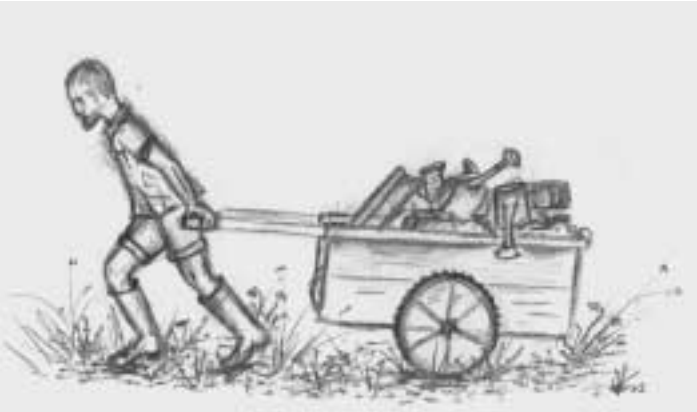

Transporting the nutrient solutions to the sites

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Bogs are humid environments

Chapter 3

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

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Abstract

We studied the effects of N on shrub-moss competition and the establishment and growth of *Betula* and *Molinia* in *Sphagnum* vegetation 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 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.

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 (e.g. 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 and Diemont, 1983, Bobbink and 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 and 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 1970's (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 (e.g. Coulson and Butterfield 1978, Bartsch and 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 approximately 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 (I) 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. (II) Vascular plants, *Betula* and *Molinia* in particular, would profit from the increased N availability in the high deposition treatment and decline at low deposition. (III) Expansion of vascular plants would result in increased shading of *Sphagnum* and thus to decreased *Sphagnum* growth.

Impression of *Sphagnum* vegetation with *Erica tetralix* and *Vaccinium oxycoccus*

Methods

Plant material

In early March 1999, 45 peat cores (diameter 34 cm, 30-35 cm deep) were cut from 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 greenhouse 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 greenhouse as the *Betula* seedlings until they had developed into a sprout with 4 to 5 leaves and were approximately 10 cm tall.

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

Experimental design

The 45 peat cores were placed into plastic containers, which were kept in an open greenhouse 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 2 sunken concrete basins and arranged in 5 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 9 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 and Christ, 1965). During the growing season, nitrogen as NH_4NO_3, dissolved in 1 l demineralised water was added once every two weeks. Both rainwater and N solution were applied with a watering can.

Measurements

Soil pore water and light

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 three months, soil moisture was sampled through vacuumed syringes, and analysed. We were careful to wait at least one week 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.

***S**phagnum* 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 x 35 cm and 20 cm deep were cut from 30 randomly selected mesocosms, and stored at 1^o 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^o C for 48 hrs before dry mass was determined.

***S**phagnum* production (g m^{-2} yr^{-1}) in the final year was calculated as:

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

With L referring to height increment in 2000; B_s, bulk density of the 1-3 cm stem fraction; $\Delta B_c/1.5$ 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, as 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, $P \leq 0.001$: 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 aboveground vegetation in all 45 mesocosms was cut flush with the top of the *Sphagnum* and sorted into species. The samples were dried at 70^{o} C for at least 48 hrs before dry mass 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.

Table 3.1 Effect of N deposition on survival of *Betula* seedlings (means ± 1SE, *n* = 5). Different letters indicate significant differences between N treatments,* $P \leq 0.05$ (1-way ANOVA).

	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	1.2 ± 0.1 ^{a}	0.5 ± 0.1 ^{b}	0.7 ± 0.1 ^{ab}	*
No. of days it took to overgrow all the *Betula* seedlings in a container after June 26	59 ± 5 ^{a}	98 ± 5 ^{b}	85 ± 5 ^{ab}	*

Data 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 post-hoc 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 waterchemistry. To discern when Molinia and N had affected interstitial water, an additional 2-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 1-way ANOVA, with N treatment as fixed factor, and for all fractions together with a 2-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 co-variable, to correct for overgrown stems and leaves (Svensson, 1995). As this co-variable had no significant effects, we omitted it from the design to be able to perform a post-hoc test. Differences between the three N treatments were analysed using a Tukey post-hoc test. Spearman's rho was used for correlation analysis.

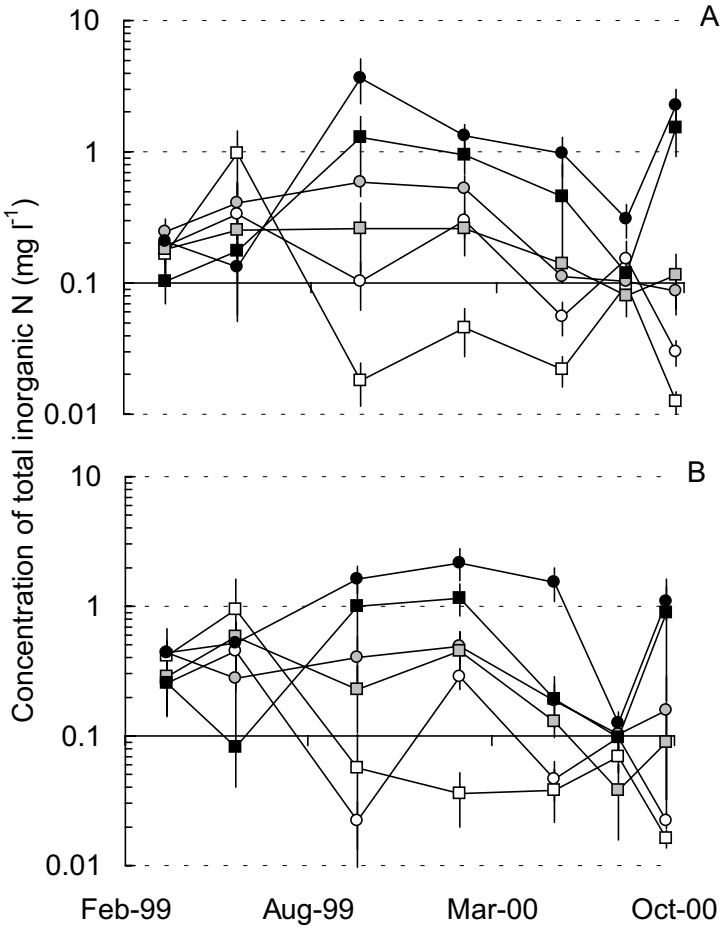

Figure 3.1 The effect of N deposition on the inorganic N concentration in interstitial water (means ± 1SE, *n* = 5) at (A) 0-5 cm and (B) 10-15 cm depth. Circles refer to mesocosms without *Molinia*, squares to mesocosms with *Molinia*. Colours of symbols refer to the fertilisation treatments with open symbols to 0 kg N ha $^{-1}$ yr $^{-1}$, closed grey to 40 kg N ha $^{-1}$ yr $^{-1}$ and black to 80 kg N ha $^{-1}$ yr $^{-1}$. Application of nutrients started early June 1999.

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: $P \leq 0.001$, 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 (Figures 3.1A and B). The differences between the N treatments had become significant at the end of the 1999 growing season (N effect: $P \leq 0.05$, n = 15, 2-way ANOVA). These differences were sustained throughout the following year, though 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 N. P concentrations hardly ever reached the detection limit of 0.01 mg l^{-1} (data not shown).

The presence of *Molinia* (Figure 3.1A and B) resulted in a significantly lower N concentration in the interstitial water at both depths (*Molinia* effect: $P \leq 0.05$, $n_{+Molinia} = 15$, $n_{-Molinia} = 30$, RM-ANOVA). This effect was most consistent in the highest N treatment at 10-15 cm depth.

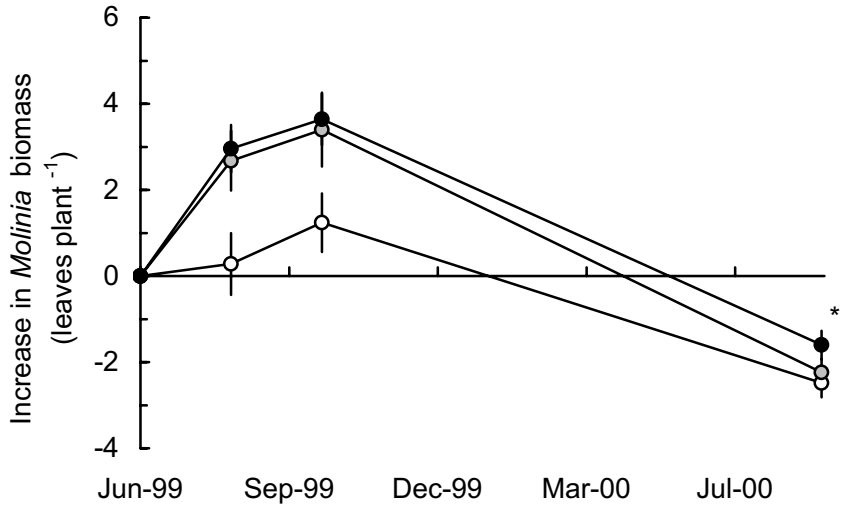

Figure 3.2 The effect of N deposition on the increase in number of green leaves of *Molinia caerulea* (means ± 1SE, $n = 5$). Open circles: 0 kg N ha^{-1} yr^{-1}, Closed grey: 40 kg N ha^{-1} yr^{-1}, closed black: 80 kg N ha^{-1} yr^{-1}. N effect: $P \leq 0.05$ (RM-ANOVA).

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. Adding N (Table 3.1), however influenced the time it took to overgrow the seedlings. In the 0 kg N treatment it took about two months for all the seedlings to die, in the 40 and 80 kg N treatments, they survived one month longer. *Molinia* fared better. Growth, as expressed by the increase in number of leaves, strongly depended on year (Figure 3.2). In 1999, growth was retarded in the 0 kg N treatment, but did not differ between the 40 kg N and 80 kg N treatments. 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 3.2). Nevertheless, 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 (Figure 3.3). 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 for this species (Table 3.2). A similar 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.

Table 3.2 The effect of N deposition on shoot biomass of vascular plants (means ± 1 SE). Different letters indicate significant differences between N treatments, ns = $P > 0.05$, * $P \leq 0.05$, *** $P \leq 0.001$ (1-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 ± 13.3^{a}	182.5 ± 18.6^{a}	290.6 ± 31.8^{b}	***
Vaccinium	g m $^{-2}$	15	9.0 ± 0.9^{a}	8.3 ± 1.0^{a}	11.6 ± 0.9^{b}	*

Total vascular plant biomass had increased in the 80 kg N treatment ($P \leq 0.001$, $n = 15$: 1-way ANOVA) and was positively correlated with interception of light ($R^2 = 0.22$, $P \leq 0.05$: data not shown).

In all species, except the deep-rooting *Eriophorum angustifolium*, N tissue concentration increased with N supply (Table 3.3). Due to increasing N concentrations and slightly decreasing P concentrations, a significant N effect was found on the N:P quotient 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 co-

limitation (Koerselman and Meuleman, 1996). *Erica* and *Molinia* showed a remarkably high N:P quotient, exceeding 30 and 45 respectively.

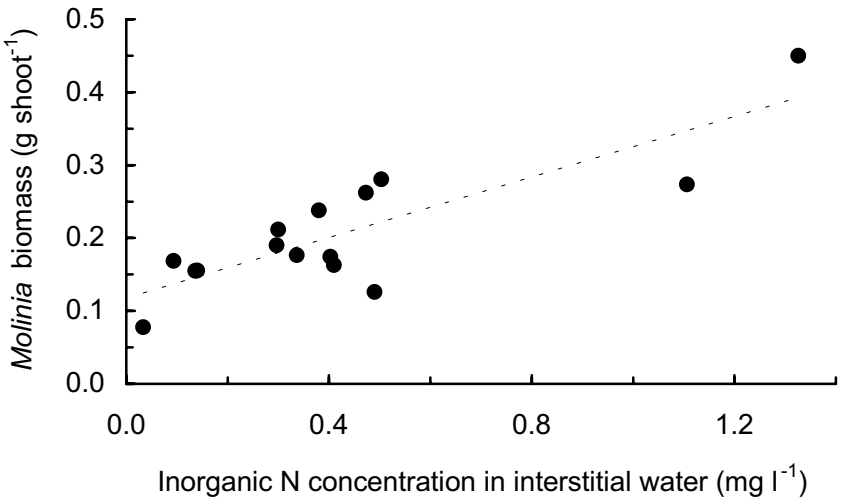

Figure 3.3 The relationship between *Molinia* shoot biomass in 2000 and the average inorganic N concentration in interstitial water at 10-15 cm depth at 3 sampling dates in the growing season of the previous year. Correlation: $P \leq 0.01$, $R^2 = 0.44$ (Spearman's rho).

Sphagnum

The cumulative height increment of *Sphagnum* over the experimental period was depressed by adding N in surplus of field background deposition ($P \leq 0.01$, $n = 15$, RM-ANOVA) and amounted to 6 cm for the 0 and 40 kg N treatments and 4 cm for the 80 kg N treatment (Figure 3.4). The N effect on *Sphagnum* production was less clear, due to considerable variability in capitulum and stem dry mass (data not shown), but it was still significant ($P \leq 0.05$, $n = 10$, 1-way ANOVA). The height increment of *Sphagnum* showed a quadratic relationship with light interception by the canopy (Figure 3.5). An interception of *c*. 53% was the threshold above which height increment decreased; production remained unaffected.

N concentration in *Sphagnum* differed between all three N treatments (Table 3.4), resulting in distinctive C:N quotients. Values for the stem fractions 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 quotient was significantly lower than the C:N quotient 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 3.4) and was higher in the capitulum fraction than in the stem fractions (fraction effect: $P \leq 0.001$, $n = 30$, 2-way ANOVA). The mean N:P quotients 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 and Meuleman 1996).

Table 3.3 The effect of N deposition on the N and P concentrations and the N:P quotient of vascular plant shoots (means ± 1SE). Different letters indicate significant differences between N treatments, ns = $P > 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ (1-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 ± 0.62^{a}	18.64 ± 0.98^{b}	23.08 ± 0.92^{c}	***
P mg g $^{-1}$	15	1.25 ± 0.09	1.23 ± 0.05	1.11 ± 0.05	ns
N:P quotient	15	12 ± 1^{a}	15 ± 1^{b}	21 ± 1^{c}	***
Erica					
N mg g $^{-1}$	15	7.47 ± 0.50^{a}	9.78 ± 0.44^{b}	13.51 ± 0.52^{c}	***
P mg g $^{-1}$	15	0.27 ± 0.03	0.28 ± 0.02	0.21 ± 0.01	ns
N:P quotient	15	31 ± 3^{a}	37 ± 3^{a}	66 ± 5^{b}	***
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 quotient	15	28 ± 5	25 ± 2	25 ± 1	ns
Molinia					
N mg g $^{-1}$	5	8.04 ± 0.24^{a}	10.50 ± 0.74^{ab}	12.99 ±1.14^{b}	**
P mg g $^{-1}$	5	0.17 ± 0.01	0.16 ± 0.01	0.15 ± 0.02	ns
N:P quotient	5	45 ± 6^{a}	64 ± 5^{ab}	95 ± 3^{b}	**
Rhynchospora					
N mg g $^{-1}$	15	8.20 ± 0.24^{a}	9.68 ± 0.38^{b}	10.18 ± 0.37^{b}	***
P mg g $^{-1}$	15	0.56 ± 0.03^{a}	0.51 ± 0.02^{a}	0.41 ± 0.03^{b}	**
N:P quotient	15	15 ± 1^{a}	19 ± 1^{a}	27 ± 2^{b}	***
Vaccinium					
N mg g $^{-1}$	15	9.40 ± 0.32^{a}	10.38 ± 0.29^{a}	12.62 ± 0.41^{b}	***
P mg g $^{-1}$	15	0.50 ± 0.03	0.43 ± 0.02	0.49 ± 0.04	ns
N:P quotient	15	19 ± 1^{a}	24 ±1^{b}	27 ± 1^{b}	***

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 (Figures 1A and 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 3.2). *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 3.3). This observation, coupled with the high N:P quotient in most vascular plant species and *Sphagnum* (Tables 3.3 and 3.4), suggests that those species that did not expand their biomass after adding N were limited by P (Verhoeven and Schmitz 1991, Koerselman and Meuleman 1996).

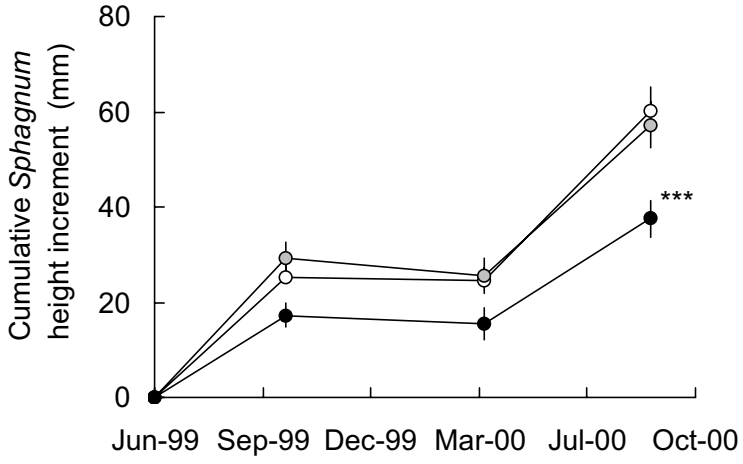

Figure 3.4 The effect of N deposition on the cumulative height increment of *Sphagnum magellanicum* (means ± 1SE, *n* =15). Open circles, 0 kg N ha $^{-1}$ yr $^{-1}$; Closed grey, 40 kg N ha $^{-1}$ yr $^{-1}$; closed black, 80 kg N ha $^{-1}$ yr $^{-1}$.
N effect: $P \leq 0.001$ (RM-ANOVA).

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 (Figure 3.4) cannot be wholly explained by increased shading (Figure 3.5): only less than half the data points indicating a combination of dense shade and short height increment coincide with the 80 kg N treatment (Figure 3.5). 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 (Press *et al.* 1986, Gunnarsson and Rydin 2000, chapter 5). The hump shaped relationship we found between shading and height increment (Figure 3.5) 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. The above suggests a decreased production, as has been observed by Hayward and Clymo (1983).

Table 3.4 Effect of N deposition on the N and P concentrations and the C:N and N:P quotients of *Sphagnum* (means ± 1SE, n = 10). Different letters indicate significant differences between N treatments, ns = $P > 0.05$, *** $P \leq 0.001$ (1-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 ± 0.15^{a}	13.19 ± 0.41^{b}	16.33 ± 0.49^{c}	***
P mg g $^{-1}$	0.41 ± 0.02	0.43 ± 0.02	0.45 ± 0.04	ns
C:N quotient	48 ± 1^{a}	34 ± 1^{b}	28 ± 1^{c}	***
N:P quotient	23 ± 1^{a}	31 ± 1^{b}	38 ± 3^{c}	***
1-2 cm stem				
N mg g $^{-1}$	8.32 ± 0.22^{a}	13.03 ± 0.16^{b}	17.86 ± 0.60^{c}	***
P mg g $^{-1}$	0.28 ± 0.02	0.26 ± 0.01	0.31 ± 0.03	ns
C:N quotient	56 ± 1^{a}	34 ± 1^{b}	28 ± 3^{b}	***
N:P quotient	31 ± 1^{a}	50 ± 2^{b}	62 ± 6^{b}	***
2-3 cm stem				
N mg g $^{-1}$	7.85 ± 0.30^{a}	12.82 ± 0.28^{b}	17.75 ± 0.67^{c}	***
P mg g $^{-1}$	0.23 ± 0.02	0.25 ± 0.01	0.27 ± 0.02	ns
C:N quotient	58 ± 2^{a}	36 ± 1^{b}	26 ± 1^{c}	***
N:P quotient	35 ± 2^{a}	52 ± 3^{b}	70 ± 6^{c}	***

There are some further discrepancies between our study and the conceptual model proposed by Lamers *et al.* (2000). In our study (Table 3.4), 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 and 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 N g $^{-1}$. For *S. magellanicum* the maximum value recorded was 24.3 (± 0.06) mg N g $^{-1}$ 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 to 3 years. Experimentally fertilised systems undergo forced rapid change, whereas peat bogs have been subject to at least 10 years 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 much differently under experimentally fertilised conditions.

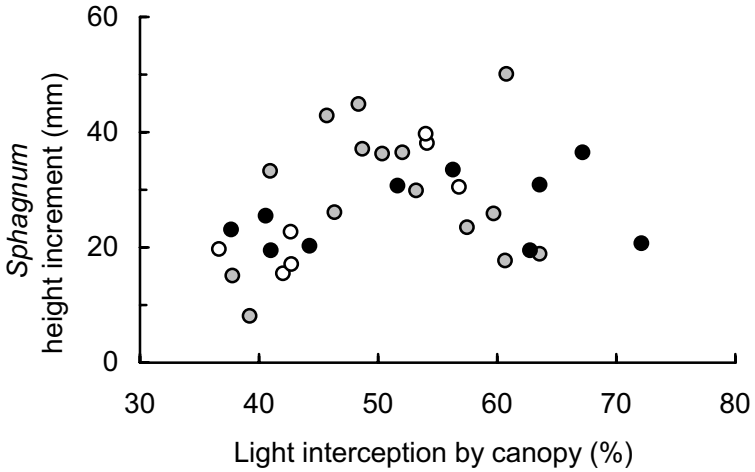

Figure 3.5 The relationship between shading and height increment of *Sphagnum* in 2000. Open circles: 0 kg N ha^{-1} yr^{-1}, closed grey 40 kg N ha^{-1} yr^{-1}, closed black: 80 kg N ha^{-1} yr^{-1}. Quadratic regression: R^2 = 0.31, *P* = 0.005. Mesocosms with unevenly clumped distribution of vascular plants have been omitted.

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 (Figures 3.1A and B) and the increased concentration of N in vascular plants (Table 3.3). It seems more likely that, as tissue N concentration of *Sphagnum* increases, its capability to absorb N declines, as shown by Woodin and 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 level and the occurrence of extreme climatic conditions (Hayward and Clymo 1983, Malmer 1988, Takagi *et al.* 1999, Aerts *et al.* 1992 and 2001, 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 between phase 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 (Figures 3.2 and 3.3, Table 3.1). 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 co-determine 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* did profit from adding N (Figure 3.2) and took up a significant part of the N available in the interstitial water (Figure 3.1B). At the beginning of the following year, the amount of N taken up in the previous year was used to form new leaves (Thornton and Millard 1993). Between May and June 2000, the presence of *Molinia* no longer suppressed N availability in the interstitial water (Figure 3.1B). 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 3.2). The extremely high N:P quotient of *Molinia* (Table 3.3) indicates limitation by P (Verhoeven and Schmitz 1991, Koerselman and 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 and 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 and 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 the interstitial water (Figures 3.1A and B) and the N tissue concentrations of both vascular plants and *Sphagnum* (Tables 3.3 and 3.4) in the 0 kg N treatment. In addition, the poor performance of *Betula* and *Molinia* in this treatment (Figure 3.2, Table 3.1) shows that when N availability decreases, survival of invasive species becomes challenged. These findings combined with the vigorous *Sphagnum* growth (6 cm in two years, Figure 3.4), 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 and Yudina (1999), who mention a similar reversibility of fertilisation effects. They observed that the vegetation had almost completely recovered 18 years after a three-year 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.

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Chapter 4

The interaction between epiphytic algae, a parasitic fungus and *Sphagnum* as affected by N and P

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Abstract

Changes in species interactions may affect ecosystem stability. These changes may be driven by anthropogenic influences such as enhanced N deposition. We report the effects of fertilisation with N and P on the infection of *Sphagnum* by its fungal parasite *Lyophyllum palustre,* the expansion of epiphytic algae and the interaction between the latter two from 1998 to 2001. We added 40 kg N ha^{-1} yr^{-1} or 3 kg P ha^{-1} yr^{-1} in a full factorial design at 4 field sites. In a greenhouse experiment we reinoculated *Sphagnum* to verify the identity of the fungus and its necrotic effect on *Sphagnum*. *L. palustre* was responsible for the necrosis of *Sphagnum* in our experiments. Adding N induced complete necrosis of *Sphagnum cuspidatum* by *L. palustre*, whereas adding P decreased the area of necrotic tissue. Disease severity was related to the N concentration in the *Sphagnum* capitula. In *Sphagnum magellanicum* and *S. papillosum*, infection with *L. palustre* resulted in defoliation of stem sections. At all sites, N addition stimulated the expansion of algae, reducing the volume of photosynthetic tissue in *Sphagnum.* The density of the film of algae in the treatments receiving N was a function of the frequency of defoliated *Sphagnum* stems. We conclude that infection with parasitic fungi and, in humid microhabitats, the expansion of epiphytic algae, may produce stochastic effects that aggravate the impact of increased N deposition or of other ecosystem disturbances that affect nutrient availability.

Introduction

Many ecosystems, including raised bogs, have evolved under low nitrogen (N) inputs and as a result, both species composition and ecosystem processes are tuned to these nutrient-poor conditions (e.g. Van Breemen 1995, Chapin *et al.* 1997). When the system is disturbed, for example by increased N deposition from anthropogenic sources, the competitive balance between species is altered and previously subordinate species may become invasive (Heil and Diemont 1983, Bobbink and Willems 1987, Lee 1998, Turkington *et al.* 1998, Nordin *et al.* 2002). The subsequent changes in competition between species and eventual species replacement have usually been attributed to interspecies differences in the ability to acquire and use different nutrient sources, potential growth rate and rates of nutrient loss (Berendse and Aerts 1987, Aerts and Berendse 1988, Berendse and Elberse 1990, Aerts and Chapin 2000).

Not all the changes in the community assemblage can be wholly explained by interspecific competition, however. Changes in multitrophic interactions, such as plant–insect (Brunsting and Heil 1985, Berdowski and Zeilinga 1987, Aerts *et al.* 1990), plant–fungus (Packer and Clay 2000, Strengbom *et al.* 2002) and plant–algae (Wear *et al.* 1999, Fong *et al.* 2000) associations, may contribute to the observed shifts in species composition. N-induced changes in the nutrient concentration and palatability of the plants play a major role in the bitrophic interactions mentioned above. These changes may involve an increase in the N tissue concentration and subsequent leaching of easily degradable N compounds (Ohlson *et al.* 1995, Flückiger and Braun 1998, Peveling *et al.* 2002) and possibly, a decrease in the concentration of secondary metabolites (e.g. Balsberg-Påhlsson 1992, Hättenschwiler and Vitousek 2000).

In this study we focus on interactions of the plant–fungus and plant–algae type in raised bogs. Raised bogs are unique in that the bryophyte component, mainly consisting of *Sphagnum* mosses, is crucial in the maintenance of the whole system. *Sphagnum* is an ecosystem engineer that shapes its own environment, making it acidic, wet and nutrient-poor (Malmer 1994, Van Breemen 1995). Thus, any disturbance that seriously interferes with *Sphagnum* growth is a threat to the survival of the bog system. Heavy N deposition poses such a threat, as it depresses *Sphagnum* growth either directly through toxic effects or indirectly by increased shading from the N-induced growth of vascular plants (e.g. Gunnarsson and Rydin 2000, Heijmans *et al.* 2000, Van der Heijden *et al.* 2000, Berendse *et al.* 2001).

We expected that in addition to the effects mentioned above, increased fungal infection and algal cover would aggravate the detrimental effects of increased N deposition on *Sphagnum* dominated vegetation. *Lyophyllum palustre* (Peck) Singer, also indicated with *Tephrocybe palustris* (Peck) Donk (Arnolds *et al.* 1995), is a fungal species (Figure 4.1A) fairly common in bogs in the northern hemisphere and known to cause necrosis (Figure 4.1B) in *Sphagnum*

(Redhead 1979, Untiedt and Müller 1984). The species belongs to the chemo-ecological group of 'ammonia fungi', which means that amending the substrate with ammonium or amino acids stimulates growth of mycelia and subsequent fructification (Sagara 1975, Yamanaka 2001). As high N deposition may enhance both the total N concentration in *Sphagnum* tissue and the concentration of free amino acids (e.g. Baxter *et al.* 1992, Jauhiainen *et al.* 1998, Tomassen *et al.* 2000), we hypothesised that (I) growth of *Lyophyllum palustre*, and thus area of necrosis, would increase with N deposition and concomitant tissue N concentration. Epiphytic algae are known to profit from enhanced nutrient availability in aquatic or humid environments and have the potential to seriously reduce the photosynthetic capacity and, ultimately, the growth of the plant acting as substrate (Wear *et al.* 1999, Fong *et al.* 2000, Peveling 2002). As bogs contain a wealth of humid microhabitats, and thus offer suitable conditions for the growth of algae (Hooper 1981), we expected (II) algae to expand with increased N deposition, reducing the photosynthetic capacity of *Sphagnum*. To test these two hypotheses, we established an experiment in which N and P were applied in a full factorial design at four field sites. In an additional greenhouse experiment we reinoculated *Sphagnum* to verify that the necrosis we found was indeed caused by *L. palustre* and was related to tissue N content.

Photographing the patches of dead *Sphagnum*

Methods

Site description

The experiment was carried out at three sites in the Netherlands and one site in Ireland, all situated in extremely poor fens or raised bogs. Although not all the sites had a continuous cover of *Sphagnum*, the individual plots were selected in areas with 100% *Sphagnum* cover. Two of the Dutch sites were less than 200 m apart and were situated in the Bargerveen, which is a reserve in the north-east of the Netherlands (52°42'N, 7°03'E). One site, Bargerveen-Sp was chosen in an area dominated by *Sphagnum papillosum* (Lindb.), the other site, Bargerveen-Sc, was on a floating raft of *Sphagnum cuspidatum* (Hoffm.). Our experimental site at Reigersplas (52°50'N, 6°27'E) was dominated by *Sphagnum magellanicum* (Brid.) and *S. papillosum*. Adjacent to this location we collected the *S. magellanicum* used in the greenhouse experiment. Clara bog (53°20'N, 7°36'E) in Ireland was chosen as a reference for our Dutch sites with regard to N deposition; deposition in the Irish Midlands is approximately half that in the north-eastern Netherlands (chapter 2). At this site, *S. papillosum, S. magellanicum* and *Sphagnum rubellum* (Wils.) were most abundant.

Field experiment

The field experiment was set up at the Dutch and Irish sites in May and July 1998, respectively. At each site, 20 plots, measuring 1 x 1 m, were laid out in 5 replicated blocks. Treatments were randomly assigned to the plots, and consisted of a control treatment that received demineralised water only, an N treatment (40 kg N ha^{-1} yr^{-1}), a P treatment (3 kg P ha^{-1} yr^{-1}), and a combination of the latter two. The nutrients, NH_4NO_3 and $NaH_2PO_4.2H_2O$, were dissolved in 2 litres demineralised water for the Dutch sites and in sieved bog water for the Irish site. The extra N and P the Irish plots received through the sieved bog water was negligible; the amount of N was equivalent to 0.5% of the N in the local bulk deposition, whereas P remained below the detection limit of 0.01 g l^{-1} (chapter 2). The nutrients were applied by watering, using a separate watering can for each treatment. The fertilisation treatments commenced on June 12th in the Dutch sites and on July 13th in the Irish site. Nutrients were added 6 times a year between March and September. We were careful to add the nutrients immediately prior to, during, or just after rainy weather. Because of the late start of the experiment, only half of the yearly doses were applied in 1998. The last treatments were given to all sites in July 2001. The Dutch sites were harvested in the second week of August 2001, the Irish site two weeks later.

Sphagnum growth, expressed as height increment, was measured using a variation of the cranked wire method (Clymo 1970). Four plastic rods per plot were inserted to a depth of *c*. 8 cm, and anchored by plastic broom bristles. When *Sphagnum* threatened to overgrow a rod, a new one was attached to the old one by fitting a piece of plastic tube over both rod ends. The length of the rod extending above the moss surface was measured twice a year, in March–April and September–November. The rods had a diameter of 1.5 mm and did not seem to interfere with the growth of the surrounding *Sphagnum* plants.

Necrosis was visible as discolored patches of dead *Sphagnum* (Figure 4.1B) and was measured by photographing each plot at regular intervals between November 2000 and August 2001. As the conditions at the site did not allow vertical photographs to be taken, we included a frame of known dimensions in our pictures. The pictures were digitised and the areas of the discolored patches and of the frame were calculated with the graphic program Image Pro Plus (2.0). The calculated area of the latter was used to correct the area of necrosis for the oblique angle.

At the end of the experiment, columns 10 cm in diameter and 10 cm deep, were cut around a cranked wire with a sharp knife. The number of columns cut per plot depended on the variation in the *Sphagnum* present. If the moss layer consisted of a homogeneous sward of one species identical in morphology, only one column was cut. If the condition, the morphology or the dominant species differed, up to three columns were cut to catch the variation. In the latter case, data on each column were treated as a separate measurement, but were corrected for in the statistical analyses by including the number of columns per plot as a co-variable. Per day, only one site was harvested. The columns were stored at 1 ^{o}C for no longer than three weeks. Algal cover and signs of previous infection by *L. palustre* were assessed after the *Sphagnum* had been removed from the columns. For both parameters we predefined 6 categories to describe the degree to which *Sphagnum* was either algae-covered or defoliated by *L. palustre* (Table 4.1). We assessed the frequency of defoliated stem parts in living green or red tissue as well as in 'dead' brown tissue. For Bargerveen-Sc we did not find defoliated stems; the current year's tissue was either white and dead, or uninfected and, as is usual for *S. cuspidatum*, all the older tissue had already disintegrated. In addition to quantifying algae and *L. palustre*, we measured the length of the coloured part of *c*. 50 *Sphagnum* individuals in each column. For *S. papillosum* this was the part of the stem that still had a green tinge, for *S. magellanicum* it was the part still tinged either green or red. The length of coloured tissue was used as an indication of the volume of photosynthetically active tissue. Capitula (0–1 cm) were cut off to analyse tissue nutrient contents.

The *Sphagnum* capitula were oven dried at 70^{o} C for 48 hrs before the material was pulverised with a ball mill. Subsequently, C and N were measured with an elemental analyser (Fisions Instruments EA 1108, Milan Italy) and used to calculate the N:C quotient of the capitula. In addition, a part of the dry milled samples were digested with sulphuric acid, salicylic acid, hydrogen peroxide and selenium. In these acidic extracts the N and P

concentrations were measured colorimetrically, using a continuous flow analyser (SKALAR SAN plus system, the Netherlands). These values were used to obtain N:P quotients.

Table 4.1. Overview of categories used to describe the frequency of defoliated stem sections (signs of previous infection) and the algal cover at the field sites.

Category	Infection frequency	Cover of algae
0	no signs	no signs
1	< 10 % of individuals in column had at least 1 cm defoliated stem	< 10 % of individuals in column visually covered with algae
2	10 to 25 % of individuals had at least 1 cm defoliated stem	10 to 25 % of individuals visually covered
3	25 to 50 % of individuals had at least 1 cm defoliated stem	25 to 50 % of individuals visually covered
4	50 to 100 % of individuals had at least 1 cm defoliated stem	50 to 100 % of individuals visually covered
5	all individual had a piece of defoliated stem of 1 cm or more	all individuals completely covered with thick layer of algae

Greenhouse experiment

In March 2000, *S. magellanicum* was collected from containers used in a previous experiment, where it had been subjected to three N deposition loads (0, 40 and 80 kg N ha^{-1} yr^{-1}) for two years. These treatments resulted in the concentrations of free amino acid N in the *Sphagnum* differing between the 0 kg N treatment and the 40 and 80 kg N treatments. Total N concentration differed between all three treatments, with the highest concentrations found in the 80 kg N treatment (chapters 3 and 5). Mycelium of *Lyophyllum palustre* (= *Tephrocybe palustris* L.) was isolated from *Sphagnum* showing signs of necrosis and transferred to Petri plates filled with Leonian's medium amended with tetracycline (Redhead 1979). Molecular techniques were used to verify the identity of the mycelium (see next caption).

When the hyphal front of the sterile cultures had nearly reached the edges of the Petri plates, we suspended the agar containing the non-sporulating mycelia in demineralised water and used it for inoculation. We inoculated twice, with two weeks in between, to make sure that enough inoculum was present to induce infection. The *Sphagnum* individuals were cut to 8 cm length and placed in groups of 35 in plastic tubes of 3.5 cm diameter. Each tube was placed in a separate container in such a way that water could move between the outer container and the inner tube. The water level in the outer containers was maintained at 5 cm below the capitula using an artificial rainwater solution (Garrels and Christ 1965). The fertilisation treatments were started after an acclimatisation period of three days. In effect, the *Sphagnum* received the same treatments as previously, the only difference being that we included one additional treatment of 3 kg P ha^{-1} yr^{-1} plus 80 kg N ha^{-1} yr^{-1}. We did so

because we assumed that as *Sphagnum* seemed to be limited by P, adding P would reduce the N tissue concentration, thus making the moss less attractive to *L. palustre*. The nutrients were dripped onto the capitula with a pipette once a week. Shortly after the fertilisation treatments had commenced, we inoculated half of the containers with a suspension of *L. palustre* mycelia, again using a pipette. A factorial block design was used, with 10 replicates for each treatment. The experiment was conducted in a climate-controlled greenhouse in which the day temperature could be kept below 25 ^{o}C and air humidity around 90%. After 16 weeks the experiment was harvested in July 2001.

At final harvest, the *Sphagnum* plants and capitula were counted and length was measured. To assess disease incidence we counted the number of *Sphagnum* individuals showing signs of infection, and to assess disease severity, we recorded the number of defoliated branches per container. In addition, we measured the total length of defoliated stems per container. The moss material was oven dried at 70^{o} C for 48 hrs before dry mass was determined; the infected *Sphagnum* individuals were set aside.

Molecular identification fungal mycelium

Molecular analysis was applied on mycelium obtained from *Sphagnum* showing signs of necrosis to confirm that infection was caused by *Lyophyllum palustre.* DNA was extracted from the fungal hyphae by the CTAB-method. Subsequently, polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) were applied (Gardes and Bruns 1996). The fungal hyphae were identified by utilising sequence differences in the internal transcribed spacer (ITS) of the nuclear rRNA repeat using the basidiomycete specific primer pair ITS-1f and ITS-4B (Gardes and Bruns 1996). The fungal ITS-RFLP patterns were produced using the restriction enzymes AluI, HinfI and MboI (MBI-Fermentas). The obtained RFLP-patterns, that is the pattern of bands on the gel, of the mycelium were compared to those from collected sporocarps of *L. palustre* to see if they were identical, thus proving that hyphae and sporocarps belonged to the same species (Baar *et al.* 1999).

Data analysis

To test our hypothesis that necrosis of *Sphagnum* would be affected by N and P fertilisation, we used a 2-way repeated measures analysis (RM-ANOVA) on repeated measurements of the necrotic area of *Sphagnum.* As the effects of N and P did not interact, we treated the N&P treatment as the result of separate N and P effects, and thus used N and P fertilisation as independent (fixed) factors. As the data did not meet the requirement of homosphericity (identified by Mauchly's test of sphericity), degrees of freedom were adjusted by the

Huynh–Feldt epsilon (Potvin *et al*. 1990). The area of dead *Sphagnum* was transformed logarithmically to achieve homogeneous variances.

Whether algal cover affected the length of coloured *Sphagnum* was tested with a 2-way ANCOVA, with treatment and site as fixed factors, the number of columns per plot as a random factor nested within treatment, and algal cover as a co-variable. *Sphagnum* columns with a dense cover of algae usually had a high frequency of defoliated stem parts. To investigate the effect of defoliated stem frequency on the algal cover we used an ANCOVA with treatment and site as fixed factors, the number of columns per plot as a random factor nested within treatment, and frequency of defoliated stems as a co-variable. The distribution of the data on algae over the treatments over the sites (Figure 4.4), was such that in order to obtain a balanced design in which we could focus on the effects of the defoliated stem parts we had to use data from the N and N&P treatments only.

For correlation analysis we used Pearson's test when data were distributed equally along the x-axis and Spearman correlation when this was not the case. All statistical analyses were performed using SPSS for Windows (10.0).

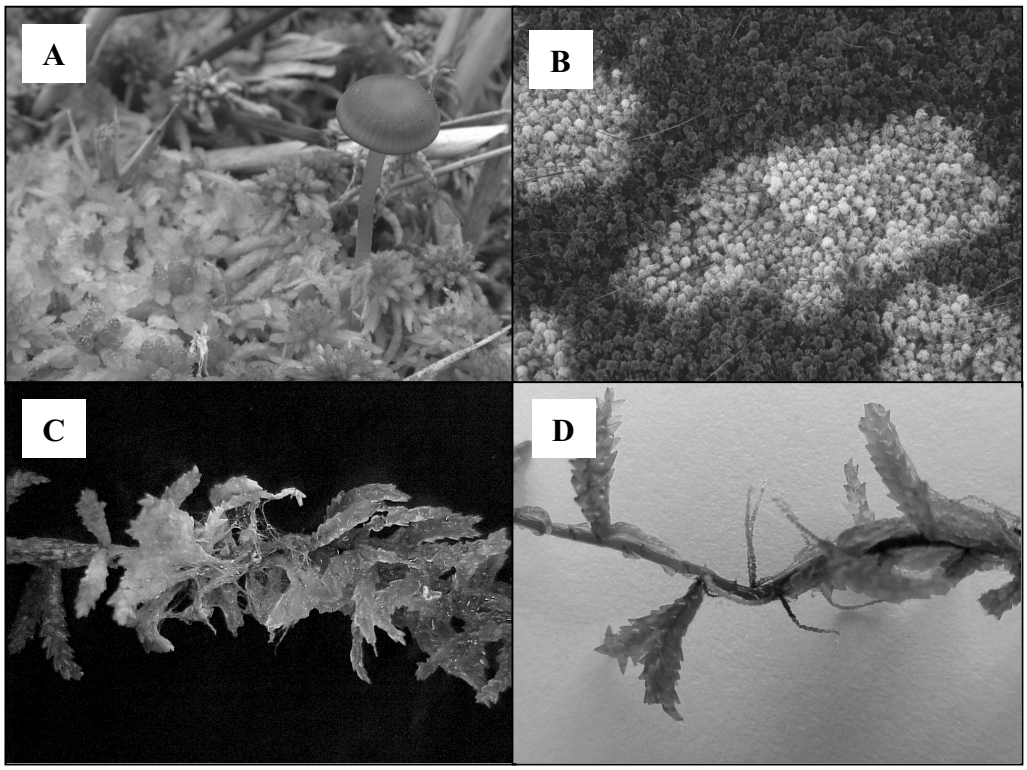

Figure 4.1. **A** Fruiting body of *Lyophyllum palustre,* **B** necrotic *Sphagnum cuspidatum*, **C** early infection around stem of *S. papillosum*, **D** defoliated stem part of *S. papillosum*, also referred to as sign of previous infection.

Results

Fungal effects on *Sphagnum*

In the greenhouse experiment, the first signs of infection were found one month after inoculation. In some of the inoculated containers a few hyphae became visible, typically 1-3 cm below the capitula. Over time, the visible mycelium multiplied and the stem of the infected *Sphagnum* plants turned yellowish-white (Figure 4.1C). In most of the infected containers, the infection did not progress and the mycelia seemed to disappear, leaving a defoliated dead section of stem (Figure 4.1D) which will be referred to as the sign of previous infection. At the end of the experiment, 82.5% of the inoculated containers and 5% of the uninoculated containers showed signs of infection, indicating that the mycelium we used for inoculation was responsible for the infection observed. Moreover, molecular analysis showed that the RFLP-patterns of the fungal mycelium (Table 4.2) were identical to those of sporocarps of *Lyophyllum palustre*. This confirmed that the fungal hyphae we isolated from *Sphagnum* showing signs of necrosis belonged to *L. palustre*.

Table 4.2. The obtained RFLP-patterns of *Lyophyllum palustre* in the internal transcribed spacer (ITS) of the nuclear rRNA repeat, using the basidiomycete specific primer pair ITS-1f and ITS-4B.

Restriction enzymes	Position bands on gel		
AluI	336	230	180
HinfI	388	335	135
MboI	325	260	205

The intensity of infection did not affect the increment of *Sphagnum* length, but it did affect biomass positively (data not shown). Infected individuals had more stem biomass per unit of length as well as more capitulum biomass compared to uninfected individuals from the same container ($t = 2.54$, $P = 0.016$ for the stem and $t = 5.4$, $P < 0.001$ for the capitulum: paired t-test). This unexpected result was probably the consequence of increased fungal biomass associated with infected *Sphagnum* tissue; the difference in biomass between infected and uninfected individuals from the same container was linearly related to the total length of infection ($R^2 = 0.51$, $P < 0.001$: linear regression) and the number of defoliated branches per container ($R^2 = 0.30$, $P < 0.001$: linear regression). Surprisingly, we found no effect of the fertilisation treatments on *Sphagnum* length increment, *Sphagnum* biomass, the total length of dead stems or the number of defoliated branches (data not shown).

In the field experiment, adding N or P did not affect the frequency of signs of previous infection. Necrosis, however, was influenced by the fertilisation treatments. Only at the Bargerveen-Sc site the necrotic spots became frequent and large enough to be measured by our methods. Thus, all measurements of necrosis are from this site. At the other three sites, necrosis was too infrequent to be used for analyses, although when necrotic spots did occur, they were restricted to the plots receiving N alone. In Bargerveen-Sc,

adding N increased the susceptibility of *Sphagnum* to infection; in the plots receiving N alone, most of the *Sphagnum* had been killed by August 2001 (Figure 4.2, Table 4.3). When seemingly uninfected *Sphagnum* tried to recolonise the dead areas from the edges, it soon became necrotic. Adding P counteracted the effects of N; there were hardly any necrotic spots (Figure 4.2, Table 4.3), and when they did occur, *Sphagnum* quickly recolonised the bare patches. The extent of necrosis in August 2001 was positively related to the N concentration in the capitulum at final harvest and to the N:C quotient (Figure 4.3A) and N:P quotient of the capitula (Figure 4.3B). When we compared the nutrient quotients of the capitula in the control plots from the different sites, we found that the N:C quotient of *Sphagnum* from Bargerveen-Sc was similar to that of Clara bog and its N:P quotient to that of Clara bog and Reigersplas (data not shown).

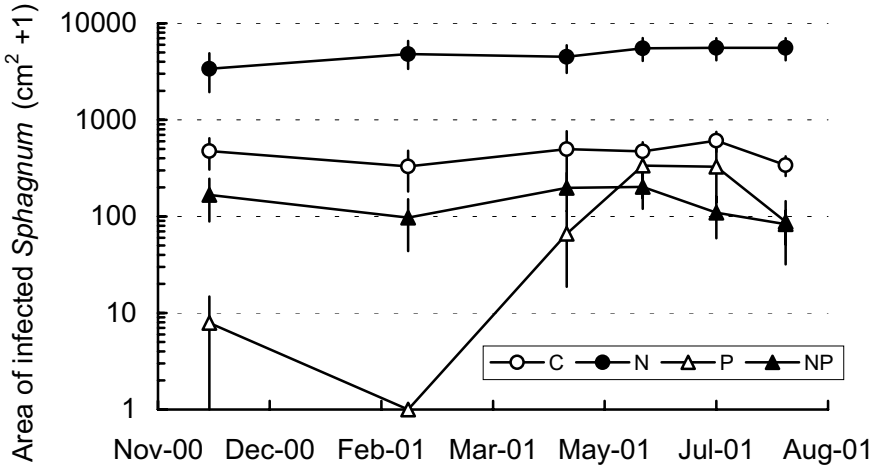

Figure 4.2. The effect of fertilisation with N and P on the area and development of necrosis by *Lyophyllum palustre* in Bargerveen-Sc in $1m^2$ plots (means ± 1SE). C = control, N = 40 kg N ha^{-1} yr^{-1}, P = 3 kg P ha^{-1} yr^{-1}. For statistics see Table 4.3.

Algal effects on *Sphagnum* and interaction with fungus

In the course of 2000, we observed patchwise colonisation and subsequent expansion of epiphytic algae at all sites in the plots receiving N. One year later, algae were more equally distributed in most of the plots that received N alone. Adding N clearly stimulated the growth of algae, whereas adding P hampered that growth (Figure 4.4). The expansion of algae affected potential photosynthesis, expressed as the length of *Sphagnum* still coloured; that length decreased once 25% or more of the individuals in a column had become covered with algae (Figure 4.5). The length of coloured *Sphagnum* also differed between the sites; *Sphagnum* from Clara Bog had a shorter coloured length than the same species from Reigersplas and Bargerveen-Sp. The height increment of *Sphagnum* was not affected by the presence of

algae, although the fertilisation treatments did have a significant effect. Adding N depressed *Sphagnum* growth, whereas adding P either stimulated growth or had no effect (chapter 2).

The frequency of defoliated stem parts largely defined the algal cover in the plots receiving either N or N&P at Clara bog, Reigersplas and Bargerveen-Sp; the higher the frequency of defoliated stem parts, the more extensive the algal cover (Figure 4.6). This effect was diminished by adding P together with N. Presence of defoliated stem parts had no effect on the algal cover in the C and P treatments.

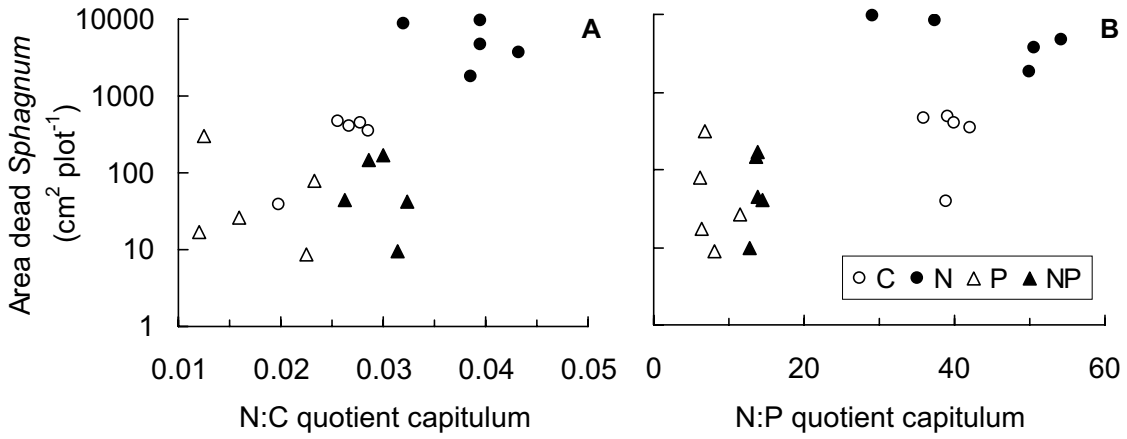

Figure 4.3. Relationship between the **A,** N:C quotient and the **B,** N:P quotient of the capitulum at final harvest and the area of dead *Sphagnum* in August 2001 in Bargerveen-Sc. C = control, N = 40 kg N $^{-1}$ yr $^{-1}$, P = 3 kg P ha $^{-1}$ yr $^{-1}$. Correlation coefficient N:C = 0.55, *P* = 0.40 (Pearson); Correlation coefficient N:P = 0.65, *P* = 0.011 (Spearman's rho).

Source	d.f.	MS	*F*	*P*
Between subjects				
N	1	110.792	392.225	0.002
P	1	441.803	13.736	0.000
N*P	1	6.764	54.774	0.373
Error	16	8.066	0.839	
Within subjects				
Time	2.172	13.957	4.027	0.024
Time*N	2.172	7.527	2.172	0.125
Time*P	2.172	6.753	1.948	0.155
Time*N*P	2.172	9.306	2.685	0.078
Error	34.750	3.466		

Table 4.3. Within and between subject effects of 2-way RM-ANOVA to test the effect of fertilisation with N and P on necrosis by *L. palustre* in Bargerveen-Sc.

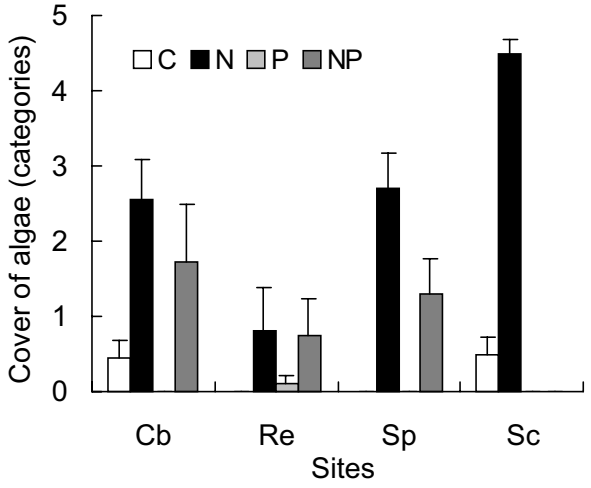

Figure 4.4. Effect of N and P on algal cover (means + 1SE) per site in 2001. C = control, N = 40 kg N ha $^{-1}$ yr $^{-1}$, P = 3 kg P ha $^{-1}$ yr $^{-1}$. Cb = Clara bog, Re = Reigersplas, Sp = Bargerveen-Sp, Sc = Bargerveen-Sc. Treatment effect: x^2 = 60.5, P < 0.001 (KRUSKAL-WALLIS).

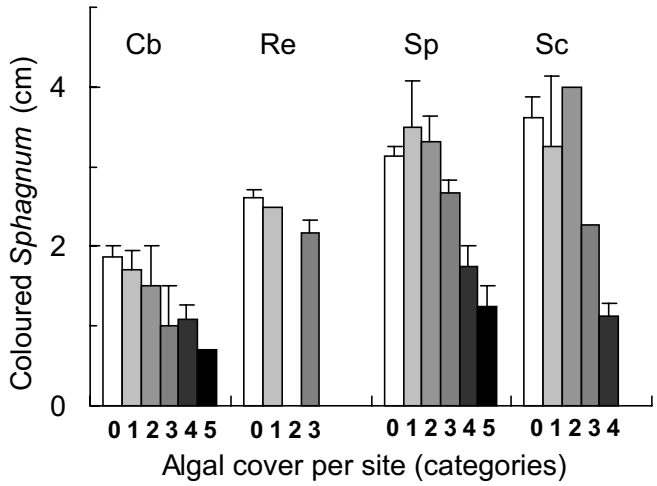

Figure 4.5. Effect of the algal cover on the length of coloured *Sphagnum* (means + 1SE) per site. Cb = Clara bog, Re = Reigersplas, Sp = Bargerveen-Sp, Sc = Bargerveen-Sc. Effect of algae: F = 26.0, P < *0.001*; Site effect: F = 40.3, P < *0.001*; no. columns per plot (treatment) effect: F = 2.5, P = 0.011; n = 5-10 (2-way ANOVA). Only significant effects are shown.

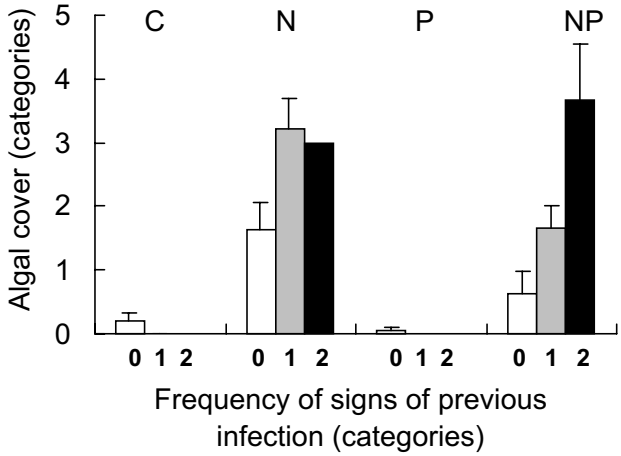

Figure 4.6. Combined effects of treatment and frequency of previous signs of infection on the algal cover (means + 1SE). C = control, N = 40 kg N ha $^{-1}$ yr $^{-1}$, P = 3 kg P ha $^{-1}$ yr $^{-1}$. Frequency: 0 = no signs of infection, 1 = less than 10%, 2 = 10-25% of the *Sphagnum* in a column had at least 1 cm of defoliated stem. For statistics we used data from the N and N&P treatments only. Treatment effect: F = 4.6, P = 0.039; *Lyophyllum palustre* effect: F = 11.9, P = 0.001; n = 5-10 (2-way ANOVA). Only significant effects are shown.

Discussion

Our results from the greenhouse experiment and from the molecular analysis (Table 4.2) show that *L. palustre* is indeed responsible for the necrotic patches observed in *Sphagnum* (Figure 4.1B), although infection does not necessarily result in necrosis. It seems that in most cases the effect of infection is restricted to defoliation of part of the stem (Figure 4.1D). It is still unclear what causes the infection to become fatal. From the extensive necrosis in the plots treated with N (Figure 4.2), we conclude that the severity of infection is connected with the N supply to the *Sphagnum*. The relationship we found between the area of dead *Sphagnum* and both the C:N and N:P quotients (Figures 3A and B) further supports our hypothesis that infection intensity increases with tissue N content. Similar relationships between plants and their fungal parasites have also been described in other studies (Anderson and Dean 1986, Van Dijk *et al.* 1991, Marschner 1995, Flückiger and Braun 1999, Strengbom *et al.* 2002). However, if the severity of infection in our study was only a function of the N concentration in *Sphagnum*, one would expect the *Sphagnum* at Bargerveen-Sc to have a much higher N:C or N:P quotient than at the other 3 sites where necrosis was barely observed; this was not the case. It is possible that *S. cuspidatum* is more susceptible to infection than *S. papillosum* and *S. magellanicum.* Such increased susceptibility might arise from a lower concentration of secondary metabolites (Hättenschwiler and Vitousek 2000) or a lesser ability to develop morphological defence structures (Oulette 1981, Kost 1988, Hassel and Kost 1998).

As expected, algal cover reduces the potential photosynthesis of *Sphagnum* (Figure 4.5). At first glance it may seem strange that this was not reflected in a reduced height increment. However, this discrepancy can be explained, if we take into account both the distributions of algae within a plot and the way in which height increment was averaged over a plot: the 4 cranked wires did not always coincide with a patch of algae.

The expansion of algae was clearly related to an increase in N availability (Figure 4.4). It seems that as *Sphagnum* becomes saturated with N, the N leaks from the cells and becomes available for other organisms such as vascular plants (Lamers *et al.* 2000, Berendse *et al.* 2001) and algae (Rudolph and Voigt 1986, this study). It seems plausible that defoliation of stems and stem mortality (Figures 4.1D and 4.6) interferes with the capacity of *Sphagnum* to sequester N, thus enhancing N availability even more. Such interference may involve removal of essential nutrients, such as P, by the fungus, a reduction in the capacity of *Sphagnum* to actively reallocate nutrients (Rydin and Clymo 1989) or an obstruction of the extracellular flow of water (Clymo and Hayward 1982), resulting in a sub-optimal cell water content (e.g. Titus *et al.* 1983). Our failure to find a relationship between *Sphagnum* growth and total length of infected stem in the greenhouse

experiment, might have been a result of the experiment being too short and the water supply being optimal.

To our knowledge, this is the first time that an interaction has been reported between a bryophyte, a parasitic fungus and epiphytic algae in the field (Figure 4.6). This finding shows that the mechanisms behind the effect of N deposition on ecosystem processes may be complex and extend over trophic levels. On a large scale, effects may seem identical in their outcome (e.g. loss of biodiversity, species replacement), but the mechanisms bringing about these effects may differ from site to site. Infection with parasites and, in humid environments, the expansion of epiphytic algae, may account for stochastic effects that aggravate the impact of increased N deposition by generating positive feedback loops, making nutrients even more available. In nutrient-poor ecosystems this could create further opportunities for invasive species to establish and expand.

Acknowledgements

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Chapter 5

Growth reduction of *Sphagnum magellanicum* subjected to high N deposition: the role of amino acid N concentration

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Abstract

We tested the relationship between *Sphagnum* growth and the amount of N stored in free amino acids in a fertilisation experiment with intact peat monoliths in an open greenhouse. Three N deposition scenarios were used: no N deposition, field conditions and a doubling of the latter, corresponding with 0, 40 and 80 kg N ha^{-1} yr^{-1}. Growth of *Sphagnum* was reduced in the 80 kg N treatment, but showed no correlation with the total N tissue concentration or with the concentration of individual or pooled free amino acids. The amount of N stored in free amino acids increased concomitantly with deposition, although it lagged more and more behind the total N concentration, pointing to the accumulation of unmeasured N compounds. Asparagine clearly acted as the major storage compound for N in *Sphagnum* stem tissue, whereas arginine fulfilled this function to a lesser extent in the capitulum. It appears that N-induced growth inhibition of *Sphagnum* is related to acclimation rather than to certain threshold concentrations of amino N or total N. We propose that when *Sphagnum* is exposed to a step increase of N, its N metabolism does not adapt fast enough to keep up with the enhanced uptake rate. This imbalance between N uptake and assimilation may lead to an accumulation of toxic NH_4^+ in the cell and a subsequent reduction in growth.

Introduction

In the last ten years, the effect of enhanced N deposition on the growth of *Sphagnum* has received much experimental attention. The effects of N on *Sphagnum* are not consistent in the various studies, although most studies report a negative relationship between height increment and elevated N concentration in the substrate or watering solution. The N effect in these studies varied with the species of *Sphagnum* (Jauhiainen *et al.* 1994, Jauhiainen *et al.* 1999, Gunnarson and Rydin 2000, Nordin and Gunnarsson 2000), water table depth (Twenhöven 1992a, Williams *et al.* 1999), the background deposition at the site of origin (Aerts *et al.* 1992, Gunnarsson and Rydin 2000), the amounts of available P and CO_2 (Aerts *et al.* 1992, Risager 1998, Jauhiainen *et al.* 1994) and the cover of vascular plants (Heijmans *et al.* 2001, Berendse *et al.* 2001, chapters 2 and 3). However, the mechanism behind the observed reduction in growth remains unclear. Increased shading of the moss surface because of N-induced stimulation of vascular plant growth (Lamers *et al.* 2000, Heijmans *et al.* 2001, Berendse *et al.* 2001) seems to account for the main effects on intact ecosystems. Additionally, light competition with unicellular algae residing in the hyaline cells of *Sphagnum* (Rudolph *et al.* 1993) might partly explain growth reduction of *Sphagnum* in the absence of vascular plants. However, some cases still remain where neither vascular plants nor enough algae were present to account for reduced *Sphagnum* growth (e.g. Van der Heijden *et al.* 2000). A feasible hypothesis is that the N-induced inhibition of growth is connected to the uptake and subsequent incorporation of N in *Sphagnum* tissue.

The N uptake in *Sphagnum* largely mirrors the uptake in vascular plants (Rudolph *et al.* 1993, Heeschen *et al.* 1997). NH_4^+ is taken up from the environment and assimilated into glutamine, whereas NO_3^- is reduced to NH_4^+ by N reductase (NR) prior to assimilation. Subsequently, glutamine is converted into other amino acids (Rudolph *et al.* 1993, Kahl *et al.* 1997). Free NH_4^+ in cells is toxic, as it threatens cell homeostasis, and is rapidly assimilated into glutamine (Lea and Miflin 1980). The uptake of NH_4^+ and NO_3^- takes place across the whole moss surface: N deposition is largely taken up by the capitulum and the stem mainly takes up N dissolved in the aquatic environment (Clymo and Hayward 1982, Rudolph and Voigt 1986). When N deposition increases, and N is no longer limiting for growth and maintenance, *Sphagnum* shows luxury consumption. The assimilation of N continues, and free amino acids accumulate (Thönes and Rudolph 1983, Baxter *et al.* 1992, Nordin and Gunnarsson 2000, Tomassen *et al.* 2000). The enlarged pool of amino acids consists mainly of the N-rich free amino acids arginine, asparagine and glutamine that also fulfil an N storage function in most vascular plants (Lea and Miflin 1980, Näsholm *et al.*1994, Calanni *et al.* 1999). Asparagine has been distinguished as the major storage compound of N in *Sphagnum* (Thönes and Rudolph 1983, Rudolph *et al.* 1993, Kahl *et al.* 1997); it is located mainly in living stem tissue. In contrast, arginine was found to accumulate chiefly in the capitulum (Baxter *et al.* 1992, Karisto *et al.* 1996,

Nordin and Gunnarson 2000). Because the assimilation of N-rich free amino acids requires energy and carbon, which then cannot be used for growth, Baxter *et al.* (1992) suggested a negative relationship between the accumulation of free amino acids in *Sphagnum* and *Sphagnum* height growth. Nordin and Gunnarsson (2000) tested this hypothesis in a fertilisation experiment and found that above a concentration of 2 mg amino acid N g^{-1} dry mass in the capitulum *Sphagnum* height growth was reduced. This value corresponded with an N deposition load exceeding 30 kg ha^{-1} yr^{-1}.

Our aim in this study was to investigate the negative relationship between amino acid N concentration and *Sphagnum* growth at high N deposition. Additionally, we wanted to study to what extent *Sphagnum* amino acid concentration was influenced by the N concentration of the surrounding pore water. To this end, a fertilisation experiment was conducted using peat monoliths with intact raised bog vegetation. Three N deposition scenarios were used: no N deposition (0 kg N ha^{-1} yr^{-1}), field conditions (40 kg N ha^{-1} yr^{-1}), and double this amount (80 kg N ha^{-1} yr^{-1}).

Methods

Plant material

In early March 1999, 15 peat cores (diameter 34 cm, 30-35 cm long) were cut from 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 *S. 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. Occasional plants of *S. papillosum* Lind. were also present. Background deposition in this part of the Netherlands is 39 kg N ha^{-1} yr^{-1} (RIVM 1999).

Experimental design

The cores were put into plastic containers, which were kept in an open greenhouse with a transparent plastic roof (light transmission of 80%, LICOR probe) and walls of coarse shade mesh, thus allowing for some air movement. The treatments, consisting of N additions at rates of 0, 40 and 80 kg N ha^{-1} yr^{-1}, were randomly assigned to the containers, which were placed in 2 sunken concrete basins and arranged in 5 replicated blocks. The basins were filled with water in order to keep the temperature within the peat columns as close to natural as possible.

After an acclimatisation period of three months, the fertilisation treatments were started in early June 1999. The water level in the containers was adjusted to 5 cm below the capitula twice a week, using an artificial rainwater solution (Garrels and Christ 1965). Once every two weeks during the growing season between March and October, N was added as dissolved NH_4NO_3. Both rainwater and nutrient solution were added with a watering can. The experiment lasted for approximately 18 months and was harvested in the second week of October 2000.

Measurements

Sphagnum growth

The height increment of *Sphagnum* was measured twice a year by using two metal rods, which 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, the distance between the bar and the *Sphagnum* surface could be measured using a ruler.

At final harvest, *Sphagnum* sods of 5 x 35 cm and 20 cm deep were cut from the *Sphagnum* cores and stored at 1^{o} C, until further analysis. After the capitula had been counted, *Sphagnum* was separated into one capitulum (0-1 cm) and two stem fractions (1-2 and 2-3 cm). In this study, data on both stem fractions have been averaged. The *Sphagnum* samples were oven dried at 70^{o} C for 48 hrs before dry mass was determined.

Soil pore water

In each container a Rhizon soil moisture sampler (Eijkelkamp Agrisearch Equipment, Giesbeek the Netherlands) with a porous length of 5 cm was inserted to a depth of 0-5 cm. Approximately once every three months, soil moisture was sampled through vacuumed syringes, and analysed. We were careful to wait at least one week between N addition and water sampling.

Amino acid extraction and analysis

At final harvest, approximately 20 individuals of *Sphagnum magellanicum* were collected from each replicate container and divided into capitulum and stem fractions. The material was blotted dry and half of it was used to determine the fresh to dry weight ratio. The other half was used for amino acid extraction, for which we adapted the methods from Bieleski and Turner (1966). The plant material was ground in a mortar, using liquid N and a pestle, and subsequently dissolved in an extraction solution containing 70 mM P-buffer (pH = 7), 1 mM DTT and a mixture of water, methanol and chloroform in a volumetric ratio of 3:12:5. Extraction took place through centrifugation at 3000 rpm for 30 minutes at 4^{o} C. The supernatant was decanted and the pellet was twice re-suspended in 2 ml distilled water and re-centrifuged. Subsequently, the supernatant was freeze dried overnight. The residue was re-suspended in 0.5 ml distilled water, briefly centrifuged to obtain a clear sample, and kept on ice.

The amino acid extracts (40 µL samples) were analysed by automated amino acid analysis (Biochrom 2000) using a lithium-based buffer system. For instrument calibration and peak identification we used an amino acid standard solution (SIGMA, NR A-6407) which was augmented with tryptophan, glutamine and norleucine. Percentage recovery was determined by addition of a known amount of a pure preparation of amino acid to each sample, immediately prior to grinding. To assess recovery, 20 µL 2 mM norleucine was used per 100 g dry mass. Nineteen amino acids were detected: lysine, threonine, methionine, isoleucine, tryptophan, phenylalanine, tyrosine, alanine, valine, leucine, proline, glycine, serine, asparagine, aspartic acid, glutamine, glutamic acid, histidine and arginine.

Other chemical analysis

The concentrations of NH_4^+ and NO_3^- in the soil moisture samples (pore water) were analysed colorimetrically with a continuous flow analyser (SKALAR, SAN plus system, the Netherlands). Vegetation samples were pulverised with a ball mill and digested with sulphuric acid, salicylic acid, hydrogen peroxide and selenium. N was analysed colorimetrically.

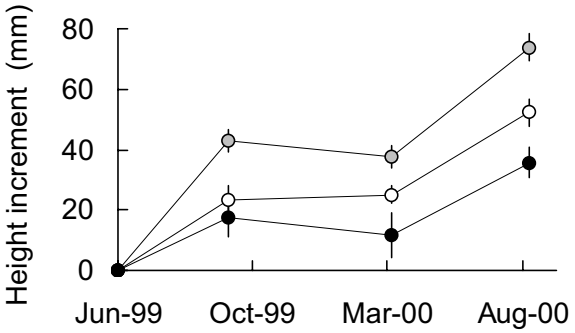

Figure 5.1 The effect of N deposition on the cumulative height increment of *Sphagnum magellanicum* (means ± 1SE) from June 1999 to September 2000. Open circles: 0 kg N ha $^{-1}$ yr $^{-1}$, closed grey 40 kg N ha $^{-1}$ yr $^{-1}$, closed black: 80 kg N ha $^{-1}$ yr $^{-1}$.

Data analysis

Data were tested for normality and equality of variance and, when necessary, were ln-transformed prior to analysis. Most of the data were tested with a 1-way ANOVA, with N treatment as the fixed factor and block as random factor. 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 post-hoc test. As the capitulum fraction of *Sphagnum* behaved differently from the stem fraction for most of the amino acids, data for each fraction were tested separately. Differences between the N treatments were determined by a Tukey post-hoc test. The cumulative height increment of *Sphagnum* was tested with a RM-ANOVA, with N treatment as the fixed factor. Logarithmic regression analysis was performed using the 'curve estimation' option in SPSS. All analyses were performed using the SPSS package 10.0 for Windows.

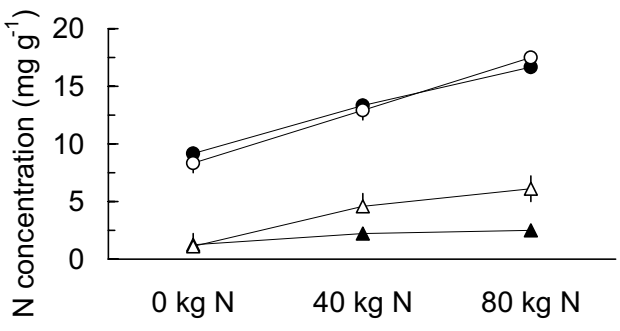

Figure 5.2 The effect of N deposition on the total tissue N concentration (circles, means ± 1SE) and the concentration of N, stored in free amino acids (triangles, means ± 1SE). Solid symbols represent the capitulum, open symbols represent the stem.

Results

Sphagnum growth as expressed by height increment, differed between all three N treatments (N effect: $P < 0.001$, RM-ANOVA) and was greatest at the field deposition level of 40 kg N ha^{-1} yr^{-1}. It appears that the growth effect in the 40 kg N treatment was primarily an initial effect within the first 4 months, after which there was no or little effect (Figure 5.1). The observed growth reduction in the 80 kg N treatment persisted throughout the experiment.

The total N tissue concentration did not differ between the stem and the capitulum and showed an almost linear increase with N deposition (N effect: $P < 0.001$, Figure 5.2). Mean values ranged from 7 mg g^{-1} in the 0 kg N treatment to 17 mg g^{-1} in the 80 kg N treatment. There was no effect of N tissue concentration on *Sphagnum* height increment (Figure 5.4A).

The amount of N stored in free amino acids increased with N deposition in both the capitulum and the stem (N effects: $P < 0.01$ and $P < 0.001$ respectively, Figure 5.2), and showed a positive relationship with the total N tissue concentration (Figure 5.3A). The latter relationship was most pronounced in the stem. The increase in *Sphagnum* amino N concentration from the 40 kg N to the 80 kg N treatment was significantly smaller than the increase from the 0 kg N to the 40 kg N treatment, however (Figure 5.2). The major part of the observed changes in amino N concentration was due to arginine in the capitulum (Figure 5.3D) and asparagine, glutamine and arginine in the stem (Figures 5.3B, C and D).

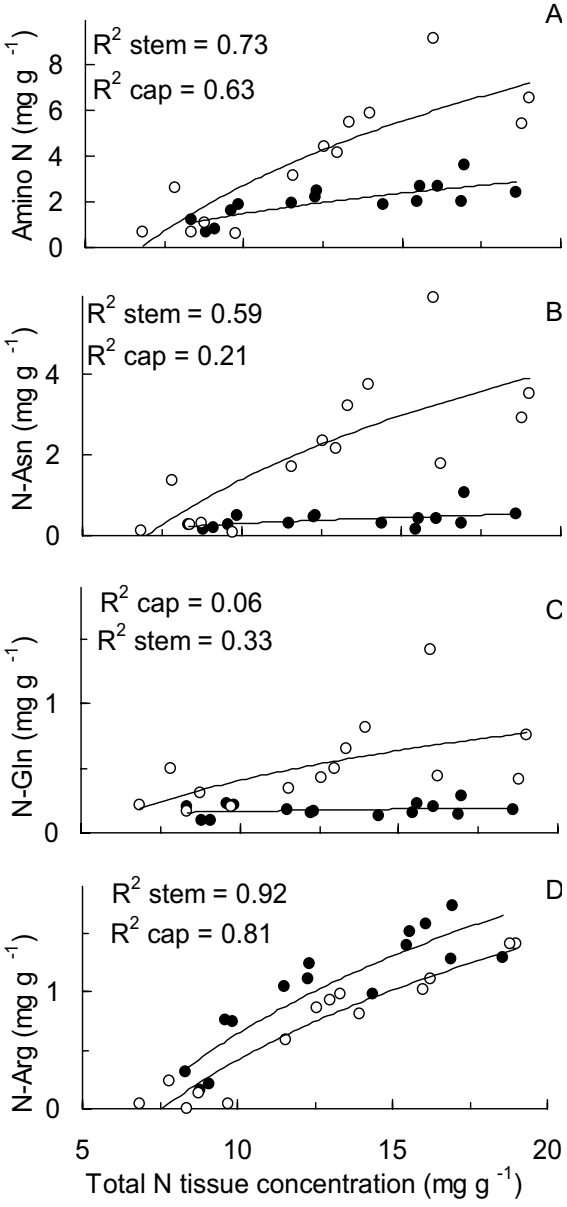

Figure 5.3 The relationship between the total N tissue concentration and N stored in all measured free amino acids ($P < 0.001$ stem & capitulum), asparagine ($P < 0.001$ stem), glutamine ($P \leq 0.05$ stem) and arginine ($P < 0.001$ stem & capitulum). ● capitulum, ○ stem (Logistic regression).

Asparagine in the stem acted as the major storage compound of N and was responsible for 21% of the total N concentration in the stem in the 80 kg N treatment. In contrast, N stored in arginine contributed to only 7% of the total N concentration in the stem and 9% in the capitulum.

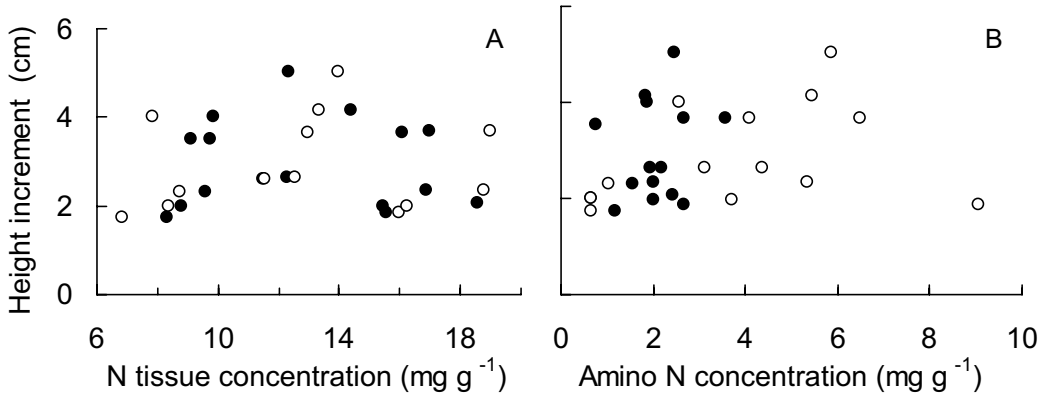

Figure 5.4 The relationship between the height increment of *Sphagnum* from early April to late September 2000 and **A**, the total N tissue concentration and **B**, the amino N concentration. ● capitulum, ○ stem.

Table 5.1 The effect of N deposition on the concentration of N stored in individual free amino acids in µg g $^{-1}$ (means ± 1SE). Only the amino acids significantly influenced by the N treatments are shown. n.s. = $P > 0.05$, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ (1-way ANOVA).

	0 kg N	40 kg N	80 kg N	N effect	Block
Capitulum					
N-Arginine	431.6 ± 129.0^{a}	1189.9 ± 104.8^{b}	1438.2 ± 84.2^{b}	***	ns
N-Glutamic acid	156.9 ± 16.0	229.3 ± 14.1	188.3 ± 17.0	***	**
N-Lysine	6.3 ± 1.2^{a}	10.8 ± 1.2^{b}	7.0 ± 0.9^{a}	*	ns
N-Phenylalanine	1.7 ± 0.5^{a}	4.2 ± 0.8^{b}	3.2 ± 0.5^{ab}	*	ns
Stem					
N-Asparagine	422.3 ± 239.6^{a}	2619.3 ± 366.8^{b}	3506.5 ± 857.5^{b}	***	ns
N-Aspartic acid	76.4 ± 13.2	135.8 ± 11.5	126.1 ± 36.8	*	*
N-Arginine	88.1 ± 41.4^{a}	832.2 ± 67.2^{b}	1232.7 ± 100.8^{c}	***	ns
N-Glutamine	275.3 ± 59.2	544.1 ± 83.4	757.1 ± 234.3	**	*
N-Isoleucine	1.0 ± 0.6^{a}	19.0 ± 4.8^{b}	7.5 ± 6.5^{ab}	*	ns
N-Phenylalanine	2.9 ± 0.9^{a}	7.3 ± 1.5^{b}	5.2 ± 0.5^{ab}	*	ns
N-Proline	1.0 ± 0.6^{a}	3.8 ± 0.2^{b}	4.8 ± 1.4^{b}	*	ns
N-Threonine	12.8 ± 3.3	29.2 ± 3.7	40.9 ± 11.6	**	*
N-Tryptophan	18.7 ± 11.8^{a}	96.8 ± 18.3b^{b}	101.8 ± 15.2^{b}	**	ns

The effect of N addition on the other free amino acids was much less pronounced, but was nevertheless significant for eight additional amino acids (Table 5.1). In general, the concentration in the stem was elevated in the 40 and 80 kg N treatments, although the 80 kg N treatment not always resulted in higher concentrations than the 40 kg N treatment. Neither the individual nor the pooled amino acid concentrations were correlated with *Sphagnum* growth in 2000 (Figure 5.4B), nor was there any correlation between *Sphagnum* growth and the total tissue N concentration minus the amino N concentration.

In the course of the experiment, the concentration of inorganic N in the pore water seemed to increase with N addition, although the treatments only differed significantly near the end of the experiment in September 2000 (P = 0.035, Table 5.2). When averaged over 2000, the concentration of inorganic N was positively correlated with the individual concentrations of asparagine, glutamine and arginine in the stem (Figures 5.5A, B and C). No such correlation was found for the capitulum.

Table 5.2 The effect of N deposition on the concentration of inorganic N (mg l^{-1}) of the pore water at 0-5 cm depth. ns = $P > 0.05$, * $P \leq 0.05$.

Month	0 kg N	40 kg N	80 kg N	N effect
Mar-99	0.19 ± 0.03	0.25 ± 0.06	0.21 ± 0.06	ns
Jun-99	0.33 ± 0.23	0.40 ± 0.19	0.13 ± 0.08	ns
Oct-99	0.10 ± 0.04	0.59 ± 0.13	3.68 ± 1.35	ns
Feb-00	0.30 ± 0.08	0.52 ± 0.14	1.32 ± 0.28	ns
May-00	0.06 ± 0.02	0.11 ± 0.01	0.96 ± 0.33	ns
Jul-00	0.15 ± 0.05	0.10 ± 0.01	0.31 ± 0.09	ns
Sep-00	0.03 ± 0.01[a]	0.09 ± 0.03[a]	2.28 ± 0.74[b]	*

Discussion

Depression of *Sphagnum* growth

Although we found depressed *Sphagnum* growth and elevated amino N concentration in the 80 kg N treatment, the two parameters were not correlated (Figure 5.4), not even when the concentrations of amino N in the 40 and 80 kg N treatments were well above the 2 mg g^{-1} threshold value (Nordin and Gunnarsson 2000). Hence, although our results clearly show that *Sphagnum* growth can be affected by enhanced N availability, neither the total N concentration nor the amino acid concentrations seem to be responsible for the growth reduction, though they do appear interrelated.

A reduction of growth seems to take place when *Sphagnum* experiences a considerable increase in N availability, relative to conditions at the site from which it was obtained. The previous is illustrated in our study, where growth was not influenced by the field deposition level of 40 kg N ha^{-1} yr^{-1}, but did suffer from an increase to the double (Figure 5.1). That *Sphagnum* growth was not inhibited by the 40 kg N load also seems to indicate that *Sphagnum* can adapt to enhanced N deposition. From literature the same patterns can be deducted. *S. cuspidatum* from sites differing in background deposition showed a dissimilar response to the same N concentration in a culture solution (Baxter *et al.* 1992). When the strength of the N solution was increased, *S. cuspidatum* from the low deposition site showed a reduction in growth whereas growth of specimens from the high deposition site was stimulated.

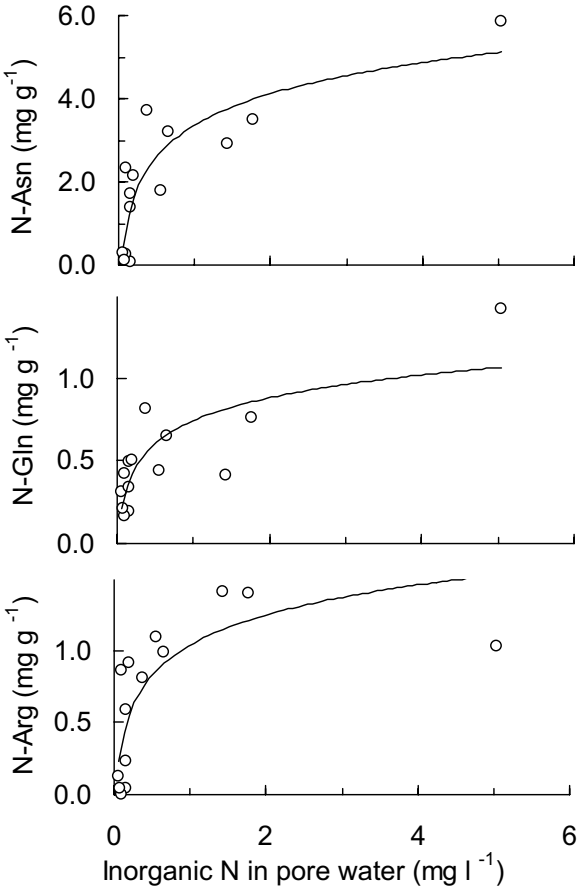

Figure 5.5 The relationship between the inorganic N concentration in soil pore water at 5 cm depth and N stored in asparagine, glutamine, and arginine in the stem. The inorganic N concentration is the mean of 4 sampling dates in February, May, July and September 2000. When the point in the right hand corner is omitted, regression yields R^2= 0.60, P = 0.002; R^2 = 0.44, P = 0.013 and R^2 = 0.74, P = 0.001.

Both the chlorophyll concentration and the initial arginine concentration in the capitulum were greatest in the *Sphagnum* from the high deposition site.

The background deposition level at the site of origin can influence not only the growth response of *Sphagnum* but also the uptake of N, as has been shown by Press *et al.* (1986). *S. cuspidatum* plants from sites differing in background deposition were kept in beakers with deionised water on the bog surface. The beakers were open to the air, and thus subjected to deposition. For a 10-day period, they measured the concentration of inorganic N in the water. The *Sphagnum* from the low deposition site had the greatest total N uptake and the highest N uptake rate. The initial N concentration of this *Sphagnum* was 12-13 mg g^{-1} as opposed to 32-33 mg g^{-1} in the *Sphagnum* from the high deposition site. When we compare the NH_4^+ uptake rates of two other studies, we again find an indication of an effect of *Sphagnum* origin: the NH_4^+ uptake rates of both *S. magellanicum* and *S. fallax* from Sweden far exceeded those of German *S. magellanicum* and *S. fallax*, whereas the uptake rates of NO_3^- were comparable (Jauhiainen *et al.* 1998, Twenhöven 1992a). The background deposition at the Swedish site was about half that of the German site, and the initial N concentrations of the *Sphagnum* capitula were approximately 10% lower (Twenhöven 1992b, Gunnarsson and Rydin 2000).

The above implies that given enough time, *Sphagnum* can adapt to a broad range in deposition loads, providing there is no light competition with green algae (Rudolph and Voigt 1986) or with vascular plants (Heijmans *et al.* 2001, Berendse *et al.* 2001). The adaptation may involve a higher tissue N content (e.g. Baxter *et al.* 1992), a reduction of the amount of N relocated from stem to capitulum (Figure 5.2), a reduced NH_4^+ uptake rate (Press *et al.* 1986; Twenhöven 1992a, Jauhiainen *et al.* 1998) or a lower nitrate reductase activity (Press and Lee 1982, Woodin *et al.* 1985, Woodin and Lee 1987, Morgan *et al.* 1992). When *Sphagnum* is exposed to a step increase of N, as is usually the case in an experimental setting, its uptake rate could exceed its assimilation rate, leading to an accumulation of toxic NH_4^+ in the cell and a subsequent reduction in growth. The extent of the latter may be influenced by the rate at which NH_4^+ can be assimilated into organic compounds (production) and may thus depend on factors the availability of P and CO_2 in the growth environment.

Evidence to support the above hypothesis can be found in our study, where the increasing difference between the total N and the amino N concentrations (Figure 5.2) points to the accumulation of some unmeasured N compounds. Additionally, the susceptibility of *Sphagnum* to high concentrations of external ammonium (Press *et al.* 1986, Rudolph and Voigt 1986) seems to confirm our suggestion of the role of NH_4^+ toxicity in *Sphagnum* growth inhibition. Further evidence is that those *Sphagnum* species most susceptible to high N loads, such as *S. fuscum*, also have the greatest NH_4^+ uptake efficiency (Jauhiainen *et al.* 1998). Ammonium toxicity as a stress factor resulting in reduced growth has been reported for a wide range of species (e.g. Fangmeier *et al.* 1994, Gerendas *et al.* 1997) including aquatic species such as *Zostera marina* (van Katwijk *et al.* 1997), and *Stratiotes alloides* (Smolders *et al.* 2000).

N uptake and relocation

Our study demonstrates the N storage function of the stem in *Sphagnum*. The concentrations of N in capitulum and stem were comparable (Figure 5.2), whereas in most other studies the concentration in the capitulum exceeded that in the stem (e.g. Gunnarsson and Rydin 2000, Van der Heijden *et al.* 2000). Moreover, we found a higher concentration of free amino acids in the stem than in the capitulum at enhanced N deposition (Figures 5.2 and 5.3). From this we infer that the degree, in which *Sphagnum* relocates N from stem to capitulum, shifts with the N loads; at increasing N load, *Sphagnum* relocates less N towards the capitulum, and a considerable amount is retained in the stem.

When N deposition increases, N is taken up, assimilated into glutamine, and used to enhance the chlorophyll content of the cells, thus enlarging photosynthetic capacity (Rudolph and Voigt 1986, Baxter *et al.* 1992, Van der Heijden *et al.* 2000). The excess N is probably converted into N storage compounds like arginine in the capitulum, and asparagine and arginine in the stem (Table 5.1). Though asparagine is clearly the major storage compound for N in *Sphagnum*, arginine responds best to an increase in N availability (Figures 5.3 and 5.5).

Sphagnum does not take up all deposited N, however. As N deposition increases, the pore water surrounding the *Sphagnum* becomes enriched with N (Williams *et al.* 1999, Table 5.2).

Acknowledgements

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Chapter 6

Expansion of *Sphagnum fallax* in bogs: striking the balance between N and P availability

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Abstract

Nitrogen deposition may cause shifts in the *Sphagnum* species composition of bogs, ultimately affecting the conservation value of these systems. We studied the effects of N and P on the expansion of *S. fallax* and *S. flexuosum* in bogs. We related historical census data of *S. fallax, S. flexuosum* and four of their accompanying species to changes in N deposition. In addition, we conducted two fertilisation experiments with N and P; one at a low deposition site with *S. flexuosum* and one at two high deposition sites with *S. fallax*. Finally, we related existing data on capitulum N and P concentrations of *S. fallax* to its abundance in the field. A relative increase in observed frequency of *S. fallax* coincided with an historical increase in N deposition in the Netherlands. There was no indication that *S. fallax* consistently outcompeted one of the other five *Sphagnum* species; the observed frequency of the *Sphagnum* species analysed was rather stable. The census data on *S. flexuosum* did not indicate a response to N deposition, but in the fertilisation experiment the species expanded at the low N deposition site when extra N was applied. In contrast, the expansion of *S. fallax* at the high deposition sites was limited by P. Plant nutrient concentrations suggested that when *S. fallax* can maintain a capitulum N concentration of 7 mg g^{-1} or higher and a P concentration of 0.7 mg g^{-1} or higher the species can grow to dominate. We conclude that *S. fallax* will gradually colonise an increasing number of new habitats in areas with a low, albeit increasing, N deposition, but will only grow to dominate when P supply is adequate. Then, the expansion of *S. fallax* may lead to ousting of the other *Sphagnum* species present.

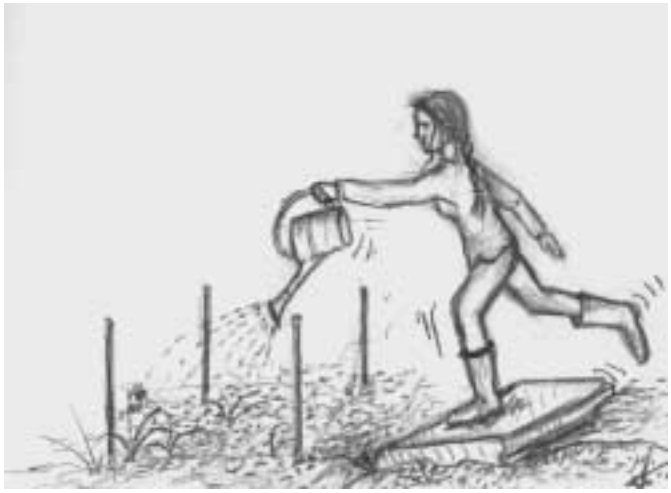
Introduction

Shifts in species composition in a settled community are often preceded by abrupt or gradual changes in the abiotic environment. These changes may be natural, for example a decrease in pH following expansion of *Sphagnum* in fens (Zobel 1988, Van Breemen 1995) or induced by man, such as an increase in N deposition (e.g. Berendse *et al.* 2001 Nordin *et al.* 2002). In nutrient poor ecosystems, a continuous supply of a limiting nutrient, such as N, may overwhelm existing spatial heterogeneity in soil N availability, ultimately affecting species co-existence and diversity (Reynolds *et al.* 1997, Plotnick and Gardner 2002). Species less adapted to nutrient-poor conditions, which are often present at the fringe of these systems, may take advantage of an increase in nutrients and spread, gradually suppressing slower growing species. In time, the community would be expected to reach a new equilibrium in species composition when other nutrients or factors, such as water availability, become limiting (e.g. Lee and Caporn 1998).

Since bryophytes do not have roots or a cuticle, they are generally more sensitive to changes in the atmospheric environment than vascular plants. Consequently, atmospheric pollution will probably be expressed first in the species assemblage of the bryophyte community, especially in ombrotrophic habitats such as raised bogs, as argued by Lee and Studholme (1992). They discussed the expansion of the minerotrophic species *Sphagnum fallax* in both fen and bog communities in the previous century, mainly attributing its success to its indifference to N pollution. An experimental study by Twenhöven (1992) confirmed that *S. fallax* is more tolerant to N deposition than *Sphagnum magellanicum,* and grows faster than this species in the lower parts of the hummock-hollow gradient. From this result the author predicted that, in time, *S. fallax* would oust *S. magellanicum* from these habitats. For this to come about however, one must assume that either N availability continues to limit growth of *S. fallax,* and not P as has been shown for areas with high N deposition (Aerts *et al.* 1992, Kooijman and Kanne 1993), or that N depresses growth of *S. magellanicum* relatively more than growth of *S. fallax*.

In this study we pursued two different objectives. Our main objective was to examine the effects of N and P on the expansion of *S. fallax* in *S. magellanicum* or *S. papillosum* vegetation. The second objective was to test if *S. flexuosum* var*. flexuosum* would show a similar response to N and P. Both *S. fallax* and *S. flexuosum* belong to the *S. recurvum* species complex and are either described as two separate species (Flatberg 1992) or varieties (Smith 1977). In a number of ecological studies, authors refer to the name of the complex and do not make further taxonomic distinction (e.g. Clymo and Hayward 1982, Anderson *et al.* 1995, Williams and Silcock 2001). The two species occupy habitats with a similar range in pH (Andrus 1986), although *S. flexuosum* usually grows in more fen-like habitats than *S. fallax* (Koerselman and Verhoeven 1992).

We hypothesised that (I) as *S. fallax* and *S. flexuosum* are rather similar in morphology and taxonomically closely related, they would not differ in their ecological response to enhanced N deposition and, as such, we could treat them as one species. We expected (II) that *S. fallax* and *S. flexuosum* would only expand at the expense of surrounding *Sphagnum* species when both N and P would be available in sufficient quantities. As a consequence, expansion of these species at a high N deposition site would be determined by P, and at a low deposition site by N supply. To test these hypotheses, we related census data of these species to changes in N deposition in the Netherlands and conducted two separate fertilisation experiments with N and P in the field. In addition, we related existing data on capitulum N and P concentrations of *S. fallax* to its abundance in the field.



Nutrients were applied with a watering can

Methods

Analysis of census data

Data on historical and recent observations of *Sphagnum fallax* (Klinggr) Klinggr., *Sphagnum flexuosum* Dozy & Molk., *Sphagnum magellanicum* Brid., *Sphagnum papillosum* Lindb., *Sphagnum palustre* L. and *Sphagnum teres* (Schimp.) Ångstr. were obtained from the database of the Dutch Bryological Society. In this database all data, from the 1800s onwards, on bryophyte observations in the Netherlands from both amateurs and professionals are amassed. We required an overview encompassing rare and common *Sphagnum* species as well as species from ombrotrophic and minerotrophic habitats. We hereby assumed that by looking at the cumulative observations in a set of rare and common *Sphagnum* species, we would circumvent problems arising from different census efforts. We selected only those observations that could be supported by herbarium material and checked microscopically by an expert (Table 6.1).

Table 6.1 General information on the observations we selected from the database of the Dutch Bryological Society for our analyses. No. observations used, refers to the total number of observations we selected from the database. No. observations yr^{-1} indicates the range in the selected number of observations per year.

Species	No. observations used	No. observations yr^{-1}
S. fallax	692	0 - 32
S. magellanicum	227	0 - 10
S. papillosum	475	0 - 20
S. flexuosum	216	0 - 25
S. palustre	863	0 - 44
S. teres	119	0 - 23

Data on total N deposition before 1980 refer to modelled values (Van Oene *et al*. 1999), and data after 1980 represent actual values (RIVM 2000, unpublished). Both these data sets are concerned with the Veluwe area, in the centre of the Netherlands. We assumed that, although actual values for total deposition may differ between the different parts of the Netherlands (RIVM 1999), their course over time would be similar to that of the Veluwe.

Site descriptions

We studied the effects of adding N and P on *S. flexuosum* at one site in Ireland, whereas the effects on *S. fallax* were studied at two sites in the

Netherlands. Background N deposition for the Irish site was approximately one third of that for the Dutch sites (chapter 2).

The Irish site, Clara Bog (53°20'N, 7°36'E), was situated in the Irish Midlands. We established our plots in two former pools with a floating peat layer of 20–50 cm, supporting *S. magellanicum* lawn vegetation. Vascular plants formed a sparse cover of less than 5%.

The first Dutch site, Bargerveen (52°42'N, 7°03'E), was chosen in an area with a solid peat layer dominated by a hummock vegetation of *Sphagnum papillosum* and *Erica tetralix* (L). In most plots, 1 to 10 scattered individuals of *S. fallax* were present. The total vascular plant cover ranged from 25% to 50%.

The second Dutch site, Reigersplas (52°50'N, 6°27'E), was established on a floating raft (20–50 cm thick) dominated by a low hummock–lawn vegetation of *S. magellanicum* and *S. papillosum* with a sparse cover of vascular plants, which varied between 10% and 25%. In most plots, 1 to 40 scattered individuals of *S. fallax* were present.

Experiment 1: effects of N and P on *S. flexuosum*

In October 1998, we established 10 plots measuring 1 x 1 m at the Irish location. From the middle of each plot, we cut a sod of *S. magellanicum* vegetation (15 x 15 cm wide and 20 cm deep) and replaced it with a similar sod of *S. flexuosum*, previously collected from a soak on the same bog. The fertilisation treatments were randomly assigned to the plots, and consisted of a control treatment that received bog water only, and an N treatment (40 kg N ha^{-1} yr^{-1}). N was applied as NH_4NO_3 dissolved in 2 litres sieved bog water with a watering can; we used a separate watering can for each treatment. Nutrients were added 6 times a year between February and October. We were careful to add the nutrients immediately prior to, during, or just after rainy weather. The extra N the plots received through the sieved bog water was negligible; the amount of N was equivalent to *c*. 0.5% of the N in the local bulk deposition (chapter 2). As a result of the belated start of the experiment, the plots received only one sixth of the yearly N doses in 1998. By the end of 2000, the area of *S. flexuosum* was receding in both the control and N plots, suggesting that expansion was limited by another factor than N. We therefore decided to add an extra P treatment in addition to the existing treatments in February 2001. Across each plot, we inserted plastic sheets (1 m x 30 cm) unto a depth of 25 cm to create a split plot. Subsequently, the plot halves received an extra litre of bog water in addition to the existing treatments with or without an equivalent of 3 kg P ha^{-1} yr^{-1} in the form of $NaH_2PO_4.2H_2O$. The last nutrients were applied in July 2002.

The area of *S. flexuosum* was measured by photographing each plot at regular time intervals between October 1998 and August 2001. As the conditions at the site did not allow vertical photographs to be taken, we included a frame of known dimensions in our pictures. The pictures were digitised and the areas of the *S. flexuosum* patches and of the frame were calculated with the graphic program Image Pro Plus (2.0). The calculated area

of the latter was used to correct the area of *S. flexuosum* for the oblique angle.

In September 2001 we harvested the experiment and we cut one column with a diameter and depth of 10 cm per species per plot half. *Sphagnum* was removed from the columns, sorted into species and divided into a capitulum (0–1 cm) and stem fraction (1–2 cm). The *Sphagnum* was oven dried at 70^{o} C for 48 hrs before chemical analysis. The dried *Sphagnum* material was pulverised and digested with sulphuric acid, salicylic acid, hydrogen peroxide and selenium. Subsequently, the N and P concentrations were measured colorimetrically, using a continuous flow analyser (SKALAR SAN plus system, the Netherlands).

Experiment 2: Effects of N and P on *S. fallax*

The experiment was established in May 1998 at two Dutch sites and was carried out parallel with a study on the general effects of N and P on bog vegetation; this is the reason that the set-up of this experiment diverges from experiment 1. At both sites, 20 plots, measuring 1 x 1 m, were laid out in 5 replicated blocks. Treatments were randomly assigned to the plots, and consisted of a control treatment that received demineralised water only, an N treatment (40 kg N ha $^{-1}$ yr $^{-1}$), a P treatment (3 kg P ha $^{-1}$ yr $^{-1}$), and a combination of the latter two. The nutrients, NH_4NO_3 and $NaH_2PO_4.2H_2O$, were dissolved in 2 litres demineralised water. They were watered on, using a separate watering can for each treatment. The fertilisation treatments commenced on June 12th 1998. Nutrients were added 6 times a year between March and September. We were careful to add the nutrients immediately prior to, during, or just after rainy weather. Because of the late start of the experiment, only half of the yearly doses were applied in 1998. The last treatments were given in July 2001. The experiment was harvested in the second week of August 2001.

We used the point-intercept method (Jonasson 1988) to measure the cover of the different *Sphagnum* species in marked representative subplots. This entailed fixing a frame of 25 cm x 37.5 cm and with a 2.5 cm grid above this subplot. At 150 points, a stainless steel needle could be lowered to the moss surface. We recorded each species that was touched with the point of the needle. Cover was measured at the start of the experiment (June–July 1998) and each year between August and early September thereafter. The consequence of using subplots to record changes in scattered *S. fallax* was, that in half of the cases, despite the presence of *S. fallax* in the larger plot, it was not recorded in the subplot. At the end of the experiment, we had recorded *S. fallax* for 9 plots at the Bargerveen site and for 11 plots at the Reigersplas site.

At harvest, columns with a diameter and depth of 10 cm, were cut from the *Sphagnum* layer. The number of columns cut per plot depended on the

variation in the *Sphagnum* present. If the moss layer consisted of a homogeneous sward of one species identical in morphology, only one column was cut. If the condition, the morphology or the dominant species differed, up to four columns were cut to catch the variation. The columns were stored at 1 ^{o}C for no longer than three weeks. The rest of the preparation and analyses proceeded in the same way as in the *S. flexuosum* experiment.

Tissue N, P concentrations & natural abundance of *S. fallax*

To investigate whether natural abundance of *S. fallax* could be related to the N and P availability in the field, we also gathered data on capitulum N and P concentrations from literature (Brock and Bregman 1989, Aerts *et al*. 1992, Risager 1998, Gunnarsson and Rydin 2000 and Hoosbeek *et al*. 2002) thereby assuming that nutrient concentration in the capitulum is a good measure for nutrient availability of the growth environment. We distinguished bogs without *S. fallax*, bogs with *S. fallax* growing scattered or in patches within vegetation dominated by either *S. papillosum* or *S. magellanicum* and bogs wholly dominated (total cover of more than 60%) by *S. fallax*. As we could not find differences in the capitulum nutrient concentration of *S. fallax* and *S. magellanicum* or *S. papillosum* when growing in mixed stands in our experiment 2, we used capitulum nutrient concentration of *S. magellanicum* for those raised bogs where *S. fallax* was absent. We only used nutrient concentrations of *Sphagnum* from bogs that had not been subjected to fertilisation treatments, and had been collected in the fall. An exception to the first, were our own data concerning the P and N&P plots in which *S. fallax* had expanded and the control plots in which nothing changed during experiment 2. These data were portrayed as separate categories.

Data analysis

We tested the effect of the fertilisation treatments on the expansion of *S. flexuosum*, expressed as the change in cover, with a 1-way RM-ANOVA, with treatment as a fixed factor. As the data did not meet the requirement of homosphericity (identified by Mauchly's test of sphericity), degrees of freedom were adjusted by the Huynh–Feldt epsilon. To test how the treatments affected capitulum N and P concentrations of *S. flexuosum* and *S. magellanicum*, we used a 2-way ANOVA, with species and treatment as fixed factors. To examine the same for the *S. fallax* experiment, we included site as a random factor in the design.

Expansion of *S. fallax*, expressed as the change in covered plot area, was tested with a 2-way RM-ANOVA with N and P application as fixed factors, and site as a random factor. As the effects of N and P did not interact, we treated the N&P treatment as the result of separate N and P effects, and thus used N

and P fertilisation as independent (fixed) factors, as stated above. To obtain a balanced design, we selected those plots that had at least one recorded *S. fallax* individual in the point-quadrat subplot at the start of the experiment. This had as a consequence that we omitted one N&P plot from the analyses for each site. The plots used for statistical analysis were divided over the treatments as follows: for Bargerveen 3xC, 2xN, 2xP, 0xNP and 2xC, 3xN, 3xP & 2xNP for Reigersplas. All statistics were calculated using SPSS for Windows (10.0).

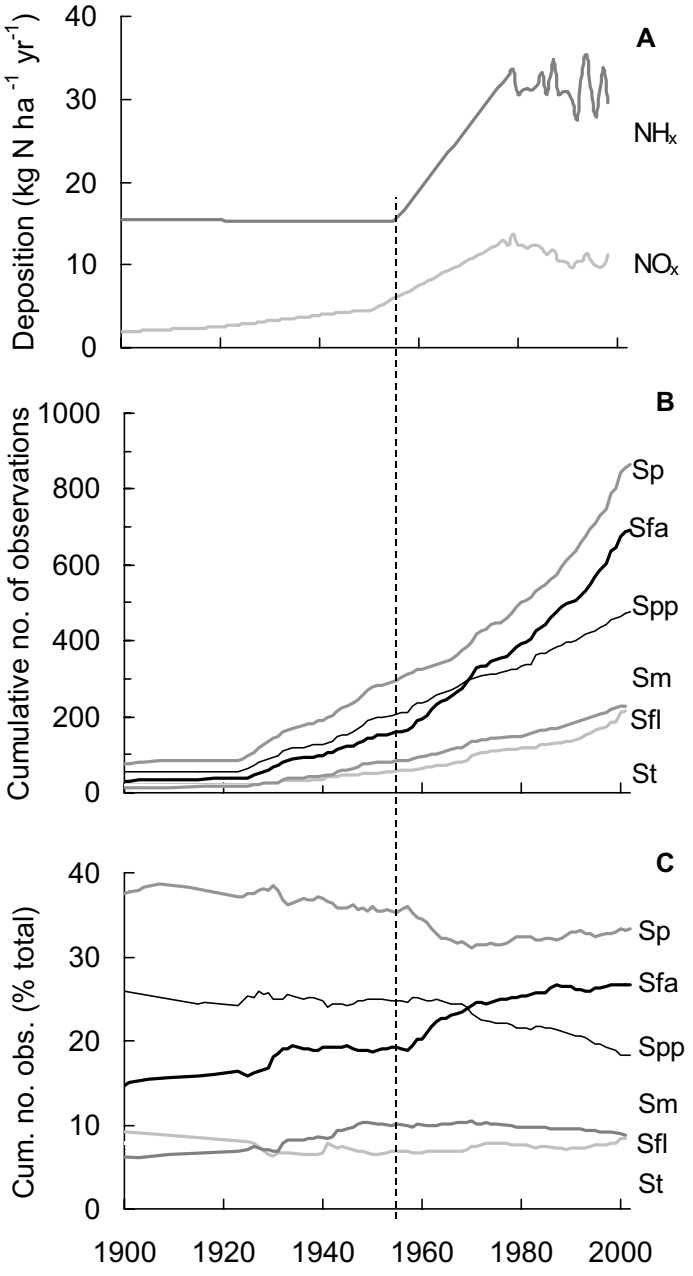


Figure 6.1 A Progress of total NO_x and NH_x depositions over time, **B** the cumulative number of observations per *Sphagnum* species, **C** as B, but expressed as percentage of the cumulative total of all 6 species. Sp = *Sphagnum palustre*, Sfa = *S. fallax*, Spp = *S. papillosum*, Sm = *S. magellanicum*, Sfl = *S. flexuosum* and St = *S. teres*. The dashed line indicates the onset of the increase in NH_x deposition in the Netherlands.

Results

Census data

From the 1920s onward, the census effort increased notably: the number of observations per species steadily inclined until the second half of the 1950's (Figure 6.1B). From then on the cumulative number of *S. fallax* observations started to incline at a steeper slope than before (Figures 6.1B and C), coinciding with the increase in ammonium deposition (Figure 6.1A, Van Oene *et al*. 1999). For the other *Sphagnum* species, including *S. flexuosum*, no consistent shifts in the cumulative number of observations were recorded; the relative number of observation of these species remained rather constant over time (Figure 6.1C).

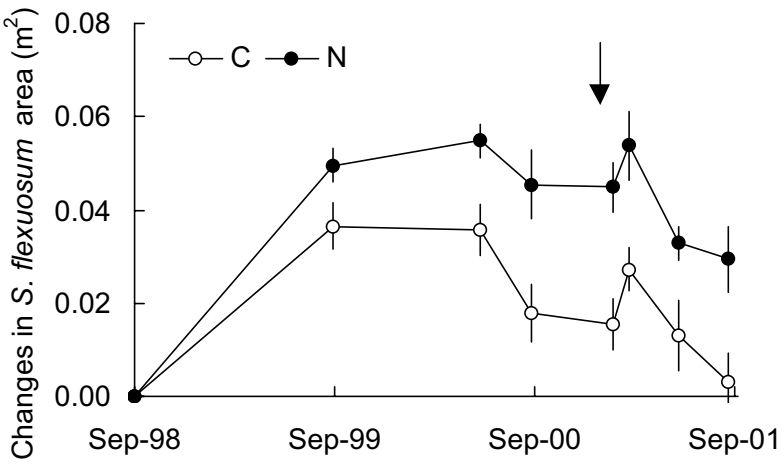


Figure 6.2 The effect of 3 years of N fertilisation (40 kg N ha $^{-1}$ yr $^{-1}$) on the expansion of *Sphagnum flexuosum* transplanted in *S. magellanicum* vegetation, expressed as the change in area (means ± 1SE). The arrow indicates the insertion of plastic sheets to create split plots. Data on *S. flexuosum* area of the split plots were pooled, as no effects of adding P could be detected. For statistics, see Table 6.2.

Experiment 1: *S. flexuosum*

Shortly after we had transplanted *S. flexuosum*, the species expanded steadily at the expense of the surrounding *S. magellanicum* (Figure 6.2, Table 6.2). Adding N further encouraged this expansion, resulting in a significantly greater *S. flexuosum* cover in the plots treated with N after one year. Adding N also affected the colour of the *S. flexuosum*; instead of yellowish green, the species turned a bright darker green. Over time, the *S. flexuosum* area decreased in both treatments with the sharpest decrease found in the control

treatment. Early February 2001, we inserted the plastic sheets in the plots and started applying P. Regardless of nutrient treatment, *S. flexuosum* responded to the placement of sheets by a short increase in cover after which the species reverted to its steady decrease in cover until the end of the experiment, 7 months later. At harvest time, the *S. flexuosum* patch in the control treatment had shrunken to the same size as at the start of the experiment.

Table 6.2 Between and within subject effects of RM-ANOVA to test the effects of N fertilisation for 3 years on the expansion of *Sphagnum flexuosum* in *S. magellanicum* vegetation. The areas of *S. flexuosum* within the split plots were pooled, as no effects of adding P could be detected.

Source	d.f.	MS	*F*	*P*
Between subjects				
N	1	720677.819	10.495	0.012
Error	8	68665.765		
Within subjects				
Time	6	267364.756	50.807	0.000
Time*N	6	28274.681	5.373	0.000
Error	48	5262.373		

Adding N and P affected the nutrient concentrations of both *S. flexuosum* and *S. magellanicum* similarly (Table 6.3): adding N increased the N concentration in the capitulum, whereas P concentration remained unaffected in both species. In turn, adding P resulted in increased P concentrations, but did not affect the N concentrations. Adding a combination of N and P resulted in lower capitulum P concentrations in comparison with *Sphagnum* treated with P alone; the N concentrations seemed lower, albeit not significantly so, than *Sphagnum* receiving N only. The N concentration itself did not differ between the species, but the P concentration did show some peculiar species differences. When either no nutrients or only N were added, *S. flexuosum* had a higher capitulum P concentration than *S. magellanicum* (Species effect: F = 8.701, P = 0.009, N effect: ns, 2-way ANOVA). When P was added, alone or in combination with N, *S. magellanicum* had the highest P concentration (Species effect: F = 23.430, P = 0.000, N effect: F = 28.570, P = 0.000, 2-way ANOVA).

Table 6.3 The effects of adding N for 3 years and P for one year on the concentrations of N and P in the capitula of *Sphagnum magellanicum* and *S. flexuosum* (means ± 1SE). Different letters indicate significant differences between the treatments for the pooled species), ns = P > 0.05, *** P < 0.000. (2-way ANOVA, n = 10 for treatment effect and n = 20 for species effect)

	C	N	P	NP	Spec. effect	Treat. effect	S*T
S. magellanicum							
N capitulum (mg g^{-1})	9.24 ± 0.16^{a}	19.12 ± 1.09^{b}	8.61 ± 0.49^{a}	16.71 ± 1.27^{b}	ns	***	ns
P capitulum (mg g^{-1})	0.39 ± 0.07^{a}	0.32 ± 0.03^{a}	1.35 ± 0.07^{b}	0.98 ± 0.05^{c}	ns	***	***
S. flexuosum							
N capitulum (mg g^{-1})	6.86 ± 0.45^{a}	21.52 ± 1.54^{b}	8.55 ± 0.85^{a}	18.67 ± 2.67^{b}	ns	***	ns
P capitulum (mg g^{-1})	0.55 ± 0.05^{a}	0.55 ± 0.09^{a}	1.01 ± 0.07^{b}	0.71 ± 0.06^{c}	ns	***	***

Experiment **2**: *S. fallax*

The expansion of *S. fallax* was encouraged by adding P at both sites (Figure 6.3, Table 6.4), and proceeded as follows. First, the greater length increment of the *S. fallax* individuals resulted in an elevation of the capitula above of the *Sphagnum* carpet (Figure 6.4A), then, as support of the surrounding *Sphagna* diminished, the top-heavy and often branching individuals collapsed over the surrounding *S. magellanicum* or *S. papillosum*, ultimately outshading them (Figure 6.4B). Adding N alone did not affect *S. fallax*, but when applied with P, it seemed to induce an extra growth response compared to P alone at the Reigersplas site; the increase in area was not significant, however (Figure 6.3). We found new establishment of *S. fallax* in two N&P subplots in 2000 and 2001 for Reigersplas and Bargerveen, respectively. Whether *S. fallax* established from spores or from a fragment of stem we cannot say; expansion seemed the result of branching only.

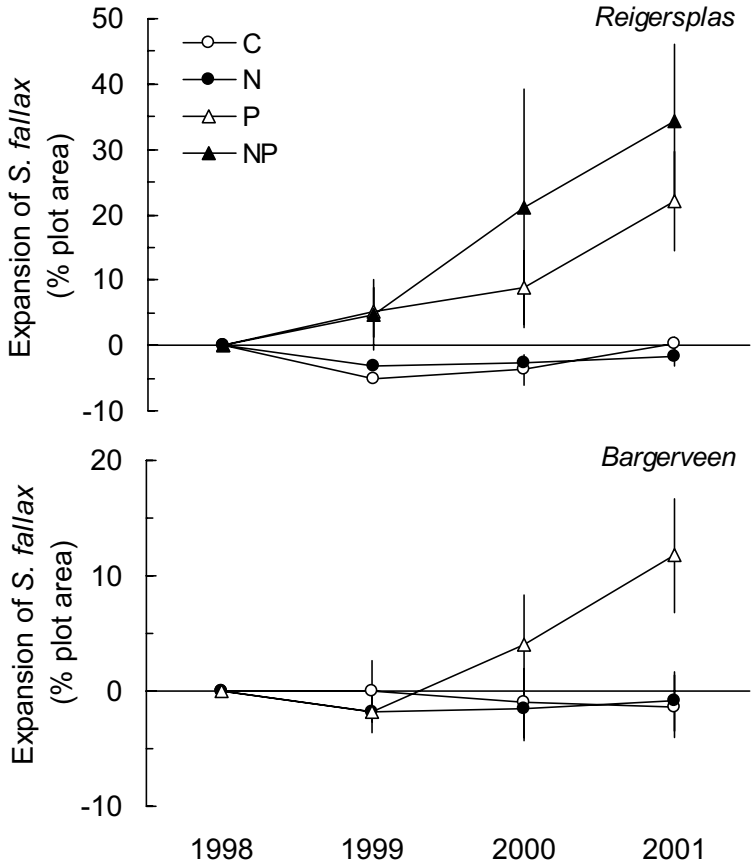


Figure 6.3 The effects of 3 years of N and P fertilisation on the expansion of *Sphagnum fallax* in *S. magellanicum* and *S. papillosum* vegetation, expressed as the change in covered plot area, at two sites (means ± 1SE). C = control, N = 40 kg N ha $^{-1}$ yr $^{-1}$, P = 3 kg P ha $^{-1}$ yr $^{-1}$. Only data on those plots where *S. fallax* was recorded with the point–intercept measurements at the start of the experiment, are included. For statistics, see Table 6.4.

Capitulum N and P concentrations (Figure 6.5 for *S. fallax*) did not differ between *S. fallax*, *S. papillosum* and *S. magellanicum*, and showed similar responses to the nutrient treatments as described for the previous experiment.

Source	d.f.	MS	*F*	*P*
Between subjects				
N	1	3.157	1.642	0.226
P	1	20.028	10.419	0.008
Site	1	6.487	3.375	0.093
N*P	1	7.785	4.05	0.069
Site*N	1	8.389	4.364	0.061
Site*P	1	1.127	0.586	0.460
Error	11	21.145		
Within subjects				
Time	3	1.915	1.915	0.011
Time*N	3	0.285	0.648	0.590
Time*P	3	2.254	5.125	0.005
Time*Site	3	0.0257	0.058	0.981
Error	33	0.44		

Table 6.4 Within and between subject effects of RM-ANOVA to test the effects of N and P fertilisation for 3 years on the expansion of *Sphagnum fallax* in *S. magellanicum* and *S. papillosum* vegetation at two sites. Only main effects and their 2-way interactions are shown

Tissue N, P concentrations & natural abundance of *S. fallax*

When we consider the N and P concentrations in the capitulum of *S. fallax* (Figure 6.5), it seems that the three categories that we distinguished in the abundance of *S. fallax,* were reflected in the capitulum nutrient concentrations. First, N availability seemed to restrict the occurrence of *S. fallax*, corresponding with N capitulum concentrations up to 7 mg N g^{-1}. Then, once nutrient supply was such that capitulum N concentrations exceeded 7 mg N g^{-1}, *S. fallax* was found co-occuring with other *Sphagnum* species, but did not dominate the vegetation. Finally, when P availability allowed for P concentrations of 0.7 mg g^{-1} or higher, *S. fallax* started to dominate the *Sphagnum* vegetation. The capitulum concentrations of *S. fallax* in our second fertilisation experiment corresponded with the stages described; the non-expanding *S. fallax* had extremely low P concentrations, whereas the expanding *S. fallax* had high P concentrations (Figure 6.5).

Discussion

***S**. flexuosum*

We predicted that *S. flexuosum* would not differ from *S. fallax* in its response to N and P. It is obvious that *S. flexuosum* did not respond to the increase in N deposition in the Netherlands in the same way as *S. fallax* (Figure 6.1), thus seemingly proving our hypothesis wrong. However, adding N in our transplantation experiment in Ireland, did encourage *S. flexuosum* to expand (Figure 6.2, Table 6.2), as predicted for an area with lower N deposition. This would suggest that *S. flexuosum* can outcompete *S. magellanicum* when nutrient availability allows. Our finding that disturbances of the bog surface, such as sod cutting or inserting sheets, favoured *S. flexuosum* (Figure 6.2), also points in that direction, since disturbance of soils and thus the microbial community, may coincide with a temporary release of nutrients (Bhatti *et al*. 2002).

The decline in *S. flexuosum* area we found in the course of 2000 and 2001 despite the adequate nutrient supply (Table 6.3), may be related to the infrequent summer precipitation in the Irish Midlands in these years (Limpens, unpublished); reduced water availability might have shifted the competitive balance between *S. flexuosum* and *S. magellanicum* towards the latter, resulting in a net decrease of the area covered by *S. flexuosum* (Rydin 1993). All in all, the response of *S. flexuosum* to nutrient addition seems similar to that of *S. fallax*: when water availability is adequate, the availability of nutrients determines whether the species can or cannot expand in *S. magellanicum* vegetation. Still, the question remains why the response of *S. flexuosum* to the historical increase in N deposition differed from that of *S. fallax* (Figure 6.1).

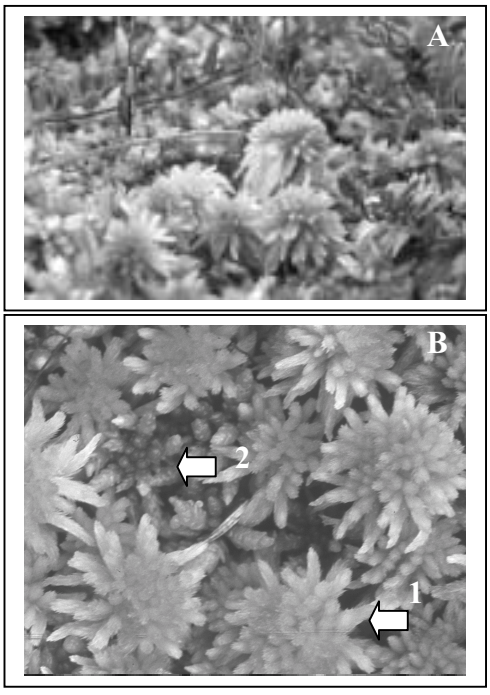


Figure 6.4 Phosphorus induced expansion of *Sphagnum fallax*. **A** *S. fallax* capitula thrusting out of *S. magellanicum* vegetation, **B** Continued expansion of *S. fallax* through frequent branching (1) leads to overtopping and subsequent outshading of *S. papillosum* (2).

As *S. flexuosum* was and is less common than *S. fallax*, at least in Europe (Daniels and Eddy 1990) it is possible that its capability to disperse and colonise new habitats is inferior to that of the latter species, or that the elevation in N deposition has a relative minor impact on the more minerotrophic and fen-like (*sensu* Bridgham *et al.* 1996) niche that *S. flexuosum* occupies in the Netherlands (Koerselman and Verhoeven 1992).

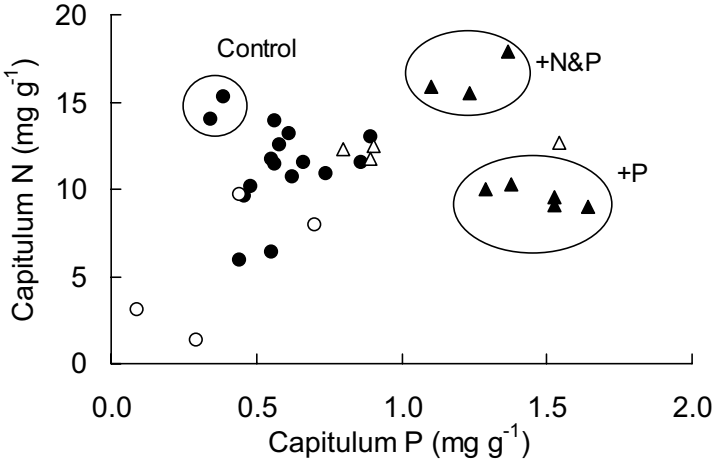


Figure 6.5 The relationship between the N and P concentrations in *Sphagnum fallax* capitula (0-1 cm) and the dominance of *S. fallax* in the field. ○ *S. fallax* absent from bog, values are for *S. magellanicum* (sources: Aerts *et al.* 1992, Gunnarsson and Rydin 2000, Limpens unpublished data), ● *S. fallax* co-occurring with *S. magellanicum* (sources: Risager 1998, *S. fallax* experiment in this study), △ *S. fallax* dominant (sources: Brock and Bregman 1989, 0-4 cm fraction!, Hoosbeek *et al*. 2002, 0-3 cm fraction!, Limpens unpublished data, 0-1 cm fraction), ▲ expanding *S. fallax* vegetation (source: *S. fallax* experiment). P, N&P and control indicate the treatments used in experiment 2 in this study.

S. fallax

Our results give the first strong indications at the natural scale that the increase in prominence of *S. fallax* in the last few decades, is related to N deposition (Figure 6.1); a relationship previously suggested by a number of authors (Ferguson and Lee 1983, Tüxen 1983, Voigt and Johnson 1987, Lee and Studholme 1992) but actually studied at only one site (Twenhöven 1992). However, after N deposition has increased to a high level, as nowadays is the case in the Netherlands (Figure 6.1), expansion of *S. fallax* from subordinate to dominant species, seems to be determined by P rather than by N availability (Figures 6.3 and 6.5, Table 6.4), which is in accordance with our hypothesis. We suggest that, as N deposition increases, pockets in bogs which have both an ample P availability and suitable hydrological conditions,

will gradually become colonised by *S. fallax*. These pockets would probably comprise flushed habitats, lagg zones and edges of hollows or pools. These observations are in agreement with the results of Twenhöven (1992), who reported that a simulated increase in N deposition only stimulated growth of *S. fallax* in hollows and partly in lawn vegetation. Apart from being wet, these habitat types tend to have a higher P availability, if only because of a higher nutrient influx from run-off (Damman 1986).

A time lag between the appearance of suitable habitats and their colonisation by *S. fallax* would explain the continued increase in observations of this species (Figure 6.1) instead of a levelling off, which one would expect when N availability would no longer constrain expansion. Only bogs or acidic fens with a high P availability, be it from internal nutrient release after recent re-wetting (Chepkwony *et al.* 2001, Venterink *et al.* 2002), or external input through agricultural run-off, deposition, in-blown leaves or sand, are likely to develop dominance of *S. fallax* (Figure 6.5). It seems that when nutrient availability is such that *S. fallax* can maintain a capitulum N concentration of 7 mg g^{-1} or higher and a P concentration of 0.7 mg g^{-1} or higher (Figure 6.5), the species becomes dominant. Once dominant, the relatively high degradability of *S. fallax* tissue (Coulson and Butterfield 1978, Twenhöven 1992, Lütt 1992) would help to maintain the high nutrient availability in its environment, thus securing an extended period of supremacy. The expansion of *S. fallax* in hummock vegetation at the Bargerveen site (summer water table –50 cm) was rather surprising, because, as a hollow species, it should not be able to survive in the drier hummock habitat (Andrus 1986, Rydin 1985, Li *et al*. 1992). This discrepancy can probably be explained by the high frequency of summer showers during the experimental period and the conservative water use of the surrounding *S. papillosum* (Rydin 1985, Li *et al*. 1992); the combination likely decreased drought stress in *S. fallax*. In the long term, it is unlikely that *S. fallax* is able to maintain a high cover in such a habitat.

Competition

Our results (Figures 6.2 and 6.3) show that changes in nutrient availability can result in one species (*S. fallax, S. flexuosum*) overtopping and overgrowing another *(S. papillosum, S. magellanicum*). This suggests that a change in the physical environment may induce a shift in the relative competitive ability between *Sphagnum* species, as has been shown for vascular plants (e.g. Berendse and Elberse 1990) and discussed for bryophytes (Rydin 1997). Availability of nutrients clearly restricted growth of *S. fallax* and *S. flexuosum* in our study, which is in accordance with their minerotrophic nature. Once this restriction was lifted, the species could expand and win the competition for light with the surrounding *Sphagnum* species. In the long term, competitive exclusion only seems possible in a narrow band dictated by both water and nutrient availability, however. When these specific conditions are not met, it is likely that co-existence will occur, with the abundance of the separate species

fluctuating over time, depending on whether the environmental conditions favour the one or the other (Kosiba and Sarosiek 1991, Rydin 1993).

Conclusions

We conclude that *S. fallax* will gradually colonise an increasing number of new habitats in areas with a low, albeit increasing, N deposition, but will only grow to dominate when P supply and water availability are adequate. Then, the expansion of *S. fallax* may lead to actual ousting of the other *Sphagnum* species present. We suggest that the N and P concentrations in the capitulum may be used to predict whether *S. fallax* has the potential to become dominant or not.

Acknowledgements

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Using the point-intercept method to measure abundance of *Sphagnum* species

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Chapter 7

How litter quality affects mass loss and N loss from decomposing *Sphagnum*

Juul Limpens and Frank Berendse (SUBMITTED)

Abstract

N deposition may affect litter decomposition and may thus have an impact on the rate of carbon (C) sequestration in *Sphagnum* peatlands. We present results from four separate experiments aimed at delineating the effects of litter N-enrichment, *Sphagnum* species, stem part of *Sphagnum* and place of incubation on decomposition rate and N release. We measured mass loss and N loss from litterbags incubated at 10-15 cm in the field for one year. Mass loss was positively related to the N:C quotient of the litter, but depended strongly on the range in N:C quotients observed; only a distinctive difference in N:C quotients affected mass loss. Although hummock species decayed at a slower rate than hollow species, the differences between the species became less pronounced for older, brown stem parts and for N-enriched litter. Brown stem parts decayed at a slower rate than green stem parts, except for *S. papillosum*. Neither position of incubation (low hummock or hollow), nor the inorganic N concentration of the incubation environment affected mass loss. N loss was mainly determined by, and positively related to, the N:C quotient of the litter; species and stem part had minor effects. Above a N:C quotient of *c.* 0.015, net N loss was observed for all species. We conclude that decomposition of *Sphagnum* is stimulated by N deposition. As the latter also affects litter N concentration and thus N release, we think that positive feedbacks through changing litter quality should be taken into account when modelling the effects of N deposition on *Sphagnum* peatlands and C sequestration in these systems.

Introduction

Approximately a quarter of the world's pool of soil organic C is stored in peatlands in the northern hemisphere (Gorham 1991, O'Neill 2000). Furthermore, these systems act as a sink for atmospheric C, sequestering about 12% of current human emissions (Clymo 1998). The rate of C accumulation in peatlands is governed by low decomposition rates rather than by high productivity (Clymo 1983); the bulk of accumulated C is sequestered in highly resistant *Sphagnum* litter (Verhoeven and Liefveld 1997). Changes in litter decomposability thus may affect the carbon balance of peatlands and ultimately impact on the global carbon budget (Gorham 1991, Chapin *et al.* 1992, Woodwell 1994, Moore 2002).

Changes in the quality of soil organic matter may be brought about by N-induced shifts in the abundance's of species differing in litter quality (e.g. Berendse *et al.* 1989, Van Vuuren *et al.* 1992), or by an alteration of the litter quality of the species themselves (Aerts 1997, Van der Krift *et al.* 2000). In turn, quality, and thus degradability of litter may be a function of the concentration of resistant compounds (e.g. Horner *et al.* 1988, Northup *et al.* 1998) or the concentration of nutrients (Aerts 1989; Szumigalski and Bayley 1996, Aerts and de Caluwe 1999).

In peatlands in north-western Europe, the historic increase in atmospheric N deposition has resulted in changes in species composition, favouring vascular plants above *Sphagnum*, and enhanced organic N concentrations of *Sphagnum* (e.g. Malmer 1990, Pitcairn *et al.* 1995, Risager 1998, Aerts *et al.* 2001, Berendse *et al.* 2001). Although it is generally accepted that a shift from *Sphagnum* dominated to vascular plant dominated litter slows down the rate of C sequestration in peatlands (Heal *et al.* 1978, Hobbie 1996), the effects of N enriched litter on decomposition and nutrient cycling are less understood and, as yet, remain a matter of debate. On the one hand N-enriched *Sphagnum* seems to have a higher decay potential (Coulson and Butterfield 1978, Szumalski and Bayley 1996, Aerts *et al.* 2001), on the other hand it has been suggested that a shortage of degradable C or P and not N limits decay of *Sphagnum* litter (Damman 1988, Hogg *et al.* 1994).

In this study, we focussed on the effects of litter N-enrichment on decomposition rates and N mineralisation for different *Sphagnum* species in contrasting incubation environments in bogs. Our main hypothesis was, that (I) both mass loss and N loss over time would be positively related to the N:C quotient of the incubated litter. As intrinsic litter quality seems to overrule effects of microhabitat (Johnson and Damman 1991) and hummock species seem to decay faster than hollow species (Johnson and Damman 1993), we further hypothesised that (II) hummock species, such as *S. magellanicum* and *S. papillosum*, would decay slower and have a lower N turn-over rate than hollow species, such as *S. cuspidatum* and *S. fallax*, and that (III) neither place of incubation nor N availability in the incubation environment would affect the rate of decay. As the rate of decomposition tends to decrease over

time, as the more degradable fractions are respired first (Fyles and McGill 1987, Hogg 1993), we expected that (IV) young, still green or red *Sphagnum* stems would decay at a higher rate than older, brown stems. To test these hypotheses, we established four separate litterbag experiments in which we followed mass loss and N loss from litterbags incubated in intact bog vegetation for one year.

Carrying *Sphagnum* and water samples (with chest waders)

Methods

Experimental design

Experiment I

The objective of the first experiment was to test the effect of experimentally modified N:C quotients on mass loss from *Sphagnum* material. In September 1998, *Sphagnum magellanicum* Brid. was collected from outdoor mesocosms that had been subjected to three years of enhanced N deposition (+ 50 kg N ha^{-1} yr^{-1}), elevated atmospheric CO_2 concentration (560 ppmv), using Free Air CO_2 Enrichment (FACE) technology, and a control treatment (Heijmans *et al.* 2001a). Each treatment caused distinctive tissue N:C quotients, with the lowest quotient found for the *Sphagnum* in the CO_2 treatment (Heijmans *et al.* 2001a).

We extracted *Sphagnum* individuals from the collected sods, cut off the capitula (0–1 cm) and divided the stems into a still green part (1–3 cm from top of capitulum) and a brown, older part with branches and leaves still intact (5-7 cm from top of capitulum). The separate materials were mixed thoroughly and were oven-dried for at least 96 hours at 30 ^{o}C, before they were divided in portions of *c.* 3 g, weighed and put into litterbags. Subsequently, we enclosed an encoded plastic tag, sealed the litterbags and stored them at room temperature until incubation. The litterbags (3 by 7 cm) were manufactured from polypropylene mesh with a mesh size of 74 microns (B. Henr. Lampe b.v. Sneek, the Netherlands). Mesh of this size allows microfauna and fungi to enter, but excludes macrofauna and prevents loss of separated *Sphagnum* leaves.

In January 1999 (Table 7.1), all litterbags were taken into the field and one sample for each type of material was inserted 10–15 cm below the moss surface within a 50 cm radius from a pole in low hummock vegetation of *S. magellanicum* in the Netherlands (Reigersplas, 52^{o}50'N, 6^{o}27'E). There were 10 poles: the experimental blocks. We used stainless steel wire to secure the litterbags to their pole. After one year all litterbags attached to the pole were harvested.

Experiment II

This experiment was established to test the effects of *Sphagnum* species and position of incubation (hummock, hollow) on mass loss. We collected sods of two hummock species, *S. magellanicum* and *S. papillosum* Lindb., and of two hollow species, *S. cuspidatum* Hoffm. and *S. fallax* (Klinggr.) Klinggr., from a bog in Ireland (Clara bog, 53^{o}20'N, 7^{o}36'E) in September 1998. The subsequent processing of the *Sphagnum* and the manufacturing of the litterbags proceeded as in experiment I.

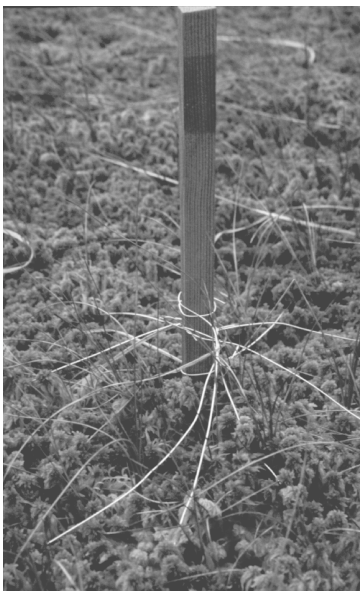
In May 1999 (Table 7.1), one sample for each type of material was inserted at 10-15 cm depth around 20 poles, half of which were situated in low hummock vegetation of *S. papillosum*; the other half in hollow vegetation of *S. cuspidatum* in the Netherlands (Bargerveen, 52°42'N, 7°03'E).

Experiment III

The aim of the third experiment was to test the effect of background N deposition level on mass loss for three contrasting litter types: a hummock *Sphagnum*, a hollow *Sphagnum* and a vascular plant species. We collected *S. papillosum*, *S. fallax* as sods and *Eriophorum angustifolium* L. as leaf litter at a high deposition site in the Netherlands (Reigersplas) and at a low deposition site in Ireland (Clara bog) in August 1999. The subsequent processing of the *Sphagnum* and the manufacturing of the litterbags proceeded as in experiment I; *Eriophorum* litter was cut in 2 cm fragments and treated the same as *Sphagnum* thereafter.

In December 1999 (Table 7.1), litterbags containing stems of *Sphagnum* and leaf litter of *Eriophorum* were incubated around 10 poles in low hummock vegetation of *S. papillosum* (Bargerveen).

In August 2001, samples of *S. magellanicum*, *S. papillosum* (1–2 cm stem) and *E. angustifolium* (leaf litter) were collected in Ireland and the Netherlands to measure N, P and K concentrations in plant materials from sites contrasting in N deposition.



Pole with stainless steel wires leading to the burrowed litterbags.

Experiment IV

This experiment was established to study the effect of inorganic N concentration in the incubation environment on mass loss. For our litterbags, we used part of the same litters as in experiment III: Dutch *S. papillosum, S. fallax* and *E. angustifolium* litters and Irish *S. fallax* (Table 7.1). The *Sphagnum* and *Eriophorum* litters were treated the same as in the previous experiments, but incubation proceeded slightly differently.

In December 1999 (Table 7.1), the litterbags were incubated at 10–15 cm depth in containers with bog vegetation and kept under a roof. The containers were part of an ongoing experiment in which *S. magellanicum* vegetation had been subjected to a simulated N deposition of 0, 40 and 80 kg N ha^{-1} yr^{-1}. For the year that the litterbags were incubated, the inorganic N concentration of the soil pore water at 10–15 cm depth differed significantly between the 0

kg N treatment on the one hand, and the 40 and 80 kg N treatments on the other hand (chapter 3).

Contrary to the other experiments, we had five instead of ten replicates for each treatment and/or place of incubation combination.

Measurements

At the end of each incubation year the harvested litterbags were oven-dried for at least 96 hours at 30°C. Ingrown roots were removed before weighing the contents of the litterbags, and then the plant material was oven-dried again for 48 hrs at 70°C. Subsequently, it was stored in an airtight bag at room temperature until chemical analyses were made. Additional material (20 samples for each species) was used to determine the weight ratio between litter dried at 30°C and litter dried at 70°C. The ratio was the same for all species and did not differ between young or old stem parts of *Sphagnum* (T_{70} = 0.92 * T_{30}; R^2 = 0.999 linear regression).

Mass loss (%) after one year of incubation was calculated for all experiments and expressed as a percentage of the weight before incubation:

$$\text{Mass loss} = ((W30_{t=0} - W30_{t=1})/ W30_{t=0}) * 100$$

$W30_{t=0}$ refers to the weight of the contents of a litterbag before incubation dried at 30 °C and $W30_{t=1}$ refers to the weight of the same contents after one year (365 days) incubation in the field.

N loss (mg gDW^{-1} yr^{-1}) from the litterbags was calculated for experiments I-III and treated as an indicator for N mineralisation:

$$\text{N loss} = (([N]_{t=0} * W30_{t=0}) - ([N]_{t=1} * W30_{t=1}))* W70/W30$$

$[N]_{t=0}$ refers to the N concentration of the plant material in the non-incubated litterbags and $[N]_{t=1}$ to the N concentration of the plant material left in the litterbags after one year (365 days) incubation in the field. W70 = the weight of plant material dried at 70 °C.

Chemical analysis

The dried plant material (70 °C) was pulverised and the C and N concentrations were measured using an elemental analyser (Fisions Instruments EA 1108, Milan Italy). Data on C and N concentrations were corrected for water and ash content, both of which were determined from sub-samples after subsequent drying at 105 °C and igniting at 550 °C. For N, P and K analyses, dried and pulverised material was digested with sulphuric acid, salicylic acid, hydrogen peroxide and selenium. Subsequently, the N and

P concentrations were measured colorimetrically, using a continuous flow analyser (SKALAR SAN plus system, the Netherlands). K was measured by flame atomic emission spectroscopy (AES).

Data analysis

Data were tested for normality and equality of variance and, when necessary, were $\log_e$-transformed prior to analysis. All analyses were conducted using the SPSS statistical package for Windows (10.0). Differences within factors were analysed with a post-hoc Tuckey test.

The factors affecting mass loss were tested for each experiment separately with variations of the same multi-factor ANOVA model. For experiment I, we used stem part and pre-treatment as fixed factors and block structure (block) as a random factor. In experiment II, an ANOVA was used with species, stem part and place of incubation as fixed factors and block as a random factor, nested within place of incubation. For experiment III, we included country, species and stem part as fixed factor with block as a random factor. For experiment IV we chose an ANOVA with N treatment and species as fixed factors. Owing to significant interactions between species and stem part, and species and country for experiments II and III, we also performed some extra 2-way and 1-way ANOVA's for these experiments to distinguish between the separate effects.

To resolve whether mass loss could be related to tissue N:C quotient, we pooled the data from the first three experiments, and tested the relationship for each species separately. For this test we did not include data from experiment IV, because in this experiment litterbags were not incubated in the field. To distinguish effects of experiment, pre-treatment and country where the litter had been collected from effects of tissue quality, we first made an indicator, separately numbering each type of material differing in these three factors. We used an ANCOVA with this indicator and stem part as fixed factors and N:C quotient of the plant material before incubation as a co-variable.

To investigate to what extent N loss from the litters could be explained by its N:C quotient before incubation, we performed an ANCOVA for each species separately, with stem part and the 'Experiment–Pre-treatment–Country' indicator as fixed factors and the N:C quotient as a co-variable. To test whether species differed in N loss per unit N, we used a 3-way ANOVA with species, plant part and the 'Experiment–Pre-treatment–Country' indicator as fixed factors.

Table 7.1 General information on the different experiments (Exp) discussed in this study. Sm = *Sphagnum magellanicum*, Sp = *S. papillosum*, Sc = *S. cuspidatum*, Sf = *S. fallax*, Ea = *Eriophorum angustifolium*, G = green or red stem parts, B = brown stem parts or leaf litter. Hum = hummock, hol. = hollow. The experiments lasted for 1 year.

Exp	*n*	Species	Colour	Country	Collected	Pre-treatment	Start	Incubation
I	10	Sm	G & B	NL	Sep-98	C, N, CO_2	Jan-99	Sm lawn
II	10	Sm,Sp,Sc,Sf	G & B	Ire	Sep-98	none	May-99	Sp hum. & Sc hol.
III	10	Sp, Sf	G & B	NL & Ire	Aug-99	none	Nov-99	Sp hummock
III	10	Ea	B	NL& Ire	Aug-99	none	Nov-99	Sp hummock
IV	5	Sp	G	NL	Aug-99	none	Dec-99	Sm mesocosm
IV	5	Sf	G	NL & Ire	Aug-99	none	Dec-99	Sm mesocosm
IV	5	Ea	B	NL	Aug-99	none	Dec-99	Sm mesocosm

Results

Mass loss

Experiment I

N-enriched *S. magellanicum* tissue had a higher mass-loss than untreated material, which was the same for both green and brown stem parts (Figure 7.1). Enrichment with CO_2 did not lead to the expected reduction in mass loss, but seemed to either increase it or have no effect; the CO_2-enriched green stem parts seemed to decay at a faster, albeit not significant, rate than the untreated material, whereas mass loss from the CO_2-enriched brown stem parts did not differ from the untreated material.

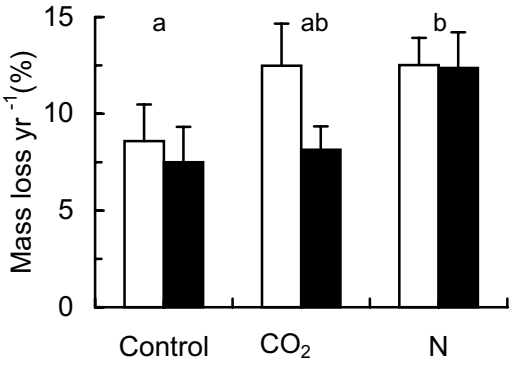


Figure 7.1 Experiment I: mass loss of *Sphagnum magellanicum* material expressed as percentage of the initial mass (means + 1SE). Control, CO_2 (560 ppmv CO_2) and N (+ 50 kg N ha $^{-1}$ yr $^{-1}$) refer to the 3 years of experimental treatments that the *Sphagnum* had received prior to incubation (Heijmans *et al.* 2001a). White and black bars indicate red and brown stem parts, respectively. Different letters above the bars indicate significant differences between the pre-treated litters (2-way ANOVA, effect pre-treatment n = 20: F = 5.475, P = 0.008, effect block n = 6: F = 4.456, P = 0.001, only significant P < 0.05 effects are shown).

Experiment II

Species (F = 81.781, P < 0.001) and stem part (F = 171.709, P < 0.001) both affected mass loss, whereas position of incubation had no effect (Figure 7.2). We also found a significant interaction between species and stem part (F = 22.678, P < 0.001), which made it necessary to analyse the species effects for green and brown parts separately. Green stem parts of the two hummock species, *S. magellanicum* and *S. papillosum*, decayed at a slower rate than those of the two hollow species, *S. cuspidatum* and *S. fallax*. In turn, the latter decayed faster than *S. cuspidatum*.

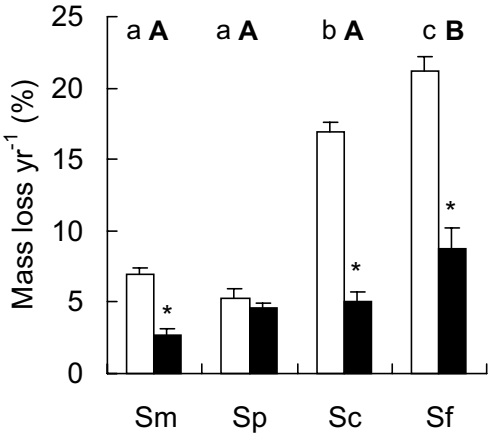


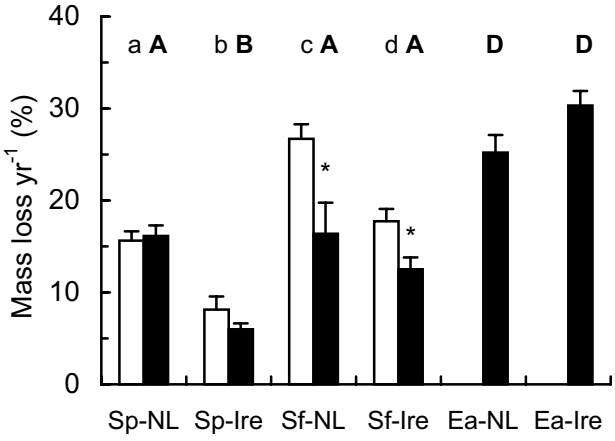
Figure 7.2 Experiment II: mass loss of *Sphagnum magellanicum* (Sm), *S. papillosum* (Sp), *S. cuspidatum* (Sc) and *S. fallax* (Sf) material expressed as percentage of the initial mass (means + 1SE). Light and dark bars indicate red/green and brown stem parts respectively, data for both incubation environments were pooled. Different letters above bars denote differences between species, with low case referring to green stem parts and capitals referring to brown stem parts. * indicates differences between stem parts. Green stem parts: species effect: F = 146.505, P < 0.001; Brown stem parts: species effect: F = 11.580, P < 0.001 (n = 20, 2-way ANOVA, only P < 0.10

The differences in mass loss between the species mostly disappeared when decay rates of the older, brown stem parts were considered; only *S. fallax* decayed at a significantly higher rate than the other species. Brown stem parts of *S. magellanicum, S. cuspidatum* and *S. fallax* decayed at approximately half the rate of green stem parts; for *S. papillosum* we found no such disparity (Figure 7.2).

Experiment III

We found significant effects of country (F = 29.076, P < 0.001), species (F = 81.781, P < 0.001) and stem part (F = 171.709, P < 0.001) on mass loss, as well as from interactions between country and species (F = 14.677, P < 0.001) and between species and plant part (F = 22.678, P < 0.001). In regard to these interactions, we analysed the effects of species and country for green and brown stem parts separately.

Figure 7.3 Experiment III: mass loss of *Sphagnum papillosum* (Sp), *S. fallax* (Sf) and *Eriophorum angustifolium* expressed as percentage of the initial mass (means + 1SE). NL (the Netherlands) and Ire (Ireland) refer to litter provenance. White & black bars indicate green & brown stem parts/leaf litter, respectively. Different letters above bars denote differences between species; low case = green & capitals = brown stem parts. * differences between stem parts. Green stem parts: country effect F = 96.252, P < 0.001; species effect F = 61.870, P < 0.001 (n = 20, 2-way ANOVA). Brown litter Ire: species F = 90.937, P < 0.001, NL: species F = 3.562, P = 0.061. *S. papillosum*: country F = 97.995, P < 0.001 (n = 10, 1-way ANOVA, only P < 0.10 effects are shown).



For both *S. papillosum* and *S. fallax*, green stem parts collected at a high deposition site decayed faster than those from a low deposition site (Figure 7.3), and, regardless of country, green stem parts of *S. papillosum* decayed at a slower rate than those of *S. fallax*, mirroring the species effect we found in experiment II.

For brown stem parts, the effects of species (F = 39.997, P < 0.001) and country (F = 23.587, P < 0.001) were slightly different and showed a significant interaction (F = 10.829, P = 0.002). *S. papillosum* collected at a high deposition site decayed at a higher rate than stem parts from a low deposition site, but we failed to find such a country effect for *S. fallax* and *E. angustifolium* (Figure 7.3). Differences between species were most pronounced for material from the low deposition site: mass loss differed between all three species with *E. angustifolium* leaf litter decaying fastest. For plant material collected at a high deposition site, however, we only found a marginal difference between the species, with *E. angustifolium* decaying at a slightly higher rate than *S. papillosum* (Figure 7.3).

When we compared decay of brown stem parts to that of green stem parts, we found a similar species effect as in experiment II, irrespective of country: mass loss from brown stem parts was lower than that for green stem parts for *S. fallax* and did not differ for *S. papillosum*.

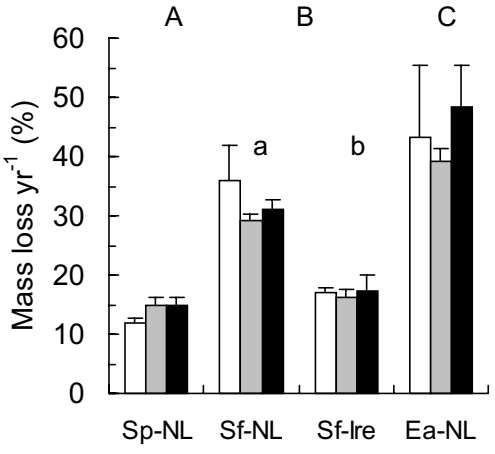


Figure 7.4 Experiment IV: mass loss of green stem parts of *Sphagnum papillosum* (Sp) and *S. fallax* (Sf) and leaf litter of *Eriophorum angustifolium* expressed as percentage of initial mass (means + 1SE). NL (the Netherlands) and Ire (Ireland) refer to litter provenance. White (0 kg N ha $^{-1}$ yr $^{-1}$), grey (40 kg N ha $^{-1}$ yr $^{-1}$) and black (80 kg N ha $^{-1}$ yr $^{-1}$) bars indicate the treatments that the incubation environments were subjected to. Different capitals above bars indicate differences between species, low case letters indicate a country effect within a species (3-way ANOVA, effect species n = 30,15: F = 40.167, P = 0.000, effect country(species) n = 15: F = 21.271, P = 0.000, only P < 0.10 effects are shown).

Experiment IV

N concentration of the incubation environment did not affect mass loss (Figure 7.4), regardless of litter type. We did find an effect of species on mass loss, which was similar to the effects described for the green stem parts in Experiment III*. E. angustifolium* leaf litter had the highest rate of decay, followed by green stem parts of *S. fallax* collected at a high deposition site. Green stem parts of *S. papillosum* collected at the same location had the lowest mass loss, and decayed at approximately the same rate as *S. fallax* from a low deposition site (Figure 7.4).

Litter quality, mass loss and N loss

The contrasting levels of N deposition between the low and high deposition sites resulted in differences in litter quality; litter from the high deposition site turned out to have a consistently higher N concentration than material from the low deposition site (Table 7.2), whereas P concentrations were similar. Irish *E. angustifolium* had a slightly lower K concentration.

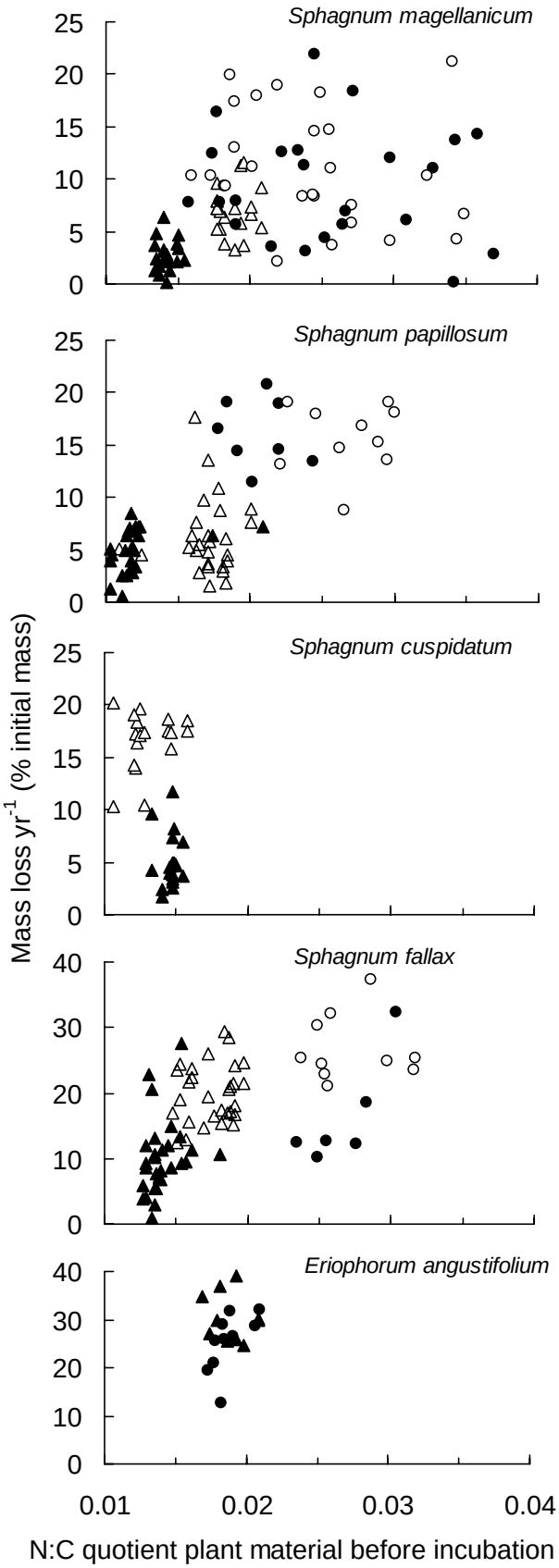
Table 7.2 The N, P and K concentrations (means ± 1SE) of the 1-2 cm stem fractions of *Sphagnum magellanicum* (Sm) and *S. papillosum* (Sp) and the litter of *Eriophorum angustifolium* (Ea) for the Netherlands and Ireland. Different letters indicate significant differences between countries (Students t-test, n = 4-15, $P < 0.05$).

	N (mg g^{-1})		P (mg g^{-1})		K (mg g^{-1})	
	NL	Ire	NL	Ire	NL	Ire
Sm	13.84 ± 1.11[a]	8.44 ± 0.75[b]	0.28 ± 0.03[a]	0.30 ± 0.06[a]	3.77 ± 0.02[a]	5.62 ± 1.63[a]
Sp	12.08 ± 0.57[a]	5.96 ± 1.51[b]	0.31 ± 0.02[a]	0.28 ± 0.02[a]	2.95 ± 0.23[a]	2.91 ± 0.28[a]
Ea	10.42 ± 0.94[a]	7.31 ± 0.72[b]	0.30 ± 0.07[a]	0.16 ± 0.02[a]	3.67 ± 0.92[a]	1.39 ± 0.18[b]

Although the N:C quotient of the litter before incubation was not the main factor explaining mass loss for the species studied, we did find a strong positive relationship between the N:C quotient and mass loss for both *S. magellanicum* and *S. fallax* (Figure 7.5, Table 7.3). The main factors explaining mass loss for most species, however, were stem part and experiment-related factors, such as country and pre-treatment (Table 7.3).

Table 7.3 Factors and co-variables that affected ($P < 0.10$) mass loss from litterbags per species after one year. Data on experiments (exp) I, II and III were pooled. E.Pt.C refers to combined effects of experiment (E), pre-treatment (Pt) or country (C).

					ANCOVA model	
Species	Sign effects	df	*F*	*P*	Co-variables	Factors
S.mag	N:C	1	8.101	0.006	N:C	Stem part, E.Pt.
	Stem part	1	12.691	0.001		
	E.Pt.	3	18.350	0.000		
	Stempart * E.Pt.	3	5.167	0.003		
S.pap	E.C	2	15.358	0.000	N:C	Stem part, E.C
S.fal	N:C	1	3.974	0.050	N:C	Stem part, E.C
	Stem part	1	25.126	0.000		
	Stem part * E.C	2	3.107	0.051		
S.cus	Stem part	1	128.562	0.000	N:C	Stem part
E.ang	Country	1	3.969	0.063	N:C	Country



In contrast, the N:C quotient of the litter before incubation was the main factor explaining N loss from litter for all species studied (Figure 7.6, Table 7.4). The N:C quotient from which net N release, or mineralisation, was observed for the first time (the critical N:C quotient) had a minimum of about 0.015 (C:N quotient of 67) for all species. N loss per unit of N was highest for *S. fallax*; N loss for the other species, *S. magellanicum, S. papillosum, S. cuspidatum* and *E. angustifolium*, did not differ significantly (species effect: F = 3.574, P = 0.015, 3-way ANOVA).

Figure 7.5 Experiments I-III: relationship between the N:C quotient of the litters before incubation and mass loss for each species. Triangles denote litters from a low deposition site, circles denote litters from a high deposition site. Open symbols refer to green stem parts and filled symbols to brown stem parts or leaf litter. For statistics see Table 7.3.

Discussion

Relationship N:C quotient and mass loss

Our results for *S. magellanicum* and *S. fallax* supported our main hypothesis that mass loss, and thus C release, are affected by litter N:C quotient (Figure 7.5, Table 7.3). The distinct differences in mass loss between the stem parts of *S. papillosum* from a low and a high deposition site (Figures 7.3, 7.4 and 7.5), together with the finding that only the N concentration, not those of P or K, differed between these litters (Figure 7.5, Table 7.2) suggest that a similar effect may apply for this species as well. The reason that we failed to find such an N effect (Table 7.3) for *S. papillosum* may be linked to the necessity of including country as a factor in our general model (Table 7.3), thus limiting the range in N concentrations over which the relationship between mass loss and N concentration could be tested. The narrow range in N:C quotients of the *S. cuspidatum* and *E. angustifolium* litters we used (Figure 7.5, Table 7.3) might likewise explain the absence of an N effect for these species. Such a dependency on the range of N:C quotients studied could account for why only few authors (Coulson and Butterfield 1978, Hogg *et al.* 1994) report a relationship between the N litter concentration and mass loss, whereas most do not (e.g. Bartsch and Moore 1985, Damman 1988, Hoosbeek *et al.* 2002).

Table 7.4 Factors and co-variables that affected ($P < 0.10$) N loss from litterbags per species after one year. Data on experiments (exp) I, II and III were pooled. E.Pt.C refers to combined effects of experiment (E), pre-treatment (Pt) or country (C).

					ANCOVA model	
Species	Sign. effects	df	F	P	Co-variable	Factors
S. mag	N:C	1	142.716	0.000	N:C	Stem part,
	E.Pt	3	6.239	0.001		E.Pt
S. pap	N:C	1	129.174	0.000	N:C	Stem part, E.C
	Stem part	1	10.167	0.002		
	E.C	2	88.495	0.000		
	Stem part* E.C	1	10.631	0.000		
S.fal	N:C	1	77.197	0.000	N:C	Stem part, E.C
	Stem part	1	12.059	0.001		
	E.C	2	31.114	0.000		
	Stem part * E.C	1	6.471	0.003		
S. cus	N:C	1	6.324	0.017	N:C	Stem part
	Stem part	1	3.484	0.071		
E. ang	N:C	1	31.747	0.000	N:C	Country
	Country	1	10.128	0.005		

The higher decomposition rate of *Sphagnum* subjected to a high N supply could be a result of the higher tissue N concentration itself, making the material more attractive to the microbial community. When faced with a high N supply, *Sphagnum* stores a considerable part of the N surplus in free amino acids (e.g. Rudolph *et al.* 1993, Nordin and Gunnarsson 2001, chapter 5). As these compounds are easily degradable and relatively mobile, they are likely to either leach from the cells or become respired soon after fresh material is incubated; this might explain part of the higher mass loss of the N-enriched green stem parts (Figures 7.1 and 7.3). However, it does not explain why the N effect is sustained in the older, brown material as measured for N-enriched *S. magellanicum* (Figure 7.1) and *S. papillosum* (Figure 7.3), nor why the difference in the rate of decay between brown stem parts of *S. papillosum* and *S. fallax* seems to be diminished by N (Figure 7.3). An additional possibility may be that the high N supply decreased the concentration or interfered with the function of some organic compound responsible for decay resistance.

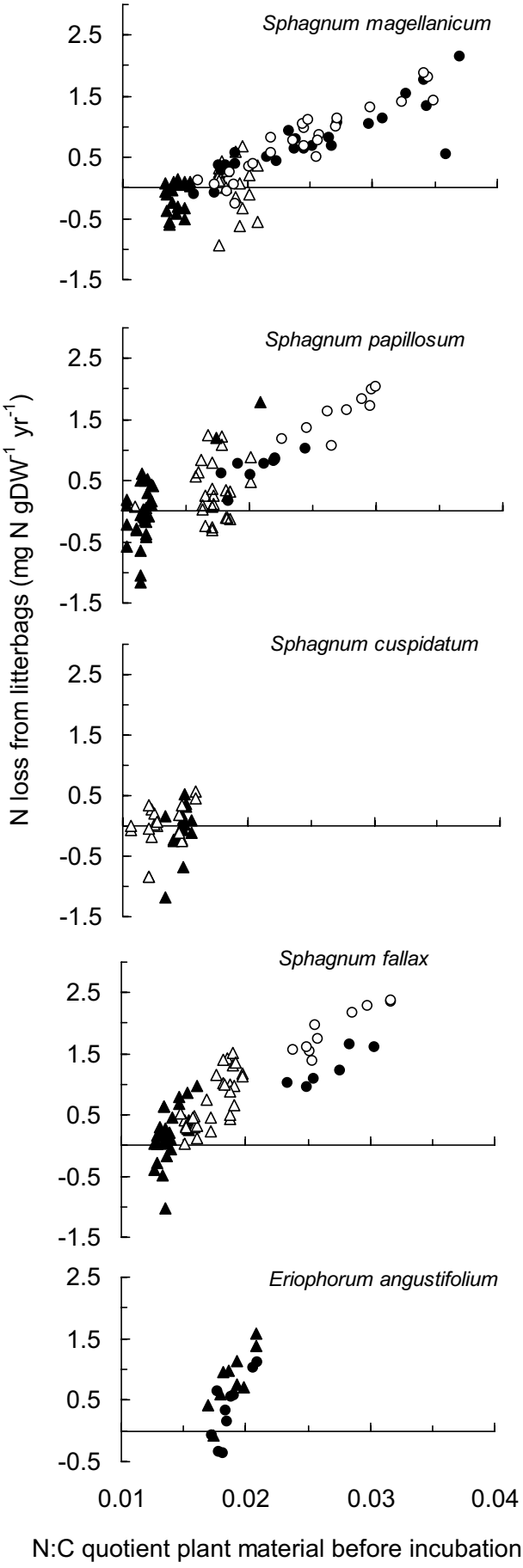


Figure 7.6 Experiments I-III: relationship between the N:C quotient of the litters before incubation and N loss for each species. Triangles denote litters from a low deposition site, circles denote litters from a high deposition site. Open symbols refer to green stem parts and filled symbols to brown stem parts or leaf litter. For statistics see Table 7.4.

The latter is supported by our failure (Figure 7.4), and that of others (Clymo 1965, Rochefort *et al.* 1990), to find a relationship between decay and the N concentration in the incubation environment. Since, if N would limit the microbial or fungal community, and thus decay, one would expect a higher N concentration in the incubation environment to stimulate decay. *Sphagnum* is known to produce a whole suite of compounds slowing down decay, some of which are associated with the cell wall (Verhoeven and Liefveld 1997, Børsheim *et al.* 2001). To what extent elevated N deposition affects the concentrations of these compounds in *Sphagnum* is unknown, although there is evidence from other species that an increase in N supply can either suppress the production of carbon-based secondary compounds or result in a dilution of these compounds, owing to an increase in growth (Koricheva *et al.* 1998, Hamilton *et al.* 2001).

The unanticipated increase in decomposition rate of *S. magellanicum* (Figure 7.1), previously subjected to an elevated CO_2 supply for three years (Heijmans *et al.* 2001a), indicates that the high C content of this material was probably the result of an accumulation of easily degradable compounds such as soluble sugars (Van der Heijden *et al.* 2000). The latter would explain why the brown stem parts decomposed at the same rate as the control material, whereas the green stem parts seemed to decompose faster.

Other factors affecting mass loss

We anticipated that hummock species would decay at a slower rate than hollow species, as has been shown before by Lütt (1992) and Johnson and Damman (1993). Although this assumption was proven right by our results for green stem parts (Figures 7.2, 7.3 and 7.4), our results for brown stem parts (Figures 7.2 and 7.3) indicate that species differences in the later stages of decay may be less distinct than supposed, something earlier suggested by Hogg (1993).

As expected, the brown stem parts generally decayed at a slower rate than the green stem parts (Figures 7.2 and 7.3). This difference was affected by N, however (Figures 7.1 and 7.3, Table 7.4), as discussed under the previous caption. What we did not expect, and for which we have no explanation, is the similarity in decay between green and brown stem parts of *S. papillosum* (Figures 7.2 and 7.3). As we found a similar pattern for three separate litters, this anomalous response can hardly be attributed to chance.

Neither the position of incubation (Figure 7.2) nor the N concentration in the incubation environment (Figure 7.4) affected mass loss, which is in accordance with our hypothesis. However, since we did find a significant block effect in experiment I (Figure 7.1), and mass loss of the same species showed considerable variation between the separate experiments (Table 7.3), there must have been some minor effects of environment, such as temperature (Hobbie 1996, Chapman and Thurlow 1998) or moisture (Hiroki and Watanabe 1996) on mass loss.

Factors affecting N loss and implications

N loss did proceed as expected and was positively related to the N:C quotient of the litter (Figure 7.6, Table 7.4). Remarkably, this relationship was approximately the same for all species, be it *Sphagnum* or *Eriophorum*. The accelerated release of N from N-enriched litter in the early stages of decay shows that we cannot expect that the excess N present in litter will just be removed from the N cycle by long-term storage in peat (Malmer 1993). More N becomes available in the rhizosphere instead, and since at least part of the vascular plants in bogs is still limited by N (Berendse *et al.* 2001, Heijmans *et al.* 2001a, chapters 2 and 3), the enhanced N supply is likely to result in an increased cover of vascular plants. In turn, the latter may ultimately lead to the outshading of *Sphagnum* (e.g. Hogg *et al.* 1995, Berendse *et al.* 2001). In regard to the considerable evaporation of moist *Sphagnum* carpets (Heijmans *et al.* 2001b, Kellner 2001), it is also reasonable to assume that a higher N availability in the rhizosphere would result in an increased N flux from the rhizosphere to *Sphagnum*. Such an excess in N supply to *Sphagnum* could result in toxic effects (e.g. Baxter *et al.* 1992, Nordin and Gunnarsson 2000, chapter 5), make *Sphagnum* more susceptible to parasitic fungi, or encourage growth of epiphytic algae (Coker 1966, Rudolph and Voigt 1986, chapter 4).

Acknowledgements

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Chapter 8

General discussion

How N affects *Sphagnum*

Our results unambiguously showed that a simulated increase in N deposition depressed *Sphagnum* (chapters 2 and 3, Figure 8.1). How this decreased vitality came about, is not so straightforward, however, and can only be partly explained by increased shading of expanding vascular plants, and not at all by an increased metabolic effort to incorporate the excess N into N rich free amino acids, as was previously hypothesised (chapter 1).

It seems that we can distinguish two types of N effects. A direct toxic effect that seems to be linked to the N metabolism of *Sphagnum* (chapter 5), and an indirect effect brought about by intensified interactions with other organisms, such as vascular plants, epiphytic algae and fungi (chapters 2, 3 and 4). A considerable part of both types of effect is influenced by the amount of deposited N that *Sphagnum* can incorporate in its tissue and by the resulting tissue N concentration. As such, the impact of a high N supply is not so much determined by the level of N deposition *per se* than by the balance between the negative effects of N on the one hand and the supply of potentially growth-limiting factors such as water, P, CO_2, light and temperature on the other hand. The above is supported by the additive nature of N and P effects on *Sphagnum* (chapters 2 and 4). When P is added, the negative effect of N is tempered, *Sphagnum* growth is stimulated, the tissue N concentration decreases, the availability of N to other organisms is suppressed (chapter 2) and *Sphagnum* becomes less susceptible to parasitic fungi (chapter 4).

When we related *Sphagnum* production in the field to background wet N deposition, we observed an initial increase in production with wet deposition up to 15 kg N ha^{-1} yr^{-1} (Table 8.1). From then on, the average production seemed to decrease, even if potential production was still high. Although it is difficult to estimate the resultant total N deposition, it is reasonable to assume that when wet deposition is *c.* 15 kg N ha^{-1} yr^{-1}, total deposition will vary between 20 and 30 kg N ha^{-1} yr^{-1} (Bobbink *et al.* 1992). This range corresponds remarkably well with the N supply that, under optimal growth conditions, has been shown to induce maximum biomass production of *S. fuscum* (30 kg N ha^{-1} yr^{-1}, Jauhianen *et al.* 1994) and *S. magellanicum* (24 kg N ha^{-1} yr^{-1}, Rudolph and Voigt 1986). The above seems to confirm our opinion that *Sphagnum* can cope fairly well with an increase in N deposition,

as long as the moss does not face too much N at once (chapter 5), the overall growing conditions are near optimal and it does not suffer from negative interactions with other organisms (chapter 4).

A high N deposition does not seem to alter the competitive balance between the *Sphagnum* species studied. It seems that only in combination with a high P supply, *S. fallax* can grow to dominate (chapter 6).

Table 8.1 The average *Sphagnum* production (g dry mass m^{-2} yr^{-1}) for bogs subject to different background N deposition. Minimum and maximum production values are in brackets. n indicates the number of sources the data were derived from. The literature sources used have been marked with * in the reference list. Our own data concerning the production in the control plots of our field fertilisation experiment (chapter 2) were included in the two last columns; data for different sites were treated as separate sources.

	Wet deposition (kg N ha^{-1} yr^{-1})							
Section	**0 – 5**	*n*	**5 – 10**	*n*	**10 – 15**	*n*	**15 –**	*n*
Sphagnum	174 (35-340)	3	189 (55-615)	10	274 (73-725)	6	163 (0-348)	3
Acutifolia	181 (42-492)	6	150 (55-330)	8	360 (332-454)	3	-	-
Cuspidata	132 (45-501)	4	223 (35-600)	4	253 (0-463)	7	92 (0-233)	3

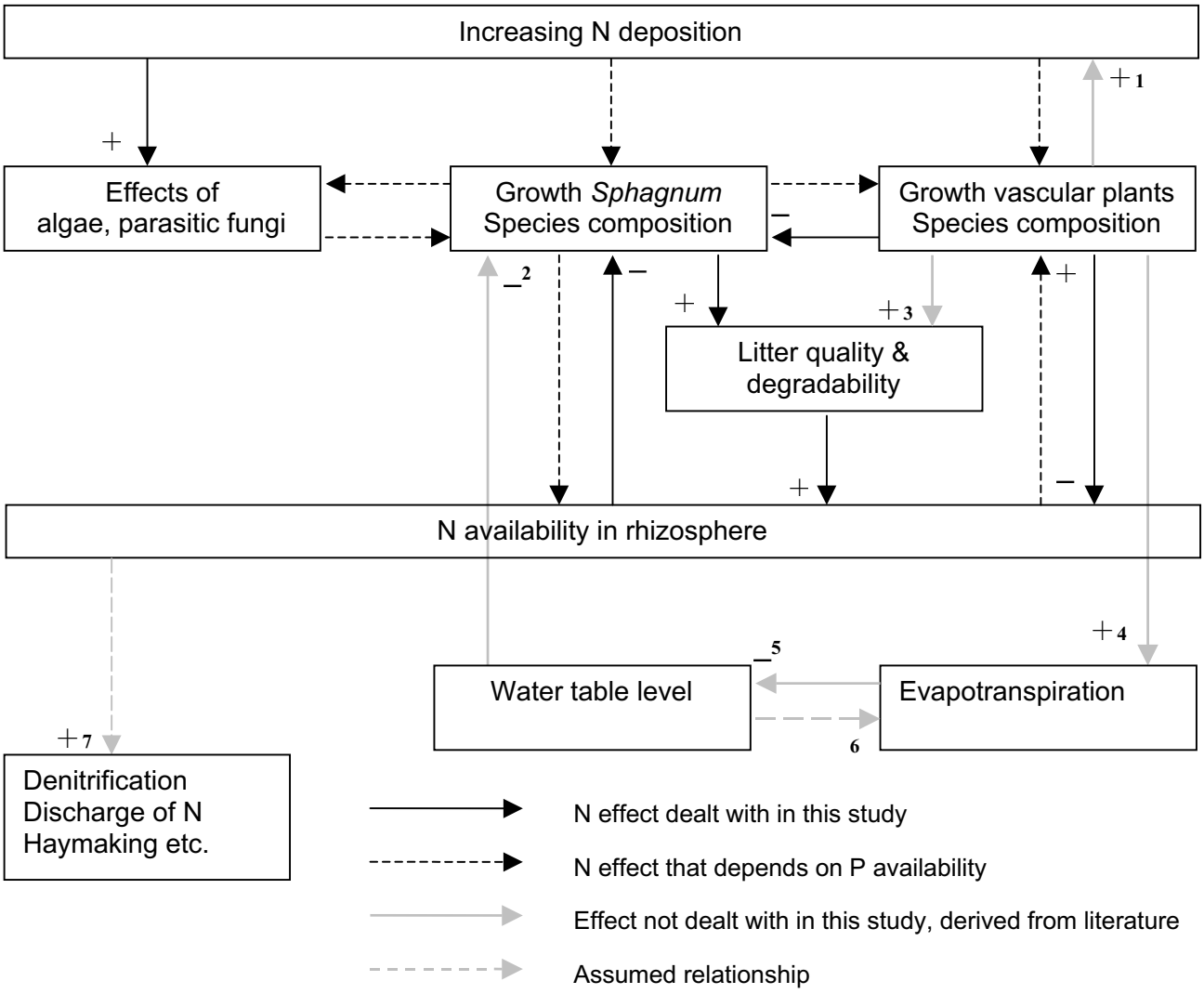


Figure 8.1 Schematic summary of the effects of an increasing N supply on bog vegetation dominated by *Sphagnum.* Numbers next to arrows refer to the notes below.

Notes

1. When vascular plants expand, the deposition surface increases, and as a result, the canopy captures more atmospheric N (e.g. Heil et al. 1988).
2. A decrease in the water table reduces water availability to *Sphagnum*, and thus may suppress *Sphagnum* production (e.g. Hayward and Clymo 1982, Titus *et al.* 1983).
3. Vascular plant litter decomposes more readily than *Sphagnum* litter (Coulson and Butterfield 1978, Hobbie 1996).
4. A dense vascular plant cover intercepts more precipitation than a sparse cover (see also chapter 2), resulting in an increased evaporation from the leaf surface. Moreover, a denser cover may also lead to a higher net transpiration in dry periods (Schouwenaars 1993, Spieksma *et al.* 1997, Kellner 2001).
5. An increase in evapotranspiration may affect the water balance of bogs, resulting in more pronounced fluctuations in the water table, and on average, a lower water table in summer (Schouwenaars 1993).
6. As a marked decrease in the water table reduces *Sphagnum* evaporation (Overbeck and Happach 1957, Williams and Flanagan 1996), and does either not affect or suppress transpiration of vascular plants (Kentaro *et al.* 1999), the net result is probably a decrease in evapotranspiration (Van der Schaaf 1999).
7. Nitrogen losses from the system may be brought about by denitrification (e.g. Urban *et al.* 1988), removal with run-off, or removal with plant material after haymaking or sod cutting (Koerselman and Verhoeven 1992).

How N affects vascular plants

Total vascular plant cover increased with N deposition (chapters 2 and 3, Figure 8.1) indicating that vascular plant growth is still limited by N at sites with intermediate to high N deposition, as was hypothesised (chapter 1). The response of the individual vascular plant species, however, suggests that at least some of the more shallow rooting species, such as *V. oxycoccus*, are limited by P (chapter 2, Table 8.2) or, possibly, by potassium (Hoosbeek *et al.* 2002). Nevertheless, as long as there are some sub-dominant to dominant vascular plant species that are still limited by N, an increase in N supply may lead to an increase in total vascular plant cover and thus deteriorate growth conditions for *Sphagnum* (chapter 2).

Table 8.2 Nutrient limitation of individual vascular plant species per site. The data refer to a field fertilisation experiment with N and P (chapter 2). N or P were assumed to be limiting growth, when the cover of the individual species increased after adding these nutrients (RM-ANOVA, n = 10).

Site	Species	N limited	P limited
Clara bog	*Vaccinium oxycoccus*	+	+
	Erica tetralix/Calluna vulgaris	+	0
	Eriophorum angustifolium	0	0
Reigersplas	*Vaccinium oxycoccus*	0	+
	Erica tetralix/Rhynchospora alba/Andromeda polifolia	+	0
	Molinia caerulea	(+)	0
	Eriophorum angustifolium	0	0
Bargerveen-Sp	*Vaccinium oxycoccus*	0	+
	Erica tetralix/Calluna vulgaris	+	0
	Molinia caerulea	(+)	0
	Eriophorum angustifolium	0	0
Bargerveen-Sc	*Molinia caerulea*	(+)	+
	Eriophorum angustifolium	0	0
Harkeven	*Vaccinium oxycoccus*	(+)	(+)
	Eriophorum vaginatum/Empetrum nigrum	(+)	0
Rundeven	*Vaccinium oxycoccus*	+	0

According to theory (Malmer *et al.* 1994, Lamers *et al.* 2000, Berendse *et al.* 2001) and our hypothesis (chapter 1), an increase in atmospheric N supply enhanced the availability of N in the rhizosphere (chapter 2 and 3, Figure 8.1). Vascular plants seemed to profit from this enrichment, as indicated by enhanced growth or increased tissue N concentration (chapters 2 and 3). It is likely, that vascular plants also profited directly from the applied N, by taking it up through their leaves; we measured lower atmospheric N inputs below the canopy than above the canopy (chapter 2).

Although the experiments presented in this thesis were lengthy compared to most previous studies (chapter 1), they are too short to accurately predict long term effects of N. Nevertheless, the combination of N-limited vascular plant growth (chapter 2, Table 8.2) and the accelerated release of N from N-enriched litter (chapter 7, Figure 8.1) do not bode well for the prospect of *Sphagnum* vegetation subject to high N deposition. Additionally, the influence

of stochastic effects, such as extreme climatic conditions (Aerts *et al.* 2001) or infection of *Sphagnum* by *T. palustris* (chapter 4) may gain in importance and further disrupt the increasingly delicate balance between *Sphagnum* and vascular plants.

As vascular plants expand, it is likely that evapotranspiration increases concomitantly (e.g. Heijmans *et al.* 2001a, Figure 8.1). Ultimately, the higher water losses may affect the water balance of bogs and initiate the change of bogs into wet heaths (Risager 1998) or forest (Ohlson *et al.* 2001).

Restoration perspectives

Can bogs continue to exist in areas subject to high N deposition? The answer to this question must be a tentative yes, since we still have raised bog vegetation in the Netherlands where N deposition has exceeded 30 kg N ha^{-1} yr^{-1} since the 1970s (e.g. Van Oene 1999). However, we cannot discount the strain N puts on the competitive balance between *Sphagnum* and vascular plants, or how a high N supply initiates positive feedback processes (Figure 8.1). In this regard, it is well possible that N deposition can render initially successful restoration efforts futile.

The production of *Sphagnum* and the role of this species as ecosystem engineer (Van Breemen 1995) seem the key to the continued existence of bog vegetation subject to high N deposition. When *Sphagnum* grows vigorously, it keeps down its own tissue N concentration, thus reducing disease incidence and severity (chapter 4). Furthermore, a high *Sphagnum* production diminishes the amount of N available to vascular plants. *Sphagnum* achieves the latter by absorbing atmospherically derived nutrients, tuning down decomposition processes through its badly decomposable litter and by creating a wet and acidic environment (e.g. Malmer *et al.* 1994, Van Breemen 1995). Excretion of presumed allopathic substances further adds to the severe climate for vascular plant growth (Johnson and Damman 1993, Verhoeven and Liefveld 1995). Additionally, a growing *Sphagnum* carpet forms an ever-changing substrate, on which expansion of epiphytic algae and establishment of vascular plants can be a difficult process (chapters 3 and 4).
When we would assume that (I) 10 mg g^{-1} (1% dry mass) is a sound target tissue N concentration for *Sphagnum* (chapters 4 and 7), that (II) relocation of N within *Sphagnum* is negligible (chapter 3), that (III) there are no major ecosystem disturbances, that (IV) the average *Sphagnum* production would be 250 g m^{-2} yr^{-1} (chapter 2, Table 8.1), and finally, that (V) *Sphagnum* cover is near continuous, then the *Sphagnum* layer in this virtual bog would be able to cope with a total N deposition of *c*. 25 kg N ha^{-1} yr^{-1}, keeping the N available to vascular plants minimal; a higher N load would require a higher average production. From the above, we infer it may be possible to circumvent an important part of the negative N effects by carefully choosing the sites where restoration measures are taken, and possibly, by additional management aimed at optimising overall growth conditions for *Sphagnum*.

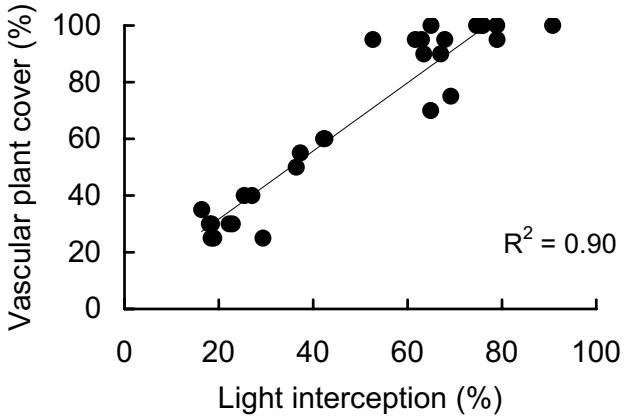
Below, we shall shortly elaborate on which factors should be taken into account when choosing sites for restoration measures.

The obvious way of dealing with the negative effects of a high N deposition is to continue on our way to reduce the emission of N compounds. As a marked reduction is not achievable in the short term, it is advisable to focus restoration efforts in areas with a relatively low background deposition. That a reduction in deposition may be effective in the short term, is confirmed by our finding that, once the source of enrichment is taken away, *Sphagnum* vegetation returns to its nutrient poor status within two years (chapter 3).

Water availability, and thus water table level, are crucial for a high *Sphagnum* production (Hayward and Clymo 1982, Titus *et al.* 1983). A water table of 5 to 10 cm below the moss surface yields the highest production of *S. papillosum* and *S. magellanicum* (Clymo 1973, Hayward and Clymo 1983), which are the dominant peat forming *Sphagnum* species in the Netherlands. Furthermore, a high and fairly constant water table tempers the rate of decomposition, and thus nutrient release, by lowering the soil temperature and reducing the depth of the oxic layer (Sikora *et al.* 1993, Belyea 1996, Koerselman *et al.* 1993).

As *Sphagnum* growth in the Netherlands is rather limited by P than by N (chapter 2, 3 and 6), the amount of available P co-determines potential production. At the same time however, P also limits growth of a number of vascular plant species (chapter 3, Table 8.1), and may influence the species-composition of the *Sphagnum* layer, by encouraging *S. fallax* (chapter 6). On account of the above, adding P to stimulate *Sphagnum* growth is not recommendable.

Figure 8.2 The relationship between light interception by the canopy and the estimated cover of vascular plants. Data were taken from Van Eekelen (unpublished), and were collected in the Bargerveen reserve in vegetation dominated by *Erica* in February 2001. Each data point was calculated from the average of two PAR measurements for an area of 1 x 1 m taken at *Sphagnum* surface with a light probe (2 cm x 1 m light sensitive area), corrected for the PAR outside the canopy (SunScan Canopy Analyses system, Delta –T Devices Ltd UK).



A continuous high supply of CO_2, derived from decomposing deeper peat (Lamers *et al.* 1999, Turetski *et al.* 1999, Smolders *et al.* 2001) or from run-off, seems to stimulate production of both hummock and hollow species. In this regard, the age, that is the stage of decay, of the underlying peat and the direction of superficial water flow should be taken into account (Tomassen, unpublished).

Density of vascular plants should not be or become too high. It has proven difficult to pinpoint from which density on vascular plants have an adverse effect on *Sphagnum*, because the effect of shading seems to depend on the overall growing conditions (Hayward and Clymo 1983, chapter 2). When the latter is favourable, light interception of more than *c*. 50% seems to depress *Sphagnum* growth (Hayward and Clymo 1983, chapter 3). For vegetation dominated by *Erica*, this value corresponds with an estimated cover of 70% (Figure 8.2).

We conclude that, despite the possibilities to get round part of the negative N effects, we must realise that the resilience of the bog ecosystem and the range of conditions under which *Sphagnum* bogs can survive, decrease with N deposition, and thus, are not infinite. The higher the N deposition level, the lower the number of potential restoration sites that will meet the requirements mentioned above, and the lower the capacity of the system to overcome disturbances, such as extremely dry summers. Therefore, the best future of *Sphagnum* bogs in the Netherlands undoubtedly lies in further reducing the emission of N compounds.

***V**accinium oxycoccus* berry on mixed vegetation of *Sphagnum magellanicum* and *S. papillosum*

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Summary

The research presented in this thesis had as its aim to investigate whether high nitrogen (N) deposition could effect *Sphagnum* bogs to such an extent, that initially successful restoration efforts in the Netherlands would be rendered futile. We give experimental evidence for and elaborate on the proposed mechanisms by which N affects intact bog vegetation. Furthermore, we paid special attention to interactions between species as well as to physiological effects of a high N supply on *Sphagnum*. We also tried to get an impression of long term N effects on bog vegetation by studying the impact of an elevated N supply on litter quality and decomposition rate.

To elucidate which nutrient, N or P, limits growth of *Sphagnum* and vascular plants in five *Sphagnum* bogs in the Netherlands subject to high N deposition, and one bog in Ireland subject to intermediate deposition (chapter 2), we conducted a 4-year fertilisation experiment with N and P. The effects of the applied N and P turned out to be additive in nature. Adding N increased the concentration of inorganic N in the rhizosphere, encouraged vascular plants to grow, and depressed *Sphagnum* height increment and production. Increased shading by vascular plants explained part of the negative N effects on *Sphagnum*. Adding P decreased the inorganic N concentration and increased the P concentration in the rhizosphere. Furthermore, P stimulated *Sphagnum* growth and *Sphagnum* production, whereas vascular plant growth remained largely unaffected. The results show that when *Sphagnum* production is stimulated, here by lifting the limitation by P, the negative effects of N are tempered and the capacity of *Sphagnum* to withhold N for other organisms increases.

To better understand the relationships between N deposition, *Sphagnum* growth, N availability in the rhizosphere and vascular plant growth, we manipulated the level of N deposition to mesocosms with intact bog vegetation (chapter 3). In addition, we wanted to test whether the establishment of *Betula* sp. and *Molinia caerulea*, would be improved by an increase in N availability and impaired by a decrease in N availability relative to field conditions. 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 interstitial water and in *Sphagnum* tissue decreased when N input ceased and increased when N input was doubled. Growth of *Molinia* 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, indicating a negative effect of shading when vascular plant cover is dense. We give evidence that N deposition encouraged vascular plants to grow by enhancing N availability in the rhizosphere. Moreover, our results suggest that these negative N effects are reversible in a short time span, once deposition is reduced.

In the fourth chapter we focussed on the negative effects of N on *Sphagnum*, and investigated how N affects the interactions between *Sphagnum*, epiphytic algae and a parasitic fungus that can induce necrosis in *Sphagnum*. We added 40 kg N ha^{-1} yr^{-1} or 3 kg P ha^{-1} yr^{-1} in a full factorial design at 4 field sites (chapter 2), and followed expansion of epiphytic algae and necrotic *Sphagnum*. In a greenhouse experiment we reinoculated *Sphagnum* to verify the identity of the fungus and its necrotic effect on *Sphagnum*. *Lyophyllum palustre* turned out to be responsible for the necrosis of *Sphagnum* in our experiments. Adding N induced complete necrosis of *Sphagnum cuspidatum* by *L. palustre*, whereas adding P decreased the area of necrotic tissue. Disease severity seemed to be related to the N concentration in the *Sphagnum* capitula. In *Sphagnum magellanicum* and *S. papillosum*, infection with *L. palustre* resulted in defoliation of stem sections. At all sites, N deposition stimulated the expansion of algae, reducing the photosynthetic area of *Sphagnum.* The density of the film of algae in the treatments receiving N was a function of the frequency of defoliated *Sphagnum* stems.
This study shows that field experiments in intact systems are important to fully appreciate the diverse ways in which excess of a previously limiting nutrient can unhinge the system.

To investigate whether the N-induced accumulation of N-rich free amino acids depresses *Sphagnum* growth, we tested the relationship between *Sphagnum* growth and the amount of nitrogen stored in free amino acids in a fertilisation experiment, imitating 3 levels of N deposition (chapter 5). *Sphagnum* growth was not correlated with the total N tissue concentration or with the concentration of individual or pooled free amino acids. The amount of N stored in free amino acids increased with deposition, although it lagged more and more behind the total N concentration, the latter pointing to the accumulation of unmeasured N compounds. It appears that N-induced growth inhibition of *Sphagnum* is a result of its slow physiological response to a sudden increase in N, rather than a result of toxic effects of a high concentration of amino N or total N in its tissue. We propose that when *Sphagnum* is exposed to a step increase of N, its N metabolism does not adapt fast enough to keep up with the enhanced uptake rate. This imbalance between N uptake and assimilation may lead to an accumulation of toxic NH_4^+ in the cell and a subsequent reduction in growth.

After having dealt with the effects of N on *Sphagnum* in general, we proceeded (chapter 6) with the consequences of nutrient enrichment on the competitive balance between *Sphagnum* species. We studied the effects of N and P on the expansion of *S. fallax* and *S. flexuosum* in bogs. We related historical census data of *S. fallax, S. flexuosum* and four of their accompanying species to changes in N deposition. Additionally, we conducted two fertilisation experiments with N and P; one at an intermediate (Irish) deposition site with *S. flexuosum* and one at two high deposition (Dutch) sites with *S. fallax*. Finally, we related existing data on capitulum N and P concentrations of *S. fallax* to its abundance in the field. A relative increase in observed frequency of *S. fallax* coincided with an historical increase in N deposition in the Netherlands. There was no indication that *S. fallax*

consistently outcompeted one of the other five *Sphagnum* species. The census data on *S. flexuosum* did not indicate a response to the historical increase in N deposition, but in the fertilisation experiment, the species expanded at the intermediate N deposition site when extra N was applied. In contrast, the expansion of *S. fallax* at the high deposition sites was limited by P. Plant nutrient concentrations suggested that when *S. fallax* can maintain a capitulum N concentration of 7 mg g^{-1} or higher and a P concentration of 0.7 mg g^{-1} or higher the species can grow to dominate. We conclude that *S. fallax* will gradually colonise an increasing number of new habitats in areas with a low, albeit increasing, N deposition, but will only grow to dominate when P supply is adequate. Then, the expansion of *S. fallax* may lead to ousting of the other *Sphagnum* species present.

In chapter 7 we present results from four separate experiments aimed at delineating the effects of litter N-enrichment, *Sphagnum* species, stem part of *Sphagnum* and place of incubation on decomposition rate and N release. We measured mass loss and N loss from litterbags incubated at 10-15 cm in the field for one year. Mass loss was positively related to the N:C quotient of the litter, but depended strongly on the range in N:C quotients observed; only a distinctive difference in N:C quotients affected mass loss. Although hummock species decayed at a slower rate than hollow species, the differences between the species became less pronounced for older, brown stem parts and for N-enriched litter. Brown stem parts decayed at a slower rate than green stem parts, except for *S. papillosum*. Neither position of incubation (low hummock or hollow), nor the inorganic N concentration of the incubation environment affected mass loss. N loss was mainly determined by, and positively related to, the N:C quotient of the litter; species and stem part had minor effects. Above a N:C quotient of *c*. 0.015, net N loss was observed for all species. We conclude that decomposition of *Sphagnum* is stimulated by N deposition. As the latter also affects litter N concentration and thus N release, we think that positive feedback through changing litter quality should be taken into account when modelling the effects of N deposition on *Sphagnum* peatlands and C sequestration in these systems.

Can the high N deposition in the Netherlands render initially successful restoration efforts futile? We certainly cannot discount the strain N puts on the competitive balance between *Sphagnum* and vascular plants, or how an increase in N deposition initiates positive feedback processes. However, the impact of a high N supply seems to depend on *Sphagnum* production, which is not so much determined by the level of N deposition *per se* than by the balance between the negative effects of N on the one hand and the supply of potentially growth-limiting factors such as water, P, CO_2, light and temperature on the other hand. When *Sphagnum* grows vigorously, it keeps down its own tissue N concentration, thus reducing disease incidence and severity. Furthermore, a high *Sphagnum* production diminishes the amount of N available to vascular plants. From this we infer that we may be able to circumvent an important part of the negative N effects described in this thesis by carefully choosing the sites where restoration measures are taken, and possibly, by additional management aimed at optimising overall growth conditions for *Sphagnum.* Nevertheless, we must realise that the resilience of

the ecosystem and the range of conditions under which *Sphagnum* bogs can survive, decreases with N deposition, and as such, are not infinite. The higher the N deposition level, the lower the number of potential restoration sites that will meet the requirements and the higher the vulnerability of the system to disturbances.

On account of the above we conclude, with the risk of being blatantly obvious, that the best way to preserve *Sphagnum* bogs in the Netherlands, is to continue on our way to reduce N deposition. As the potential production of *Sphagnum* seems to peak between 10 and 15 kg N ha^{-1} yr^{-1} wet deposition, this seems to be both a sensible and politically feasible range for a target deposition.

Samenvatting

Het proefschrift dat nu voor u ligt, heeft tot doel na te gaan in hoeverre hoogveen te lijden heeft van de aanzienlijke stikstofverrijking van de atmosfeer hier in Nederland. Omdat hoogveen behoort tot de voedselarmste ecosystemen in ons land, viel te verwachten dat een hoge stikstofbelasting een verstorend effect heeft op de vegetatiesamenstelling. In hoeverre dit een succesvol herstelbeheer van hoogveen in de weg zou kunnen staan, was echter nog grotendeels onduidelijk. Het uitgevoerde onderzoek richtte zich op de effecten van stikstof op veenmos zelf en op de interacties tussen veenmos en andere groepen organismen, zoals hogere planten, paddestoelen en algen. Om een idee te krijgen van de gevolgen voor de nutriëntenkringloop in hoogveen, onderzochten wij eveneens de invloed van stikstof op de afbraaksnelheid van dood veenmos.

In hoeverre stikstof de samenstelling van een vegetatie kan beïnvloeden, is afhankelijk van de mate waarin de groei van de afzonderlijke plantensoorten bepaald wordt door de stikstofbeschikbaarheid. Hiernaast speelt voor hoogvenen de groei van veenmos een belangrijke rol, omdat alle voedingsstoffen die vanuit de atmosfeer op een hoogveen terecht komen (depositie), eerst de veenmoslaag moeten passeren voordat ze door kruiden en grasachtigen opgenomen kunnen worden. Hoe hoger de veenmos productie, hoe meer voedingsstoffen het mos kan opnemen en hoe minder beschikbaar blijft voor deze hogere planten. Om te onderzoeken welke voedingsstof, stikstof of fosfor, de groei van veenmos en hogere planten in Nederland het meest bepaalt, werd een 4-jarig bemestingsexperiment opgezet in 5 venen in Drenthe en 1 veen in de Ierse Midlands (hoofdstuk 2). Dit Ierse veen diende als referentie voor gebieden met een lage achtergronddepositie (de depositie van stikstof in Ierland is ongeveer drie keer zo laag als in Nederland). Uit de resultaten bleek dat de afzonderlijke effecten van stikstof en fosfor additief van aard waren en geen interactie vertoonden. De gesimuleerde toename in stikstofdepositie had een verrijking van het oppervlakkige bodemwater met anorganische stikstof tot gevolg. Verder nam de bedekking van kruiden en grasachtigen toe en namen groei en productie van veenmos af. De intensere beschaduwing door hogere planten bleek slechts voor een klein deel verantwoordelijk voor de geobserveerde afname in veenmosgroei. Bemesting met fosfor leidde tot een daling van de anorganische stikstofconcentratie en tot een stijging van de anorganische fosforconcentratie in het bodemwater. Ook stimuleerde fosfor de groei van veenmos, terwijl de groei van hogere planten nauwelijks beïnvloed werd. De resultaten laten zien dat de negatieve effecten van stikstof verminderd kunnen worden door andere groeilimiterende factoren op te heffen.

Om de onderlinge samenhang tussen stikstofdepositie, de groei van veenmos, de concentratie van anorganische stikstof in het bodemwater en de groei van hogere planten beter te begrijpen, werden emmers veenvegetatie onder één dak geplaatst en vervolgens blootgesteld aan 3 niveaus van

stikstofdepositie: 0, 40[1] en 80 kg stikstof per hectare per jaar (hoofdstuk 3). In een deel van de emmers werden jonge scheuten van *Molinia caerulea* (pijpenstrootje) en kiemplanten van *Betula* sp. (berk, kruising tussen zachte en ruwe berk) geplaatst om uit te zoeken of deze soorten reageren op veranderingen in stikstofaanbod. Na 2 jaar bleek dat de stikstofconcentraties in zowel het bodemwater als in het veenmos waren afgenomen in de veentjes waarin de stikstofdepositie was teruggebracht. In de veentjes die een verdubbeling van het depositie niveau hadden ondergaan, waren beiden toegenomen. Ook bleek de groei van pijpenstrootje positief gecorreleerd te zijn met de concentratie anorganische stikstof in het bodemwater op 10-15 cm diepte. In het eerste jaar, leidde bemesting met stikstof tot een toename in de productie van pijpenstrootje en een hogere overlevingskans voor de kiemplanten van berk. Hoewel we geen duidelijke relatie vonden met de biomassa van de hogere planten, bleek de hoogtegroei van veenmos boven een beschaduwingsintensiteit van 53% af te nemen. De resultaten laten zien dat een verhoging van de stikstofdepositie de groei van hogere planten mede stimuleert door de beschikbaarheid van stikstof in het bodemwater te verhogen. Verder geven ze aan dat, indien de depositie wordt teruggebracht, de naijleffecten relatief gering zijn en het systeem op korte termijn terugkeert naar een voedselarme status.

In hoofdstuk 4 wordt een onderzoek beschreven naar de effecten van een hoge stikstofbelasting op de interacties tussen veenmos, epiphytische[2] algen en een op veenmos parasiterende schimmel. In een bemestingsexperiment met stikstof en fosfor (beschreven in hoofdstuk 2), volgden we op 4 locaties de infectieverschijnselen in het veenmos en de uitbreiding van epiphytische algen. Daarnaast voerden we een kasexperiment uit om de identiteit van de parasitische schimmel en haar effect op veenmos te verifiëren. Hiervoor behandelden we veenmos met een schimmelextract dat eerder uit geïnfecteerd mos was geïsoleerd. Uit dit laatste experiment bleek *Lyophyllum palustre*, ook wel bekend als *Tephrocybe palustris* (veenmosgrauwkop), inderdaad verantwoordelijk voor de waargenomen veenmossterfte (necrose). Uit het veldexperiment werd duidelijk dat stikstof de gevoeligheid van *Sphagnum cuspidatum* (waterveenmos) voor deze parasiet verhoogde, terwijl bemesting met fosfor deze gevoeligheid juist deed afnemen. De infectieintensiteit leek positief gecorreleerd met de stikstofconcentratie in het veenmos. Infectie met de veenmosgrauwkop leidde bij *Sphagnum papillosum* (wrattig veenmos) en *Sphagnum magellanicum* (hoogveenveenmos) niet tot volledige necrose, zoals bij waterveenmos, maar tot ontbladering van delen van de stengel. De groei van epiphytische algen werd op alle locaties sterk gestimuleerd door het toedienen van stikstof. Bovendien bleek de algenbedekking het dichtst op veenmos dat eerder door de veenmosgrauwkop aangetast was. Dit onderzoek laat zien hoe belangrijk veldexperimenten zijn om een indruk te krijgen van de uiteenlopende wijze waarop een overmatig aanbod van voedingsstoffen een ecosysteem kan beïnvloeden.

[1] 40 kg stikstof per hectare per jaar komt overeen met de gemiddelde stikstofdepositie in Nederland.
[2] Epiphytisch algen zijn algen die op het bladoppervlak groeien, hier blaadjes van veenmos

In het volgende hoofdstuk gaan we na of de door stikstoftoediening veroorzaakte afname in veenmosgroei in verband gebracht kan worden met de ophoping van vrije aminozuren. Voor dit onderzoek maakten we gebruik van veenmosweefsel dat afkomstig was uit het in hoofdstuk 3 beschreven kasexperiment. We konden geen verband vinden tussen de groei van veenmos en de totale stikstofconcentratie in het veenmosweefsel, de totale concentratie vrije aminozuren, of hun afzonderlijke concentraties. Hoewel de concentratie van het in vrije aminozuren opgeslagen stikstof toenam met de stikstofdepositie, was deze stijging minder dan op basis van de totale stikstofconcentratie in het weefsel verwacht kon worden. Dit laatste duidt op de ophoping van één of meerdere stikstofverbindingen die we niet hebben gemeten. De resultaten doen vermoeden dat het waargenomen negatieve effect van stikstof op de veenmosgroei eerder het gevolg is van een (te) trage fysiologische aanpassing aan een abrupte verhoging van het stikstofaanbod, dan van een toxisch effect na overschrijding van een bepaalde (drempel)waarde voor de stikstofconcentratie in het veenmos. Het is mogelijk dat, indien veenmos ineens wordt blootgesteld aan een sterke toename in het stikstofaanbod, het stikstofmetabolisme de verhoogde stikstofopname niet kan bijhouden, waardoor giftig ammonium zich in de cel kan ophopen. Dit laatste zou de afname van de groei wellicht kunnen verklaren.

Het zesde hoofdstuk beschrijft de gevolgen van een toename in het aanbod van stikstof en fosfor op de uitbreiding van *Sphagnum fallax* (fraai veenmos) en de nauw verwante *S. flexuosum* (slank veenmos) in veenmosveen. We onderzochten of het aantal waarnemingen van fraai en slank veenmos en vier andere veenmossoorten in verband kon worden gebracht met de ontwikkeling van de stikstofdepositie in Nederland vanaf 1900. Ook voerden we twee bemestingsexperimenten met stikstof en fosfor uit. Eén experiment werd uitgevoerd met slank veenmos op een Ierse locatie met een relatief lage achtergronddepositie, het andere experiment werd met fraai veenmos uitgevoerd op twee Nederlandse locaties. Hiernaast probeerden we een relatie te leggen tussen de concentraties van stikstof en fosfor in veenmos en de relatieve bedekking van fraai veenmos in het veld. Uit de resultaten bleek dat het aantal waarnemingen van fraai veenmos relatief meer toenam met de historische stijging van de stikstofdepositie in Nederland dan die van de overige veenmossoorten, slank veenmos incluis. Hoewel de ontwikkeling in het aantal waarnemingen van slank veenmos geen indicatie gaf voor een respons op stikstofdepositie, nam de bedekking van deze soort in het bemestingsexperiment op de Ierse locatie wel toe na toediening van stikstof. De uitbreiding van fraai veenmos op de Nederlandse locaties, leek daarentegen juist bepaald door het aanbod van fosfor. De stikstof- en fosforconcentraties in het veenmos doen vermoeden, dat op locaties waar fraai veenmos een weefselconcentratie van 7 milligram stikstof en 0.7 milligram fosfor per gram drooggewicht kan handhaven, de kans groot is dat deze soort zal gaan domineren. We concluderen dan ook dat fraai veenmos zich weliswaar zal uitbreiden in gebieden met een stijgende stikstofdepositie, maar alleen dominant zal worden wanneer het fosforaanbod toereikend is. In dit laatste geval kan de uitbreiding van fraai veenmos leiden tot verdringing van de reeds aanwezige veenmossoorten.

In hoofdstuk 7 besteden we in vier experimenten aandacht aan de effecten van de stikstofconcentratie in veenmos, het soort veenmos, de ouderdom en het abiotisch milieu op de afbraaksnelheid van veenmos en de consequenties hiervan voor de stikstofcyclus. Voor de experimenten werden zakjes gemaakt van fijnmazig gaas, die werden gevuld met stukjes veenmosstengel. De zakjes werden vervolgens ingegraven op 10-15 cm diepte in veenmosvegetatie. Een jaar later werden zowel het massaverlies als het stikstofverlies van het ingegraven veenmos bepaald. Verrijking met stikstof stimuleerde de afbraak van veenmos, hoewel dit pas duidelijk naar voren kwam wanneer materialen met groot verschil in stikstof:koolstof verhouding werden vergeleken. Stengels van slenkbewonende veenmossoorten braken sneller af dan stengels van bultbewonende veenmossoorten. Dit verschil werd echter kleiner, of verdween zelfs, bij ouder of met stikstof verrijkt stengelmateriaal. Met uitzondering van *Sphagnum papillosum* (wrattig veenmos), braken oudere, reeds bruin verkleurde, stengeldelen van veenmos langzamer af dan jonge, nog niet verkleurde, delen van de stengel. Het abiotisch milieu leek de afbraak nauwelijks te beïnvloeden. Het maakte geen verschil of zakjes werden ingegraven in een veenmosslenk of een veenmosbult, of in een al dan niet met stikstof verrijkt milieu. Het stikstofverlies bleek duidelijk een functie van de stikstofconcentratie. Hoe hoger de stikstof:koolstof verhouding van het ingegraven materiaal, des te groter was het stikstofverlies. Boven een stikstof:koolstof verhouding van ongeveer 0.015 werd een netto stikstofverlies waargenomen voor alle onderzochte soorten. Gezien een stijging van de stikstofdepositie leidt tot een hogere stikstofconcentratie in het veenmosweefsel, en dit op zijn beurt weer tot gevolg heeft dat zowel de afbraaksnelheid als de hoeveelheid stikstof die vrijkomt tijdens het afbraakproces toenemen, concluderen we dat een hoge stikstofdepositie de stikstofkringloop versnelt. Een dergelijke positieve terugkoppeling[1] kan ertoe leiden dat de beschikbaarheid van stikstof veel sneller toeneemt dan je op basis van de toename in stikstofdepositie zou verwachten, met alle gevolgen van dien.

Heeft herstel en ontwikkeling van hoogveen in Nederland wel zin bij het huidige hoge stikstofdepositieniveau? De negatieve effecten van stikstof, zoals de stimulering van de groei van hogere planten, of het in gang zetten van positieve terugkoppelingen, kunnen zeker niet gebagatelliseerd worden. Maar een belangrijk deel van de negatieve invloed van stikstof hangt af van de stikstofconcentratie in het veenmosweefsel en het vermogen van veenmos om de beschikbaarheid van stikstof voor andere organismen laag te houden. Beide zijn afhankelijk van de veenmosproductie, die op zijn beurt bepaald wordt door de balans tussen de negatieve effecten van stikstof enerzijds en de factoren die de veenmosgroei bevorderen anderzijds. Dit zou betekenen, dat een belangrijk deel van de negatieve invloed van stikstofdepositie op hoogveen omzeild kan worden door met zorg de locaties te kiezen waar herstelprojecten worden uitgevoerd, en eventueel, door het nemen van extra beheersmaatregelen om de productiviteit van veenmos te bevorderen. Desalniettemin moeten we ons realiseren dat naarmate de stikstofdepositie toeneemt het (a)biotische bereik, waarbinnen een optimale veenmosgroei

[1] Positieve terugkoppeling: een zichzelf versterkend proces

mogelijk is, kleiner wordt. Bovendien neemt bij een stijgende stikstofbelasting het vermogen van het hoogveensysteem om verstoringen te doorstaan af.

We concluderen dat hoogveen(herstel) in Nederland pas een zekere toekomst heeft wanneer we doorgaan met het terugbrengen van de stikstofdepositie. Aangezien het kritisch depositie niveau van 5 kg stikstof per hectare per jaar niet tot de politieke mogelijkheden lijkt te behoren, is, in aanmerking nemend dat veenmosproductie een maximum bereikt in venen met een bulkdepositie van 10-15 kg stikstof per hectare per jaar, een minimum van 15 kg stikstof per hectare per jaar via bulkdepositie een redelijk compromis voor de middellange termijn.

Curriculum vitae

Juul (of Jules) Limpens werd geboren op 31 juli 1974 te Heerlen. In 1991 behaalde zij het Atheneum-B diploma aan het Romboutscollege te Brunssum, waarna zij in september dat jaar aanving met de studie Biologie aan de Katholieke Universiteit te Nijmegen (KUN). Na aanvankelijk getwijfeld te hebben tussen de Biochemische of Ecologische richting, werd na veel laboratorium- en veldpractica het pleit beslist in het voordeel van de ecologie: het veld lokte meer dan de protocollen.

Tijdens de doctoraalfase participeerde zij in lopend onderzoek naar veenmosverlanding in het Pikmeeuwenwater (Afdeling Aquatische Oecologie en Milieubiologie, KUN), waar zij extra aandacht besteedde aan de vegetatiesamenstelling, de waterchemie en het effect van de CO_2 concentratie in bodemwater op het drijfvermogen van *Sphagnum cuspidatum* (waterveenmos). Hierna keerde zij natte ecosystemen voor korte tijd de rug toe en verrichtte onderzoek aan de effecten van *Rhinanthus minor* (kleine ratelaar) op de vegetatiedynamiek van zich van agrarisch gebruik herstellend kalkgrasland (Leerstoelgroep Vegetatie-ecologie, Universiteit te Utrecht). Vervolgens deed zij ervaring op in het natuurbeleid, door een beheerplan te schrijven voor de 'achtertuin' van haar ouders, de Schinveldse bossen (Natuurmonumenten en de afdeling Milieukunde, KUN). Tussentijds verrichte zij karteringswerkzaamheden in het rivierengebied in opdracht van een ecologisch onderzoeksbureau, Ecoconsult.

In 1997 sloot zij de studie Biologie af en begon met haar werkzaamheden aan de Interactieve Flora van Nederland en Vlaanderen, een samenwerkingsproject van de lerarenopleiding Biologie van de KUN en uitgeverij Malmberg. Na bijna twee jaar met de toegepaste kant van de Biologie bezig te zijn geweest, deed zich de gelegenheid voor om terug te keren naar de meer fundamentele kant, en begon zij in 1998 met haar promotieonderzoek (AIO aanstelling) bij de leerstoelgroep Natuurbeheer en Plantenecologie van Wageningen Universiteit (WUR). Dit onderzoek naar de effecten van stikstofdepositie op hoogveenvegetatie maakte deel uit van een overkoepelend onderzoeksproject aan hoogveen: het Overlevingsplan Bos en Natuur (OBN) voor hoogvenen. Vanaf januari 2002, werd Juul voor halve tijd aangesteld als docent, en was betrokken bij het geven van de vakken Systeem- en Landschapsecologie en het Veldpracticum Bos- en Natuurbeheer II. Zij hoopt deze aanstelling in de loop van 2003 aan te kunnen vullen met vervolgonderzoek in het kader van het OBN hoogvenen.

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