

**Effects of elevated CO<sub>2</sub> and increased N deposition  
on bog vegetation in the Netherlands**

**Gevolgen van een verhoogde atmosferische  
CO<sub>2</sub>-concentratie en N-depositie  
voor hoogveenvegetatie in Nederland**

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**Effects of elevated CO<sub>2</sub> and increased N deposition  
on bog vegetation in the Netherlands**

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**Proefschrift**

ter verkrijging van de graad van doctor  
op gezag van de rector magnificus  
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## Stellingen

1. *Sphagnum magellanicum* heeft voordeel van een hogere CO<sub>2</sub>-concentratie in de lucht.  
*dit proefschrift*
2. De hoogveenvegetatie is een stabiele vegetatie, maar eenmaal uit evenwicht gebracht blijft er weinig van over.  
*dit proefschrift*
3. Vaatplanten hebben pas bij een hoge dichtheid een negatieve invloed op het veenmos.  
*dit proefschrift*
4. Nederlands veenmos heeft zich aangepast aan de huidige hoge stikstofdepositie.  
*dit proefschrift*
5. Veenmos is beter af met vaatplanten dan zonder.
6. De aanwezigheid van mooie hoogveenvegetaties in de veentjes in het Dwingelderveld is mede te danken aan de beschutte ligging in het bos.
7. Experimenten in hoogveen en andere laag-productieve ecosystemen zijn pas zinvol wanneer ze minimaal drie groeiseizoenen duren.
8. Kasexperimenten zijn ongeschikt voor het voorspellen van de reactie van vegetaties op veranderingen in het milieu.
9. De gedachte dat alleen een significant resultaat telt, leidt vaak tot onderzoek dat weinig met de realiteit te maken heeft.
10. Hoogvenen moeten net zoals bossen worden meegenomen in internationale regelgeving over CO<sub>2</sub>-emissies.
11. De maakbare natuur is (gelukkig) net zo'n illusie als de maakbare samenleving.
12. Het tekort aan arbeidskrachten in sommige sectoren wordt mede veroorzaakt doordat er 'maar' een beroepsopleiding voor nodig is.
13. Stabiele software is een effectievere maatregel tegen het vloeken dan het ophangen van posters.

*Stellingen behorend bij het proefschrift "Effects of elevated CO<sub>2</sub> and increased N deposition on bog vegetation in the Netherlands" van Monique Heijmans.*

*Wageningen, 18 oktober 2000*

## Abstract

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Ombrotrophic bogs are important long-term sinks for atmospheric carbon. Changes in species composition of the bog plant community may have important effects on carbon sequestration, because peat mosses (*Sphagnum*) contribute more to peat accumulation than vascular plants. The aim of this study was to investigate the effects of elevated atmospheric carbon dioxide (CO<sub>2</sub>) and increased nitrogen (N) deposition on bog vegetation in the Netherlands, with special attention to the relationship between peat mosses and vascular plants.

Three experiments were conducted, one outdoors and two in the greenhouse, in which peat monoliths were exposed to different levels of atmospheric CO<sub>2</sub> and N deposition. The outdoor experiment was part of the European BERI project, which used MiniFACE technology for creating elevated CO<sub>2</sub> conditions. The vegetation response in all three experiments was followed for two or three growing seasons. In addition, evapotranspiration and the partitioning of <sup>15</sup>N-labelled N deposition among *Sphagnum*, vascular plants and peat was measured.

The results showed, for the first time, that elevated CO<sub>2</sub> benefits growth of *Sphagnum*, but not necessarily at the cost of vascular plant growth. Increases in vascular plant biomass were non-significant, and were apparently restricted by the faster *Sphagnum* height growth and/or nutrient limitation. *Sphagnum* can take advantage of elevated CO<sub>2</sub> because its growth is less nutrient limited than that of vascular plants. Reductions in evapotranspiration at elevated CO<sub>2</sub> in summer would further benefit *Sphagnum*, as its growth is very sensitive to changes in moisture availability. During three growing seasons of N addition, the *Sphagnum* layer became saturated with N, resulting in a larger availability of N and better growth of vascular plants. After reaching a cover of about 60% vascular plants reduced *Sphagnum* growth through increased shading.

These changes in relative abundances of peat mosses versus vascular plants, in response to treatments and interactions between species, have implications for carbon sequestration in peat bogs. As elevated CO<sub>2</sub> favours *Sphagnum* growth, it is expected that carbon sequestration in bogs increases with increasing levels of atmospheric CO<sub>2</sub>. In contrast, increased N deposition will likely reduce carbon sequestration by increasing the relative abundance of vascular plants.

**Key words:** <sup>15</sup>N tracer, BERI, carbon dioxide, competitive interactions, evapotranspiration, global change, MiniFACE, nitrogen, ombrotrophic bog vegetation, plant species composition, *Sphagnum*, vascular plants

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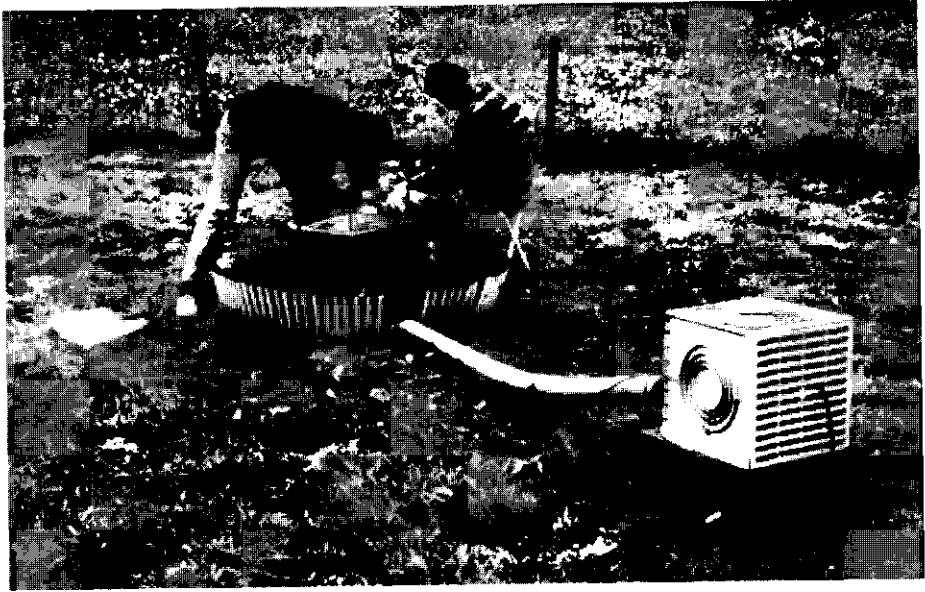
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Monique



# 1

## General introduction

### Global change

In recent decades, there has been growing concern about the effects of man-induced global environmental changes. Climate change has received most of the attention from the public, but other components of global change will have important ecological consequences as well. Three components of global change are recognised by the International Biosphere-Geosphere Programme (IGBP): 1) land-use and land-cover change; 2) changes in the atmospheric composition; 3) climate change. Recently a fourth component has been added: 4) declines in biological diversity (Walker and Steffen 1999). Two of the changes in atmospheric composition will be dealt with in this thesis: increasing carbon dioxide (CO<sub>2</sub>) concentrations and increasing nitrogen (N) deposition.

Many people believe that climate change is uncertain, but most other components of global are hardly subject to controversies, and it is well established that they are human-caused (Vitousek 1994). While there is uncertainty about the contribution of human factors to the recent observed increase in global mean temperature, in 1996 the Intergovernmental Panel on Climate Change (IPCC) concluded that "... *the balance of evidence suggests that there is a discernible human influence on global climate*" (IPCC 1996). What we do not know and requires further research are the ecological consequences of these changes, the interactions among components of change, and the local and global feedbacks resulting from alterations to ecological systems (Vitousek 1994).

The best documented aspect of global change is the increasing concentration of CO<sub>2</sub> in the atmosphere. The atmospheric CO<sub>2</sub> level increased from 280 ppmv (1 ppmv = 1 part per million by volume) in pre-industrial times to 360 ppmv nowadays and continues to increase steadily at a rate of 1.5 ppmv per year as a result of human activities (IPCC 1994). The increase in atmospheric CO<sub>2</sub> concentration in the past century is unusually fast (Webb and Bartlein 1992; Raynaud *et al.* 1993). When looking at longer time scales, measurements of trapped air from a deep Antarctic ice core demonstrate that CO<sub>2</sub> concentrations have been fluctuating on the time scale of glacial-interglacial cycles, with low concentrations during glacial periods and relatively high concentrations during interglacial times. However, the recent increase is already nearly as large as the range between glacial minima and interglacial maxima, and took place 5-10 times faster than observed in the ice-core record (Vitousek 1994). This recent increase in CO<sub>2</sub> concentrations has been mainly caused by fossil fuel combustion (Table 1.1). Another important source of CO<sub>2</sub> is changes in land use and land management, especially deforestation (Table 1.1). The increase in atmospheric CO<sub>2</sub> concentration is expected to continue, and a stabilisation of concentration will not

occur before 2100, even in a scenario of low population growth and low economic growth (IPCC 1994).

**Table 1.1** Fluxes and reservoir changes of carbon expressed in Gt C year<sup>-1</sup> for 1980-1989. From Schimel (1995).

CO <sub>2</sub> sources	Gt C year <sup>-1</sup>
Emissions from fossil fuel combustion and cement production	5.5 ± 0.5
Net emissions from changes in tropical land use	1.6 ± 1.0
Total anthropogenic emissions	7.1 ± 1.1
Partitioning among reservoirs	
Storage in the atmosphere	3.2 ± 0.2
Oceanic uptake	2.0 ± 0.8
Uptake by Northern Hemisphere forest regrowth	0.5 ± 0.5
CO <sub>2</sub> fertilisation	1.0 ± 0.5
N deposition	0.6 ± 0.3
Residual (source)	(0.2 ± 2.0)

The impacts of human activity on the global N cycle have been even more pronounced. Currently, more N is released into the environment by human-driven processes (agriculture, fossil fuel combustion) than by natural processes (N fixation by microbes, lightning) (Vitousek 1994). As a result atmospheric N deposition rates have increased; in Europe from 2-6 kg N ha<sup>-1</sup> year<sup>-1</sup> to 15-60 kg N ha<sup>-1</sup> year<sup>-1</sup> (Pitcairn *et al.* 1995). In contrast to CO<sub>2</sub>, which is well-mixed in the atmosphere, there are large regional variations in rates of N deposition. In the Netherlands N deposition sharply increased since 1950 (Van Oene *et al.* 1999) to high levels of 30-60 kg N ha<sup>-1</sup> year<sup>-1</sup> (RIVM 1999). Agriculture (mainly intensive livestock farming) contributes most (55%) to N deposition in the Netherlands by emitting NH<sub>3</sub> (RIVM 1999).

Both increased atmospheric concentrations of CO<sub>2</sub> and some gaseous forms of N (N<sub>2</sub>O and NO<sub>x</sub>) may contribute to global warming. The greenhouse gases absorb long-wave radiation emitted from the earth's surface and re-emit it, which has the effect of warming the earth's surface and lower atmosphere. Increases in concentrations will change the heat balance at the earth's surface. This enhanced "greenhouse effect" is the basis for predictions of increases in global mean temperatures. The effect these gases have in altering the energy balance of the earth-atmosphere system is named "radiative forcing". CO<sub>2</sub> is the most important greenhouse gas, in terms of radiative forcing, its increase since pre-industrial times contributing more than half to the greenhouse effect (IPCC 1996). The "Global Warming Potential" (GWP) compares the relative warming effect of different gases. Other greenhouse gases (methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O), Ozone (O<sub>3</sub>) and halocarbons) occur in much lower concentrations in the atmosphere than CO<sub>2</sub>, but have much larger GWPs. The concentrations of these gases in the atmosphere have also been increasing (IPCC 1996), with the increase in atmospheric N<sub>2</sub>O concentration having occurred concurrently with the increased anthropogenic N emissions (Vitousek 1994).

## Global change and terrestrial ecosystems

Both carbon and nitrogen are essential elements for all organisms. A change in supply will therefore have profound effects on ecosystems. Elevated CO<sub>2</sub> as well as increased N deposition change the chemical composition of plant tissues, thereby potentially affecting herbivory and decomposition processes. In addition, both global changes have the potential to alter species composition of plant communities as a result of changed competitive relationships between species. There are many ways in which changes in tissue chemistry and plant species composition will affect the functioning of plant communities or ecosystems as there are many indirect effects and feedback mechanisms, which will not be discussed here.

That increased N deposition can change species composition has been shown by several field experiments. In plant communities where N supply limits primary production, adding N leads to an increase in net primary production and biomass, and a decrease in overall species richness (Vermeer 1986; Tilman 1987; Berendse and Elberse 1990). N enrichment can be expected to favour plants with high maximum growth rates, which will increase in abundance, resulting in elimination of slow-growing species (Jefferies and Maron 1997). In the Netherlands, where the level of N deposition is among the highest in Europe, shifts in species composition in heathlands, weakly buffered waters and chalk grasslands have been attributed to the increased N deposition (Berendse *et al.* 1993; Bobbink *et al.* 1997).

For CO<sub>2</sub>, only a limited number of experimental studies has been conducted in natural plant communities. For individual plant species it has been shown that elevated CO<sub>2</sub> commonly increases photosynthetic rates and growth (at least in the short term) and reduces transpiration rates. However, plant responses do not directly translate into vegetation responses. Field studies showed much smaller (if any) increases in aboveground biomass than observed for individual species grown in pots. One of the consistent findings is that species respond differently (Körner 1996), resulting in changed competitive relationships in the long term.

The effects of elevated CO<sub>2</sub> and increased N deposition are likely to interact. Studies under field conditions suggest that the vegetation response to elevated CO<sub>2</sub> depends largely on interactions with other environmental factors, such as water, nutrient availability and temperature. In nutrient poor ecosystems such as arctic tundra, nutrient availability and/or uptake must increase before plants are able to benefit from CO<sub>2</sub> fertilisation (Oechel and Vourlitis 1994). A compilation of ecosystem level CO<sub>2</sub> responses (Körner 1996) revealed that almost all low fertility natural or model ecosystems, in contrast to high fertility systems, showed no or little stimulation of biomass under elevated CO<sub>2</sub>, which stresses the importance of nutritional status.

Not only does global change affect terrestrial ecosystems, the terrestrial biosphere is also a major player in the exchange of greenhouse gases between the earth and the atmosphere, thereby influencing global change. By absorbing significant amounts of CO<sub>2</sub>, terrestrial ecosystems can slow down the build up of CO<sub>2</sub> in the atmosphere and consequently reduce the rate of climate change (Schimel 1995). The terrestrial biosphere is one of the four main reservoirs of carbon (others are fossil fuel, the atmosphere and the oceans). As the fluxes among the four pools should balance, the average 1980s values

(Table 1.1) lead to a sink of 1.4 Gt C year<sup>-1</sup> in terrestrial ecosystems. Plant growth responses to increased levels of atmospheric CO<sub>2</sub> and N deposition are thought to have increased carbon storage in terrestrial ecosystems (Field *et al.* 1992). However there are large uncertainties in these numbers that are calculated from ecosystem models, often lacking essential feedbacks, and they are hardly tested under field conditions. The lack of knowledge about positive and negative feedbacks from the biosphere is a major factor limiting better simulations of future atmospheric CO<sub>2</sub> concentrations (Schimel 1995).

Although short-term individual plant responses to components of global change have been well documented, long-term ecosystem responses are largely unknown. Resource limitations and interactions with other species may constrain the response of plants in field conditions as compared with observations in single-species studies under controlled conditions. Reviews on the response of terrestrial ecosystems to elevated CO<sub>2</sub> strongly emphasise the need for more multi-year studies on natural vegetation (Koch and Mooney 1996; Körner 1996).

## Global change and peat bogs

With respect to the terrestrial carbon sink most of the focus has been on forest ecosystems. Remarkably, until recently, peatlands did not receive much attention, in spite of peat being a large and long-lasting sink for carbon. Large areas in Russia, Scandinavia and Canada are covered with peat. An enormous carbon pool of 455 Pg (1Pg = 10<sup>15</sup> g) - about one-third of the world's pool of soil organic carbon - has accumulated in northern peatlands (tundra and boreal forest regions) over thousands of years (Gorham 1991). Changes in these ecosystems, for example brought about by climate change, can have important consequences for the global carbon cycle. However, little is known about the effects of global change on mire ecosystems.

The basic subdivision of mires (peat-forming ecosystems) is into bogs and fens. Bogs (corresponding with "hoogveen" (high mire) in Dutch) are ombrotrophic systems fed by rainwater only and dominated by peat mosses (*Sphagnum*). Fens (corresponding with "laagveen" (low mire) in Dutch) are minerotrophic peatlands fed by groundwater and/or surface water in addition to rainwater. It is the *Sphagnum*-dominated bogs that have the greatest peat accumulation rates due to extremely low rates of decomposition (Thormann *et al.* 1999).

The amount of carbon being sequestered in peatlands depends on the balance between the production and decomposition of plant material. Changes in plant species composition may have important effects on this balance because species differ strongly in both productivity and decomposability. This certainly holds for bog plant communities, which are composed of peat mosses and vascular plants. *Sphagnum* species in particular are capable of storing large amounts of carbon, because they decompose slowly (Clymo and Hayward 1982). Litter of *Sphagnum* is more recalcitrant than that of vascular plants and forms the bulk of bog peat (Coulson and Butterfield 1978). Vascular plants contribute less to peat accumulation and, moreover, contribute more to methane emission. They stimulate methane production by supplying labile organic carbon through root exudation. In addition, some vascular plant species (*Eriophorum*) transport methane through aerenchymae from the anaerobic root zone to the atmosphere, thus reducing the fraction of methane that oxidises in the upper, aerobic peat layer (Saarnio *et al.* 1998; Frenzel and Rudolph 1998;

Joabsson *et al* 1999). Therefore, global warming due to greenhouse gases should be alleviated whenever *Sphagnum* expands at the expense of vascular plants, and vice versa.

Another reason for studying bogs is that they contain many locally threatened species, due to peat mining and drainage, which may become further endangered by global change. Ombrotrophic bogs are expected to be very sensitive to changes in atmospheric composition as they depend on the atmospheric supply of nutrients. As such, the bog vegetation experiences extremely nutrient poor conditions. The peat mosses and vascular plants use different sources of mineral nutrients. Where *Sphagnum* largely depends on nutrients from atmospheric deposition, the rooted vascular plants mainly capture nutrients that are mineralised from organic matter. *Sphagnum* is very effective in intercepting the atmospheric deposition (Woodin and Lee 1987; Williams *et al.* 1999) and thereby reduces this supply of nutrients to vascular plants. In addition, *Sphagnum* slows down decomposition by producing slowly decaying litter and creating a harsh (wet, acid, mineral-poor) environment for decomposers (Van Breemen 1995). Bog plant species deal with these nutrient poor conditions by being very economical with their nutrients. In addition, the vascular plants have to adapt to the upward growth of *Sphagnum* plants in the order of 1-10 cm each year. Only few plant (and animal) species are able to cope with the harsh conditions created by the *Sphagnum* layer, making bog vegetation generally species-poor.

Where CO<sub>2</sub> experiments were already scarce for terrestrial ecosystems in general, CO<sub>2</sub> enrichment studies in bogs are completely lacking. Only measurements on individual *Sphagnum* species grown at several CO<sub>2</sub> levels in growth chamber and greenhouse have been performed (Silvola 1985; Jauhiainen *et al.* 1994, 1997, 1998a; Jauhiainen and Silvola 1999). Although it has been recognised for some time that high N deposition poses a threat to bog vegetation, there has been only one field experiment studying the vegetation response to changes in N deposition in intact bog vegetation for more than one season (Lütke Twenhöven 1992a, 1992b). The large-scale decline of *Sphagnum* species in the southern Pennines in England has been attributed to air pollution (Lee and Studholme 1992), but the mosses had been exposed to other pollutants besides N. Both sulphur (Ferguson and Lee 1979) and nitrogen (Press *et al.* 1986) have been shown to inhibit *Sphagnum* growth. The specific N deposition rates in the Netherlands make it interesting to study the response of bog vegetation to global change in our country too.

## Bog vegetation in the Netherlands

Mires once covered a major part of the land surface in the Netherlands, but most of this has been lost as a result of reclamation and drainage, peat cutting and peat dredging, leaving only about 7000 ha of bog. Most of the remaining bogs have degenerated due to cutting, burning and drainage, so that at present truly undisturbed bog vegetation covers less than 5 ha (Barkman 1992). Actively growing bog vegetation is also found in heath pools that have become filled with peat. Barkman (1992) estimates the present surface of ombrotrophic bog vegetation in heath pools at about 50 ha, much more than is left in bog remnants. Also in these heath pools peat was cut by farmers, but not as extensively as in the bog areas, resulting in a random mosaic of different succession stages of bog vegetation in the heath pools nowadays. Obviously, all characteristic bog species are threatened in the Netherlands.

## Aims and approach

The aim of this study is to investigate the effects of elevated atmospheric CO<sub>2</sub> and increased N deposition on bog vegetation in the Netherlands, with emphasis on the relationship between peat mosses and vascular plants. The general hypothesis is that elevated CO<sub>2</sub> will favour growth of *Sphagnum* over that of vascular plants and that increased N deposition will result in the opposite effect, i.e. a change in the competitive balance between mosses and vascular plants to the benefit of vascular plants. The study is part of the European BERI (Bog Ecosystem Research Initiative) project in which groups from Finland, Sweden, Switzerland, England and The Netherlands were involved (Hoosbeek *et al* 1995). At five bogs in these countries similar field experiments were set up.

In the Netherlands large peat monoliths including intact bog vegetation were transferred from the field to an experimental garden where they were placed in large containers. The monoliths were exposed to two CO<sub>2</sub> treatments (ambient and elevated (560 ppmv) CO<sub>2</sub>) and two N treatments (ambient and increased N deposition (+ 5 g N m<sup>-2</sup> year<sup>-1</sup>)) for three growing seasons. The target CO<sub>2</sub> concentration of 560 ppmv corresponds with a doubling of pre-industrial CO<sub>2</sub> concentrations. Adding 5 g N m<sup>-2</sup> year<sup>-1</sup> means a doubling of the ambient N deposition in the Netherlands. The experiments were not conducted in the field for logistical reasons (lack of electricity nearby) and to prevent major disturbance of this extremely rare type of vegetation. This thesis describes the results from the Dutch site only. Results from the other sites will be touched upon briefly in the General discussion.

The CO<sub>2</sub> treatments were established using FACE (Free Air CO<sub>2</sub> Enrichment) technology, which has been considered as the best technology for investigating the responses of natural ecosystems to elevated CO<sub>2</sub> (Schulze *et al.* 1999). In FACE systems, CO<sub>2</sub> concentrations within the experimental plots are elevated by supplying CO<sub>2</sub>-enriched air from a series of vertical pipes arranged around each plot. Wind direction, wind speed and CO<sub>2</sub> sensors within the plots are used to control which pipes emit CO<sub>2</sub>. The main advantage of FACE systems is that virtually all environmental factors other than CO<sub>2</sub> concentration are unaffected, since no chamber or barrier is constructed around the experimental plots. In contrast, chamber effects in Open Top Chambers (OTCs), which have been used in many elevated CO<sub>2</sub> experiments, result in alteration of the radiation and wind regimes and pollinator access. The major disadvantage of FACE is the high costs linked with the large amounts of CO<sub>2</sub> required. As a consequence only a few treatments and a limited number of replicates can be included in the experiments. For this reason the BERI experiments consisted of separate CO<sub>2</sub> and N treatments, as opposed to a factorial design.

For the BERI experiments in bog vegetation relatively small (1.1 m diameter) MiniFACE systems (Miglietta *et al.* 2000) could be used, with all pipes emitting CO<sub>2</sub>-enriched air continuously. The CO<sub>2</sub> supply to the elevated CO<sub>2</sub> rings was adjusted automatically by a PC and mass flow controllers, to maintain the target concentration of 560 ppmv. The supply was based on measured wind speed and CO<sub>2</sub> concentration in the middle of the ring, as described in detail by Miglietta *et al.* (2000).

In addition to the FACE experiment ("outdoor experiment"), we conducted two greenhouse experiments. One greenhouse experiment was set up to study the vegetation



response to elevated CO<sub>2</sub> in interaction with N supply ("greenhouse experiment"), which was not possible in the outdoor experiment. The other greenhouse experiment aimed at studying competition between *Sphagnum* and vascular plants as affected by elevated CO<sub>2</sub> and increased N deposition ("competition experiment").

For all three experiments peat monoliths were used, cut from a heath pool in the State Forestry of Dwingeloo. The vegetation of all monoliths was dominated by *Sphagnum magellanicum*, with *Vaccinium oxycoccus*, *Erica tetralix*, *Eriophorum angustifolium* and *Drosera rotundifolia* as frequently co-occurring species (Table 1.2). The outdoor and greenhouse experiment used peat monoliths including intact bog vegetation, the competition experiment used the peat monoliths for creating monocultures and mixtures of *Sphagnum magellanicum* and *Eriophorum angustifolium*.

In all three experiments measurements were made of *Sphagnum* growth, relative abundance of plant species, vascular plant biomass, chemical composition of plant tissues (C, N, P, K) and water use. To get more insight in the fate of the deposited N, a small amount of <sup>15</sup>N tracer was applied to the experimental plots of the outdoor experiment and to additional plots in the untreated field site. The distribution of the <sup>15</sup>N tracer among *Sphagnum*, vascular plants and peat was determined at the final harvest.

**Table 1.2** Scientific and Dutch names of the plant species occurring in our experiments.

Scientific name	Dutch name
<b>Mosses</b>	
<i>Sphagnum magellanicum</i> Brid.	Hoogveen-veenmos
<i>Sphagnum papillosum</i> Lindb.	Wrattig veenmos
<i>Sphagnum fallax</i> (Klingr.) Klingr. or <i>S. recurvum</i> var. <i>mucronatum</i> (Russ.) Warnst.	Slank veenmos
<i>Aulacomnium palustre</i> (Hedw.) Swägr.	Rood viltmos = Veen-knopjesmos
<i>Calliergon stramineum</i> (Brid.) Kindb.	Sliertmos
<b>Vascular plants</b>	
<i>Vaccinium oxycoccus</i> L. or <i>Oxycoccus palustris</i> Pers.	Kleine veenbes
<i>Erica tetralix</i> L.	Gewone dophei
<i>Eriophorum angustifolium</i> Honck.	Veenpluis
<i>Drosera rotundifolia</i> L.	Ronde zonnedaauw
<i>Calluna vulgaris</i> (L.) Hull	Struikhei
<i>Empetrum nigrum</i> L.	Kraaihei
<i>Rhynchospora alba</i> (L.) Vahl	Witte snavelbies
<i>Eriophorum vaginatum</i> L.	Eenarig wollegras
<i>Andromeda polifolia</i> L.	Lavendelhei
<i>Pinus sylvestris</i> L.	Grove den

## Outline of the thesis

The first two chapters after this general introduction describe the vegetation response observed in the outdoor experiment and in the greenhouse experiment. The following chapters explore key processes in bog ecology, giving more insight in the mechanisms lying behind the observed changes at the vegetation level: competition between *Sphagnum* and vascular plants (Chapter 4), evapotranspiration (Chapter 5) and N capture (Chapter 6).

Chapter 2 describes the vegetation response to three growing seasons of elevated CO<sub>2</sub> or increased N deposition in the outdoor experiment. The response is described in terms of *Sphagnum* growth, above- and belowground biomass of vascular plants, shifts in relative abundance of the species and nutrient concentrations in plant tissues. It is the first description of the response of mire vegetation to elevated CO<sub>2</sub> in the world, and the first for the response to N supply for this kind of vegetation in the Netherlands.

Chapter 3 deals with the vegetation response in the greenhouse experiment in which peat monoliths with intact bog vegetation were exposed to combinations of CO<sub>2</sub> and N treatments (as opposed to the previous chapter). In other ecosystems the vegetation response to elevated CO<sub>2</sub> is often constrained by nutrient limitation, is this also the case in the nutrient poor bog ecosystem? The raised temperatures in the greenhouse gave rise to unexpected effects, which will be discussed in this chapter.

In Chapter 4 the results of the competition experiment are presented. Observing the intact bog vegetation has the great advantage of including species interactions, but does not allow separation of direct (species-specific) and indirect (as a result of competition) effects. Here, monocultures and mixtures of *Sphagnum magellanicum* and *Eriophorum angustifolium* were exposed to combinations of CO<sub>2</sub> and N treatments. Growth of both species was followed during three growing seasons.

In Chapter 5 evapotranspiration data determined in all three experiments are described and discussed. Elevated CO<sub>2</sub> is known to reduce transpiration rates at the leaf level, but does it also reduce evapotranspiration in bog vegetation, where *Sphagnum* dominates, which don't have stomata? Does N increase evapotranspiration by stimulating growth of vascular plants? Growth of *Sphagnum* is very sensitive to changes in moisture availability, which makes it important to study how evapotranspiration is affected by elevated CO<sub>2</sub> and N. As the monoliths were in containers and water gifts were recorded we were able to determine evapotranspiration in all three experiments and investigate the effects of elevated CO<sub>2</sub> and N supply.

Chapter 6 describes the fate of <sup>15</sup>N labelled N deposition in ombrotrophic bog vegetation, both at ambient and increased N deposition. It has often been suggested that at high N deposition more N becomes available to vascular plants, but this was never tested in field conditions. Here, shifts in distribution of a <sup>15</sup>N tracer among *Sphagnum*, vascular plant species and peat caused by increased N deposition are presented.

The general discussion in Chapter 7 aims at integrating the results from the different experiments and drawing general conclusions. In addition, the consequences for carbon sequestration will be discussed based on calculations for the studied bog vegetation.

## Effects of elevated CO<sub>2</sub> and increased N deposition on bog vegetation in the Netherlands

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### Summary

We studied the effects of elevated atmospheric CO<sub>2</sub> and increased N deposition on the plant species composition of a *Sphagnum*-dominated bog ecosystem in the Netherlands. Large peat monoliths (surface area 1 m<sup>2</sup>, depth 0.6 m) with intact bog vegetation were kept outdoors in large containers and were exposed to elevated CO<sub>2</sub> or increased N deposition for three growing seasons. Elevated CO<sub>2</sub> conditions (target concentration 560 μmol CO<sub>2</sub> mol<sup>-1</sup>) were created using MiniFACE technology. N deposition was increased by 5 g N m<sup>-2</sup> year<sup>-1</sup> by adding dissolved NH<sub>4</sub>NO<sub>3</sub> at three-week intervals during the growing season.

Elevated atmospheric CO<sub>2</sub> increased height growth of *Sphagnum magellanicum*, the dominant *Sphagnum* species, in the second and third growing season. Vascular plant biomass was not significantly affected by elevated CO<sub>2</sub>, but growth of species growing close to the moss surface was negatively influenced by the increased *Sphagnum* height growth. Elevated CO<sub>2</sub> did not change allocation to belowground plant parts.

Adding N increased aboveground vascular plant biomass. The shallow-rooted species *Vaccinium oxycoccus* responded most to the increased N deposition. *Sphagnum* growth was significantly reduced in the third growing season. This reduction was likely the result of the increased vascular plant cover, given the observed negative relation between vascular plant cover and *Sphagnum* growth.

The observed shifts in species composition as a result of species-specific responses to treatments and interactions between peat mosses and vascular plants will have important consequences for the sequestration of carbon in the bog ecosystem.

## Introduction

There has been a great deal of research on the effects of elevated atmospheric CO<sub>2</sub> concentrations on growth of individual plants, but relatively little on the effects on natural vegetation. Yet plant level responses to elevated CO<sub>2</sub> cannot be directly extrapolated to the ecosystem level, because interspecific interactions and resource limitation of growth come into play (Körner 1996). Clearly, elevated CO<sub>2</sub> affects ecosystems, but the long-term responses are largely unknown and the mechanisms driving the response are poorly understood. This is why reviews on the response of terrestrial ecosystems to elevated CO<sub>2</sub> strongly emphasise the need for more multi-year studies with natural vegetation (Koch and Mooney; Körner 1996).

The impact of elevated CO<sub>2</sub> on ecosystems has repercussions on CO<sub>2</sub> in the atmosphere. When terrestrial ecosystems sequester more C under elevated CO<sub>2</sub>, they will slow the accumulation of CO<sub>2</sub> in the atmosphere thus reducing the rate of climate change (Schimel 1995; Walker *et al.* 1997). Growing bog ecosystems are potentially important in this regard, because, as peat-forming systems, they constitute a long-term sink for atmospheric CO<sub>2</sub>. An enormous carbon pool of 455 Pg (1 Pg = 10<sup>15</sup> g) - about one-third of the world's pool of soil organic carbon - has accumulated in northern peatlands (tundra and boreal forest regions) over thousands of years (Gorham 1991). Within peatlands it is the *Sphagnum*-dominated bog ecosystems that have the greatest peat accumulation potential due to extremely low rates of decomposition (Thormann *et al.* 1999). Changes in these ecosystems, for example brought about by climate change, can have important consequences for the global carbon cycle. However, little is known about the effects of global change on bog ecosystems.

The amount of carbon being sequestered in peatlands depends on the balance between the production and decomposition of plant material. Changes in plant species composition may have important effects on this balance because species differ strongly in both productivity and decomposability. This certainly holds for bog vegetation, which are comprised of peat mosses (*Sphagnum*) and vascular plants. *Sphagnum* species in particular are capable of holding large amounts of carbon, because they decompose slowly (Clymo and Hayward 1982). Litter of *Sphagnum* is more recalcitrant than that of vascular plants and forms the bulk of bog peat (Coulson and Butterfield 1978). Vascular plants contribute less to peat accumulation and, moreover, contribute more to methane emission. They stimulate methane production by supplying labile organic carbon through root exudation. In addition, some vascular plant species (*Eriophorum*) transport methane through aerenchymae from the anaerobic root zone to the atmosphere, thus diminishing the fraction of methane that oxidises in the upper, aerobic, peat layer (Saarnio *et al.* 1998; Frenzel and Rudolph 1998; Joabsson *et al.* 1999). Therefore, carbon sequestration in bogs is expected to increase when *Sphagnum* gains a competitive advantage over vascular plants.

Elevated atmospheric CO<sub>2</sub> and increased N deposition are two important components of global change, which are expected to alter the competitive balance between *Sphagnum* and vascular plants in bog vegetation. Elevated CO<sub>2</sub> generally stimulates growth of individual plants, but net production of a whole plant community is not necessarily increased. One of the few consistent findings in CO<sub>2</sub>-enrichment studies in multi-species

systems is that the CO<sub>2</sub> response is species specific, with some species declining and other species gaining in abundance (Körner 1996; Warwick *et al.* 1998; Leadley *et al.* 1999).

The effect of elevated CO<sub>2</sub> on bog vegetation is largely unknown, because only measurements on individual *Sphagnum* species have been performed (Silvola 1985; Jauhiainen *et al.* 1994, 1998a; Jauhiainen and Silvola 1999). Studies in other ecosystems suggest that the vegetation response depends largely on interactions with other environmental factors, such as water, nutrient availability and temperature. In nutrient-poor ecosystems such as arctic tundra, nutrient availability and/or uptake must increase before plants are able to benefit from CO<sub>2</sub> fertilisation (Oechel and Vourlitis 1994). The only sources of mineral nutrients in ombrotrophic bogs are atmospheric deposition and the mineralisation of dead organic matter. *Sphagnum* is very effective in absorbing nutrients from atmospheric deposition (Woodin and Lee 1987; Williams *et al.* 1999), thereby reducing this supply of nutrients to vascular plants. Vascular plants largely depend on the nutrients mineralised from dead organic matter, but the slow rate of decomposition of *Sphagnum* litter depresses this supply of nutrients to vascular plants.

We hypothesise that elevated CO<sub>2</sub> will change the competitive balance between mosses and vascular plants to the benefit of *Sphagnum*, because it is less nutrient limited than vascular plants (Sveinbjörnsson and Oechel 1992; Jauhiainen *et al.* 1998a). With the high ambient N deposition in the Netherlands it is likely that *Sphagnum* growth is not N limited (Aerts *et al.* 1992), whereas vascular plant growth possibly still is, as *Sphagnum* is very effective in intercepting atmospheric N supply. Increased N deposition is expected to result in opposite effects, *i.e.* a competitive advantage for vascular plants. *Sphagnum* may no longer capture all nutrients from atmospheric deposition when N deposition is increased (Woodin and Lee 1987). Consequently more N may reach the rhizosphere, which should increase growth rates of vascular plants (Malmer *et al.* 1994). The increased shading of the moss layer caused by an increased vascular plant cover may then depress *Sphagnum* growth (Clymo 1973; Hayward and Clymo 1983). Although many studies have addressed the short-term response of *Sphagnum* to increased N deposition, experimental evidence obtained from intact bog vegetation is still scarce.

To test these hypotheses, we investigated the effects of elevated atmospheric CO<sub>2</sub> and increased N deposition on bog vegetation in the Netherlands. To do so we kept large peat monoliths with intact bog vegetation in large containers outside and exposed them to elevated CO<sub>2</sub> or increased N deposition during three growing seasons. During the experiment we followed *Sphagnum* growth (height increment) and the abundance of vascular plant species. At the final harvest, *Sphagnum* biomass growth, vascular plant biomass (above- and belowground) and nutrient concentrations in plant tissues were determined.

## Methods

### Site description

The peat monoliths used in this study were taken from a small mire in the State Forestry of Dwingeloo (52°49'N, 6°25'E) in the north of the Netherlands. This former extensive heathland with active sand dunes has been forested with pine and oak at the beginning of the 20<sup>th</sup> century. The field site is one of a series of heathland pools (0.5–1.5 m deep) that

has become filled with peat and is situated in a former glacial valley. The mire measures 50 by 150 m and is surrounded by forest. The mean annual rainfall is 840 mm and the mean annual temperature 9 °C. The surface peat layer of relatively undecomposed *Sphagnum* litter is 20 to 40 cm thick and overlies wet highly decomposed peat. The surface peat has the following physical-chemical characteristics (5-15 cm depth): bulk density 30-50 g dm<sup>-3</sup>; N concentration 11 mg N g<sup>-1</sup>; P concentration 0.4 mg P g<sup>-1</sup>; K concentration 0.6 mg K g<sup>-1</sup>. The pool bed consists of loamy sand.

Due to irregular peat cutting in the past, the vegetation is now a mosaic of secondary succession stages. The vegetation in the monoliths consisted of *Sphagnum magellanicum* Brid. lawns with *Vaccinium oxycoccus* L., *Erica tetralix* L. and *Eriophorum angustifolium* Honck. as dominant vascular plant species. Other species present were *Sphagnum papillosum* Lindb., *Sphagnum fallax* (Klinggr.) Klinggr., *Aulacomnium palustre* (Hedw.) Schwägr., *Calliergon stramineum* (Brid.) Kindb., *Drosera rotundifolia* L., *Calluna vulgaris* (L.) Hull, *Empetrum nigrum* L., *Rhynchospora alba* (L.) Vahl, *Eriophorum vaginatum* L., *Andromeda polifolia* L. and *Pinus sylvestris* L..

The experimental site is a grassland in Wageningen (51°99'N, 5°70'E), fenced to exclude rabbits. Weather conditions during the three-year experiment varied considerably (Table 2.1). The first year (1996) was very dry with the driest spring of this century, while 1998 was exceptionally wet. 1997 was a warm and sunny year. The winter of 1997/1998 was very mild. In 1998, spring and summer were cloudy.

Wet N deposition (NH<sub>4</sub> + NO<sub>3</sub>) has been measured in Witteveen (15 km from field site) and in Wageningen (1.5 km from experimental site) and ranges from 11 kg N ha<sup>-1</sup> year<sup>-1</sup> in dry 1996 to 15 kg N ha<sup>-1</sup> year<sup>-1</sup> in wet 1998 without differences between the sites (Boschloo and Stolk 1999a, 1999b, 1999c). However, total N deposition is mainly (50-60%) composed of dry NH<sub>x</sub> compounds, being higher in the region around Wageningen than in the north of the Netherlands. Dry NH<sub>x</sub> deposition is calculated from detailed emission data and by comparing measured and calculated NH<sub>3</sub> concentrations in the atmosphere. The resulting total N deposition in 1997 amounted to 52 kg N ha<sup>-1</sup> year<sup>-1</sup> in the Wageningen region and 37 kg N ha<sup>-1</sup> year<sup>-1</sup> in the area in which the field site is situated (RIVM 1999).

**Table 2.1** Weather conditions during the experiment in Wageningen. Data are seasonal averages for temperature and sums of precipitation and solar radiation, from the weather station of Wageningen University. Winter = Dec-Feb, Spring = Mar-May, Summer = Jun-Aug, Autumn = Sep-Nov.

	Temperature (°C)			Precipitation (mm)			Solar radiation (kJ cm <sup>-2</sup> )		
	1996	1997	1998	1996	1997	1998	1996	1997	1998
Winter		2	5		118	133		24	27
Spring	8	9	10	54	127	231	116	122	107
Summer	16	18	16	135	197	217	152	162	143
Autumn	9	10		226	124		61	66	

## Experimental design

Twenty peat monoliths, including the intact bog vegetation, were cut in March 1996 when the surface layer of peat was frozen. The monoliths had a diameter of 1.1 m and were about 25 cm deep. The wet highly decomposed peat below this upper peat layer was also collected. At the same day the monoliths were brought to the experimental site in Wageningen where they were placed into plastic containers (1.1 m diameter, 60 cm deep) that were buried to a depth of 50 cm in the experimental site. First the loose peat was put in the containers and tap water was added to replace the water drained from the peat. Then the intact frozen monolith was placed on top. We expected this set-up to give realistic results, because bog ecosystems are generally systems isolated from the mineral substrate, with external input only from atmospheric deposition.

The peat monoliths were used for two simultaneous experiments. The CO<sub>2</sub> experiment consisted of an ambient CO<sub>2</sub> treatment and an elevated CO<sub>2</sub> (560 µmol CO<sub>2</sub> mol<sup>-1</sup>) treatment and the N experiment consisted of an ambient N deposition treatment and an increased N deposition (5 g N m<sup>-2</sup> year<sup>-1</sup> added) treatment. Unfortunately, financial restrictions made it impossible to combine the CO<sub>2</sub> and N treatments in one experiment, as this meant a doubling of the CO<sub>2</sub> equipment and supply. All treatments were replicated five times. The 20 peat monoliths were randomly assigned to one of the four treatments. The elevated CO<sub>2</sub> plots were located at least 6 m from other plots to prevent CO<sub>2</sub> enrichment in the control plots (Miglietta *et al.* 2000). To avoid edge effects, no measurements were taken in the outer 15 cm of each plot.

The CO<sub>2</sub> treatments were established using MiniFACE technology. Each MiniFACE consisted of a ring (1.1 m in diameter) of polyethylene tubing, 5 cm in diameter, fitted with 72 venting pipes that were 18 cm tall. Each pipe had two holes, at 6 and 12 cm above the moss surface. Blowers located next to the MiniFACE rings at 50 cm above the surface supplied ambient air to the rings. The CO<sub>2</sub> concentration of the ambient air fluctuated diurnally with a mean ambient CO<sub>2</sub> concentration of 360 µmol CO<sub>2</sub> mol<sup>-1</sup> in daytime. In the elevated CO<sub>2</sub> MiniFACE rings, pure CO<sub>2</sub> was added to the airflow from the blowers. The CO<sub>2</sub> concentration at the centre of the rings at 7.5 cm above the moss surface was continuously monitored with an infrared gas analyser. The CO<sub>2</sub> supply to the elevated CO<sub>2</sub> rings was adjusted automatically by a PC and mass flow controllers, to maintain the target concentration of 560 µmol CO<sub>2</sub> mol<sup>-1</sup>. The supply was based on measured wind speed and CO<sub>2</sub> concentration in the middle of the ring, as described in more detail by Miglietta *et al.* (2000). At the Wageningen site the CO<sub>2</sub> concentration deviated less than 20% from the pre-set target concentration of 560 µmol CO<sub>2</sub> mol<sup>-1</sup> for 97.5% of the time during 1996 and 1997 (Miglietta *et al.* 2000).

Nitrogen was added in the form of NH<sub>4</sub>NO<sub>3</sub> dissolved in demineralised water. A total amount of 5 g N m<sup>-2</sup> (corresponding with 50 kg N ha<sup>-1</sup> year<sup>-1</sup>) was added each growing season to the high N plots in six applications (about every three weeks) by watering each of these plots with 2 l of N solution, simulating a rain event of 2 mm. The ambient N deposition treatment received the same amount of demineralised water. Whenever possible, the N treatments were applied during rainy weather. In dry periods the N addition was followed by an extra addition of 2 l of artificial rainwater (see below).

Water levels in the plots were allowed to fluctuate between 5 and 20 cm below moss surface. A hole in the container at 5 cm below moss surface allowed overflow into a 25-l

jerrycan. Whenever the water level dropped to the minimum level, artificial rainwater was added to increase the level to 10 cm below moss surface. In this way water levels could follow quite a natural regime, with low water levels in dry periods and high water levels in wet periods, but extreme events as flooding and drying out of the peat were prevented. The artificial rainwater was made by preparing a seawater solution of the chemical composition as given in Garrels and Christ (1965) and diluting this stock solution 8000 times with demineralised water (Table 4.1). This water is equivalent to very clean rainwater without N or P. Electrical conductivity was about  $11 \mu\text{S cm}^{-1}$  and pH was about 5.8.

The  $\text{CO}_2$  treatments started in May 1996 and the first N was added in June 1996. In the winter months December, January and February the MiniFACE system was turned off because of the low rates of biological activity. The experiment ended with a final harvest in the beginning of September 1998, immediately after peak biomass for vascular plants, and just before major senescence and reallocation of nutrients.

## Measurements

### *Species composition*

Plant species composition and abundance (both mosses and vascular plants) were measured non-destructively using the point-quadrat method (Jonasson 1988). The point-quadrat covered an area of 25 by 37.5 cm and consisted of 150 points with a grid size of 2.5 cm. The permanently marked point-quadrat subplot (pq subplot) was chosen to be representative of the type of vegetation. At the 150 points a sharpened knitting needle was lowered to the top of the moss surface and at all contacts with the vegetation the species hit was noted. The abundance of the moss species was measured in June 1996 at the beginning of the experiment, in October 1996 and 1997 at the end of the growing season for the mosses, and in late August 1998 at the end of the experiment. Point-quadrat measurements for the vascular plants were made in June 1996, in early September 1996, late August 1997 and 1998 at peak biomass.

### *Sphagnum growth*

*Sphagnum* growth was measured non-destructively during the experiment by measuring height increment using the cranked wire method (Clymo 1970). In each plot, four stainless steel wires were placed about 8 cm deep. The wires were anchored in the peat by plastic bristles from a brush attached to the lower end of the wire. The length of the wire extending to the moss surface was measured monthly, except in winter. The mosses grew faster than expected and cranked wires were replaced by new ones when they became overgrown.

At the end of the experiment *Sphagnum* bulk density was determined at each cranked wire, so that height growth could be converted into biomass growth. A column of 8 cm diameter and 10 cm deep was cut with a sharp knife around each cranked wire. The upper 3 cm of the peat cylinder was cut off and the capitulum (defined as top 1 cm) of each *Sphagnum* shoot was cut off with scissors. The capitula were counted. Vascular plant parts were removed from the samples. All sections were dried at  $70^\circ\text{C}$  for at least 48 hours and weighed. *Sphagnum* net production in 1998 ( $\text{g m}^{-2} \text{ year}^{-1}$ ) was calculated as height increment (mm) times bulk density of the 0-3 cm layer ( $\text{g dm}^{-3}$ ).



### Vascular plant biomass

The total aboveground vegetation in the pq subplots was harvested in early September 1998, one week after the last point-quadrat recordings. All vascular plants were clipped off at the moss surface. The litter on top of the moss surface was also collected. The harvested species were sorted into current-year parts, other living parts and dead parts; the living parts were sorted into leaves, stems, flowering stems, flowers and berries. All plant parts were dried at 70 °C for at least 48 hours and weighed. For all ericaceous species it was possible to separate current-year parts from older parts. The current-year parts made up an important part of the annual aboveground production, since secondary stem growth in these species is very small (Backéus 1985). *D. rotundifolia*, *E. angustifolium* and other graminoid species had no older living parts, so the aboveground biomass was equal to the current-year aboveground production.

To reconstruct the development of the aboveground vascular plant biomass during the experiment, regressions between the final point-quadrat data and the aboveground vascular plant biomass at the final harvest for each species were used to calculate aboveground vascular plant during the experiment. These regressions were highly significant ( $P < 0.001$ ,  $R^2 = 0.60-0.95$ ,  $n = 13-20$ ). Species were treated separately because the biomass per hit differed strongly between the species. Species such as *V. oxycoccus* and *D. rotundifolia* with their leaves held horizontally were 'easily' hit and had a low biomass per hit (101 mg hit<sup>-1</sup> and 32 mg hit<sup>-1</sup> respectively). A tall erect species such as *E. angustifolium* had a much higher biomass per hit (324 mg hit<sup>-1</sup>).

The belowground vascular plant biomass was determined in three peat columns of 11 cm diameter and 30 cm deep per plot. The peat columns were cut into sections 5 cm thick and all belowground stems, rhizomes and roots were extracted and partitioned into five groups: 1) *V. oxycoccus*, 2) other ericaceous species, 3) *E. angustifolium*, 4) other graminoid species and 5) *D. rotundifolia*. All fractions were dried at 70 °C for at least 48 hours and weighed.

### Nutrient concentrations

Total C, N, P and K concentrations were measured in all current-year plant parts for *S. magellanicum*, *V. oxycoccus*, *E. tetralix* and *E. angustifolium*. P and K concentrations were measured in dry (70 °C), milled samples digested with sulphuric acid, salicylic acid, hydrogen peroxide and selenium and analysed by colorimetry for P concentrations and by flame atomic emission spectroscopy for K concentrations. C and N concentrations were determined in dry, ball milled samples by a CN analyser. To determine water and ash content, subsamples were subsequently dried at 105 °C and ignited at 550 °C. The total C and N concentrations were expressed as proportion of the organic matter content.

### Data analysis

Data were tested for normality and equality of variance. Biomass data for separate species and root:shoot ratios were ln-transformed prior to analysis, as these data deviated from normality. CO<sub>2</sub> effects and N effects were analysed separately, using *t*-tests. Pearson correlation coefficients and other statistics were calculated using SPSS for Windows (8.0).

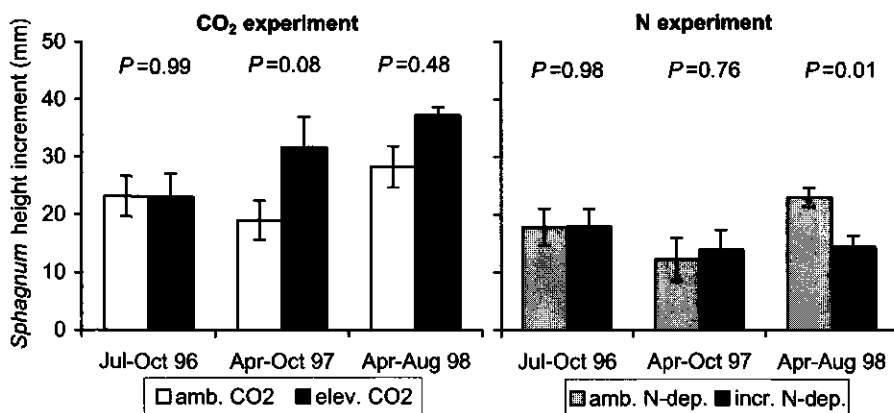
## Results

### *Sphagnum* growth

The moss layer was always dominated by *S. magellanicum*, which on average covered 95% of the moss surface. There were no significant changes in the cover of the moss species during the experiment (data not shown). *Sphagnum* height increment did not respond to the treatments in the first growing season, but was significantly increased at elevated CO<sub>2</sub> in the second and third growing season (Fig. 2.1). Over three growing seasons *Sphagnum* height increment was 70-124 mm in elevated CO<sub>2</sub> as opposed to 53-100 mm for ambient CO<sub>2</sub>. In contrast, increased N deposition resulted in a significantly reduced *Sphagnum* height increment in the third growing season. Over all treatments, height increment was highest in 1998 despite the fact that the measurements stopped at the end of August before the end of the growing season for *Sphagnum*. The wet weather in 1998 (Table 2.1) probably offered favourable conditions for *Sphagnum* growth.

Differences in height increment in *Sphagnum* between treatments were partly compensated by changes in bulk density (Table 2.2). We found a significant negative relation between *Sphagnum* bulk density 0-3 cm (g dm<sup>-3</sup>) and height increment ( $r = -0.69$ ,  $P < 0.01$ ,  $n = 20$ ). There was also a significant negative correlation between dry weight per capitulum and the number of capitula ( $r = -0.71$ ,  $P < 0.01$ ,  $n = 20$ ); thus as capitula numbers (per unit area) increased their size decreased. It seems likely that the differences in bulk density were caused by differences in morphology: we observed that under elevated CO<sub>2</sub> *Sphagnum* shoots had fewer branches per unit length of stem, whereas the shoots in the high-N treatment were very compact.

Elevated CO<sub>2</sub> resulted in a 17% higher biomass production in 1998 (Table 2.2), but, because of the relatively low bulk density, this effect was less than the increase of height increment and was not significant ( $P = 0.16$ ). However, the increased height increment itself has important consequences for the ecosystem, as will be discussed below. Increased N deposition resulted in a significantly lower (-32%) *Sphagnum* biomass production, mainly caused by the reduction in height increment.



**Figure 2.1** *Sphagnum* height increment (mm) during three growing seasons under ambient or elevated atmospheric CO<sub>2</sub> and under ambient or increased N deposition. Data are means  $\pm$  SE,  $n = 5$  plots.

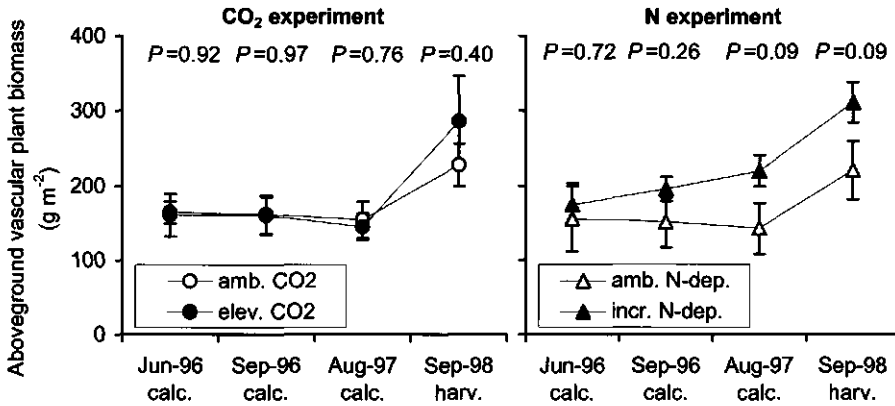
**Table 2.2** Growth characteristics of *Sphagnum*, determined after three growing seasons of CO<sub>2</sub> or N treatments in 8 cm diameter cores. Data are means  $\pm$  SE,  $n = 5$  plots, 4 cores per plot. Level of significance: \*  $P < 0.05$ .

	CO <sub>2</sub> experiment		N experiment	
	amb. CO <sub>2</sub>	elev. CO <sub>2</sub>	amb. N-dep.	incr. N-dep.
Bulk density 0-3 cm (g dm <sup>-3</sup> )	11.6 $\pm$ 1.3	9.6 $\pm$ 0.5	13.6 $\pm$ 1.4	14.2 $\pm$ 1.6
Capitula biomass (g m <sup>-2</sup> )	95 $\pm$ 9	101 $\pm$ 6	115 $\pm$ 11	93 $\pm$ 7
Number of capitula	81 $\pm$ 9	75 $\pm$ 3	90 $\pm$ 13	69 $\pm$ 7
Capitulum dry mass (mg)	6.5 $\pm$ 1.2	6.9 $\pm$ 0.5	7.0 $\pm$ 1.2	7.0 $\pm$ 0.5
Biomass production (g m <sup>-2</sup> year <sup>-1</sup> )	323 $\pm$ 29	377 $\pm$ 19	308 $\pm$ 18	211 $\pm$ 30 *

## Vascular plant biomass and species composition

### Total vascular plant biomass

Aboveground vascular plant biomass increased in most plots during the experiment, particularly in wet 1998. Elevated atmospheric CO<sub>2</sub> did not significantly affect aboveground biomass (Fig. 2.2, Table 2.3). Vascular plants were more responsive to N addition, where aboveground biomass showed increases from the beginning of the experiment (Fig. 2.2). This resulted in 54 and 41% larger aboveground peak-biomass in 1997 and 1998 respectively, but due to large between-plot variability the N treatment effect was only marginally significant (Table 2.3).



**Figure 2.2** Development of the aboveground vascular plant biomass (g m<sup>-2</sup>) during the experiment under ambient or elevated CO<sub>2</sub> and under ambient or increased N deposition. Data are means  $\pm$  SE,  $n = 5$  plots. The biomass in 1996 and 1997 was calculated from point-quadrat data, using linear regressions between number of hits at the end of August 1998 and the harvested aboveground biomass one week later for *V. oxycoccus*, *E. tetralix*, *E. angustifolium*, *D. rotundifolia* and other species.

**Table 2.3** Aboveground biomass, litter mass (including standing dead) and production (= current-year biomass) of vascular plants after three growing seasons of CO<sub>2</sub> or N treatments. Data are means ± SE, *n* = 5 plots. Level of significance: (\*) *P* < 0.10. Other species are: *Calluna vulgaris*, *Empetrum nigrum*, *Rhynchospora alba*, *Eriophorum vaginatum*, *Andromeda polifolia*, *Molinia caerulea* and *Pinus sylvestris*.

	CO <sub>2</sub> experiment		N experiment	
	amb. CO <sub>2</sub>	elev. CO <sub>2</sub>	amb. N-dep.	incr. N-dep.
<b>Aboveground biomass (g m<sup>-2</sup>)</b>				
all species	227 ± 37	286 ± 66	220 ± 49	311 ± 24 (*)
<i>Vaccinium oxycoccus</i>	102 ± 23	93 ± 21	94 ± 9	137 ± 19 (*)
<i>Erica tetralix</i>	62 ± 15	106 ± 51	82 ± 25	98 ± 14
<i>Eriophorum angustifolium</i>	37 ± 8	64 ± 21	32 ± 23	33 ± 14
<i>Drosera rotundifolia</i>	5 ± 2	1 ± 0	7 ± 2	7 ± 1
other species	21 ± 20	21 ± 15	6 ± 3	35 ± 16
<b>Aboveground litter mass (g m<sup>-2</sup>)</b>				
all species	42 ± 16	44 ± 9	26 ± 13	66 ± 16 (*)
<b>Aboveground production (g m<sup>-2</sup> year<sup>-1</sup>)</b>				
all species	184 ± 27	251 ± 48	166 ± 30	240 ± 20 (*)
<i>Vaccinium oxycoccus</i>	88 ± 20	84 ± 19	76 ± 8	107 ± 17
<i>Erica tetralix</i>	38 ± 7	81 ± 38	47 ± 13	66 ± 10
<i>Eriophorum angustifolium</i>	37 ± 8	64 ± 21	32 ± 23	33 ± 14
<i>Drosera rotundifolia</i>	5 ± 2	1 ± 0	7 ± 2	7 ± 1
other species	16 ± 15	20 ± 14	4 ± 2	27 ± 13

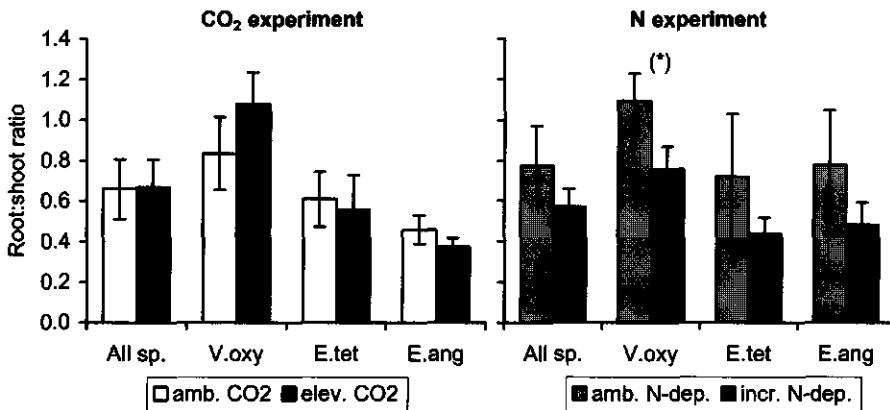
The proportion of current-year parts in aboveground biomass was large (76%) and was greatest in the elevated CO<sub>2</sub> treatment (86%). *V. oxycoccus* grows at the moss surface and in our study much of the older parts became overgrown by *Sphagnum*, particularly in the elevated CO<sub>2</sub> treatment where we measured the highest *Sphagnum* height increment. There was a significant negative correlation between *Sphagnum* height increment and the aboveground biomass of older plant parts ( $r = -0.77$ ,  $P < 0.01$ ,  $n = 20$ ). Because of the high proportion of current-year biomass, the 36% ( $P = 0.26$ ) increase in aboveground vascular plant production (Table 2.3) brought about by elevated CO<sub>2</sub> was greater than the increase in biomass (including older parts). Increased N deposition boosted aboveground production by 44%, which was statistically marginally significant ( $P = 0.08$ ).

Belowground biomass accounted for 69% of the total biomass and consisted of 26% roots. The remainder comprised subterranean stems of ericaceous species and subterranean stems and rhizomes of graminoid species. The subterranean stems of ericaceous species were old and overgrown stems, and probably were not growing or responding to CO<sub>2</sub> or N treatments. Also belowground biomass varied greatly between replicates. Elevated CO<sub>2</sub> did not significantly affect total belowground or root biomass (Table 2.4). Increased N deposition resulted in greater total belowground (29%) and root (18%) biomass, but the N treatment effect was not significant (Table 2.4).

**Table 2.4** Belowground biomass (belowground stems, rhizomes and roots) and root biomass ( $\text{g m}^{-2}$ ) of vascular plants after three growing seasons of  $\text{CO}_2$  or N treatments. Data are means  $\pm$  SE,  $n = 5$  plots, 3 cores per plot. There were no significant treatment effects.

	$\text{CO}_2$ experiment		N experiment	
	amb. $\text{CO}_2$	elev. $\text{CO}_2$	amb. N-dep.	incr. N-dep.
<b>Belowground biomass (<math>\text{g m}^{-2}</math>)</b>				
all species	607 $\pm$ 119	680 $\pm$ 114	526 $\pm$ 95	676 $\pm$ 115
<i>Vaccinium oxycoccus</i>	271 $\pm$ 84	318 $\pm$ 78	308 $\pm$ 58	319 $\pm$ 65
other ericoid species	248 $\pm$ 62	256 $\pm$ 56	172 $\pm$ 52	290 $\pm$ 68
graminoid species	86 $\pm$ 26	120 $\pm$ 15	48 $\pm$ 21	65 $\pm$ 11
<b>Roots (<math>\text{g m}^{-2}</math>)</b>				
all species	145 $\pm$ 28	168 $\pm$ 29	147 $\pm$ 24	174 $\pm$ 27
<i>Vaccinium oxycoccus</i>	81 $\pm$ 23	97 $\pm$ 25	102 $\pm$ 16	104 $\pm$ 21
other ericoid species	46 $\pm$ 18	42 $\pm$ 8	37 $\pm$ 12	50 $\pm$ 10
graminoid species	18 $\pm$ 6	27 $\pm$ 5	12 $\pm$ 4	19 $\pm$ 3

There were no indications that plant species under elevated  $\text{CO}_2$  allocated more biomass to roots (Fig. 2.3). Only *V. oxycoccus* had a slightly higher root:shoot ratio (with root biomass as root and aboveground biomass as shoot) under elevated  $\text{CO}_2$ , but this may be because a greater proportion of the aboveground biomass had been overgrown by the *Sphagna*, resulting in a relatively small aboveground biomass. Increased N deposition resulted in lower root:shoot ratios for all three species groups, in accordance with the usual response of plants to N fertilisation, but this effect was marginally significant only in *V. oxycoccus*.

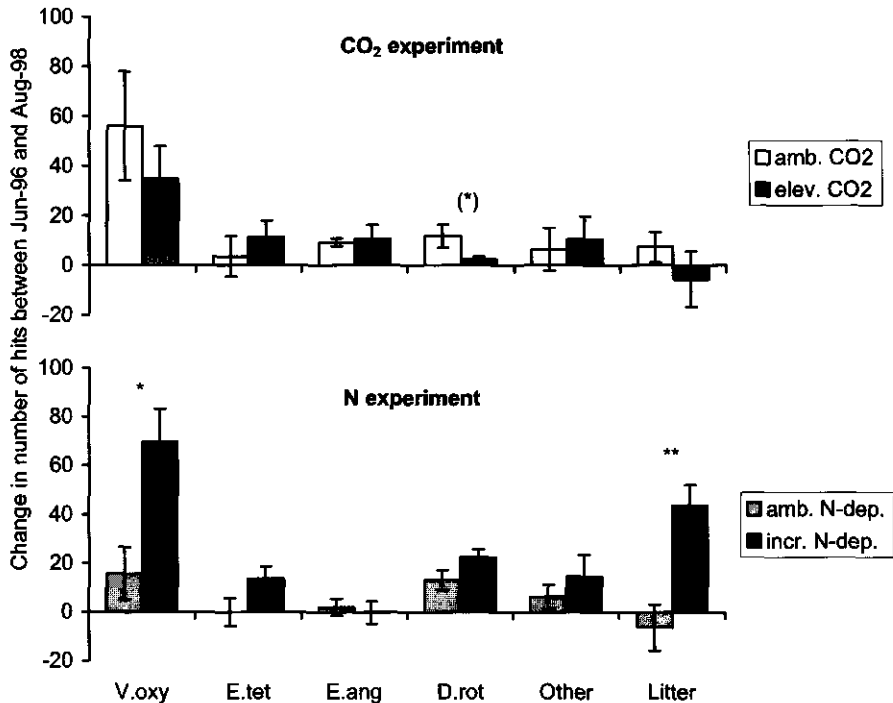


**Figure 2.3** Root:shoot ratio for all vascular species together, *V. oxycoccus*, *E. tetralix* including other ericaceous species and *E. angustifolium* including other graminoid species after three growing seasons of  $\text{CO}_2$  and N treatments. Data are means  $\pm$  SE,  $n = 5$  plots. Shoot = aboveground biomass; root = root biomass. Level of significance: (\*)  $P < 0.10$ .

## Species composition

Eleven species of vascular plants were recorded in the pq subplots. *V. oxycoccus*, *E. tetralix*, *E. angustifolium* and *D. rotundifolia* were present in all pq subplots. Point-quadrat measurements did not show major changes in species composition in the CO<sub>2</sub> experiment (Fig. 2.4). Only *D. rotundifolia* did not increase in cover in the elevated CO<sub>2</sub> plots, while it increased in all other treatments during the experiment. There was hardly any *D. rotundifolia* biomass in the elevated CO<sub>2</sub> compared to the other treatments (Table 2.3). It seems likely that this low abundance of *D. rotundifolia* in the elevated CO<sub>2</sub> plots was caused by the great height increment of *Sphagnum*. There was a significant negative correlation between *Sphagnum* height increment during the whole experiment and *D. rotundifolia* aboveground biomass ( $r = -0.57$ ,  $P = 0.01$ ,  $n = 20$ ).

Species abundance responded more strongly to the N treatment (Fig. 2.4). The abundance of all species, except of *E. angustifolium* increased more in the high N treatment than in the N control treatment. For *V. oxycoccus* the N treatment effect was significant ( $P = 0.01$ ). Although cover of *E. angustifolium* increased during the first two growing seasons, its cover declined in 1998 (data not shown), resulting in no net change from the beginning (Fig. 2.4). The large increase in litter abundance in the high N treatment (Fig. 2.4) is the result of the great abundance of *E. angustifolium* in the previous growing season. Litter mass (including standing dead) was almost significantly increased by the

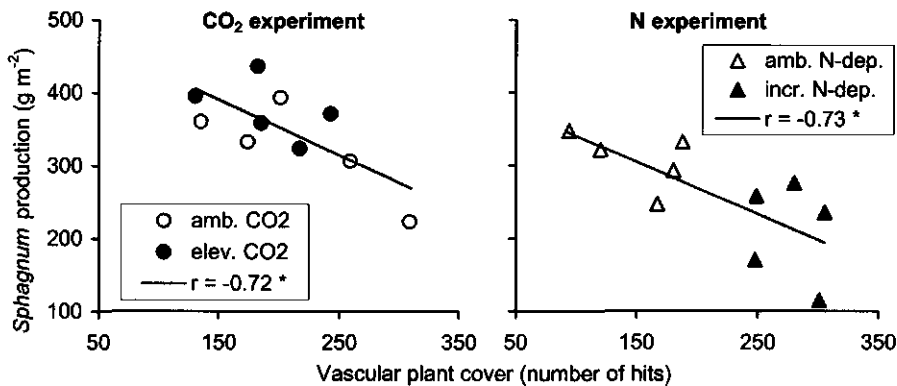


**Figure 2.4** Change in abundance (change in number of hits between Jun-96 and Aug-98) after three growing seasons of CO<sub>2</sub> or N treatments for *V. oxycoccus*, *E. tetralix*, *E. angustifolium*, *D. rotundifolia*, other vascular plant species and litter. Data are means  $\pm$  SE,  $n = 5$  plots. Levels of significance: (\*)  $P < 0.10$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ .

N addition treatment (Table 2.3). In all treatments litter was mainly from *E. angustifolium*. The point-quadrat recordings revealed that it was mainly *E. angustifolium* that caused the increased aboveground vascular plant biomass in the high N treatment in 1997, while *V. oxycoccus* responded most to N addition in 1998. Aboveground biomass of *V. oxycoccus* was increased by 46% at the final harvest in response to N fertilisation ( $P = 0.075$ ).

#### Effects of vascular plants on Sphagnum

The increased abundance of vascular plants under increased N deposition probably affected *Sphagnum* growth (Fig. 2.5). We found a significant negative correlation between vascular plant cover (expressed as total number of hits) and *Sphagnum* biomass growth in 1998 ( $r = -0.63$ ,  $P < 0.01$ ,  $n = 20$ ). There was also a relation with *Sphagnum* height increment, though it was less significant ( $r = -0.43$ ,  $P = 0.06$ ,  $n = 20$ ; not shown). Increased N deposition had a very significant effect on vascular plant cover ( $P < 0.01$ ), more significant than on (living) biomass, because litter also has a shading effect and is included in plant cover, and because *V. oxycoccus* had a high cover at relatively little biomass. Both the cover of litter and *V. oxycoccus* increased significantly under high N deposition (Fig. 2.4).



**Figure 2.5** Relation between *Sphagnum* production ( $\text{g m}^{-2}$ ) and vascular plant cover (number of hits) in 1998 after three growing seasons of CO<sub>2</sub> or N treatments. Level of significance: \*  $P < 0.05$ .

#### Nutrient concentrations in *Sphagnum* and vascular plants

Table 2.5 lists nutrient concentrations (N, P and K) and C:N ratios in the capitula (*Sphagnum*) and green leaves (vascular plants). In all four dominant species N concentrations had fallen after three seasons growing under elevated CO<sub>2</sub>. As a consequence the C:N ratio increased. Elevated CO<sub>2</sub> reduced the N concentration by 11% on average, and over all species this CO<sub>2</sub> effect was highly significant ( $P = 0.01$ ; ANOVA: CO<sub>2</sub> and species as factors, species as random factor). The same holds for the C:N ratio. The reduction in N concentration in plant tissues is one of the few consistent responses of plants to CO<sub>2</sub> enrichment (Körner 1996; Koch and Mooney 1996; Cotrufo *et al.* 1998). The reduction in N concentration in all dominant species combined with the increases in biomass (although not significant) may indicate that they all benefited from elevated CO<sub>2</sub>. N addition raised the N concentrations and reduced the C:N ratio in all dominant species

except *E. angustifolium*. In *S. magellanicum* the N concentration increased by 44%. The N effect was highly significant ( $P < 0.01$ ) for *S. magellanicum* and *E. tetralix* and marginally significant ( $P = 0.07$ ) for *V. oxycoccus*. There were no CO<sub>2</sub> or N treatment effects on P and K concentrations.

**Table 2.5** Nutrient concentrations and C:N ratios in capitula (*Sphagnum*) and current-year leaves (vascular plant species) after 3 growing seasons of CO<sub>2</sub> or N treatments. Data are means  $\pm$  SE,  $n = 5$  plots. Significance levels: (\*)  $P < 0.10$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ .

	CO <sub>2</sub> experiment		N experiment	
	amb. CO <sub>2</sub>	elev. CO <sub>2</sub>	amb. N-dep.	incr. N-dep.
<b>N concentration (mg N g<sup>-1</sup>)</b>				
<i>Sphagnum magellanicum</i>	16.8 $\pm$ 0.6	15.4 $\pm$ 0.2 *	16.8 $\pm$ 0.7	24.3 $\pm$ 0.6 **
<i>Vaccinium oxycoccus</i>	13.6 $\pm$ 0.3	12.2 $\pm$ 0.4 *	14.5 $\pm$ 1.1	17.5 $\pm$ 0.9 *
<i>Erica tetralix</i>	13.3 $\pm$ 0.6	11.8 $\pm$ 0.8	12.2 $\pm$ 0.7	18.0 $\pm$ 0.4 **
<i>Eriophorum angustifolium</i>	15.4 $\pm$ 1.0	13.4 $\pm$ 0.5	14.5 $\pm$ 0.4	14.8 $\pm$ 0.3
<b>P concentration (mg P g<sup>-1</sup>)</b>				
<i>Sphagnum magellanicum</i>	0.79 $\pm$ 0.04	0.85 $\pm$ 0.09	0.70 $\pm$ 0.05	0.68 $\pm$ 0.03
<i>Vaccinium oxycoccus</i>	0.86 $\pm$ 0.11	0.91 $\pm$ 0.16	0.57 $\pm$ 0.04	0.54 $\pm$ 0.02
<i>Erica tetralix</i>	0.71 $\pm$ 0.20	0.85 $\pm$ 0.31	0.33 $\pm$ 0.03	0.32 $\pm$ 0.02
<i>Eriophorum angustifolium</i>	0.76 $\pm$ 0.12	0.64 $\pm$ 0.04	0.59 $\pm$ 0.04	0.68 $\pm$ 0.05
<b>K concentration (mg K g<sup>-1</sup>)</b>				
<i>Sphagnum magellanicum</i>	6.5 $\pm$ 0.3	7.0 $\pm$ 0.4	6.4 $\pm$ 0.5	5.3 $\pm$ 0.2(*)
<i>Vaccinium oxycoccus</i>	6.1 $\pm$ 0.7	5.9 $\pm$ 0.5	4.4 $\pm$ 0.3	4.9 $\pm$ 0.1
<i>Erica tetralix</i>	5.0 $\pm$ 0.4	4.3 $\pm$ 0.6	3.7 $\pm$ 0.1	3.9 $\pm$ 0.3
<i>Eriophorum angustifolium</i>	8.3 $\pm$ 0.8	7.1 $\pm$ 0.8	8.5 $\pm$ 0.7	8.9 $\pm$ 0.5
<b>C:N ratio</b>				
<i>Sphagnum magellanicum</i>	29 $\pm$ 1	32 $\pm$ 0 *	29 $\pm$ 1	20 $\pm$ 0 **
<i>Vaccinium oxycoccus</i>	42 $\pm$ 1	47 $\pm$ 1 (*)	40 $\pm$ 3	33 $\pm$ 2 (*)
<i>Erica tetralix</i>	46 $\pm$ 2	53 $\pm$ 3	51 $\pm$ 3	34 $\pm$ 1 **
<i>Eriophorum angustifolium</i>	34 $\pm$ 2	38 $\pm$ 1	36 $\pm$ 1	35 $\pm$ 1

## Discussion

There was no evidence that transferring the peat monoliths had had any irreversible effect on the vegetation. We observed that the species composition in the control plots hardly changed, except for *V. oxycoccus* that increased its abundance in all treatments, mainly in 1998. This increased abundance may be the result of the wet conditions in 1998 or of the higher ambient N deposition in Wageningen than in the field site. *Sphagnum* growth might be expected to be greater in our experiment than in the field site, because we prevented severe drought, but also in our experiment *S. magellanicum* hardly grew (in height) during the warmest months with low water tables (data not shown). Height increment of *S. magellanicum* in the control plots was remarkably similar to that measured in another comparable small mire near our field site, in 1998 and 1999 (Limpen, unpublished data).



The duration of three growing seasons seemed to be sufficient to measure the effects of changed interactions between species in response to the sudden changes in CO<sub>2</sub> and N supply. The lack of response during the first growing season (Figs 2.1 and 2.2) agrees with findings from other CO<sub>2</sub> experiments in low productivity systems (Warwick *et al.* 1998; Leadley *et al.* 1999). We suggest that slow growing species that recycle a large proportion of earlier assimilated nutrients and carbon are less responsive to sudden changes in the external environment, because in the first year of the treatment the responses are buffered by the large stocks these species have built up in previous years.

### CO<sub>2</sub> effects

The CO<sub>2</sub> effect on *Sphagnum* growth measured in this study is probably a direct response to elevated CO<sub>2</sub> and not an indirect response brought about by increased moisture availability as observed in other ecosystems (Drake *et al.* 1996; Owensby *et al.* 1999). In our experiment the water level relative to the moss surface was not higher in the elevated CO<sub>2</sub> plots (measured twice a week, unpublished data), because of the increased *Sphagnum* height increment, and extreme low water levels were prevented. However, we did find a reduced water use of the bog vegetation under elevated CO<sub>2</sub> (unpublished data), which might be beneficial for *Sphagnum* growth under real field conditions, as *Sphagnum* species are very sensitive to changes in water content (Clymo and Hayward 1982; Schipperges and Rydin 1998).

We are not aware of other CO<sub>2</sub> enrichment studies in *Sphagnum*-dominated mire vegetation. In greenhouse experiments, Jauhiainen *et al.* (1994, 1998a) found CO<sub>2</sub>-induced increases in height increment and production for *S. angustifolium*, but not for *S. fuscum*. The dominant *Sphagnum* in our study, *S. magellanicum*, may have a greater diffusive resistance to CO<sub>2</sub> uptake within its leaves than other *Sphagnum* species, because its photosynthetic cells are enclosed within water-filled hyaline cells (Proctor 1982). Such resistance might cause *S. magellanicum* to be CO<sub>2</sub>-limited in the current situation and therefore to react appreciably to CO<sub>2</sub> enrichment in our study.

The failure to find any statistically significant increase in aboveground vascular plant production, despite an increase of 36%, can partly be attributed to large between-plot variability. Furthermore, differences in *Sphagnum* height growth complicated the measurement of aboveground biomass. At the final harvest we observed that even parts of current-year stems, particularly of *V. oxycoccus*, were overgrown by *Sphagnum*. Once covered by the moss, leaves soon fall off and it is almost impossible to distinguish these current-year stems from older stems. Therefore we assume that aboveground vascular plant production, determined from aboveground current-year biomass, is underestimated, particularly in the elevated CO<sub>2</sub> treatment.

The only CO<sub>2</sub> response found in a long-term tundra experiment was an increased tiller density of *Eriophorum vaginatum* (Tissue and Oechel 1987; Oechel and Vourlitis 1996). *E. vaginatum* is also a common species in bog ecosystems, but was present in only a few plots in our experiment. Of all multi-year CO<sub>2</sub>-enrichment studies we know of, the tundra ecosystem study is probably most similar to our bog study. The lack of response at the ecosystem level in the tundra study was attributed to nutrient limitation. The growing conditions in our study were probably much more favourable than in the tundra, because of the milder Dutch climate, longer growing season and much higher ambient N deposition.

## Nitrogen effects

Despite the high ambient N deposition at our experimental site (approximately  $50 \text{ kg N ha}^{-1} \text{ year}^{-1}$ ) the vegetation still responded to the added N. Under ambient N deposition *Sphagnum* growth is obviously not limited by N, given the lack of a stimulating effect of N addition on growth. That *Sphagnum* growth was not N-limited was also indicated by the high N:P ratio ( $24 \pm 1$ ) in *S. magellanicum* capitula, corresponding with findings from Aerts *et al.* (1992) who showed that growth of *S. magellanicum* from south Sweden having high N:P ratios ( $> 14$ ) was P-limited. Although N was not limiting growth of *Sphagnum*, it was still able to capture a large portion of deposited N under ambient N deposition. Approximately 60% of an aerielly deposited  $^{15}\text{N}$ -label was found in the living *Sphagnum* layer in the N control treatment fifteen months after addition (Chapter 6). Increased N deposition significantly reduced the proportion of  $^{15}\text{N}$  in *Sphagnum* and significantly increased the proportion of  $^{15}\text{N}$  in vascular plants (Chapter 6). In addition, inorganic N concentrations in soil water, sampled at 10, 30 and 60 cm depth at the end of the experiment, were significantly increased in the high N treatment (unpublished data).

It is clear that during 3 growing seasons of N addition *Sphagnum* was less able to retain the deposited N, resulting in increased N availability for vascular plants. Consequently, the shallow-rooted species *V. oxycoccus* significantly increased cover (Fig. 2.4). The other species did not respond in terms of biomass or cover, which might be attributed to the P-limiting conditions, suggested by the high N:P ratios ( $27 \pm 2$  and  $22 \pm 1$  for *E. tetralix* and *E. angustifolium* respectively in the N control treatment). N:P ratios in aboveground plant material higher than 15 indicate P limitation (Koerselman and Meuleman 1996; Verhoeven *et al.* 1996). N:P ratio in *V. oxycoccus* was significantly increased in the high N treatment ( $26 \pm 2$  versus  $19 \pm 2$  in the N control treatment), suggesting that further expansion of the cover of *V. oxycoccus* at increased N deposition might be restricted by P limitation.

Our results agree with those of Lütke Twenhöven (1992a, 1992b), who manipulated N deposition in a similar bog vegetation in Germany and reported a similar significant increase in the standing crop of *V. oxycoccus* after two years of ammonium addition. In his study, *E. angustifolium* did not respond and *S. magellanicum* was unaffected or negatively affected by the N addition.

## Indirect effects: interactions between *Sphagnum* and vascular plants

The increased height increment of *Sphagnum* under elevated  $\text{CO}_2$  affected two species that grow close to the moss surface: *D. rotundifolia* and *V. oxycoccus*. The abundance of *D. rotundifolia* increased in all treatments, but not in the elevated  $\text{CO}_2$  plots (Fig. 2.4) where its final biomass dropped to very low values (Table 2.3). It seems that the small *D. rotundifolia* plants could not keep pace with the rate of *Sphagnum* growth, which is confirmed by the inverse relation between *D. rotundifolia* biomass and *Sphagnum* height increment. Svensson (1995) observed that *D. rotundifolia* avoided being overgrown by increasing height growth when height growth of *S. fuscum* was increased, but *Sphagnum* height growth was much less than in our study. The negative correlation between the aboveground biomass of old stems of *V. oxycoccus* and *Sphagnum* height increment means that *V. oxycoccus* became overgrown faster and had to invest more in aboveground growth

to stay on top of the moss surface. Within the experimental period, however, this did not lead to a reduced aboveground biomass or production of this species.

Previous studies have suggested that shading by vascular plants may reduce *Sphagnum* growth (Clymo 1973; Hayward and Clymo 1983). In our experiment it was therefore probably the increased vascular plant cover (including litter) under increased N deposition that reduced the *Sphagnum* growth in the third growing season (Fig. 2.5). This negative correlation between vascular plant cover and *Sphagnum* growth does not exclude other factors than shading. It is also possible that added N affected *Sphagnum* growth directly. It may have taken two growing seasons to saturate the *Sphagnum* layer with N, leading to reduced *Sphagnum* growth in the third growing season, coinciding with growth stimulation of shallow-rooted species. There are studies that support this explanation: in laboratory experiments with monocultures of *Sphagnum* species, Press *et al.* (1986) and Jauhiainen *et al.* (1994, 1998a) showed a negative response to high N supply, and in a Canadian bog *S. fuscum* production decreased after the addition of 15 g N m<sup>-2</sup> in one growing season, whereas vascular plant production increased only slightly (Thormann and Bayley 1997).

Fertilisation of several oligotrophic ecosystems (mire, tundra, heathland) that had a significant non-vascular plant component (lichens and mosses) increased the abundance of vascular plants and decreased the biomass of non-vascular plants (Lütke Twenhöven 1992a; Jonasson 1992; Hogg *et al.* 1995; Chapin *et al.* 1995; Press *et al.* 1998). Two of these studies (Jonasson 1992; Hogg *et al.* 1995) included removal of vascular plants. Only in a dense *Molinia* stand did cutting of the *Molinia* benefit *Sphagnum* (Hogg *et al.* 1995). It seems that high densities of vascular plants (induced by nutrient addition) are needed for reduction of *Sphagnum* growth.

### Implications for carbon sequestration

When speculating about the consequences for carbon sequestration, we should not only consider changes in biomass production or decomposition of single species, but also changes in species composition. There are large differences between species in productivity and decomposability and therefore, in the long term, changes in species composition can result in important changes in carbon sequestration. This has been ignored in almost all models dealing with global change and terrestrial ecosystems. Changes in species composition may be the result of species-specific responses to changes in the environment and of competitive interactions between species. This experiment clearly demonstrated that both species-specific responses to treatments and interactions between peat mosses and vascular plants are important, even in a relatively short period of three growing seasons. In bog ecosystems, recalcitrant *Sphagnum* litter sequesters more carbon than the more easily decomposable litter of vascular plants (Clymo and Hayward 1982), whereas vascular plants contribute more to methane emission (Joabsson *et al.* 1999). Thus changes in relative contributions of both species groups will have consequences for the exchange of greenhouse gases between bog and atmosphere.

Now that bogs are more and more considered as important ecosystems with respect to carbon sequestration, it is remarkable that so little is known about the impact of CO<sub>2</sub> itself. Our results show a potential important feedback mechanism: elevated CO<sub>2</sub> giving a competitive advantage to *Sphagnum*, resulting in increased peat accumulation (thus C sequestration) in the long term, which feeds back to the CO<sub>2</sub> concentration in the atmosphere. However, we must be careful to draw conclusions on carbon sequestration

since the effects of elevated CO<sub>2</sub> on decomposition, an important component of the carbon balance, are largely unknown.

Increased N deposition may result in an opposite chain of effects. We observed reduced *Sphagnum* and increased vascular plant production in our experiment, which corresponds with results from other studies. This change in the competitive balance between peat mosses and vascular plants to the benefit of vascular plants is expected to decrease sequestration of CO<sub>2</sub>. Furthermore, plant tissues containing more N might be decomposed faster (Coulson and Butterfield 1978). The N addition in our experiment resulted in higher N concentrations; not only in live tissues (Table 2.5), but also in brown *Sphagnum*, 5-10 cm below moss surface (unpublished data). Therefore, we believe that the increased abundance of vascular plants and the reduced *Sphagnum* growth brought about by high N deposition will reduce carbon sequestration and increase methane emission from bog ecosystems.

## Acknowledgements

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## Differential responses of *Sphagnum* mosses and vascular plants to elevated CO<sub>2</sub> and increased N deposition

Monique Heijmans, Herman Klees, Willem de Visser and Frank Berendse

### Summary

The effect of elevated atmospheric CO<sub>2</sub> concentrations on bog vegetation is largely unknown. In other ecosystems the response of plant growth to rising CO<sub>2</sub> levels appears to depend on nutrient availability. We studied the effects of elevated CO<sub>2</sub> in combination with N supply on the growth of *Sphagnum* mosses and vascular plants in ombrotrophic bog vegetation. In a greenhouse experiment peat monoliths (34 cm diameter, 40 cm deep) with intact bog vegetation were exposed to ambient (350 ppmv) or elevated (560 ppmv) atmospheric CO<sub>2</sub> combined with low (no N addition) or high (5 g N m<sup>-2</sup> year<sup>-1</sup> added) N deposition during two growing seasons.

Elevated atmospheric CO<sub>2</sub> had unexpected deleterious effects on growth of *Sphagnum magellanicum*, the dominant *Sphagnum* species. Its growth was greatly reduced, particularly in the second growing season when the plants had an unhealthy appearance, independent of N supply. The negative CO<sub>2</sub> effect was strongest in the warmest months, suggesting a combined effect of elevated CO<sub>2</sub> and the raised temperatures in the greenhouse.

## Introduction

Ombrotrophic bogs are peat-forming systems and as such they can serve as important long-term sinks for atmospheric CO<sub>2</sub> (Gorham 1991). By absorbing significant amounts of CO<sub>2</sub>, terrestrial ecosystems can slow the build up of CO<sub>2</sub> in the atmosphere and consequently reduce the rate of climate change (Schimel 1995; Walker *et al.* 1997). Among peatlands the *Sphagnum*-dominated bogs have the greatest peat (thus carbon) accumulation potential due to low rates of decomposition (Thormann *et al.* 1999). Thus changes in these ecosystems, for example brought about by climate change, can have important consequences for global carbon cycling. However, little is known about the effects of climate change on bog ecosystems.

The amount of carbon being sequestered in peatlands depends on the balance between the production and decomposition of plant material. Changes in plant species composition may have important effects on this balance because species differ strongly in productivity and decomposability. This certainly holds for the bog vegetation, which comprises peat mosses (*Sphagnum*) and vascular plants. *Sphagnum* species form the bulk of bog peat, because *Sphagnum* litter is more recalcitrant than that of vascular plants and decomposes slowly (Coulson and Butterfield 1978; Clymo and Hayward 1982). In contrast, vascular plants contribute less to peat accumulation and, moreover, contribute more to methane emission (Joabsson *et al.* 1999).

Elevated CO<sub>2</sub> generally stimulates productivity of individual plants, but not necessarily net ecosystem production (Körner 1996; Koch and Mooney 1996). Plant level responses to elevated CO<sub>2</sub> cannot directly be extrapolated to the ecosystem level, because interactions between species and resource limitation of growth come into play (Körner 1996). The effect of elevated CO<sub>2</sub> on bog vegetation is largely unknown, because only measurements on individual *Sphagnum* species have been performed (Silvola 1985; Jauhiainen *et al.* 1994, 1997, 1998a; Jauhiainen and Silvola 1999). Studies in other ecosystems suggest that the vegetation response depends largely on interactions with other environmental factors, such as water, nutrient availability and temperature. In nutrient poor ecosystems such as arctic tundra, nutrient availability and/or uptake must increase before plants are able to benefit from CO<sub>2</sub> fertilisation (Oechel and Vourlitis 1994). A compilation of ecosystem level CO<sub>2</sub> responses (Körner 1996) revealed that almost all low fertility natural or model ecosystems, in contrast to high fertility systems, showed no or little stimulation of biomass under elevated CO<sub>2</sub>, which stresses the importance of nutritional status. This is confirmed by studies with individual plant species, which show that the CO<sub>2</sub> response often depends on the nutrient status, with no response to elevated CO<sub>2</sub> at low nutrient supply (Arp *et al.* 1998).

The only sources of mineral nutrients in ombrotrophic bogs are the atmospheric deposition and the mineralisation of dead organic matter. *Sphagnum* is very effective in absorbing nutrients from atmospheric deposition (Woodin and Lee 1987; Williams *et al.* 1999), thereby reducing this supply of nutrients to vascular plants. Vascular plants largely depend on the nutrients mineralised from dead organic matter, but the slow decomposition of *Sphagnum* litter depresses this supply of nutrients to vascular plants. A high N deposition or high rate of mineralisation would increase the N availability in the rhizosphere and should consequently increase vascular plant growth (Woodin and Lee

1987; Malmer *et al.* 1994). The resulting increased shading of the moss layer would depress *Sphagnum* growth (Clymo 1973; Hayward and Clymo 1983).

Elevated CO<sub>2</sub> and increased N deposition probably act differently on *Sphagnum* and vascular plants, which has important consequences for the exchange of CO<sub>2</sub> and CH<sub>4</sub> between bogs and the atmosphere. Because *Sphagnum* captures most nutrients from the atmosphere, it is less nutrient limited, and we hypothesised that its growth would be stimulated by elevated CO<sub>2</sub> (Sveinbjörnsson and Oechel 1992; Jauhiainen *et al.* 1998a). Vascular plant growth was expected to benefit from high N deposition, assuming that growth of vascular plants is currently limited by N. As a consequence the moss may suffer from shading, but the increased N deposition may also directly harm *Sphagnum* growth, as was shown by Press *et al.* (1986). In addition, we hypothesised that elevated CO<sub>2</sub> will affect vascular plant growth only at a high N supply.

To test these hypotheses, we investigated the effects of elevated atmospheric CO<sub>2</sub> and increased N deposition on the growth of *Sphagnum* mosses and vascular plants in bog vegetation. We conducted a greenhouse experiment using peat monoliths with intact bog vegetation to allow species interactions. The monoliths were exposed to two levels of atmospheric CO<sub>2</sub> combined with two levels of N deposition for two growing seasons.

## Methods

### Plant material

The peat monoliths used in this study were cut from a small mire in the State Forestry of Dwingeloo (52°49'N, 6°25'E) in the north of the Netherlands. This site is one of a series of heath pools (0.5-1.5 m deep) that has become filled with peat and is situated in a former valley. The heath pool measures 50 by 150 m and is surrounded by forest. The peat layer is 25 to 50 cm thick and is more or less floating. Due to irregular peat cutting in the past, the vegetation is now a mosaic of secondary succession stages.

The monoliths were cut from a lawn vegetation dominated by *Sphagnum magellanicum* Brid. and *Vaccinium oxycoccus* L.. Other vascular plant species were *Rhynchospora alba* (L.) Vahl, *Erica tetralix* L., *Eriophorum angustifolium* Honck. and *Drosera rotundifolia* L.. Other moss species were *S. papillosum* Lindb., *S. fallax* (Klinggr.) Klinggr., *Aulacomnium palustre* (Hedw.) Schwägr. and *Calliergon stramineum* (Brid.) Kindb.. Twenty peat monoliths were cut in March 1996 when the upper peat layer was still frozen. The columns were 34 cm in diameter and 40 cm deep and were placed in white plastic containers of equal size.

### Experimental design

The peat monoliths were exposed to two atmospheric CO<sub>2</sub> levels (ambient: 350 ppmv and elevated: 560 ppmv) combined with two levels of N supply (low: no N addition and high: 5 g N m<sup>-2</sup> year<sup>-1</sup> added). All treatments were replicated five times. The CO<sub>2</sub> treatment was established by placing the containers in the greenhouse in two identical compartments in which the CO<sub>2</sub> concentration could be regulated. Every two weeks the CO<sub>2</sub> level was switched between the compartments (and the containers were moved) to avoid a compartment effect. Nitrogen was added as dissolved NH<sub>4</sub>NO<sub>3</sub>. A total amount of 5 g N m<sup>-2</sup> was added each growing season to the high N containers in six applications

(about every three weeks) by watering with 250 ml of N-solution, simulating a rain event of 2.5 mm. The low N containers received the same amount of demineralised water. These gifts preceded the weekly water addition (see below). In the second growing season the N doses were included in that week's water gift.

The temperature in the greenhouse was set to simulate the daily and seasonal pattern of outdoor temperature by using the weekly average minimum and maximum temperature for the 1980s. These values were increased by 5 °C to prevent exceeding the cooling capacity of the greenhouse during warm spells. During the day the temperature was set to change every 4 hours from the minimum temperature (from 0:00 to 4:00 hour) to the maximum temperature (from 12:00 to 16:00 hour) and back. Minimum temperature ranged between 10 and 18 °C and maximum temperature ranged between 18 and 28 °C. Humidity was set at 75%. No artificial light was used.

Water level in each container was restored to 5 cm below moss surface every week by watering the experimental plots with artificial rainwater. Water levels dropped to a minimum of 10 cm below moss surface in 1996 and 15 cm below moss surface in 1997 (due to increased water use of the vascular plants and raised moss surface). The artificial rainwater was made by diluting a seawater solution of the chemical composition as given in Garrels and Christ (1965) 8000 times with demineralised water (Table 4.1). This water is equivalent to very clean rainwater without N or P.

The experiment started in June 1996. From November 1996 to March 1997 the monoliths overwintered outside underneath a transparent roof because it was not possible to have low temperatures in the greenhouse. In that period there was no CO<sub>2</sub> treatment. The experiment ended with a final harvest in the first week of October 1997.

## Measurements

### *Species abundance*

Plant species composition and abundance (both mosses and vascular plants) were measured non-destructively using the point-quadrat method (Jonasson 1988). The point-quadrat consisted of 125 points spaced at 2.5 cm intervals. At each point a sharpened knitting needle was lowered and at all contacts with the vegetation the species hit was noted. The measurements were made in July 1996, October 1996 (only mosses), July 1997 (only vascular plants) and September/October 1997.

*R. alba* became the dominant vascular plant species during the experiment. As it is a rather tall erect species, its abundance might not be measured accurately by the point-quadrat method. Therefore we counted all green shoots and measured the height of each shoot in autumn 1996 and 1997. The sum of shoot heights was used to estimate aboveground biomass in 1996 using linear regression between sum of shoot heights in 1997 and the harvested aboveground biomass ( $R^2 = 0.97$ ,  $P < 0.001$ ,  $n = 20$ ).

### *Sphagnum growth*

*Sphagnum* growth was measured non-destructively during the experiment by measuring height increment and counting shoot density. *Sphagnum* height increment was measured with the cranked wire method (Clymo 1970). In each monolith two stainless steel wires were anchored in the peat at a depth of 8 cm by plastic bristles attached to the lower end of the wire. The length of the wire extending above the moss surface was measured monthly,



except in winter. *Sphagnum* shoot density was measured as the number of capitula in one permanently marked 10 cm diameter plot. The number of capitula was counted three times during the experiment.

At the final harvest a *Sphagnum*/peat column of 10 cm diameter was cut in the middle of each monolith for measurements on individual *Sphagnum* shoots. From twenty intact *Sphagnum* individuals the length of the green shoot was measured. After removing the capitulum (defined as the top 1 cm), the next 2 cm were used to determine the shoot dry mass per unit length. Other *Sphagnum* capitula and green parts of the 10 cm core were also collected. All fractions were dried at 70 °C for at least 48 hours and weighed. We considered the harvested green biomass as a measure of *Sphagnum* biomass growth during the experiment, as the length of green shoot was about equal to measured height increment during the experiment and correlated significantly with it ( $r = 0.89$ ,  $P < 0.001$ ,  $n = 20$ ). For six containers with large height increment ( $> 5$  cm) the corresponding green length was lower, as green length appeared to have a maximum of 6 cm, and biomass growth might have been underestimated.

### Vascular plant biomass

The total aboveground vegetation in the containers was harvested in the first week of October 1997 immediately after the last point-quadrat recordings. The litter on top of the moss surface was also collected, and *Rhynchospora* buds were collected from the moss layer. The harvested species were sorted into current-year parts, other living parts and dead parts; the living parts were sorted into leaves, stems and flowering stems. For all ericaceous species it was possible to distinguish current-year parts from older parts. For *Rhynchospora* and *Drosera* the current-year biomass is equal to total (living) biomass. All plant parts were dried at 70 °C for at least 48 hours and weighed.

To calculate the increase in total aboveground vascular plant biomass during the experiment, we estimated vascular plant biomass at the beginning of the experiment, using regressions between the final point-quadrat data and the aboveground vascular plant biomass at the final harvest for each species. These regressions were highly significant ( $P < 0.001$ ,  $R^2 = 0.82-0.98$ ,  $n = 14-20$ ) except for *D. rotundifolia* ( $P = 0.07$ ), but this species contributed very little to vascular plant biomass.

Species were treated separately because the biomass per hit differed strongly between the species. Species such as *V. oxycoccus* and *D. rotundifolia* with their leaves held horizontally were 'easily' hit and had a low biomass per hit (126 mg hit<sup>-1</sup> and 92 mg hit<sup>-1</sup> respectively). A tall erect species such as *E. angustifolium* had a much higher biomass per hit (466 mg hit<sup>-1</sup>). Regressions were more significant when dead parts were included. For October 1997 these calculations resulted in a highly significant correlation ( $r = 0.93$ ,  $P < 0.001$ ,  $n = 20$ ) between the calculated and the harvested total aboveground biomass, including dead parts.

### Nutrient concentrations

C, N, P and K concentrations were measured in all current-year plant parts for *S. magellanicum*, *R. alba* and *V. oxycoccus*. Dry milled samples were digested with sulphuric acid, salicylic acid, hydrogen peroxide and selenium and analysed by colorimetry for P concentrations and by flame AES for K concentrations. The C and N concentrations were determined by a CN analyser and corrected for water and ash content.

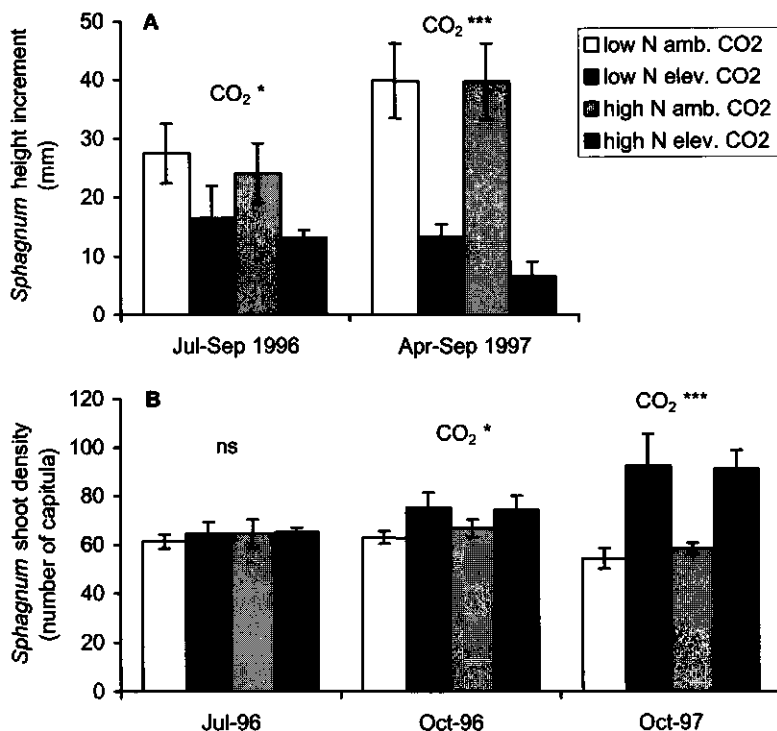
## Data analysis

Treatment effects were analysed using two-way analysis of variance (ANOVA) with CO<sub>2</sub> and N supply as independent factors. Vascular plant biomass varied greatly and appeared to be largely determined by the abundance at the beginning of the experiment. Therefore the percentage increase relative to the beginning of the experiment (as deduced from point-quadrat data) was used as variable. When necessary, data were ln-transformed to achieve homogeneous variances. Pearson correlation coefficients and other statistics were calculated using SPSS for Windows (8.0).

## Results

### *Sphagnum* growth

The moss layer was always dominated by *S. magellanicum*, which on average covered 93% of the moss surface. Its cover did not change during the experiment (data not shown). *Sphagnum* height increment was significantly reduced at elevated CO<sub>2</sub> (Fig. 3.1A), both in the first and in the second growing season. In the second growing season, height growth was only 10 mm in elevated CO<sub>2</sub> as opposed to 40 mm for ambient CO<sub>2</sub>. The reduction in



**Figure 3.1** *S. magellanicum* A) height increment (mm) and B) shoot density (number of capitula in 10 cm subplot) during two growing seasons of combined CO<sub>2</sub> and N treatments. Data are means  $\pm$  SE ( $n = 5$  replicates). Significant treatment effects (tested with 2  $\times$  2 ANOVA) for each season (A) or date (B) are included. Level of significance: \*  $P < 0.05$ , \*\*\*  $P < 0.001$ .

height growth under elevated CO<sub>2</sub> was partly compensated by an increase in *Sphagnum* shoot density (Fig. 3.1B). At elevated CO<sub>2</sub> *Sphagnum* shoot dry mass per unit length was also higher, but the capitulum dry mass was much lower (Table 3.1).

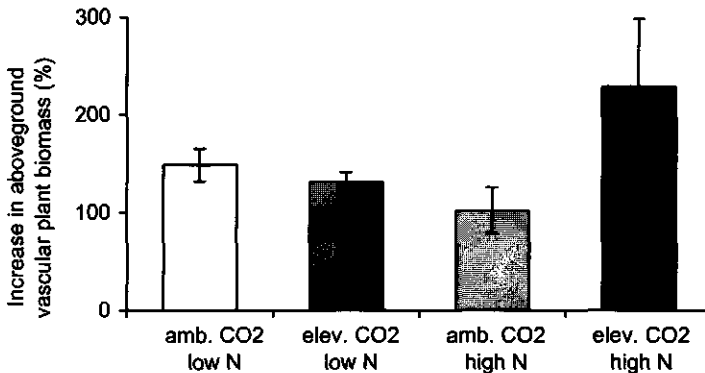
In the elevated CO<sub>2</sub> treatment many capitula were broken off and replaced by small young capitula. The *Sphagnum* shoots from the elevated CO<sub>2</sub> treatment looked compact (densely branched) and there was a negative relation between shoot dry mass per cm and length of green shoot or height increment ( $P < 0.01$ ). The *Sphagnum* green biomass was significantly reduced at elevated CO<sub>2</sub> (Table 3.1). In addition the *Sphagnum* plants of the elevated CO<sub>2</sub> treatments had an unhealthy appearance in the second growing season. Their capitula and branches broke off easily when touched. N had no effect on any measured component of *Sphagnum* growth (Fig. 3.1, Table 3.1). The CO<sub>2</sub> effects did not depend on the N supply (Fig. 3.1, Table 3.1).

**Table 3.1** *Sphagnum* growth characteristics determined after two growing seasons of combined CO<sub>2</sub> (C) and N treatments in a 10 cm diameter core. Data are means  $\pm$  SE,  $n = 5$  replicates. Level of significance: (\*)  $P < 0.10$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

	low N		high N		ANOVA		
	amb. CO <sub>2</sub>	elev. CO <sub>2</sub>	amb. CO <sub>2</sub>	elev. CO <sub>2</sub>	CO <sub>2</sub>	N	CxN
Length of green shoot (mm)	44 $\pm$ 6	24 $\pm$ 3	50 $\pm$ 3	17 $\pm$ 6	***	-	-
Shoot dry mass per cm (mg cm <sup>-1</sup> )	4.3 $\pm$ 0.5	4.5 $\pm$ 0.7	3.8 $\pm$ 0.4	5.9 $\pm$ 0.3	*	-	(*)
Capitulum dry mass (mg)	11.6 $\pm$ 0.8	4.0 $\pm$ 1.1	10.2 $\pm$ 1.3	6.2 $\pm$ 1.0	***	-	-
Green biomass (g)	1.9 $\pm$ 0.1	1.3 $\pm$ 0.2	2.0 $\pm$ 0.2	0.9 $\pm$ 0.2	**	-	-

### Vascular plant biomass and species composition

Aboveground vascular plant biomass (including dead parts) increased in all containers during the experiment and the relative increase was largest under elevated CO<sub>2</sub> combined with high N supply (Fig. 3.2). Thus, only at high N deposition elevated CO<sub>2</sub> tended to have a positive effect on vascular plant growth (C  $\times$  N interaction:  $P = 0.08$ ). Aboveground living biomass was also largest in the high N-high CO<sub>2</sub> treatment, but variation was large



**Figure 3.2** Percentage increase in total aboveground vascular plant biomass (including dead parts) during two growing seasons of combined CO<sub>2</sub> and N treatments. Data are means  $\pm$  SE,  $n = 5$  containers.

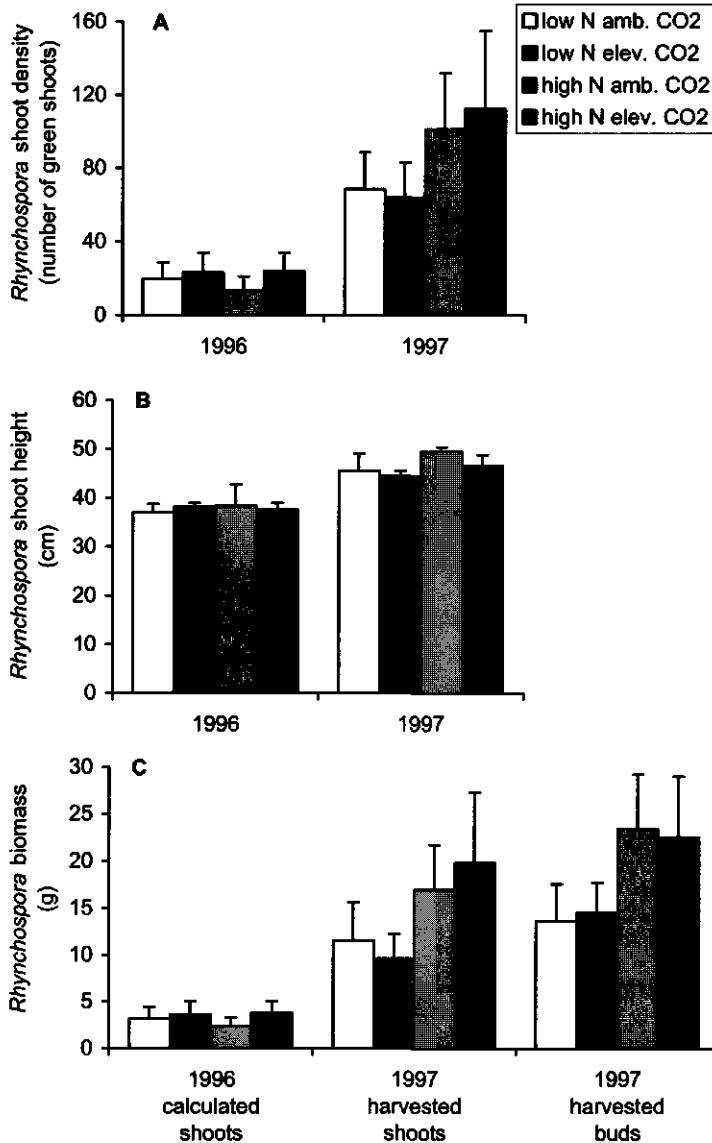
**Table 3.2** Aboveground biomass and necromass (standing dead and litter) ( $\text{g m}^{-2}$ ) of vascular plants after two growing seasons of combined  $\text{CO}_2$  and N treatments. Data are means  $\pm$  SE,  $n = 5$  containers. Other species are: *Calluna vulgaris*, *Eriophorum vaginatum*, *Carex rostrata* and *Pinus sylvestris*.

	low N		high N	
	amb. $\text{CO}_2$	elev. $\text{CO}_2$	amb. $\text{CO}_2$	elev. $\text{CO}_2$
<b>Aboveground biomass (<math>\text{g m}^{-2}</math>):</b>				
all species	279 $\pm$ 46	202 $\pm$ 24	318 $\pm$ 39	393 $\pm$ 105
<i>Rhynchospora alba</i>	127 $\pm$ 45	106 $\pm$ 29	186 $\pm$ 52	218 $\pm$ 84
<i>Vaccinium oxycoccus</i>	89 $\pm$ 26	69 $\pm$ 9	99 $\pm$ 26	104 $\pm$ 15
<i>Erica tetralix</i>	21 $\pm$ 7	5 $\pm$ 3	19 $\pm$ 14	26 $\pm$ 21
<i>Eriophorum angustifolium</i>	40 $\pm$ 31	14 $\pm$ 9	11 $\pm$ 7	19 $\pm$ 5
<i>Drosera rotundifolia</i>	2 $\pm$ 0	6 $\pm$ 2	3 $\pm$ 1	2 $\pm$ 1
other species	0 $\pm$ 0	2 $\pm$ 2	1 $\pm$ 0	25 $\pm$ 25
<b>Aboveground necromass (<math>\text{g m}^{-2}</math>):</b>				
all species	59 $\pm$ 29	20 $\pm$ 7	38 $\pm$ 9	45 $\pm$ 16

(Table 3.2). Total living vascular plant biomass consisted for 89% of current-year biomass. N addition resulted in a 48% greater living biomass on average ( $P = 0.08$ ).

Nine species of vascular plants were present in the monoliths. Only *V. oxycoccus* and *D. rotundifolia* were present in all monoliths, *R. alba*, *E. tetralix* and *E. angustifolium* were present in 14 to 18 containers. For all these species except *R. alba* (but see below), elevated  $\text{CO}_2$  combined with high N supply resulted in the largest increase in aboveground biomass (data not shown), but treatment effects were not significant. The treatments had no significant effect on necromass (Table 3.2).

*R. alba* produced more than half of the vascular plant biomass in 1997. This species expanded greatly during the experiment and became the dominant species in most containers. Countings of *Rhynchospora* shoot density (Fig. 3.3A) revealed a tripling of numbers of shoots at the low N treatment, while at the high N treatment shoot density at the end of the experiment was even almost six times that in 1996. Shoot height also increased from 1996 to 1997, without treatment effects (Fig. 3.3B). Also aboveground biomass increased more when N supply was high ( $P = 0.03$ ), resulting in a 73% greater biomass at harvest (Fig. 3.3C). The biomass of the winterbuds was even slightly higher than that of the aboveground shoots, and was greater in the high N treatments, but only marginally significantly so ( $P = 0.095$ ) because of large variability. Elevated  $\text{CO}_2$  did not affect *R. alba* density, height or biomass.



**Figure 3.3** *Rhynchospora alba* A) shoot density (number of green shoots), B) average shoot height (cm) and C) biomass of shoots (aboveground) and winterbuds in 34 cm container. Data are means  $\pm$  SE,  $n = 5$  containers. Biomass of the shoots in 1996 was calculated from the sum of shoot heights, using linear regression between sum of shoot height and harvested shoot biomass in 1997.

Nutrient concentrations in *Sphagnum* and vascular plants

Table 3.3 lists nutrient concentrations (N, P and K) and C:N ratios in the capitula of *S. magellanicum*, the leaves and buds of *R. alba* and the current-year leaves of *V. oxycoccus*. Elevated CO<sub>2</sub> is often found to reduce N concentrations in plant tissues, but this was not the case in our experiment. N concentrations in the *Sphagnum* capitula even increased under elevated CO<sub>2</sub>. This is explained by the poor growth of *Sphagnum* under elevated CO<sub>2</sub> in the second growing season, given the negative correlation between *Sphagnum* green biomass and the N concentration ( $r = 0.70$ ,  $P = 0.001$ ,  $n = 20$ ). N addition increased the N concentration and reduced the C:N ratio in all dominant species (Table 3.3). The N effect was significant ( $P < 0.01$ ) for *Sphagnum* and *Vaccinium*, but not ( $P = 0.06$ ) for *Rhynchospora*.

The nutrient concentrations in the leaves of *Rhynchospora* were very low as opposed to those in the winterbuds. Apparently this species had already allocated a large part of its nutrients from the leaves to the hibernating parts. These leaves were indeed partly senesced at the time of harvest. P was most effectively translocated, given the large difference in P concentration between leaves and buds.

**Table 3.3** Nutrient concentrations and C:N ratios in capitula of *S. magellanicum*, leaves and buds of *R. alba* and current-year leaves of *V. oxycoccus* after two growing seasons of combined CO<sub>2</sub> (C) and N treatments. Data are means  $\pm$  SE,  $n = 5$  replicates. Level of significance: (\*)  $P < 0.10$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

	low N		high N		ANOVA		
	amb. CO <sub>2</sub>	elev. CO <sub>2</sub>	amb. CO <sub>2</sub>	elev. CO <sub>2</sub>	C	N	CxN
<b>N concentration (mg N g<sup>-1</sup>)</b>							
<i>S. magellanicum</i> capitula	11.3	12.7	13.3	17.7	**	***	(*)
<i>R. alba</i> leaves	4.5	5.6	6.8	7.2	-	(*)	-
<i>R. alba</i> buds	13.4	13.3	15.2	15.0	-	(*)	-
<i>V. oxycoccus</i> leaves	10.4	8.4	10.2	11.4	-	**	**
<b>P concentration (mg P g<sup>-1</sup>)</b>							
<i>S. magellanicum</i> capitula	0.53	0.53	0.50	0.55	-	-	-
<i>R. alba</i> leaves	0.21	0.27	0.21	0.12	-	-	-
<i>R. alba</i> buds	1.41	1.69	1.26	1.16	-	*	-
<i>V. oxycoccus</i> leaves	0.42	0.39	0.43	0.38	*	-	-
<b>K concentration (mg K g<sup>-1</sup>)</b>							
<i>S. magellanicum</i> capitula	3.7	3.6	3.6	3.0	-	-	-
<i>R. alba</i> leaves	2.3	2.4	2.1	1.6	-	-	-
<i>R. alba</i> buds	7.5	7.6	6.7	6.6	-	-	-
<i>V. oxycoccus</i> leaves	5.4	5.6	5.6	5.3	-	-	-
<b>C:N ratio</b>							
<i>S. magellanicum</i> capitula	44	41	37	28	*	**	-
<i>R. alba</i> leaves	113	96	79	80	-	(*)	-
<i>R. alba</i> buds	35	36	31	33	-	(*)	-
<i>V. oxycoccus</i> leaves	52	56	48	48	-	**	-

## Discussion

### Responses of *Sphagnum*

Clearly, elevated atmospheric CO<sub>2</sub> affected *Sphagnum* growth in our study (Fig. 3.1, Table 3.1), but the negative response of *Sphagnum* was unexpected and opposite to the positive CO<sub>2</sub> effect found in the Dutch BERI experiment: a mesocosm experiment outdoors using large intact peat monoliths from the same field site (Chapter 2). In addition, no negative (nor positive) responses of *Sphagnum* growth to elevated CO<sub>2</sub> were reported from the BERI field experiments in bog sites in Finland, Sweden and Switzerland (Berendse *et al.* submitted). Moreover, we do not know of any other study that reported a negative CO<sub>2</sub> effect on *Sphagnum* growth.

Silvola (1985) measured photosynthesis in four *Sphagnum* species, including *S. magellanicum*, and found increased photosynthetic rates with rising CO<sub>2</sub> concentrations. These short-term effects may disappear during long-term exposure to elevated CO<sub>2</sub>, as observed in *Eriophorum vaginatum* (Tissue and Oechel 1987), but Sveinbjörnsson and Oechel (1992) hypothesised that for bryophytes the long-term response will resemble the short-term response. They expect this mainly because bryophytes are less nutrient limited (having low nutrient demands and being very effective in capturing atmospheric deposition) than vascular plants under nutrient poor conditions. In greenhouse experiments Jauhiainen *et al.* (1994, 1998a) found CO<sub>2</sub>-induced increases in length increment and biomass production for *S. angustifolium*, but no CO<sub>2</sub> effect on biomass production for *S. fuscum*.

As the observed negative response of *Sphagnum* to elevated CO<sub>2</sub> in this study is so deviating from that in other experiments, particularly the one with the same vegetation, we assume that the specific greenhouse conditions were responsible for that response. There are several differences between this greenhouse experiment and the Dutch BERI experiment outdoors. The outdoor experiment used much larger monoliths (110 cm diameter, 60 cm deep), received natural rainwater and had an overflow for excess rainwater. However, the main difference is believed to be the raised temperatures in the greenhouse set 5 °C higher than the average outdoor temperature. Nevertheless, *Sphagnum* grew well in the ambient CO<sub>2</sub> treatment in the greenhouse, thus there has to be an interaction with CO<sub>2</sub>. This agrees with our finding that elevated CO<sub>2</sub> had stronger negative effects on *Sphagnum* height growth in warmer months (Table 3.4).

Silvola (1985) showed a positive interaction between CO<sub>2</sub> and temperature for photosynthesis in *Sphagnum*, including *S. magellanicum*. CO<sub>2</sub>-induced increases in net photosynthetic rates were greater at high temperatures, with temperatures changing from 1 to 35 °C. This interaction between CO<sub>2</sub> and temperature has also been found for vascular plants (Morisson and Lawlor 1999). In our experiment, perhaps a large amount of photosynthesised carbon accumulated as non-structural carbon compounds, which may inhibit growth directly or indirectly when excreted by vascular plants or the *Sphagnum* plants themselves.

The negative CO<sub>2</sub> effect appeared already in the first (warm) month of measurement (Table 3.4), which suggests a direct negative effect of elevated CO<sub>2</sub> when combined with high temperatures. The deleterious effects in the second growing season (loose capitula, hardly any growth) however point in the direction of a toxic effect, perhaps through

accumulation of allelopathic substances. Experimental warming in tussock tundra reduced growth and biomass of non-vascular plant species, including *Sphagnum* species (Hobbie *et al.* 1999), which stresses the vulnerability of bryophytes for raised temperatures.

The negative response of *Sphagnum* to elevated CO<sub>2</sub> cannot be explained by the vascular plant cover, which was not affected by elevated CO<sub>2</sub>. In the Dutch BERI experiment the reduced *Sphagnum* growth under increased N deposition in the third growing season was partly explained by the increased vascular plant cover. This negative relation between *Sphagnum* growth and vascular plant cover was not observed in this greenhouse experiment, possibly because of the dominant CO<sub>2</sub> effect or the shorter duration.

Also nutrient concentrations in the capitula (Table 3.3) do not give further indications. N, P and K concentrations were not abnormal as compared to Table 2.5 (Chapter 2) and Jauhiainen *et al.* (1998b). N concentrations were higher under elevated CO<sub>2</sub>, but not as high as in the Dutch BERI experiment (Table 2.5) or in the experiments of Press *et al.* (1986) and Jauhiainen *et al.* (1998b), where high N supply reduced *Sphagnum* growth. K concentrations were rather low, but they were so in all treatments.

The CO<sub>2</sub> effect on growth of *Sphagnum* did not depend on the level of N deposition. *Sphagnum* did not respond to the N treatment, indicating that *Sphagnum* growth was not N-limited, which was expected given the high ambient N deposition in the Netherlands under which the *Sphagna* grew up. Thus, CO<sub>2</sub> responses should be possible even at low N supply rates.

**Table 3.4** *Sphagnum* height increment (mm) during two growing seasons under ambient or elevated CO<sub>2</sub> and average temperature (°C) in the greenhouse and outdoors in each measurement period. Height increment data are means of 10 plots (N treatments pooled). Outdoor temperature data are from the weather station of Wageningen University in Wageningen (2 km from greenhouse). Level of significance: \*  $P < 0.05$ , \*\*\*  $P < 0.001$ .

Period	Height increment (mm)		ANOVA CO <sub>2</sub> -effect	Temperature (°C)	
	amb. CO <sub>2</sub>	elev. CO <sub>2</sub>		greenhouse	outdoor
<b>1996</b>					
15-Jul - 21-Aug	14 ± 2	7 ± 2	*	22	18
21-Aug - 24-Sep	5 ± 1	4 ± 1	-	19	13
4-Sep - 15-Oct	8 ± 2	4 ± 1	-	17	12
15-Oct - 6-Dec	1 ± 1	0 ± 1	-		
6-Dec - 3-Apr	-5 ± 1	-5 ± 1	-		
<b>1997</b>					
3-Apr - 1-May	7 ± 1	5 ± 1	-	14	7
1-May - 2-Jun	6 ± 2	3 ± 1	-	18	13
2-Jun - 1-Jul	7 ± 2	1 ± 1	*	20	16
1-Jul - 1-Aug	10 ± 2	0 ± 1	***	22	17
1-Aug - 1-Sep	2 ± 1	-1 ± 1	*	22	20
1-Sep - 29-Sep	8 ± 2	2 ± 1	*	18	14



## Responses of vascular plants

There were large variations in aboveground vascular plant biomass between the containers from the beginning of the experiment, which obscured significant treatment effects, despite some large differences in mean biomass. In the Netherlands, where living bog vegetation is very rare (Barkman 1992), it was not possible to find more homogeneous living bog vegetation, and therefore plot size (34 cm diameter) or the number of replicates was probably too small. Still *R. alba* showed a rather clear response to the N treatments (Fig. 3.3). This species increased enormously in all treatments during the experiment, but even more so under high N supply. Ohlsson and Malmer (Ohlson and Malmer 1990) suggested that *R. alba* can occur only in sites characterised by low interspecific competition for nutrients, as it has a rather high nutrient demand compared with other bog plants. In our experiment the abundance of *Rhynchospora* also expanded greatly in containers with high initial abundance of other vascular plants. The raised temperatures in the greenhouse probably have increased nutrient mineralisation, which made it possible for *Rhynchospora* to increase at such rates.

The increase in total aboveground vascular plant biomass during the experiment showed an almost significant interaction between CO<sub>2</sub> and N treatments, the CO<sub>2</sub>-induced increase at the high N treatment being larger than at the low N treatment. At low N supply, vascular plant growth was likely N-limited, as indicated by the smaller mean aboveground biomass at low N. *Sphagnum* species are very effective in absorbing nutrients from the atmospheric deposition (Woodin and Lee 1987; Williams *et al.* 1999), thereby reducing nutrient supply to vascular plants. The Dutch BERI experiment showed that vascular plant growth was still N-limited at the ambient N deposition of about 50 kg N ha<sup>-1</sup> year<sup>-1</sup> in Wageningen (Chapter 2). As this level corresponds to the high N deposition treatment in the greenhouse (assuming that ambient N deposition in the greenhouse is negligible), vascular plant growth in the greenhouse experiment may partly have been still N-limited at the high N treatment, preventing a clear positive CO<sub>2</sub> response. Alternatively, other nutrients or resources (light) became limiting at high N supply.

Concluding, elevated CO<sub>2</sub> had negative effects on *Sphagnum* growth, whereas increased N deposition increased growth of *R. alba*. We found no significant interactions between CO<sub>2</sub> and N treatments. The negative response of *Sphagnum* to elevated CO<sub>2</sub> appears to be linked to the raised temperatures in the greenhouse, the CO<sub>2</sub> effect being strongest in the warmest months. As *Sphagnum* is the main carbon sequestering species in bogs and rising atmospheric CO<sub>2</sub> levels will likely be followed by increases in temperature, further research on the combined effects of elevated CO<sub>2</sub> and increased temperature on *Sphagnum* growth is urgently needed.

## Acknowledgements

Staatsbosbeheer, the National Forest Service in the Netherlands, is thanked for permission to take the peat monoliths from their terrain. We thank Jan van Walsem for chemical analysis and Wim Arp and Juul Limpens for helpful suggestions improving this manuscript.

## Competition between *Sphagnum magellanicum* and *Eriophorum angustifolium* as affected by elevated CO<sub>2</sub> and increased N deposition

Monique Heijmans, Herman Klees and Frank Berendse

### Summary

We studied competition between peat mosses (*Sphagnum*) and vascular plants as affected by elevated CO<sub>2</sub> and increased N deposition in a greenhouse experiment. Peat monoliths with monocultures and mixtures of *Sphagnum magellanicum* and *Eriophorum angustifolium* were exposed to ambient (350 ppmv) or elevated (560 ppmv) atmospheric CO<sub>2</sub>, combined with low (no N addition) or high (5 g N m<sup>-2</sup> year<sup>-1</sup> added) N deposition. During three growing seasons growth of *Sphagnum* and *Eriophorum* was followed.

The presence of *Eriophorum* did not affect *Sphagnum* biomass, because *Eriophorum* density did not become high enough to provide severe shading of the moss surface. In contrast, *Sphagnum* had a negative effect on *Eriophorum* biomass, particularly on the number of flowering stems. Possibly, the presence of a living *Sphagnum* layer decreased nutrient availability to *Eriophorum* by immobilisation of nutrients mineralised from the peat.

Elevated CO<sub>2</sub> and/or increased N deposition did not change these competitive relationships between *Sphagnum* and *Eriophorum*, but had independent effects. Elevated CO<sub>2</sub> had a positive effect both on *Sphagnum* and *Eriophorum* biomass. This positive CO<sub>2</sub> effect on *Eriophorum* was transient, probably because of P limitation. N addition had a direct negative effect on *Sphagnum* height growth in the first growing season, but an increased shoot density compensated this in the third year resulting in no N effect on *Sphagnum* biomass at the end of the experiment. *Eriophorum* responded little to N addition, as N availability appeared not limiting plant growth.

## Introduction

Ombrotrophic bogs are peat-forming ecosystems and as such they can serve as important long-term sinks for atmospheric CO<sub>2</sub> (Gorham 1991). Among peatlands the *Sphagnum*-dominated bogs have the greatest peat (thus carbon) accumulation potential due to low rates of litter decomposition (Thormann *et al.* 1999). Changes in these ecosystems, for example brought about by climate change, can have important consequences for global carbon cycling. However, little is known about the effects of climate change on bog ecosystems.

The amount of carbon being sequestered in peatlands depends on the balance between the production and decomposition of plant material. Changes in plant species composition may have important effects on this balance because species differ strongly in both productivity and decomposability. This certainly holds for bog vegetation, which are comprised of peat mosses (*Sphagnum*) and vascular plants. *Sphagnum* species form the bulk of bog peat, because *Sphagnum* litter is more recalcitrant than that of vascular plants and decomposes slowly (Coulson and Butterfield 1978; Clymo and Hayward 1982). Vascular plants contribute less to sequestration of carbon dioxide; moreover, they contribute more to methane emission (Joabsson *et al.* 1999). Therefore, carbon sequestration in bogs is expected to increase when *Sphagnum* gains a competitive advantage over vascular plants.

*Sphagnum* and vascular plants use different sources of mineral nutrients. Where *Sphagnum* largely depends on nutrients from atmospheric deposition, the rooted vascular plants mainly capture nutrients that are mineralised from organic matter. Although not in direct competition for nutrients, there are still several ways in which *Sphagnum* and vascular plants may interact. *Sphagnum* reduces nutrient supply to vascular plants by intercepting the atmospheric deposition (Woodin and Lee 1987; Williams *et al.* 1999) and by slowing down litter decomposition. *Sphagnum* produces a slowly decaying litter and creates a harsh (wet, acid, mineral-poor) environment for decomposers (Van Breemen 1995). Vascular plants may exert a negative effect on *Sphagnum* by shading and covering the moss layer with litter (Malmer *et al.* 1994). These negative interactions between peat mosses and vascular plants can be considered as asymmetric competition, where light competition is to the advantage of vascular plants, while nutrient competition is advantageous to *Sphagnum* (Rydin 1997). However, these relationships have rarely been investigated experimentally.

Elevated CO<sub>2</sub> and increased N deposition are two important components of global change, which are expected to alter the competitive balance between *Sphagnum* and vascular plants in bog vegetation. Elevated CO<sub>2</sub> generally stimulates growth of individual plants, but net production of a whole plant community is not necessarily increased. Nutrient limitation is often held responsible for a lack of positive CO<sub>2</sub> effects on net ecosystem production (Körner 1996; Koch and Mooney 1996). However, one of the few consistent findings in CO<sub>2</sub>-enrichment studies in natural vegetation is that the CO<sub>2</sub> response is species specific, with some species declining and other species gaining in abundance (Körner 1996; Leadley *et al.* 1999). Therefore elevated CO<sub>2</sub> may lead to changes in competitive relations between plant species, also in nutrient poor environments.

We hypothesised that elevated CO<sub>2</sub> will give a competitive advantage to *Sphagnum*, because it is less nutrient limited than vascular plants (Sveinbjörnsson and Oechel 1992;

Jauhiainen *et al.* 1998a). With the high ambient N deposition in the Netherlands it is likely that *Sphagnum* growth is not N-limited (Aerts *et al.* 1992), whereas vascular plant growth is possibly still N-limited as *Sphagnum* is very effective in intercepting atmospheric N supply. At increased N deposition *Sphagnum* may no longer capture all nutrients from atmospheric deposition. Consequently more N may reach the rhizosphere and increase growth rates of vascular plants (Woodin and Lee 1987; Malmer *et al.* 1994). The increased shading of the moss layer caused by an increased vascular plant cover may then depress *Sphagnum* growth (Clymo 1973; Hayward and Clymo 1983). We hypothesised that increased N deposition will change the competitive balance between mosses and vascular plants to the benefit of vascular plants.

We studied competition between *Sphagnum magellanicum* and *Eriophorum angustifolium*, as affected by elevated CO<sub>2</sub> and increased N deposition in a greenhouse experiment. Peat monoliths were used as a basis for monocultures and mixtures of these species. These monoliths were exposed to two levels of atmospheric CO<sub>2</sub> combined with two levels of N deposition. During three growing seasons growth of *Sphagnum* and *Eriophorum* was followed.

*S. magellanicum* is the dominant *Sphagnum* species in our field site and *E. angustifolium* is one of the important vascular plant species. This species was chosen as vascular plant as it was expected to exert a negative influence on *Sphagnum* growth through shading and by covering the moss surface with dead leaves. In addition, *E. angustifolium* may have a relatively large nutrient demand, as indicated by higher nutrient concentrations in leaves than in leaves of other (ericaceous) bog plant species (measured in field plants collected in July 1996, unpublished data), which makes it vulnerable to the competition for nutrients by *Sphagnum*.

## Methods

### Plant material

The peat monoliths used in this study were cut from a small mire in the State Forestry of Dwingeloo (52°49'N, 6°25'E) in the north of the Netherlands. This site is one of a series of heath pools (0.5-1.5 m deep) that has become filled with peat. The heath pool measures 50 by 150 m and is surrounded by forest. The peat layer is 25 to 50 cm thick and is more or less floating. The monoliths were cut from lawn vegetation dominated by *Sphagnum magellanicum* Brid.. Important co-occurring vascular plant species were *Vaccinium oxycoccus* L., *Erica tetralix* L., *Eriophorum angustifolium* Honck. and *Drosera rotundifolia* L.. Sixty peat monoliths were cut in May 1997. The columns were 24 cm in diameter and 30 cm deep and were placed in white plastic containers of equal size. Green non-flowering tillers of *E. angustifolium* were collected from the same site. Rhizomes were removed in order to obtain about equal tillers. The moss layer of the monoliths was always dominated by *S. magellanicum* with coverage of at least 75%.

### Experimental design

The peat monoliths were used for creating three modes of competition: *Sphagnum* monoculture, *Eriophorum* monoculture and *Sphagnum* + *Eriophorum* mixture. These species were exposed to two levels of atmospheric CO<sub>2</sub> (ambient: 350 ppmv and elevated:

560 ppmv) and two levels of N supply (low: no N addition and high: 5 g N m<sup>-2</sup> year<sup>-1</sup> added). All treatments were combined in a factorial design, resulting in twelve treatments, which were replicated five times.

The *Sphagnum* monoculture was established by removing (graminoid species pulled out and ericaceous species clipped off) all aboveground vascular plant parts from the monolith. In addition, mosses other than *Sphagnum* were pulled out. During the experiment regrowth of vascular plants and non-*Sphagnum* mosses was repeatedly removed. The same procedure was used for the *Sphagnum* + *Eriophorum* mixture, but in addition nine green tillers of *E. angustifolium* were planted. The *Eriophorum* monoculture was established by removing the top layer of about 5 cm of green *Sphagnum* from the monoliths. Nine green *Eriophorum* tillers were planted and a 5 cm layer of white plastic grains was added to replace the *Sphagnum* layer. At the beginning of the second growing season the top layer of the white grains was replaced by red plastic fragments to better simulate the red-coloured *S. magellanicum* surface, as the *Eriophorum* monocultures appeared to experience a higher light intensity because of the reflection by the white surface.

The CO<sub>2</sub> treatments (350 and 560 ppmv) were established by placing the containers in the greenhouse in two identical compartments in which the CO<sub>2</sub> concentration could be regulated. Every two weeks the CO<sub>2</sub> level was switched between the compartments (and the containers were moved) to avoid a compartment effect. Nitrogen was added as NH<sub>4</sub>NO<sub>3</sub> dissolved in artificial rainwater (see below). A total amount of 5 g N m<sup>-2</sup> was added each growing season to the high N plots in six applications (about every three weeks) by watering with 150 ml of N solution, simulating a rain event of 3 mm. The low N plots received the same amount of rainwater. These gifts preceded the weekly water addition.

The temperature in the greenhouse was set to simulate the daily and seasonal pattern of the outdoor temperature by using the weekly average minimum and maximum temperature for the 1980s. These values were increased by 5 °C to prevent exceeding the cooling capacity of the greenhouse during warm spells. During the day the temperature was set to change every 4 hours from the minimum temperature (from 0:00 to 4:00 hour) to the maximum temperature (from 12:00 to 16:00 hour) and back. Minimum temperature ranged between 10 and 18 °C and maximum temperature ranged between 18 and 28 °C. Humidity was set at 75%. No artificial light was used.

Water level in each container was restored to about 6 cm below moss surface every week by watering the containers with artificial rainwater to a constant weight. This weight was increased several times during the experiment to account for moss growth. Water levels never dropped more than 13 cm below moss surface. Rainwater was made by diluting an artificial seawater solution 8000 times with demineralised water. This water is equivalent to clean rainwater without N or P. The chemical composition of the artificial rainwater is given in Table 4.1.

**Table 4.1** Chemical characteristics of the artificial rainwater used in this experiment. EC = Electrical Conductivity

Na	K	Mg	Cl	HCO <sub>3</sub>	SO <sub>4</sub>	pH	EC
mg l <sup>-1</sup>	mg l <sup>-1</sup>	mg l <sup>-1</sup>	mg l <sup>-1</sup>	mg l <sup>-1</sup>	mg l <sup>-1</sup>		μS cm <sup>-1</sup>
1.4	0.05	0.16	2.5	0.02	0.34	5.8	11

The experiment started in June 1997. From November to March the monoliths wintered outside underneath a transparent roof. In that period there was no CO<sub>2</sub> treatment. The experiment ended with a final harvest in September 1999.

### *Sphagnum* growth measurements

*Sphagnum* growth was measured non-destructively during the experiment by measuring height increment and counting shoot density. *Sphagnum* height increment was measured with the cranked wire method (Clymo 1970). In each monolith four stainless steel wires were anchored in the peat at a depth of 8 cm by plastic bristles attached to the lower end of the wire. The length of the wire extending above the moss surface was measured monthly, except in winter. *Sphagnum* shoot density was measured as the number of capitula counted in one permanently marked 10 cm diameter plot in the middle of each monolith.

At the final harvest a *Sphagnum*/peat column of 11 cm diameter was cut in the middle of each monolith for measurements on individual *Sphagna*. From ten intact *Sphagnum* individuals the length of the green shoot was measured. After removing the capitulum (defined as the top 1 cm), the green parts were used to determine shoot dry mass per unit length. Other *Sphagnum* capitula and green parts from the 11 cm core were also collected to determine capitulum biomass and total green biomass. All fractions were dried at 70 °C for at least 48 hours and weighed. In one container *Sphagnum* became overgrown by a liverwort (*Odontoschisma sphagni* (Dicks.) Dum.) during the experiment. It was impossible to separate these species, and as also height increment was exceptionally small in this container, this replicate was omitted from all *Sphagnum* data analysis.

### *Eriophorum* growth measurements

During the experiment growth of *E. angustifolium* was followed by counting green, flowering and brown shoots and measuring shoot height. Shoot height was defined as the length of the longest leaf and was measured from leaf top to moss surface for each green or flowering shoot. These measurements were made at the end of each growing season.

At the end of the experiment total *Eriophorum* biomass was harvested. Aboveground parts were clipped off and belowground parts were extracted from the peat. The litter on top of the moss or grain surface was also collected. The aboveground parts were sorted into green leaves, brown leaves and flowering stems; the belowground parts were sorted into stems, rhizomes and roots. All plant parts were dried at 70 °C for at least 48 hours and weighed.

To reconstruct the development of the aboveground *Eriophorum* biomass during the experiment, aboveground biomass in the first and second growing season was calculated by linear regression from the sum of squared shoot heights. Shoot height was squared because taller shoots were also thicker (had more leaves) and it resulted in the best fit (compared with sum of shoot heights and sum of (shoot height)<sup>3</sup>). The relation between the sum of squared shoot heights and the harvested aboveground biomass was highly significant ( $P < 0.001$ ,  $R^2 = 0.88$ ,  $n = 40$ ).

### Nutrient analysis

N, P and K concentrations were measured in all plant parts. The concentrations were measured in acid extracts of dry milled samples by an auto-analyser. At the end of the

experiment water samples were collected using rhizons inserted at a depth of 0-10 and 15-20 cm below moss or grain surface.  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ , P and K concentrations were measured with an auto-analyser.

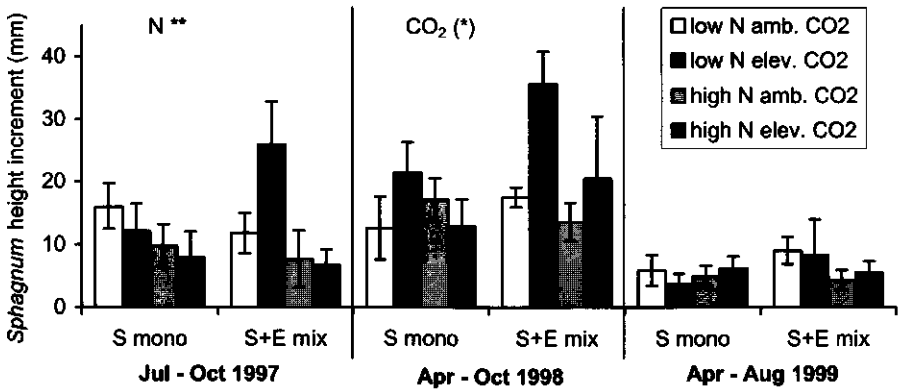
### Data analysis

Treatment effects were analysed using three-way analysis of variance (ANOVA) with species,  $\text{CO}_2$  and N supply as independent factors. In case of repeated non-destructive measurements during the experiment, a repeated measures ANOVA was performed. As interaction between year and treatments was often significant, these were followed by ANOVAs for each year. When necessary, data were ln-transformed to achieve homogeneous variances. Spearman correlation coefficients and other statistics were calculated using SPSS for Windows (8.0).

## Results

### *Sphagnum* growth

*Sphagnum* height increment during the experiment was affected both by N supply and  $\text{CO}_2$  concentration (Fig. 4.1). High N supply reduced height growth in the first season of the experiment. Elevated  $\text{CO}_2$  increased height increment almost significantly in the second growing season. These treatment effects disappeared in the third season, when mean height increment was small in all treatments. The presence of *Eriophorum* tillers in the mixtures had no significant effect on height growth (species effect:  $P = 0.13$ ), but the positive  $\text{CO}_2$  effect was more pronounced in the *Sphagnum* + *Eriophorum* mixtures (Fig. 4.1) ( $\text{CO}_2$  x species interaction:  $P = 0.08$ ).



**Figure 4.1** *Sphagnum* height increment (mm) during three growing seasons of combined  $\text{CO}_2$ , N and species treatments. Bars are means  $\pm$  SE ( $n = 4-5$  replicates). Significant treatment effects for each season are included. Level of significance: (\*)  $P < 0.10$ , \*\*  $P < 0.01$ .

*Sphagnum* shoot density, counted as the number of capitula, was without treatment effects in the first and second growing season (data not shown). In the third season the number of capitula increased under high N supply (Table 4.2). Capitulum dry mass tended

to be greater in the elevated CO<sub>2</sub> treatments. Shoot dry mass per unit length was significantly reduced in the mixtures with *Eriophorum* plants and was negatively correlated with height increment during the experiment ( $r = -0.69$ ,  $P < 0.001$ ,  $n = 39$ ). *Sphagnum* green biomass was increased under elevated CO<sub>2</sub>. Biomass was most correlated with capitulum dry mass ( $r = 0.78$ ,  $P < 0.001$ ,  $n = 39$ ), length of green shoot ( $r = 0.76$ ,  $P < 0.001$ ,  $n = 39$ ) and height increment in 1998 ( $r = 0.63$ ,  $P < 0.001$ ,  $n = 39$ ). There were no statistical significant interactions between treatments.

**Table 4.2** *Sphagnum* growth characteristics determined after three growing seasons of combined CO<sub>2</sub> (C), N and species (Sp) treatments in an 11 cm diameter core. Data are means of 4-5 replicates. Level of significance: (\*)  $P < 0.10$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ . There were no statistical interactions between treatments ( $P > 0.05$ ).

	<i>Sphagnum</i> monoculture				<i>Sphagnum</i> + <i>Eriophorum</i>				ANOVA		
	low N		high N		low N		high N		main effects		
	amb. C	elev. C	amb. C	elev. C	amb. C	elev. C	amb. C	elev. C	C	N	Sp
Number of capitula	87	91	112	144	105	95	111	156	-	*	-
Capitulum dry mass (mg)	10.4	12.8	11.5	12.6	6.4	12.9	9.4	9.7	(*)	-	-
Shoot dry mass per cm (mg cm <sup>-1</sup> )	7.8	6.7	8.5	7.8	4.7	4.2	7.0	5.0	-	-	**
Green length (cm)	3.1	3.6	3.5	3.4	3.1	4.8	3.6	3.9	-	-	-
Green biomass (g)	3.3	4.1	3.9	4.0	3.0	4.2	3.0	3.6	*	-	-

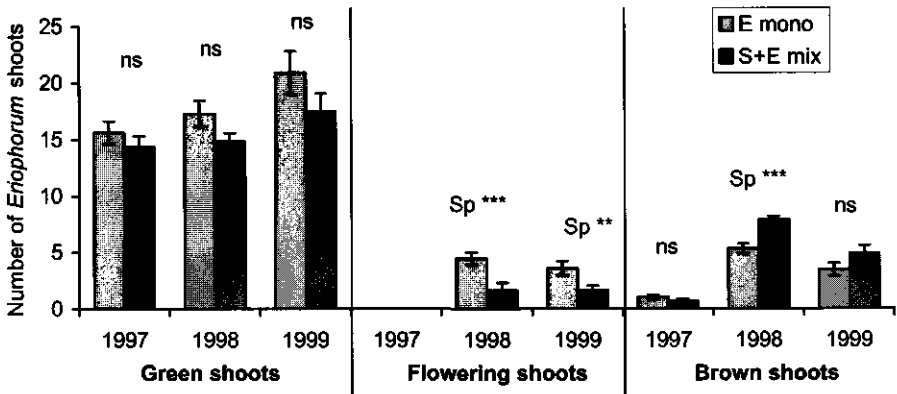
Thus, elevated CO<sub>2</sub> increased *Sphagnum* biomass through increases in height increment (mainly in the second season) and capitulum dry mass. Increased N deposition significantly reduced height increment in the first year, but an increased shoot density (number of capitula per unit area) compensated this in the third year resulting in no effect on *Sphagnum* biomass at the end of the experiment. The presence of *Eriophorum* tillers likely changed morphology of the plants (stretching, as indicated by more height increment and less shoot dry mass per unit length), but did not affect biomass.

### *Eriophorum* growth

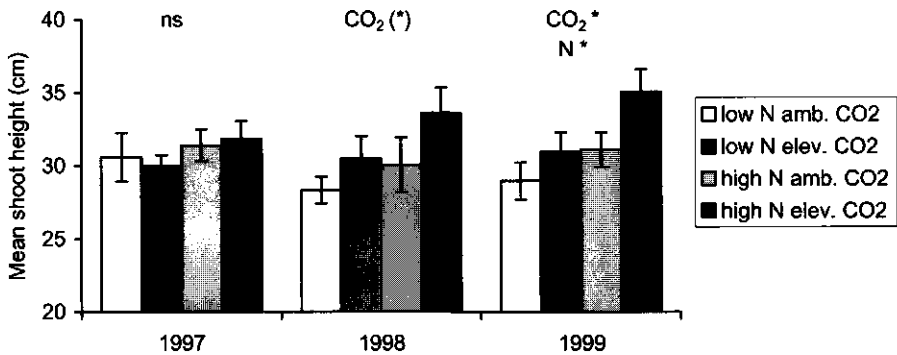
Nine green tillers were planted at the start of the experiment in June 1997. The total number of shoots increased to 16 and 26 on average at the end of the first and second season respectively, but did not increase any further in the third year. The treatments had no significant effect on total shoot density. Almost all planted tillers flowered or turned brown in the second growing season. The number of flowering shoots was greater in the *Eriophorum* monocultures, whereas the number of brown shoots increased in the *Sphagnum* + *Eriophorum* mixtures (Fig. 4.2). The number of green shoots tended to be greater in the *Eriophorum* monocultures, but not significantly so ( $P = 0.17$ ). CO<sub>2</sub> and N treatments did not affect shoot density.

In contrast, mean shoot height was affected by the CO<sub>2</sub> and N treatments, particularly in the third growing season (Fig. 4.3). Shoots were tallest in the high N-high CO<sub>2</sub> treatments with 35 cm on average, 21% larger than in the low N - low CO<sub>2</sub> treatments (29 cm). The presence of a *Sphagnum* surface had no effect on shoot height ( $P = 0.75$ ).



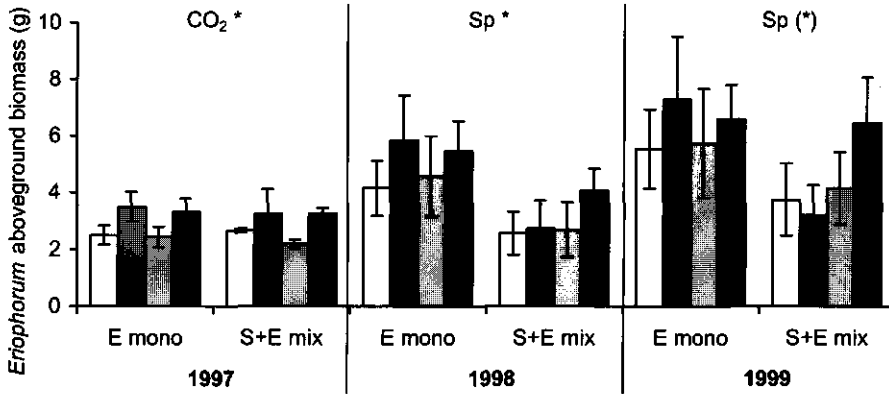


**Figure 4.2** Density of green, flowering and brown *Eriophorum* shoots during three growing seasons of combined CO<sub>2</sub>, N and species treatments. For presentation, data from the CO<sub>2</sub> and N treatments were pooled as there were only species effects. Bars are means ± SE (*n* = 20 containers). Significant species (Sp) treatment effects (tested with a 2 x 2 x 2 ANOVA) for each season are included. Level of significance: \*\* *P* < 0.01, \*\*\* *P* < 0.001. Nine green shoots were planted at the beginning of the experiment.



**Figure 4.3** Mean shoot height (cm) of *E. angustifolium* during three growing seasons of combined CO<sub>2</sub>, N and species treatments. For presentation, data from the species treatments were pooled as there were no significant species effects or interactions with species treatments. Data are means ± SE (*n* = 10 containers). Significant treatment effects (tested with a 2 x 2 x 2 ANOVA) for each season are included. Level of significance: (\*) *P* < 0.10, \* *P* < 0.05.

Aboveground biomass showed effects of CO<sub>2</sub> concentration and species composition (Fig. 4.4). Elevated CO<sub>2</sub> increased aboveground biomass by 36% in the first growing season. This enhancement became less (and non-significant) in the second and third season (29 and 23% respectively). The presence of a *Sphagnum* surface reduced aboveground biomass in the second (40%) and third (30%) growing season. There was no statistical interaction between treatments.



**Figure 4.4** Aboveground biomass of *E. angustifolium* during three growing seasons of combined CO<sub>2</sub>, N and species (Sp) treatments. Data are means ± SE (*n* = 5 containers). Significant treatment effects for each season are included. Level of significance: (\*) *P* < 0.10, \* *P* < 0.05. Biomass in 1997 and 1998 was calculated from the sum of squared shoot heights for each container, using linear regression between the sum of squared shoot heights and harvested aboveground biomass in 1999.

**Table 4.3** Above and belowground biomass and aboveground necromass of *E. angustifolium* after three growing seasons of combined CO<sub>2</sub> (C), N and species (Sp) treatments. Data are means of 5 containers. Level of significance: (\*) *P* < 0.10, \* *P* < 0.05, \*\*\* *P* < 0.001. There were no statistical interactions between treatments (*P* > 0.05).

	<i>Eriophorum</i> monoculture		<i>Sphagnum</i> + <i>Eriophorum</i>				ANOVA				
	low N		high N		low N		high N		main effects		
	amb.	elev.	amb.	elev.	amb.	elev.	amb.	elev.	C	N	Sp
<b>Aboveground biomass (g m<sup>-2</sup>)</b>											
Total	123	161	127	146	83	72	92	143	-	-	(*)
Green leaves	97	139	113	113	76	61	87	139	-	-	-
Flowering stems	26	23	14	33	7	10	5	4	-	-	***
<b>Belowground biomass (g m<sup>-2</sup>)</b>											
Total	335	355	294	311	267	267	266	367	-	-	-
Stems	154	160	147	155	129	131	135	196	-	-	-
Rhizomes	33	47	31	41	30	36	25	44	-	-	-
Roots	148	149	116	115	108	101	107	127	-	-	-
<b>Total biomass (g m<sup>-2</sup>)</b>											
Total	457	517	421	457	350	339	358	510	-	-	-
<b>Aboveground necromass (g m<sup>-2</sup>)</b>											
Brown leaves	135	165	133	181	102	125	121	161	*	-	-
Litter	9	10	8	7	5	5	6	6	-	-	*

This reduced aboveground biomass at the final harvest was mainly caused by an almost threefold reduction in biomass of flowering stems in the *Sphagnum* + *Eriophorum* mixtures (Table 4.3). The biomass of belowground parts was not significantly affected by the treatments. As a result the ratio between belowground and aboveground biomass was higher in the mixtures ( $P = 0.02$ ). Dry mass of attached brown leaves was greater in the elevated  $\text{CO}_2$  treatments (Table 4.3), which is the consequence of the greater aboveground biomass in the previous growing seasons. Litter mass (dead leaves on top of the moss or plastic grain surface) was larger in the *Eriophorum* monocultures, which can be explained by the absence of a *Sphagnum* surface overgrowing fallen dead leaves. N supply had no significant effect on total *Eriophorum* biomass or biomass of any plant part.

#### Nutrient concentrations in *Sphagnum*, *Eriophorum* and water

$\text{CO}_2$  concentration, N supply and species composition affected tissue nutrient concentrations (Table 4.4). High N supply increased N concentrations in all plant parts measured (also in belowground parts of *Eriophorum*, data not shown), except green leaves of *Eriophorum*. Elevated  $\text{CO}_2$  reduced N and K concentrations in *Sphagnum* capitula and reduced N concentrations in *Eriophorum* brown leaves. The addition of *Eriophorum* tillers reduced K concentrations in *Sphagnum* capitula. The presence of a living *Sphagnum* layer

**Table 4.4** Nutrient concentrations in capitula and brown stems of *S. magellanicum* and green and brown leaves of *E. angustifolium* after three growing seasons of combined  $\text{CO}_2$  (C), N and species (Sp) treatments. Data are means of 4-5 replicates. Nutrient concentrations in field plants collected in July 1996 are added for comparison. Level of significance: (\*)  $P < 0.10$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ . There were no statistical interactions between treatments ( $P > 0.05$ ).

	Field	S or E monoculture				S + E mixture				ANOVA		
		low N		high N		low N		high N		main effects		
		amb.	elev.	amb.	elev.	amb.	elev.	amb.	elev.	C	N	Sp
	C	C	C	C	C	C	C	C				
<b>N concentration (mg N g<sup>-1</sup>)</b>												
<i>S. mag</i> capitula	13.6	14.2	12.4	21.7	19.6	12.7	10.4	23.7	19.4	**	***	-
<i>S. mag</i> brown stems	10.9	8.5	7.4	13.4	12.3	7.6	6.7	13.0	15.1	-	***	-
<i>E. ang</i> green leaves	15.2	9.0	8.5	9.0	9.3	9.2	9.9	10.2	10.3	-	-	*
<i>E. ang</i> brown leaves	11.5	7.1	6.2	8.2	7.9	7.3	6.9	7.8	7.4	*	***	-
<b>P concentration (mg P g<sup>-1</sup>)</b>												
<i>S. mag</i> capitula	0.51	0.30	0.27	0.38	0.30	0.24	0.26	0.33	0.25	(*)	-	(*)
<i>S. mag</i> brown stems	0.31	0.28	0.22	0.31	0.25	0.19	0.20	0.28	0.28	-	-	-
<i>E. ang</i> green leaves	0.83	0.48	0.58	0.42	0.42	0.45	0.42	0.43	0.40	-	-	-
<i>E. ang</i> brown leaves	0.41	0.09	0.08	0.08	0.07	0.10	0.11	0.11	0.13	-	-	**
<b>K concentration (mg K g<sup>-1</sup>)</b>												
<i>S. mag</i> capitula	6.8	5.8	4.8	5.1	4.5	5.1	4.4	4.4	3.5	**	*	**
<i>S. mag</i> brown stems	2.7	2.6	2.1	2.0	1.5	2.0	2.6	1.3	1.4	-	**	-
<i>E. ang</i> green leaves	11.9	6.6	5.8	7.9	7.9	8.8	7.9	9.8	7.5	-	-	*
<i>E. ang</i> brown leaves	2.8	4.8	4.2	6.1	6.6	6.6	5.7	7.3	6.2	-	*	*

increased N and K concentrations in *Eriophorum* green leaves and increased P and K concentrations in brown leaves.

Nutrient concentrations in water, sampled at 15-20 cm depth, show that concentrations of K, P and to a lesser extent  $\text{NO}_3$  were much depressed in containers with *Eriophorum* compared to *Sphagnum* monocultures, indicating uptake by *Eriophorum* tillers (Table 4.5).  $\text{NH}_4$  concentrations were higher in the high N treatments, particularly in the *Eriophorum* monocultures, indicating that more  $\text{NH}_4$  was available to *Eriophorum* in the high N treatments (Table 4.6).

**Table 4.5** Nutrient concentrations in water sampled at 15 - 20 cm depth at the end of the experiment for each species treatment. Data are means ( $n = 19-20$  replicates,  $\text{CO}_2$  and N treatments pooled). Different letters indicate significant differences among species treatments (Tukey test).

	<i>Sphagnum</i> monoculture	<i>Sphagnum</i> + <i>Eriophorum</i> mixture	<i>Eriophorum</i> monoculture
$\text{NH}_4\text{-N}$ (mg $\text{l}^{-1}$ )	0.38 <sup>a</sup>	0.25 <sup>a</sup>	0.39 <sup>a</sup>
$\text{NO}_3\text{-N}$ (mg $\text{l}^{-1}$ )	0.02 <sup>a</sup>	0.01 <sup>ab</sup>	0.01 <sup>b</sup>
P (mg $\text{l}^{-1}$ )	0.07 <sup>a</sup>	0.02 <sup>b</sup>	0.02 <sup>b</sup>
K (mg $\text{l}^{-1}$ )	3.72 <sup>a</sup>	0.53 <sup>b</sup>	0.34 <sup>b</sup>

**Table 4.6**  $\text{NH}_4$  and  $\text{NO}_3$  concentrations in water sampled at 15 - 20 cm depth at the end of the experiment for each species treatment. Data are means ( $n = 9-10$  replicates,  $\text{CO}_2$  treatments pooled). Different letters indicate significant differences (Tukey test).

	<i>Sphagnum</i> monoculture		S+E mixture		<i>Eriophorum</i> monoculture	
	low N	high N	low N	high N	low N	high N
$\text{NH}_4\text{-N}$ (mg $\text{l}^{-1}$ )	0.23 <sup>cd</sup>	0.52 <sup>ab</sup>	0.15 <sup>cd</sup>	0.34 <sup>bc</sup>	0.07 <sup>d</sup>	0.72 <sup>a</sup>
$\text{NO}_3\text{-N}$ (mg $\text{l}^{-1}$ )	0.01 <sup>ab</sup>	0.02 <sup>a</sup>	0.01 <sup>ab</sup>	0.01 <sup>ab</sup>	0.01 <sup>ab</sup>	0.01 <sup>b</sup>

## Discussion

### *Eriophorum* effects on *Sphagnum*

The presence of *Eriophorum* tillers in the *Sphagnum* + *Eriophorum* mixtures did not significantly affect *Sphagnum* green biomass. The only significant effect of *Eriophorum* was a reduced stem dry mass per unit length, suggesting an etiolation (elongation) effect. This is a common response to shading for vascular plants, and has also been observed for *Sphagnum* (Murray *et al.* 1993). This effect did not result in a significantly reduced biomass, as height growth was (non-significantly) increased (33 and 40 mm over three growing seasons in monocultures and mixtures respectively) in the mixtures.

It has often been suggested that in bog vegetation *Sphagnum* will suffer from competition from vascular plants when these can increase their growth rates, *i.e.* at high

nutrient supply (Lee and Studholme 1992; Malmer *et al.* 1994; Rydin 1997; Lamers *et al.* 2000), but experimental data are scarce. In several field experiments in different oligotrophic plant communities with a surface layer of mosses and lichens (mire, tundra, heathland), fertilisation or temperature treatments resulted in reduced biomass of non-vascular plants and an increased abundance of vascular plants (Chapin and Shaver 1985; Lütke Twenhöven 1992; Jonasson 1992; Chapin *et al.* 1995; Hogg *et al.* 1995; Press *et al.* 1998; Hobbie *et al.* 1999; Maksimova and Yudina 1999).

Some authors suggest that the reduced moss biomass is a consequence of the increased vascular plant abundance. Maksimova and Yudina (1999) observed that *Sphagnum* cover declined significantly after vascular plant cover had increased to 62% after four years of fertiliser treatment in a Russian bog. However, most authors leave it open whether the experimental treatments had a direct negative effect on the non-vascular plants or whether it was the result of competition. Chapin and Shaver (1985) suggested that the negative effect on non-vascular plant growth in their experiment largely represented a direct response to the fertiliser and greenhouse treatments as vascular plant cover was always less than 60%.

Three of these studies (Jonasson 1992; Hogg *et al.* 1995; Hobbie *et al.* 1999) included removal of vascular plants. Only in a dense *Molinia* stand did cutting of the *Molinia* benefit *Sphagnum* (Hogg *et al.* 1995). In Swedish tundra, removal of shrubs had no effect on moss cover (Jonasson 1992), but from the data we could deduce that vascular plant cover in the controls was less than 60%. Hogg *et al.* (1995) and Hobbie *et al.* (1999) did not give cover data in their publications. Also in our mesocosm experiment outdoors using large intact peat monoliths from the same field site (Dutch BERI experiment, Chapter 2) *Sphagnum* growth was reduced in the third growing season in the high N treatment where vascular plant cover exceeded 60% in the second growing season. We suggest that high densities of vascular plants (cover at least 60%?) are needed for reduction of *Sphagnum* growth.

In this greenhouse experiment *Eriophorum* density was probably not high enough to reduce *Sphagnum* growth. *Eriophorum* did not expand as much as expected, possibly because of limited P availability (see below). Measurements of light intensity at the end of the experiment showed that light intensity at the moss surface was reduced only by 33% in the *Sphagnum* + *Eriophorum* mixture compared to the *Sphagnum* monocultures (data not shown). The mean aboveground biomass of 98 g m<sup>-2</sup> in the mixtures is not extremely high and corresponds with a cover of about 40% (as deduced from the Dutch BERI experiment).

Malmer *et al.* (1994) suggested that if increased vascular plant abundance inhibits growth of mosses it would be more the result of accumulation of aboveground litter covering the moss surface than of direct shading. This could be seen in Alaskan tussock tundra where abundant *Betula* litter in the fertiliser and warming treatment covered the mosses and lichens almost completely, contributing to their elimination from these plots (Chapin *et al.* 1995). In our experiment litter fall was small (Table 4.3) as the experiment started with green tillers and brown leaves remained attached to the shoots for at least one season. The small amount of dead leaves on the moss surface became easily overgrown by the *Sphagnum*.

### *Sphagnum* effects on *Eriophorum*

The most striking effect of the presence of a living *Sphagnum* layer in the mixtures was a reduced number of flowering shoots, resulting in an almost significantly reduced

aboveground biomass (Fig. 4.2, Table 4.3). This result corresponds with the observation in Alaskan tussock tundra where *Sphagnum* removal increased aboveground biomass of *Betula nana* (Hobbie *et al.* 1999). Increased flowering has been reported for other bog plant species, including *E. vaginatum*, particularly as a result of P fertilisation (Shaver and Chapin 1980; Simms 1987; Bartsch 1994). Both our observations of reduced flowering and increased ratio between belowground and aboveground parts, being general responses to reduced nutrient availability in natural vegetation (Chapin 1980), suggest that nutrient availability was reduced in the *Sphagnum* + *Eriophorum* mixtures. However, we cannot clearly demonstrate that this was the case in this experiment. N, P and K concentrations in soil water were not different between the two *Eriophorum* treatments (Table 4.5), but the concentrations in water represent only instantaneous availability. Total N, P and K pools in *Eriophorum* tillers were 15, 21 and 7% smaller in the mixtures, but not significantly so ( $P = 0.28, 0.15, 0.58$  for N, P and K respectively).

Suppose that the living *Sphagnum* layer reduced nutrient availability, resulting in reduced flowering. How could it do so? In our experiment it was not by interception of atmospheric deposition, which might be important in the field. The rainwater supplied contained neither N (except for N addition in the high N treatments) nor P and only an extremely low concentration of K (Table 4.1). The total amount of K added with rainwater was 0.02 and 0.05 g K m<sup>-2</sup> in the monocultures and mixtures respectively (the mixtures had a higher water use because of enhanced evaporation from the *Sphagnum* surface). A negligible amount compared with a pool of 2.3 g K m<sup>-2</sup> in *Eriophorum* tillers at the end of the experiment. Further, we assume that dry atmospheric deposition in the greenhouse was strongly reduced. As aboveground litter fall was also very small, nutrients required for *Eriophorum* growth had to be released by mineralisation of the peat or reallocated from senescing tissues.

Alternatively, we suggest that the living *Sphagnum* layer immobilised nutrients that were mineralised from the peat below. This assumes an upward stream of nutrients, which is reasonable because of the high evaporation of the *Sphagnum* surface (Chapter 5). Only once a week the water gift reversed the direction of water transport. Another mechanism is the insulating effect of *Sphagnum*, resulting in lower soil temperatures in summer, which might slow down mineralisation rates. However, in our experiment the peat was kept in relatively small containers, exposed on all sides to the air (not only at the top as in field conditions), and it is unlikely that there was a difference in soil temperature between the treatments.

### CO<sub>2</sub> and N effects on *Sphagnum*

The observed negative N effect on *Sphagnum* height growth was a direct effect and not affected by the presence of *Eriophorum* tillers. The negative effect was most pronounced in the first growing season and gradually became less during the experiment (Fig. 4.1). After three growing seasons of N addition *Sphagnum* green biomass was not reduced, as the number of capitula per unit area had increased in the third growing season (Table 4.2). Personal observations also suggest that *Sphagnum* recovered from the negative effects of the first season. Perhaps *Sphagnum* was able to adjust to the higher N deposition. The N supply of 5 g N m<sup>-2</sup> year<sup>-1</sup>, corresponding with an N deposition of 50 kg N ha<sup>-1</sup> year<sup>-1</sup>, is not extremely high in the Netherlands. In the Dutch BERI experiment *S. magellanicum* grew well under ambient N deposition of about 50 kg N ha<sup>-1</sup> year<sup>-1</sup> in Wageningen. The

negative response to the N addition treatment in the third growing season of this outdoor experiment corresponded with a substantial increase in vascular plant cover (Fig. 2.5). A direct negative N effect, as observed in the first year of this greenhouse experiment, has been reported earlier in relatively short-term pot experiments with *Sphagnum* monocultures (Press *et al.* 1986; Jauhiainen *et al.* 1994, 1998b), but also in field experiments (Lütke Twenhöven 1992a; Thormann and Bayley 1997).

Positive CO<sub>2</sub> effects on *Sphagnum* growth, as we observed in our study (Fig. 4.1, Table 4.2), have been reported before, in a pot experiment (Jauhiainen *et al.* 1998a) as well as in our outdoor experiment with intact bog vegetation (Chapter 2). Both CO<sub>2</sub> and N effects on height growth disappeared in the third growing season when height increment was rather small in all treatments (Fig. 4.1). Possibly P availability became limiting for *Sphagnum* growth after two seasons without additional supply of this nutrient. Extremely low P concentrations in *Sphagnum* capitula (compared with Jauhiainen *et al.* 1998b, Table 2.5, Table 3.3 and field plants) point in this direction (Table 4.4).

### CO<sub>2</sub> and N effects on *Eriophorum*

The positive CO<sub>2</sub> effect on *Eriophorum* aboveground biomass observed in the first growing season gradually declined during the experiment (Fig. 4.4). It has often been shown that the general positive CO<sub>2</sub> effect on plant growth is lacking or transient under nutrient limited conditions (Körner 1996; Arp *et al.* 1998). Probably P became the limiting nutrient for *Eriophorum* growth during this experiment. From the water quality data it is obvious that *Eriophorum* takes up P and K (Table 4.5). P concentrations in green and brown leaves were extremely low compared with field plants also sampled in summer (Table 4.4). The large difference in P concentration between green and brown leaves suggests that *Eriophorum* reallocated as much P as possible, which was not so much the case for N and K (Table 4.4). In addition, P concentrations were at a rather constant low (minimum?) level in all treatments, whereas N and K concentrations were increased (accumulated?) in green leaves in the mixtures, thus may not have been the limiting elements.

Not only P concentrations in green leaves of *Eriophorum* were low, but also N and K concentrations had decreased relative to concentrations in plants from the field site. We had not expected that nutrient availability would decrease in the greenhouse experiment, as we thought that the relatively high soil temperatures would enhance nutrient mineralisation rates in the peat (Nadelhoffer *et al.* 1991). Apparently, the rate of mineralisation became too low to meet the nutrient demand of *E. angustifolium*. The relatively small volume of the peat (20-30 cm depth) in the containers may cause this nutrient limitation. It was observed that most roots were at the bottom of the container, as apparently the roots could only grow downward.

N deposition had no effect on *Eriophorum* growth, while more NH<sub>4</sub> was available to *Eriophorum* roots in the high N treatments, also in the treatments with a *Sphagnum* surface (Table 4.6). Apparently the living *Sphagnum* layer could not capture all added N and leached NH<sub>4</sub> to the deeper peat layers, both in the *Sphagnum* monocultures and mixtures. The *Eriophorum* tillers accumulated N in all plant parts, except green leaves, but growth was not stimulated. Obviously N was not limiting growth (Fig. 4.4).

Concluding, in our experiment, the presence of *Eriophorum* tillers did not affect *Sphagnum* biomass, probably because *Eriophorum* density was not high enough, whereas the presence of a living *Sphagnum* layer had a negative effect on *Eriophorum* biomass. Possibly *Sphagnum* decreased nutrient availability in this experiment, not by interception of atmospheric supply (as atmospheric supply was much reduced), but by immobilisation of mineralised nutrients from the peat. Elevated CO<sub>2</sub> and/or increased N deposition did not change these competitive relationships between *Sphagnum* and *Eriophorum*, as indicated by the lack of statistical interactions between CO<sub>2</sub>, N and species treatments. The living *Sphagnum* layer did not fully capture the added N, but *Eriophorum* did not take advantage as its growth was not limited by N. Perhaps the *Sphagnum* layer prevented a positive CO<sub>2</sub> effect on *Eriophorum* biomass in the second and third growing season (Fig. 4.4), which means that *Sphagnum* would gain a competitive advantage under elevated CO<sub>2</sub>, but the results don't provide evidence for this theory. A long-term field experiment, including removal treatments is required to study the competitive balance between *Sphagnum* and vascular plants as influenced by environmental changes.

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## Evapotranspiration in bog vegetation: effects of elevated CO<sub>2</sub> and vascular plants

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### Summary

We determined evapotranspiration in three experiments that were set up for studying the effects of elevated CO<sub>2</sub> and increased N deposition on ombrotrophic bog vegetation. Two experiments used peat monoliths with intact bog vegetation placed in containers, one situated outdoors and the other in a greenhouse. A third experiment involved monocultures and mixtures of *Sphagnum magellanicum* and *Eriophorum angustifolium* placed in containers, situated in the same greenhouse. By measuring water in- and outputs from the containers, we determined water use of the bog vegetation in July-August for each experiment and each year. We studied the effects of elevated CO<sub>2</sub> and N supply on evapotranspiration in relation to vascular plant biomass and exposure of the moss surface (measured as height of the moss surface relative to the container edge).

Elevated CO<sub>2</sub> reduced water use of the bog vegetation in all three experiments, but the CO<sub>2</sub> effect on evapotranspiration interacted with vascular plant biomass and exposure of the moss surface. Evapotranspiration in the outdoor experiment was largely determined by evaporation from the *Sphagnum* moss surface (as affected by exposure to wind) and less so by vascular plant transpiration. Still, elevated CO<sub>2</sub> significantly reduced evapotranspiration by 9-10% in the outdoor experiment.

Vascular plants reduced evapotranspiration in the outdoor experiment, but increased water use in the greenhouse experiments. The relation between vascular plant abundance and evapotranspiration appears to depend on wind conditions, suggesting that vascular plants reduce water losses mainly by reducing wind speed at the moss surface.

*Sphagnum* growth is very sensitive to changes in water level and low water availability can have deleterious effects. As a consequence, reduced evapotranspiration in summer, whether caused by elevated CO<sub>2</sub> or by small increases in vascular plant cover, is expected to favour *Sphagnum* growth in ombrotrophic bog vegetation.

## Introduction

Ombrotrophic bogs are peat-forming ecosystems and as such they can serve as important long-term sinks for atmospheric CO<sub>2</sub> (Gorham 1991). Among peatlands the *Sphagnum*-dominated bogs have the greatest peat (thus carbon) accumulation potential due to low rates of litter decomposition (Thormann *et al.* 1999). In these wet ecosystems water availability is extremely important, particularly for *Sphagnum* growth. *Sphagnum* mosses lack the efficient water conducting system of vascular plants and are therefore more susceptible to extended periods of drought (Malmer *et al.* 1994). Large between-year variation in *Sphagnum* growth is assumed to be caused by the weather, particularly moisture conditions (Wallén *et al.* 1988; Backéus 1988). Low water availability limited moss growth in a Canadian bog (Thormann and Bayley 1997). In addition, photosynthetic rates in *Sphagnum* species have been shown to be very sensitive to tissue water content (Wallén *et al.* 1988; Schipperges and Rydin 1998). As *Sphagnum* mosses are the principle component of ombrotrophic bog vegetation, changes in water availability may have major consequences for the bog ecosystem.

Atmospheric CO<sub>2</sub> concentration affects water use of vascular plants. Leaf level transpiration is generally reduced at elevated CO<sub>2</sub> (Field *et al.* 1995), mainly through increased stomatal closure (Bettarini *et al.* 1998). However, reduced water use of a single leaf does not necessarily lead to reduced evapotranspiration at the ecosystem scale as it may be counteracted by increases in total leaf area or evaporation from the soil (Field *et al.* 1995). Still, several experiments have shown a reduction in evapotranspiration under elevated CO<sub>2</sub> (Owensby *et al.* 1997; Field *et al.* 1997; Arp *et al.* 1998). The reduced evapotranspiration resulted in increased soil moisture availability, which delayed the onset of drought stress. When stimulating effects of elevated CO<sub>2</sub> on biomass production in natural vegetation were observed, they were often obtained through an improved water status of the ecosystem and therefore more pronounced in years with frequent drought stress (Drake *et al.* 1996; Field *et al.* 1997; Owensby *et al.* 1999).

We studied the effects of elevated CO<sub>2</sub> on evapotranspiration in three experiments which were set up for investigating the effects of elevated CO<sub>2</sub> and increased N deposition on ombrotrophic bog vegetation. Two experiments used peat monoliths with intact bog vegetation, one situated outdoors (outdoor experiment) and the other in a greenhouse (greenhouse experiment). The third experiment was situated in the same greenhouse and included monocultures and mixtures of *Sphagnum magellanicum* and *Eriophorum angustifolium*. This experiment was set up to study competition between *Sphagnum* and vascular plants as affected by elevated CO<sub>2</sub> and increased N deposition (competition experiment). The N treatments of the experiments were expected to increase vascular plant biomass, thereby affecting evapotranspiration. As the monoliths were in containers and water gifts were recorded we were able to determine evapotranspiration in all three experiments and investigate the effects of elevated CO<sub>2</sub> and N.

## Methods

All peat monoliths were cut from a small mire with ombrotrophic bog vegetation in the State Forestry of Dwingeloo (52°49'N, 6°25'E) in the north of the Netherlands. This site is

one of a series of heath pools (0.5–1.5 m deep) that has become filled with peat. The heath pool measures 50 by 150 m and is surrounded by forest. The peat layer is 25 to 50 cm thick. The monoliths were cut from lawn vegetation dominated by *Sphagnum magellanicum* Brid.. Important co-occurring vascular plant species were *Vaccinium oxycoccus* L., *Erica tetralix* L., *Eriophorum angustifolium* Honck. and *Drosera rotundifolia* L..

The monoliths were transplanted to large containers (110 cm diameter, 60 cm deep) buried in a grassland (outdoor experiment) or to smaller containers (34 cm diameter, 40 cm deep and 24 cm diameter, 30 cm deep) placed in a greenhouse (greenhouse and competition experiment) in Wageningen (51°99'N, 5°70'E). They were subjected to CO<sub>2</sub> and N treatments for two or three growing seasons. As water inputs were known we could determine water use or evapotranspiration. Water use data were available for all experiments and all years only in July–August; in the outdoor experiment unknown amounts of water were lost in wet periods earlier or later in the year. Changes in evapotranspiration in these warmest months would be most relevant to *Sphagnum* growth, as then it is most prone to drought stress. All water use data were transformed to evapotranspiration in mm day<sup>-1</sup> to be able to compare the experiments. Height of the moss surface (distance from container rim) and abundance of vascular plants appeared to be very important parameters that were measured or estimated in the experiments. The experimental design and method of measuring water use, height of the moss surface and vascular plant biomass are described below for each of the three experiments.

### Outdoor experiment

Twenty large peat monoliths (110 cm diameter, 60 cm deep, cut in March 1996 when the peat was frozen to 15–20 cm below the surface) with intact bog vegetation, were used for two simultaneous experiments lasting three growing seasons. The CO<sub>2</sub> experiment involved an ambient and an elevated CO<sub>2</sub> (560 ppmv) treatment. The CO<sub>2</sub> treatments were established using MiniFACE technology (Miglietta *et al.*, 2000). Ten containers were enclosed with MiniFACE rings, five of them blowing ambient air and the other five CO<sub>2</sub>-enriched air. The N experiment consisted of an ambient and an increased N deposition (5 g N m<sup>-2</sup> year<sup>-1</sup> added) treatment. Nitrogen was added in six applications during the growing season (about every three weeks) by watering each increased N plot with 2 l of NH<sub>4</sub>NO<sub>3</sub> solution, simulating a rain event of 2 mm. The ambient N plots received the same amount of demineralised water. All treatments were replicated 5 times.

Water level in the plots was allowed to fluctuate between 5 and 20 cm below moss surface. An opening in the container at 5 cm below initial moss surface allowed overflow into a 25 l jerrycan. Whenever the water level dropped below the minimum level, artificial rainwater (Table 4.1) was added to increase the level to 10 cm below initial moss surface. In this way water levels fluctuated quite naturally from low in dry periods to high in wet periods, but extreme events as flooding and drying out of the peat were prevented.

The experiment started in May 1996. In the winter months December, January and February the MiniFACE system was turned off. The experiment ended with a final harvest in the beginning of September 1998. Water use was determined for the relatively dry period July–August each year. In 1997 a six week period was chosen as water levels in the first weeks of July were high and in many containers an unknown amount of water had flown into the connected jerrycans. For 1996 and 1998 water use could be determined for an eight week period. One plot, which had an exceptionally high water use in 1997 and 1998, was

omitted from data analysis for all years. This plot (1) was strongly exposed due to a high 'hummocky' moss surface and (2) had much moss hanging over the container edge (all containers extended about 10 cm above the soil surface), which caused water to siphon over the edge even in a relatively dry period. In March 1999 all moss hanging over the containers was cut off, rinsed, dried and weighed. The omitted plot had by far most overhanging moss and gave normal values for water loss afterwards in July-August 1999 (after the treatments had stopped). The other containers did not appear to lose water by siphoning over the container edge in the chosen periods.

Each year water was added when water levels (measured biweekly) dropped below the minimum level (4-7 times). The water level was measured before and two hours after each water addition to allow the added water to be absorbed by *Sphagnum*. The obtained relation between added amount of water and water level rise for each container was used to calculate the amount of water necessary to bring the water level to 10 cm below the moss surface and to calculate the volumes of water corresponding with differences in water level. Water use was calculated by summing the amounts of water added in the chosen period and correcting for the (small) difference in water level between the start date and the end date. This added amount (l) was divided by the surface area (m<sup>2</sup>) of the container and precipitation (mm) for the same period was added to obtain water use in mm. This total water use was divided by the number of days to obtain evapotranspiration in mm day<sup>-1</sup>. Precipitation data, as well as other meteorological data (temperature, radiation, wind speed) were obtained from the meteorological station of Wageningen University, situated at 2 km from the site.

Height of the moss surface relative to the container rim was measured four times during the experiment. An aluminium frame was fixed to the container edge and at 25 points (at 5 cm intervals) the height of the moss surface relative to this frame was measured. Also height of the container edge relative to the frame was measured at 4 points. Subtracting average container height from average moss surface height resulted in the average height of the moss surface relative to the container edge. Only measurements on dates on which water level in the containers was similar to water levels in July-August were used. The height of the moss surface moved up and down by 1-3 cm with the water table, with some plots showing more movement than others. For 1996 moss height measured in May was used and for 1998 moss height was measured in August. In 1997 moss height was not measured and was estimated as the average of 1996 and 1998.

Aboveground vascular plant biomass was determined at the final harvest in a subplot of 25 by 37.5 cm. Aboveground biomass was estimated for the previous years using linear regressions between abundance data (from point-quadrat measurements in the same subplot) and the harvested aboveground biomass for each species (see Chapter 2 for details)

### Greenhouse experiment

Twenty peat monoliths (34 cm diameter, 40 cm deep) with intact bog vegetation were exposed to two atmospheric CO<sub>2</sub> levels (ambient: 350 ppmv and elevated: 560 ppmv) combined with two levels of N supply (low: no N addition and high: 5 g N m<sup>-2</sup> year<sup>-1</sup> added) in a greenhouse during two growing seasons. The CO<sub>2</sub> treatments were established by placing the containers in the greenhouse in two identical compartments in which the CO<sub>2</sub> concentration could be regulated. Every two weeks the CO<sub>2</sub> level was switched between the compartments (and the containers were moved) to avoid a compartment effect.

Nitrogen was added by watering an ammoniumnitrate solution in six applications (about every three weeks) during the growing season.

The temperature in the greenhouse was set to simulate the daily and seasonal pattern of outdoor temperature by using the weekly average minimum and maximum temperature for the 1980s. These values were increased by 5 °C to prevent exceeding the cooling capacity of the greenhouse during warm spells. During the day the temperature was set to change every 4 hours from the minimum temperature (from 0:00 to 4:00 hour) to the maximum temperature (from 12:00 to 16:00 hour) and back. Humidity was set at 75%. No artificial light was used.

Water level in each container was restored to 5 cm below initial moss surface every week by watering the experimental plots with artificial rainwater. Water levels dropped to a minimum of 10 cm below moss surface in 1996 and 15 cm below moss surface in 1997 (due to increased water use of the vascular plants and moss growth). The experiment started in June 1996. From November 1996 to March 1997 the monoliths wintered outside underneath a transparent roof. In that period there was no CO<sub>2</sub> treatment. The experiment ended in October 1997.

Water use was determined by summing the amounts of water added in an 8-week period in July-August each year. The containers were an equal number of weeks in both compartments of the greenhouse to prevent compartment effects. This was necessary, as water use was most of the time higher in one of the two compartments, despite similar settings in temperature, light and humidity. Height of the moss surface relative to the container edge was estimated only at the beginning and end of the experiment. Aboveground vascular plant biomass was determined at the final harvest by clipping off aboveground plant parts in the whole container. Aboveground biomass was estimated for the previous year in the same way as for the outdoor experiment.

### Competition experiment

Sixty peat monoliths (24 cm diameter, 30 cm deep) were used for creating three competition modes: *Sphagnum* monoculture, *Eriophorum* monoculture and *Sphagnum* + *Eriophorum* mixture. These species were subjected to the same CO<sub>2</sub> and N treatments as in the greenhouse experiment. All treatments were combined in a factorial design, resulting in twelve treatments, which were replicated five times.

The *Sphagnum* monoculture was established by removing all aboveground vascular plant parts from the monolith. In addition, mosses other than *Sphagnum* were pulled out. The same procedure was used for the *Sphagnum* + *Eriophorum* mixture, but in addition nine green tillers of *Eriophorum angustifolium* were planted. The *Eriophorum* monoculture was established by removing the top layer of about 5 cm of green *Sphagnum* from the monoliths. Nine green *Eriophorum* tillers were planted and a 5 cm layer of plastic grains was added to replace the *Sphagnum* layer.

The CO<sub>2</sub> and N treatments were established in the same way as in the greenhouse experiment. Also the climate settings in the greenhouse were the same as in the greenhouse experiment and in 1997 both experiments were in the same compartments. Water level in each container was restored to about 6 cm below moss surface every week by watering the containers with artificial rainwater to a constant weight. This target weight was increased several times during the experiment to account for moss growth. Each year water levels

dropped to a minimum of 13 cm below moss surface in treatments with a moss surface and to 6 - 8 cm below the plastic surface in the *Eriophorum* monocultures.

The experiment started in June 1997. From November to March the monoliths wintered outside underneath a transparent roof. The experiment ended with a final harvest in September 1999. Water use was determined in the same way as for the greenhouse experiment. Height of the moss surface relative to the container edge was estimated several times during the experiment. Aboveground biomass of *Eriophorum angustifolium* was determined at the final harvest. Aboveground biomass in the previous years was estimated using linear regression between the sum of squared shoot heights (measured each year) and the harvested aboveground biomass ( $R^2 = 0.88$ ,  $n = 40$ ,  $P < 0.001$ ).

## Results

### Outdoor experiment

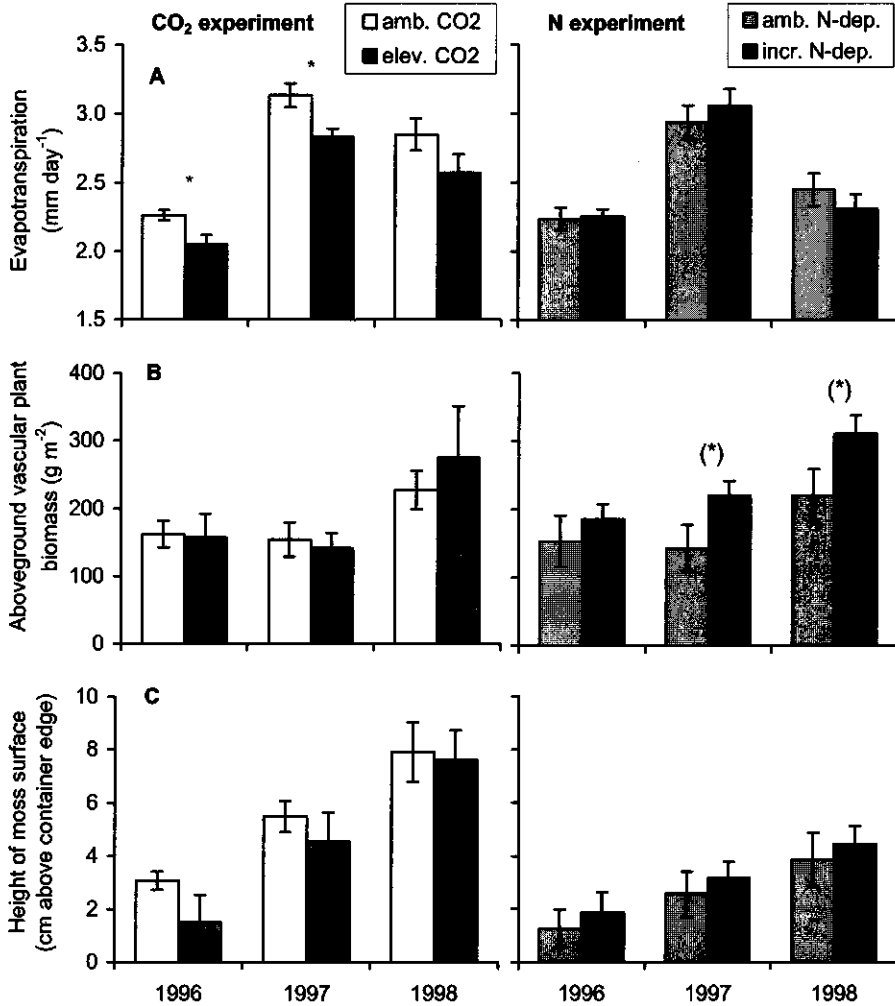
Evapotranspiration was highest in 1997 (Fig. 5.1A) when July and August were warm and sunny (Table 5.1). The weather conditions in July-August 1996 and 1998 were almost similar, but evapotranspiration was higher in 1998, particularly in the CO<sub>2</sub> experiment. This can be explained by the higher moss surface in 1998 (Fig. 5.1C). Evapotranspiration strongly increased with increasing height of the moss surface in 1997 and 1998 (Fig. 5.2, Table 5.2).

Elevated CO<sub>2</sub> significantly reduced evapotranspiration in the first two summers of the outdoor experiment (Fig 5.1A, Table 5.2). In the third year the CO<sub>2</sub> effect was almost significant when taking into account the effects of vascular plants and moss height (Table 5.2). In all three summers, elevated CO<sub>2</sub> reduced evapotranspiration by 9-10%.

Adding N deposition increased vascular plant abundance (Fig. 5.1B), but had no effect on evapotranspiration (Fig. 5.1A). The vascular plant canopy reduced evapotranspiration, particularly in 1996 (Fig. 5.3, Table 5.2). Vascular plant abundance increased in the third season of the experiment in all treatments, but even then vascular plants did not contribute positively to evapotranspiration. In the N experiment, where moss height was lower, vascular plant abundance seemed to reduce evapotranspiration up to an aboveground biomass of 325 g m<sup>-2</sup> (Fig. 5.3).

**Table 5.1** Weather conditions and average water table depth (cm below moss surface) during the periods for which evapotranspiration was determined. Weather data are mean daily averages (temperature, wind speed) or mean daily sums (solar radiation, precipitation). Weather data are from the weather station of Wageningen University in Wageningen.

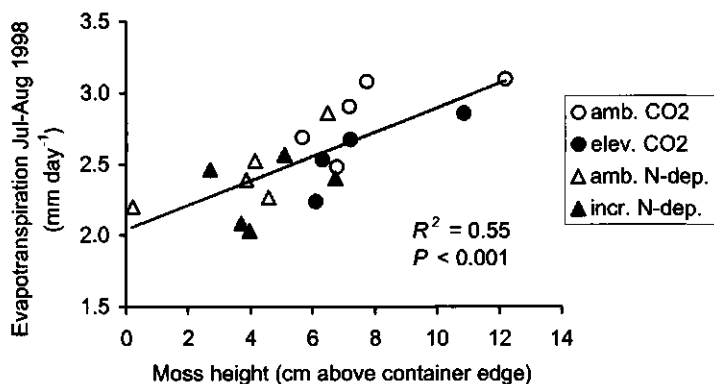
	1996	1997	1998
Temperature (°C)	16.8	19.4	16.5
Solar radiation (J cm <sup>-2</sup> )	1596	1882	1569
Wind speed (m s <sup>-1</sup> )	2.5	2.2	2.8
Precipitation (mm day <sup>-1</sup> )	1.3	1.2	1.6
Water table (cm below moss surface)	11	14	15



**Figure 5.1** A) evapotranspiration ( $\text{mm day}^{-1}$ ), B) aboveground vascular plant biomass ( $\text{g m}^{-2}$ ) and C) height of the moss surface (cm above container edge) in July-August each year of the outdoor CO<sub>2</sub> and N experiment. Data are means  $\pm$  SE ( $n = 4-5$  containers). Significant treatment effects ( $t$ -test) are included: (\*)  $P < 0.10$ , \*  $P < 0.05$ .

**Table 5.2** ANOVA results for the effects of CO<sub>2</sub>, N, vascular plants and moss height on evapotranspiration in the outdoor experiment. CO<sub>2</sub> and N experiment were analysed separately. Aboveground vascular plant biomass (vasc. pl.) and height of moss surface (moss) were included as covariables. (\*)  $P < 0.10$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ .

Year	CO <sub>2</sub> experiment			N experiment		
	vasc. pl.	moss	CO <sub>2</sub>	vasc. pl.	moss	N
1996	(*)	-	*	**	(*)	-
1997	-	(*)	*	-	(*)	-
1998	-	*	(*)	-	(*)	-



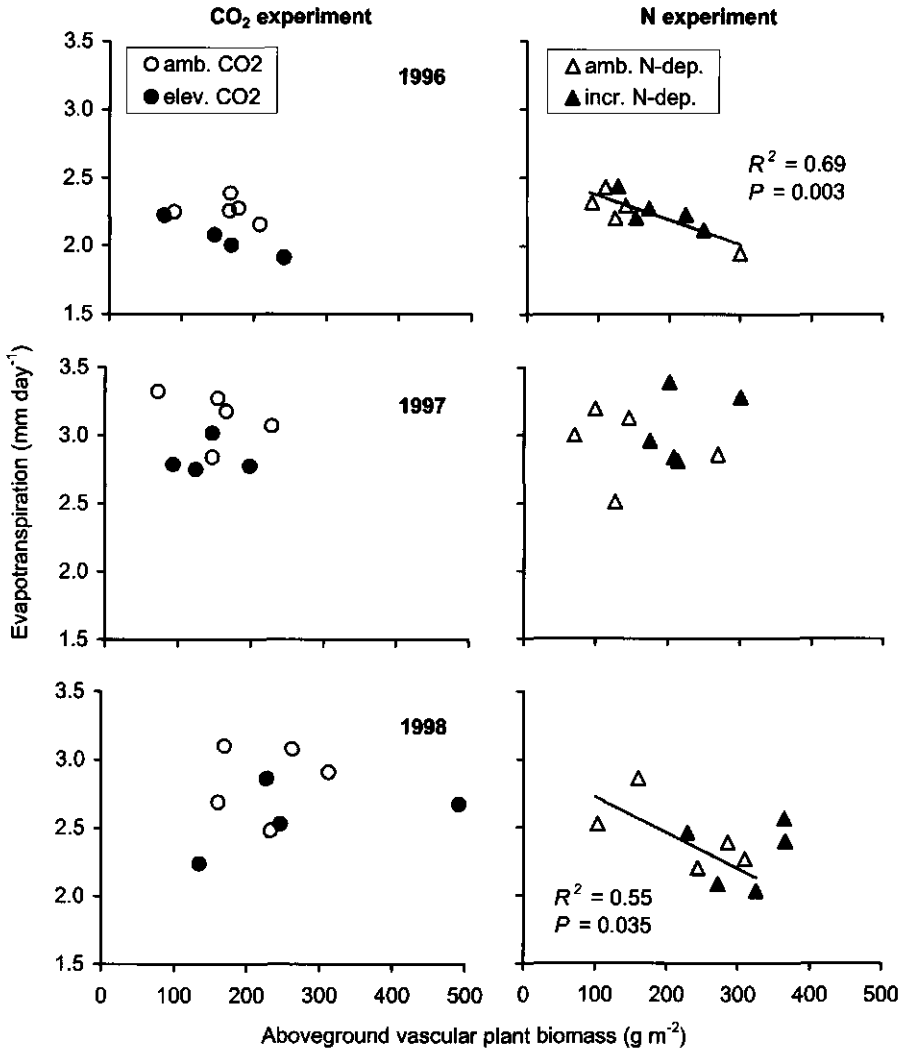
**Figure 5.2** Relation between evapotranspiration ( $\text{mm day}^{-1}$ ) and height of the moss surface (cm above container edge) in 1998 in the outdoor experiment.

### Greenhouse experiment

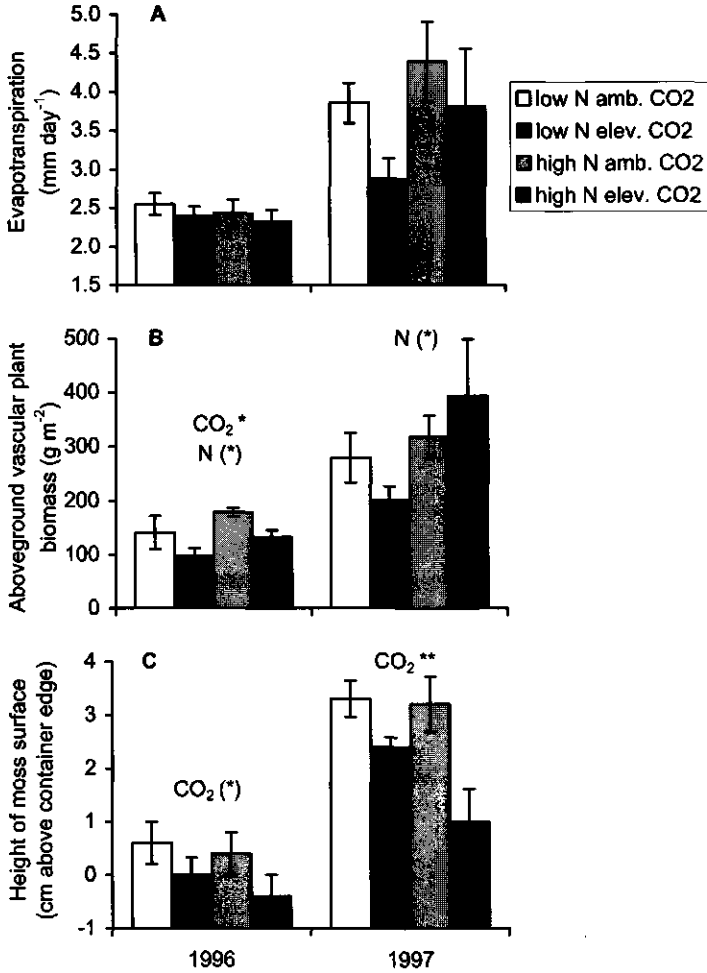
Evapotranspiration was higher in the second growing season when vascular plant abundance had greatly increased (Fig. 5.4A, B). In the second season we observed a positive relationship between evapotranspiration and aboveground vascular plant biomass (Fig. 5.5). When the effects of vascular plants were taken into account, elevated  $\text{CO}_2$  significantly reduced evapotranspiration in the second year (Fig. 5.4A, Table 5.3).

Evapotranspiration in the greenhouse was higher than outdoors. Although wind is lacking (but there is air circulation), mean temperature was higher in the greenhouse ( $22^\circ\text{C}$  in July-August) and vascular plant biomass in the second season was much greater than outdoors.





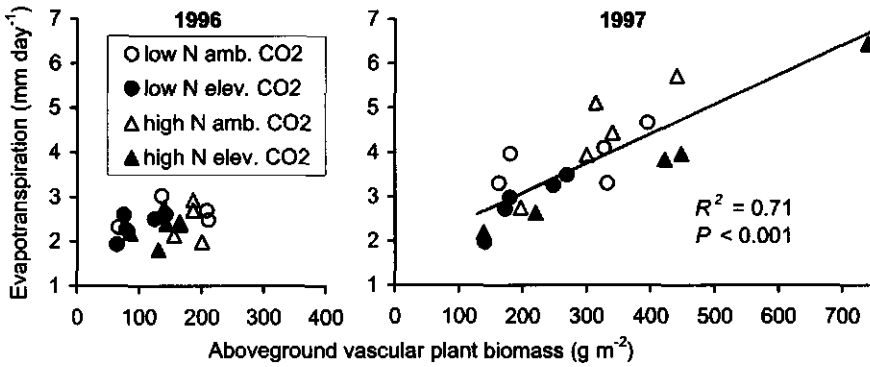
**Figure 5.3** Relation between evapotranspiration (mm day<sup>-1</sup>) and aboveground vascular plant biomass (g m<sup>-2</sup>) in each year for the CO<sub>2</sub> and N experiment outdoors. Drawn separately for the CO<sub>2</sub> and N experiment because height of the moss surface was much lower in the N experiment (Fig. 5.1).



**Figure 5.4** A) evapotranspiration (mm day<sup>-1</sup>), B) aboveground vascular plant biomass (g m<sup>-2</sup>) and C) height of the moss surface (cm above container edge) in July-August each year of the greenhouse experiment. Data are means ± SE (n = 5 containers). Significant treatment effects (tested with 2 x 2 ANOVA) are included: (\*) P < 0.10, \* P < 0.05

**Table 5.3** ANOVA results for the effects of CO<sub>2</sub>, N, vascular plants and moss height on evapotranspiration in the greenhouse experiment. Aboveground vascular plant biomass (vasc. pl.) and height of moss surface (moss) were included as covariables. \* P < 0.05, \*\*\* P < 0.001.

year	vasc. pl.	moss	CO <sub>2</sub>	N	CO <sub>2</sub> x N
1996	-	*	-	-	-
1997	***	-	*	-	-



**Figure 5.5** Relation between evapotranspiration ( $\text{mm day}^{-1}$ ) and aboveground vascular plant biomass ( $\text{g m}^{-2}$ ) in 1996 and 1997 in the greenhouse experiment.

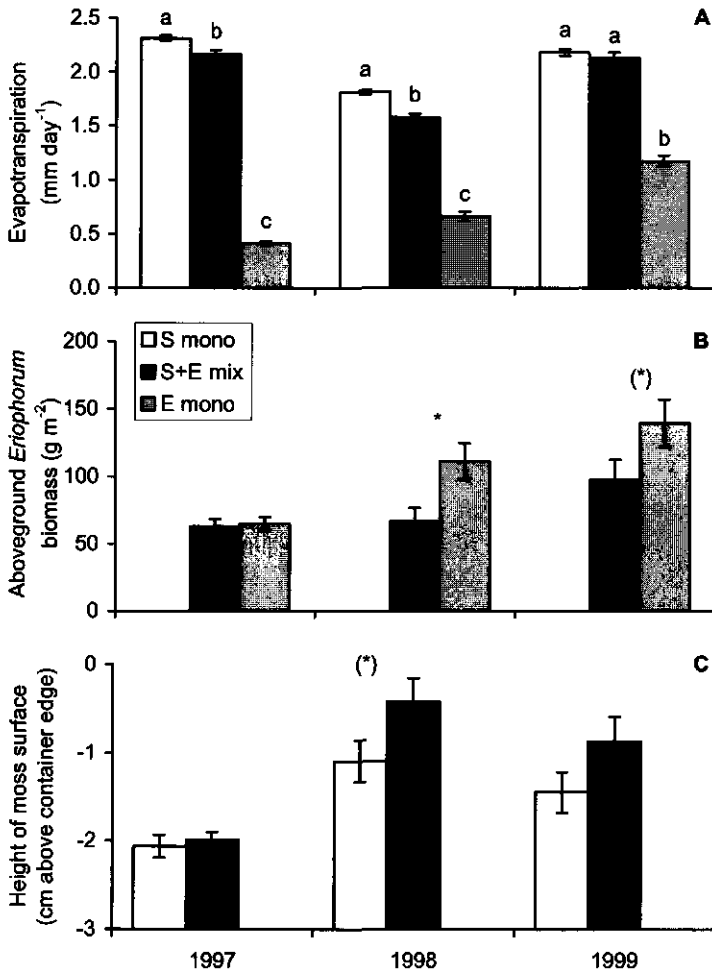
### Competition experiment

Weather conditions outside even played a role in the greenhouse. Evapotranspiration in the containers with a *Sphagnum* layer was lower in the cool, cloudy summer of 1998 (Fig. 5.6A). Both 1997 and 1999 had warm summers and the greenhouse had to ventilate much of the time to keep the temperature at the target temperature. Evapotranspiration was lower in the *Sphagnum* + *Eriophorum* mixtures than in the *Sphagnum* monocultures in the first two summers (Fig. 5.6A). Evapotranspiration of the *Eriophorum* monocultures was much less than that of the *Sphagnum* monocultures (Fig. 5.6A).

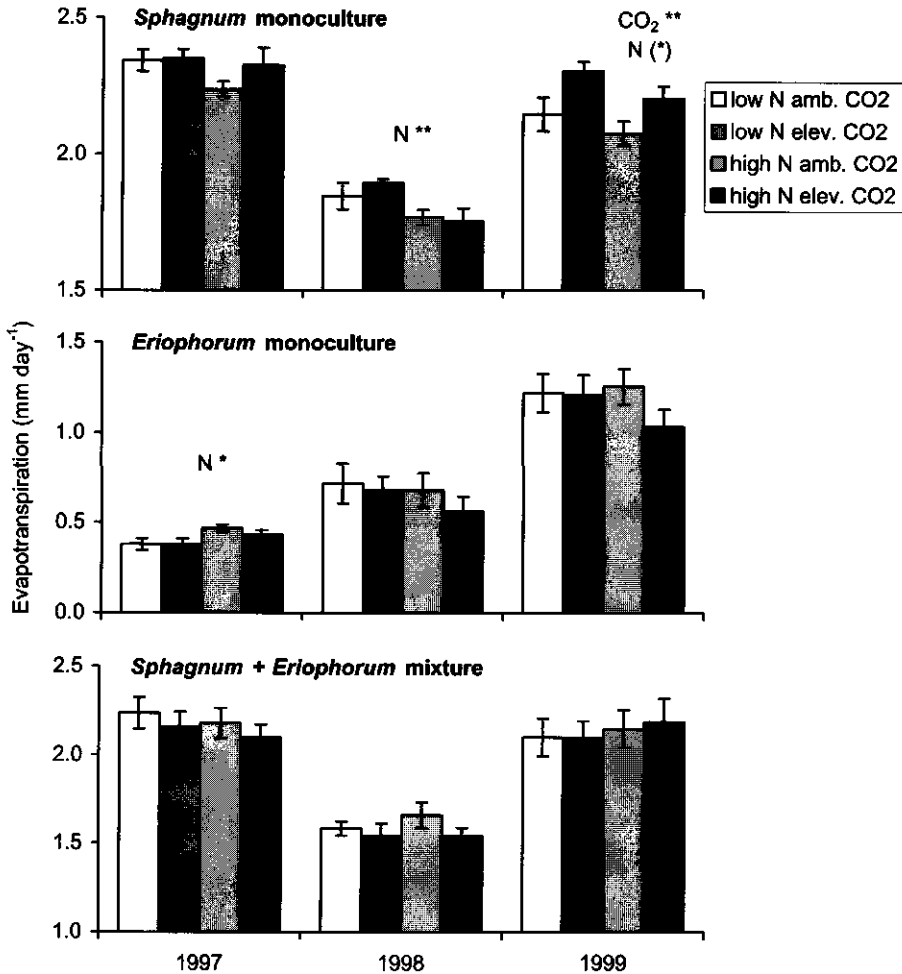
Elevated  $\text{CO}_2$  reduced evapotranspiration in the *Eriophorum* monocultures in the second and third growing season after biomass had increased (Fig. 5.7, Table 5.4), and in the *Sphagnum* + *Eriophorum* mixtures, but only in the second season. In the *Eriophorum* monocultures water use increased with increasing biomass (Fig. 5.8). In the mixtures *Eriophorum* biomass contributed positively to evapotranspiration after biomass had increased (Fig. 5.8, Table 5.4).

**Table 5.4** ANOVA results for the effects of  $\text{CO}_2$ , N, vascular plants and moss height on evapotranspiration in the competition experiment. Aboveground *Eriophorum* biomass (vasc. pl.) and height of moss surface (moss) were included as covariables. (\*)  $P < 0.10$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , n.a. not applicable.

species	year	vasc. pl.	moss	$\text{CO}_2$	N	$\text{CO}_2 \times \text{N}$
<i>Sphagnum</i> monoculture	1997	n.a.	-	-	-	-
	1998	n.a.	-	-	*	-
	1999	n.a.	-	*	-	-
<i>Eriophorum</i> monoculture	1997	*	n.a.	-	*	-
	1998	***	n.a.	***	*	-
	1999	***	n.a.	**	-	(*)
<i>Sphagnum</i> + <i>Eriophorum</i> mixture	1997	-	**	-	-	-
	1998	**	**	**	-	-
	1999	**	*	-	-	-

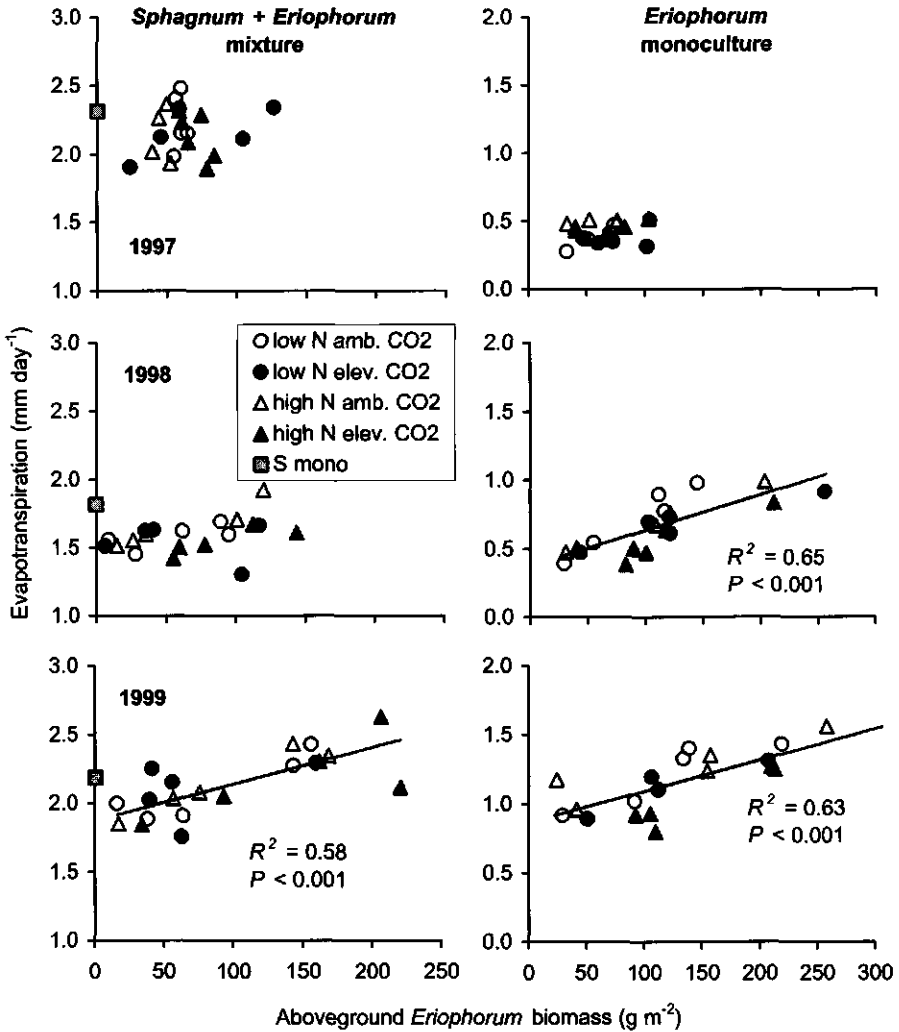


**Fig. 5.6** A) evapotranspiration ( $\text{mm day}^{-1}$ ), B) aboveground vascular plant (= *Eriophorum*) biomass ( $\text{g m}^{-2}$ ) and C) height of the moss surface (cm above container edge) in July-August each year of the competition experiment. For presentation, data from the  $\text{CO}_2$  and N treatments were pooled. Data are means  $\pm$  SE ( $n = 20$  containers). Significant effects of species composition (tested with  $3 \times 2 \times 2$  ANOVA) are included. For evapo-transpiration Tukey's post-hoc test was performed. Within each year different letters indicate significant differences. (\*)  $P < 0.10$ , \*  $P < 0.05$ .



**Figure 5.7** Evapotranspiration ( $\text{mm day}^{-1}$ ) in each year for each species treatment in the competition experiment. Data are means  $\pm$  SE ( $n = 5$  containers). Significant treatment effects (tested with 2 x 2 ANOVA) are included: (\*)  $P < 0.10$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ .

The  $\text{CO}_2$  effects were only significant when covariables were included in the statistical analysis, as elevated  $\text{CO}_2$  had a positive effect on *Eriophorum* biomass (Chapter 4). Elevated  $\text{CO}_2$  even increased evapotranspiration in the *Sphagnum* monocultures (Fig. 5.7). This could partly be explained by differences in height of the moss surface, as the significance of the  $\text{CO}_2$  effect was reduced after inclusion of moss height as covariable, but still left a significant  $\text{CO}_2$  effect (Table 5.4). The effect of N is not clear: N addition increased water use in the *Eriophorum* monocultures in 1997, but had a negative effect in 1998 (Fig. 5.7, Table 5.4).



**Figure 5.8** Relation between evapotranspiration and aboveground *Eriophorum* biomass in the *Sphagnum* + *Eriophorum* mixtures (left) and *Eriophorum* monocultures (right) for each year in the competition experiment. Note different scale Y-axes. Mean value ( $n = 20$ ) for *Sphagnum* monocultures (S mono) is included.

## Discussion

### Evapotranspiration in bog vegetation

In all three experiments we were able to determine evapotranspiration in July-August and to assess effects of elevated  $\text{CO}_2$  and vascular plant cover. The rate of evapotranspiration of the intact bog vegetation of the outdoor experiment in 1996 and 1998 (N experiment) was comparable to that measured in two Irish bogs (Van der Schaaf 1999). Evapotranspiration in our experiment was highest in 1997, which can be attributed to the extremely warm and sunny weather that summer.

Evapotranspiration is considered as the sum of evaporation from the moss surface and transpiration by vascular plants. Our experiments demonstrate that evapotranspiration in a bog is largely determined by evaporation from the moss surface and less by vascular plant transpiration. In the outdoor experiment total evapotranspiration was closely related to exposure of the moss surface to wind, which directly affects *Sphagnum* evaporation. Van der Schaaf (1999) also concluded that evapotranspiration in two Irish bogs was mainly determined by evaporation from the *Sphagnum* surface, and higher water levels resulted in increased evaporation. In contrast, we observed a negative relation between evapotranspiration and height of the water table (data not shown), which means that in our experiment the relationship was reversed, and water tables were determined by water losses. However, in our experiment water levels were more or less controlled and did not fall below 20 cm below moss surface, which is considered as the critical level for *Sphagnum magellanicum*, below which evaporation from the *Sphagnum* surface will be reduced (Van der Schaaf 1999). Instead of water level, weather conditions and exposure of the moss surface to wind explained evapotranspiration differences between years.

### Effects of elevated $\text{CO}_2$

Elevated  $\text{CO}_2$  affected water use of the vegetation in all three experiments, but the  $\text{CO}_2$  effect on evapotranspiration interacted with vascular plant abundance and exposure of the moss surface. In two of the three years, elevated  $\text{CO}_2$  significantly reduced evapotranspiration in the outdoor experiment. The reduced evapotranspiration can be explained by reduced vascular plant transpiration through increased stomatal closure under elevated  $\text{CO}_2$ . Increased water use efficiency (expressed as amount of water used to produce a unit of plant biomass) is a common response to elevated  $\text{CO}_2$  (Arp *et al.* 1998). Reduced water loss due to increased stomatal closure under elevated  $\text{CO}_2$  (Bettarini *et al.* 1998) does not apply to *Sphagnum* plants that lack stomata and do not actively transport water. The competition experiment demonstrates that indeed evapotranspiration in the *Sphagnum* monocultures was not reduced by elevated  $\text{CO}_2$ . The increase in evapotranspiration at elevated  $\text{CO}_2$  in 1999 (Fig. 5.7, Table 5.4) was probably caused by the increased *Sphagnum* biomass under elevated  $\text{CO}_2$  (Table 4.2).

Water is mainly transported externally to the apical parts of *Sphagnum* individuals through capillary rise between pendant branches and stems (Hayward and Clymo 1982). Dense growth forms (such as in *Sphagnum* species from hummocks) help *Sphagnum* survive dry periods (Schipperges and Rydin 1998). Unless *Sphagnum* morphology or density changes in response to the level of atmospheric  $\text{CO}_2$ , it cannot be expected that

*Sphagnum* evaporation will be affected by elevated CO<sub>2</sub>. Only in the greenhouse experiment *Sphagnum* shoot density changed significantly in response to elevated CO<sub>2</sub> (Fig. 3.1). The denser growth form under elevated CO<sub>2</sub> in this experiment may have contributed to the reductions in evapotranspiration.

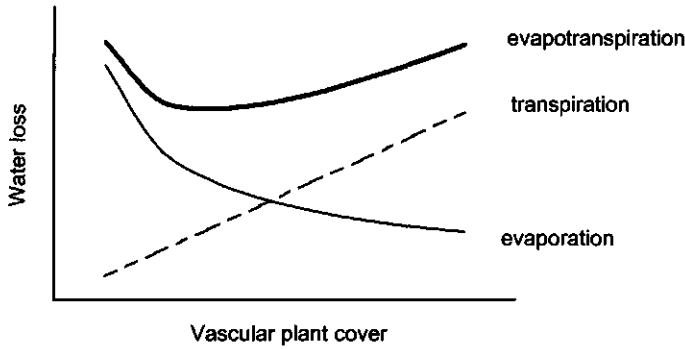
As the CO<sub>2</sub>-induced reductions in water use are likely caused by reductions in vascular plant transpiration, the CO<sub>2</sub> effect is expected to be most pronounced in the summer months when vascular plants are most active and have the largest leaf area. We found a CO<sub>2</sub>-induced reduction in evapotranspiration of 9-10% in July-August in the outdoor experiment. Although on a yearly basis the reduction will likely be smaller, the changes in water loss in the warmest and driest months are most relevant to *Sphagnum* growth (see below).

### Effects of vascular plants

Usually evapotranspiration increases with increasing plant cover, as vascular plant transpiration is governed by leaf area (and stomatal conductance), although there may be exceptions at extreme low and high plant cover (Schulze *et al.* 1994). Leaf area was not measured in our experiments, but changes in aboveground biomass are expected to reflect changes in total leaf area. In the absence of a *Sphagnum* layer water losses were greater with increasing *Eriophorum* biomass in the *Eriophorum* monocultures (Fig. 5.8), confirming a linear relationship between vascular plant cover and transpiration (Fig. 5.9). However, when water loss was mainly determined by evaporation of *Sphagnum*, as in the outdoor experiment, vascular plants reduced evapotranspiration. Total water use of the bog vegetation showed a negative relation with vascular plant abundance in the outdoor experiment (Fig. 5.3). Evapotranspiration was also lower in the *Sphagnum* + *Eriophorum* mixtures than in the *Sphagnum* monocultures (Fig. 5.6), but only when *Eriophorum* cover was relatively low. Similarly, in two Irish bogs evapotranspiration in July and August was higher in pure *Sphagnum* lysimeters (monoliths in containers inserted in the peat) than in lysimeters with vascular plants (except for the *Molinia* lysimeters) (Van der Schaaf 1999).

Vascular plants can reduce *Sphagnum* evaporation by obstruction of wind, reducing wind speed at the moss surface, and by interception of solar radiation, reducing temperature and radiation at the moss surface. This evaporation-reducing effect of vascular plants may be most pronounced at low cover, and decrease with increasing cover (Fig. 5.9). In the outdoor experiment evapotranspiration was reduced by vascular plants up to an aboveground biomass of 325 g m<sup>-2</sup> (Fig. 5.3). In the greenhouse vascular plants contributed positively to evapotranspiration after they had increased in abundance (Figs 5.5 and 5.8). Only at low abundance *Eriophorum* could reduce water losses in the *Sphagnum* + *Eriophorum* mixtures in the greenhouse (Fig. 5.6). Takagi *et al.* (1999) observed a positive contribution of vascular plants to evapotranspiration in a part of the bog where vascular plants completely covered the ground surface compared with the adjoining undisturbed *Sphagnum*-dominated bog. However, even in this vascular plant dominated vegetation the positive contribution was only observed late in the growing season when vascular plants were at peak biomass. It is suggested that at low vascular plant cover, increases in cover will reduce evapotranspiration, as the evaporation from the *Sphagnum* surface is more reduced than vascular plant transpiration is increased. However, at high vascular plant cover, increases in cover will increase evapotranspiration, as the increase in vascular plant transpiration is larger than the reduction in evaporation (Fig. 5.9).





**Figure 5.9** Suggested relation between vascular plant cover and evapotranspiration, as the sum of vascular plant transpiration and evaporation from the moss surface. The relation for transpiration may not be valid at extreme high leaf area, but this does not occur in bog vegetation. The effect of elevated  $\text{CO}_2$  will be a decrease in the slope of the transpiration line. Weather condition will have a greater impact on the evaporation line.

The exact form of this relation between vascular plant abundance and evapotranspiration probably depends on weather conditions, particularly wind. It may explain that in the calm summer of 1997 (Table 5.1) the negative relation disappeared, while it re-appeared in 1998 in the N experiment outdoors (Fig. 5.3, correlation coefficient: -0.85, 0.15 and -0.43 in 1996, 1997 and 1998 respectively for the relation between evapotranspiration and aboveground vascular plant biomass in the N experiment). In the greenhouse with less wind vascular plants had mainly an evapotranspiration-increasing effect in contrast to the vascular plants outdoors. This dependence of the relation between vascular plants and evapotranspiration on wind, suggests that vascular plants reduce water losses mainly by reducing wind speed at the moss surface.

Species composition may also play a role in the relationship between evapotranspiration and vascular plant biomass, as it is likely that species differ in their capacity to obstruct wind and to intercept solar radiation, as well as in transpiration rate. Transpiration can be expected to be related to leaf area, and obstruction and interception to plant architecture. From our experiments with mixed species composition or only one vascular plant species (competition experiment) we cannot deduce how species properties affect evapotranspiration.

### Implications for bog vegetation

Changes in evapotranspiration are expected to have important consequences for *Sphagnum* growth. Low water levels inhibit capillary rise of water to the apical parts of the *Sphagnum* individuals, causing drought stress and reduced growth (Wallén *et al.* 1988). Most vascular plants in bogs are more drought resistant than the mosses because their root systems penetrate down to the water saturated layers lower in the peat profile. Exceptions are a few shallow rooted species, *Vaccinium oxycoccus* and *Drosera rotundifolia*, which rely on the water-conducting capacity of the *Sphagnum* mosses for their water supply (Malmer *et al.* 1994). *Sphagnum magellanicum*, the dominant *Sphagnum* species in our experiments, may be very sensitive to changes in water availability. Schipperges and Rydin (1997) showed that *S. magellanicum* had lower photosynthetic rates than other *Sphagnum* species at low

tissue water contents. However, extremely high water content of the capitulum (associated with water levels near the moss surface) also reduced photosynthetic rates, particularly in *S. magellanicum* (Wallén *et al.* 1988). Increased diffusion resistance likely causes this reduced CO<sub>2</sub> uptake at saturating tissue water contents.

In our experiments *S. magellanicum* height growth increased in response to elevated CO<sub>2</sub> in the outdoor (Chapter 2, Fig. 2.1) and in the competition experiment (Chapter 4, Fig. 4.1). However, it is unlikely that these positive responses were the result of reduced drought stress, because water levels did not fall below the critical 20 cm level (Ivanov 1981). In our experiments increased height growth immediately fed back through increased exposure of the moss surface to wind and thus increased evaporation, particularly in the outdoor experiment. In the outdoor experiment water levels relative to the moss surface were not significantly different between the CO<sub>2</sub> treatments. Also in the *Sphagnum* monocultures (without a transpiration-reducing effect of CO<sub>2</sub>) elevated CO<sub>2</sub> tended to stimulate height and biomass growth. In field conditions increased *Sphagnum* height growth will not result in increased moss exposure when it occurs over the entire area, thus elevated CO<sub>2</sub> may well reduce evapotranspiration. Consequently, reduced evapotranspiration, whether caused by elevated CO<sub>2</sub> or small increases in vascular plant cover, may delay the onset of drought stress (as observed in dry ecosystems) and favour *Sphagnum* growth. Small increases in vascular plant cover provide more favourable conditions for *Sphagnum* growth, contributing to a stable equilibrium between growth of *Sphagnum* and vascular plants. However, large increases in vascular plant cover (as seen for *Molinia caerulea* in many Dutch bogs) increase evapotranspiration in summer (Schouwenaars 1993), contributing to poor growth of *Sphagnum* and ultimately to elimination of the *Sphagnum* layer. At our field site, the sheltered location in a forest gap probably contributes to the good condition of the bog vegetation. These heath pools of State Forestry Dwingeloo are among the few sites in the Netherlands where well developed ombrotrophic bog vegetation including *S. magellanicum* nowadays occurs.

## Acknowledgements

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## Effects of increased N deposition on the distribution of $^{15}\text{N}$ -labelled N between *Sphagnum* and vascular plants

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### Summary

We investigated the fate of  $^{15}\text{N}$ -labelled N deposition in ombrotrophic bog vegetation in the Netherlands, both at ambient and increased N deposition. We doubled N deposition, by adding  $5 \text{ g N m}^{-2} \text{ year}^{-1}$  as dissolved  $\text{NH}_4\text{NO}_3$ , during three growing seasons in large peat monoliths (1.1 m diameter) with intact bog vegetation kept in large containers outdoors ("N experiment"). A small amount ( $25 \text{ mg N m}^{-2}$ ) of  $^{15}\text{N}$  tracer was applied at the start of the second growing season by watering a highly  $^{15}\text{N}$ -enriched ammoniumsulphate solution over the vegetation. The distribution of the  $^{15}\text{N}$  tracer among *Sphagnum*, vascular plant species and peat was determined at the end of the third growing season. The  $^{15}\text{N}$  tracer was also applied to additional plots in the untreated field site to check for initial distribution ("field site").

One week after addition 79% of the total amount of  $^{15}\text{N}$  retrieved was found in the living *Sphagnum* layer. Vascular plants had captured only 8% at the beginning, increasing to 11% during the two growing seasons following addition. Both at the field site and the N experiment,  $^{15}\text{N}$  enrichment showed a gradient over depth with highest enrichment in *Sphagnum* followed by the vascular plants (from shallow-rooted to deep-rooted species) and the lowest or no enrichment in the deep peat layer.

In the N experiment, total recovery of the  $^{15}\text{N}$  tracer was 96% on average with no difference between the ambient and increased N deposition treatment. At ambient N deposition, still 63% of the total amount of  $^{15}\text{N}$  retrieved was found in the living *Sphagnum* layer fifteen months after  $^{15}\text{N}$  addition. Increased N deposition significantly increased the availability of N to vascular plants, as shown by the reduced proportion of  $^{15}\text{N}$  in *Sphagnum* and increased amount of  $^{15}\text{N}$  in vascular plants. Deep-rooting vascular plant species were significantly more  $^{15}\text{N}$ -enriched at increased N deposition, suggesting that N penetrates deeper into the peat at higher atmospheric inputs.

## Introduction

Bog ecosystems are expected to be very sensitive to high levels of N deposition. Ombrotrophic bogs depend on atmospheric supply of nutrients and as such they are considered to be nutrient-poor ecosystems. The bog vegetation is composed of peat mosses (*Sphagnum*) and vascular plants, which use different sources of mineral nutrients. While *Sphagnum* largely depends on nutrients from atmospheric deposition, the rooted vascular plants mainly capture nutrients that are mineralised from organic matter. *Sphagnum* is very effective in intercepting the atmospheric deposition (Woodin and Lee 1987; Williams *et al.* 1999) and thereby reduces this supply of nutrients to vascular plants. In addition, *Sphagnum* slows down decomposition by producing slowly decaying litter and creating a harsh (wet, acid, mineral-poor) environment for decomposers (Van Breemen 1995). Bog plant species deal with these nutrient-poor conditions by being very economical with their nutrients, resulting in high nutrient use efficiency compared to species from other ecosystems (Aerts *et al.* 1999).

It has often been suggested that *Sphagnum* will suffer from competition from vascular plants when these increase their growth rates at high N deposition (Lee and Studholme 1992; Malmer *et al.* 1994; Rydin 1997; Lamers *et al.* 2000). At high N deposition growth of *Sphagnum* is no longer N-limited (Aerts *et al.* 1992) and the moss may no longer capture and retain all N from atmospheric deposition. Consequently more N may reach the rhizosphere and increase the growth rates of vascular plants (Woodin and Lee 1987; Malmer *et al.* 1994). Next, the increased shading of the moss layer by an increased vascular plant cover could depress *Sphagnum* growth (Clymo 1973; Hayward and Clymo 1983). However, this sequence of events has never been investigated in field conditions. To understand the response of the bog vegetation to increased N deposition, better insight in the distribution of N between *Sphagnum* and vascular plants as affected by increased N deposition is essential.

In this study, we investigated the fate of  $^{15}\text{N}$ -labelled N deposition in ombrotrophic bog vegetation in the Netherlands, both at ambient and increased N deposition. We used large peat monoliths with intact bog vegetation kept in large containers outdoors and exposed them to doubled N deposition for three growing seasons. During the experiment we followed *Sphagnum* growth and the abundance of vascular plant species. These data can be found in Chapter 2. Here, we present the distribution of  $^{15}\text{N}$  and total N among *Sphagnum*, vascular plant species and peat. We hypothesise that increased N deposition will change the distribution of  $^{15}\text{N}$ -labelled N between *Sphagnum* and vascular plants to the benefit of vascular plants. The  $^{15}\text{N}$  tracer was also applied to additional plots at the field site to check for the  $^{15}\text{N}$  distribution one week after application.

## Methods

### Site description

The field site is a small mire with ombrotrophic bog vegetation in the State Forestry of Dwingeloo (52°49'N, 6°25'E) in the north of the Netherlands. This site is one of a series of heath pools (0.5-1.5 m deep) that has become filled with peat. The heath pool measures 50

by 150 m and is surrounded by forest. The peat layer is 30 to 50 cm thick and is highly decomposed at the bottom. The monoliths for the N experiment were cut from lawn vegetation dominated by *Sphagnum magellanicum* Brid.. Important co-occurring vascular plant species were *Vaccinium oxycoccus* L., *Erica tetralix* L., *Eriophorum angustifolium* Honck. and *Drosera rotundifolia* L.. Additional plots at the field site were laid out in the same vegetation. The monoliths were transplanted to large containers (110 cm diameter, 60 cm deep) buried in a grassland in Wageningen (51°99'N, 5°70'E) in the centre of the Netherlands.

Wet N deposition ( $\text{NH}_4 + \text{NO}_3$ ), measured in Witteveen (15 km from field site) and in Wageningen (1.5 km from experimental site), ranged from 11 kg N  $\text{ha}^{-1}$  year $^{-1}$  in dry 1996 to 15 kg N  $\text{ha}^{-1}$  year $^{-1}$  in wet 1998 without differences between the sites (Boschloo and Stolk 1999a, 1999b, 1999c). However, total N deposition is mainly (50-60%) composed of dry  $\text{NH}_x$  compounds. Dry  $\text{NH}_x$  deposition is estimated from detailed emission data. The resulting total N deposition in 1997 amounted to 52 kg N  $\text{ha}^{-1}$  year $^{-1}$  near Wageningen and 37 kg N  $\text{ha}^{-1}$  year $^{-1}$  near the field site (RIVM 1999).

### Experimental design

Ten large monoliths (110 cm diameter) with intact bog vegetation were used for the N experiment in Wageningen. They were subjected to an ambient or an increased N deposition (5 g N  $\text{m}^{-2}$  year $^{-1}$  added) treatment for three growing seasons. Nitrogen was added in six applications during the growing season (about every three weeks) by watering each high N plot with 2 l of  $\text{NH}_4\text{NO}_3$  solution, simulating a rain event of 2 mm. The N control plots received the same amount of demineralised water. The treatments were replicated 5 times.

Water levels in the plots were allowed to fluctuate between 5 and 20 cm below moss surface. An opening in the container at 5 cm below initial moss surface allowed overflow into a 25-l jerrycan. Whenever the water level dropped to the minimum level, artificial rainwater was added. In this way water levels fluctuated quite naturally, but extreme events as flooding and drying out of the peat were prevented. This experiment started in May 1996 and ended with a final harvest in September 1998.

### $^{15}\text{N}$ labelling

In May 1997, at the beginning of the second growing season, 25 mg  $^{15}\text{N}$   $\text{m}^{-2}$  was added to all plots of the N experiment in Wageningen and to 10 additional plots of 50 x 50 cm, marked with wooden poles, at the field site in Dwingeloo. The purpose of these plots was to measure the change in distribution over time of the  $^{15}\text{N}$  tracer, as it was not possible to take in-between harvests in Wageningen, because of the limited area of the experimental plots. Highly enriched ( $^{15}\text{NH}_4$ ) $_2\text{SO}_4$  (99%) was dissolved in demineralised water and applied by simulating a rain event of 2.5 l  $\text{m}^{-2}$  (equivalent to 2.5 mm rain). The solution was added homogeneously to the whole experimental plot with a small watering can. Only 25 mg N  $\text{m}^{-2}$  was added, corresponding with 0.5-1% of the Dutch annual N deposition, to minimise an N fertilisation effect.

We choose for  $^{15}\text{N}$ -labelled  $\text{NH}_4$  instead of  $\text{NO}_3$  to prevent losses through denitrification and leaching. The  $^{15}\text{N}$  solution was added together with the normal three-weekly N gift to the high N plots. The  $^{15}\text{N}$  solution was added during rainy weather in

Dwingeloo. This has probably reduced the amount of  $^{15}\text{N}$  directly taken up by the leaves of vascular plants. In Wageningen  $^{15}\text{N}$  was added one day later under dry conditions and the  $^{15}\text{N}$  addition was followed by another  $2.5 \text{ l m}^{-2}$  of demineralised water.

### Sampling

Five plots at the field site in Dwingeloo were sampled in May 1997 one week after the  $^{15}\text{N}$  addition. The other five plots in Dwingeloo were sampled at the end of August 1998. At the same time reference samples were collected in untreated similar vegetation for determination of the natural  $^{15}\text{N}$  abundance. The plots in Wageningen were harvested one week later at the beginning of September 1998.

In Dwingeloo, the central part (20 x 20 cm) of each plot (50 x 50 cm) was cut to a depth of about 30 cm for collecting vascular plants. In addition, in each plot four columns of 5 x 5 cm were cut for *Sphagnum* and peat sampling. In 1998 two blocks of 10 x 10 cm were cut for more accurate sampling. All peat cores were divided into three depths: 0-5 (green *Sphagnum*), 5-15 (brown *Sphagnum*) and 15-30 (peat) cm and cores from each plot were combined to form one sample per plot and per depth. All samples were stored in a dark, cold room at 1°C till sorting up to two months later. Litter on top of the moss surface of the 20 x 20 cm block was collected and dead *Eriophorum* and *Erica* shoots were included. Vascular plants were sorted into *V. oxycoccus*, *E. tetralix*, *E. angustifolium* and other species. Each species was then sorted into current-year leaves, roots (including belowground stems for the ericaceous species) and other plant parts. From the peat samples, all living vascular plant parts were removed. The top layer of 5 cm consisted of living *Sphagnum*. The second layer of 5-15 cm contained brown *Sphagnum* (structure still intact) and dead stems of ericaceous species. The third layer of 15 to about 30 cm was more compact peat, including dead stems of ericaceous species. No other mosses than *Sphagnum* were present in the peat cores. The reference samples were treated in the same way.

The harvesting procedure in the N experiment in Wageningen is described in detail in Chapter 2. Aboveground vascular plant parts were collected from a subplot of 25 x 37.5 cm in which vascular plant abundance was followed during the experiment. *Sphagnum*, peat and belowground vascular plant parts were sampled in three cores of 11 cm diameter in each plot. The cores were frozen and sawn in slices of 5 cm depth. These samples were stored in the freezer till sorting. The Wageningen samples were sorted into more fractions than those from the field site, and after drying, weighing and taking subsamples for N, P and K analyses, fractions were combined to obtain the same compartments as in Dwingeloo for  $^{15}\text{N}$  analysis.

### Analyses and calculations

All fractions were dried at 70 °C for at least 48 hours. Samples were ground with a ball mill to obtain a very fine, homogeneous sample. Total N concentration and  $^{15}\text{N}$  abundance were determined by an elemental analyser coupled with an isotope ratio mass spectrometer. The total N concentration was corrected for moisture content, as determined by drying at 105 °C for the Wageningen samples.

The total N pools of the compartments of the ecosystem were calculated by multiplying total N concentration with biomass (in  $\text{g m}^{-2}$ ).  $^{15}\text{N}$  abundance was given in atom% ( $= \frac{^{15}\text{N}}{(^{15}\text{N}+^{14}\text{N})} \cdot 100$ ). The natural  $^{15}\text{N}$  abundance measured in the reference samples

(mean from  $n = 5$ ) was subtracted. Multiplying this  $^{15}\text{N}$  enrichment (in atom%) with the N pool (in mol N  $\text{m}^{-2}$ ) resulted in the amount of  $^{15}\text{N}$  retrieved per unit area (mol  $^{15}\text{N}$   $\text{m}^{-2}$ ). For presenting the partitioning among compartments of the ecosystem, the amount of  $^{15}\text{N}$  of each compartment was divided by the total amount of  $^{15}\text{N}$  retrieved for each plot. One plot from the field site, harvested one week after  $^{15}\text{N}$  addition, was left out from calculations, because it hardly contained green *Sphagnum*.

## Results

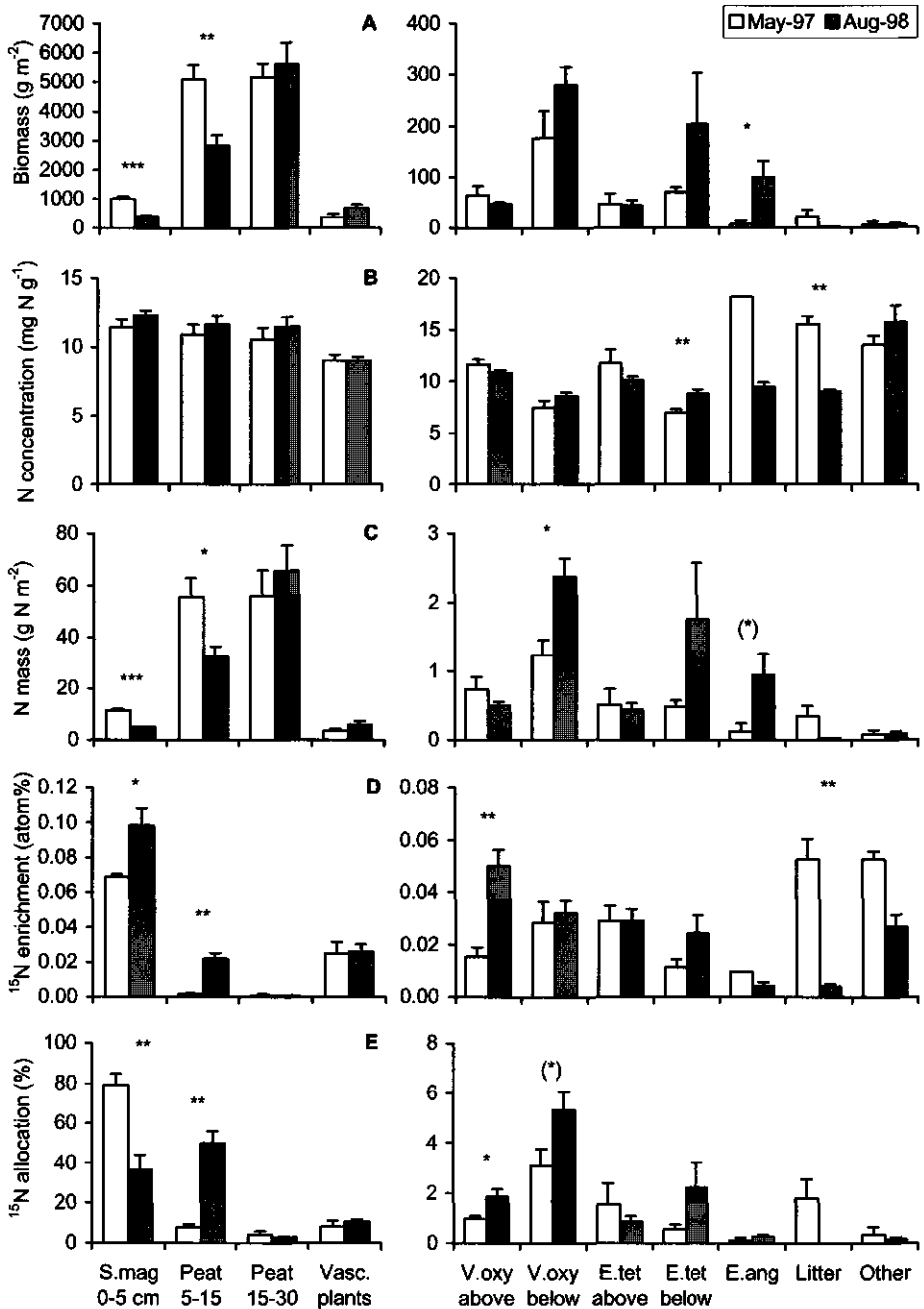
### Distribution of $^{15}\text{N}$ at the field site (Dwingeloo)

Total recovery of the  $^{15}\text{N}$  tracer at the field site ranged between 37 and 72%, being smaller than in Wageningen where nearly all tracer was retrieved (Table 6.1). Total recovery one week after addition was lower than fifteen months later ( $P = 0.02$ ,  $t$ -test). One week after addition (May 1997), 79% of the total amount of  $^{15}\text{N}$  retrieved was contained by the living *Sphagnum* (Fig. 6.1E). Only 8% was taken up by the vascular plants, mainly by the shallow-rooted *V. oxycoccus* (Fig. 6.1E). All compartments, except for the peat layer of 15-30 cm with its large N pool, were significantly  $^{15}\text{N}$ -enriched both in 1997 and 1998 when compared with reference samples ( $t$ -test). Within the vascular plants, the categories litter and other species (mainly *Drosera rotundifolia*) were most enriched (Fig. 6.1D), but because of their small biomass only a small proportion of the added  $^{15}\text{N}$  was allocated to these categories (Fig. 6.1E).

Fifteen months later, half of the total amount of  $^{15}\text{N}$  tracer retrieved was found in the *Sphagnum*/peat layer of 5-15 cm depth, corresponding with a large reduction in the amount of  $^{15}\text{N}$  in the top layer of 0-5 cm (Fig. 6.1E). This does not necessarily mean that the tracer moved to deeper layers. It is possible that the added  $^{15}\text{N}$  is still in the same *Sphagnum* tissues as where it was captured, but because *Sphagnum* grew in height the position of the tracer changed. In the control plots of the N experiment, *Sphagnum* grew 3-5 cm in height between May 1997 and August 1998. This shift in  $^{15}\text{N}$  allocation from the 0-5 to the 5-15 cm layer means that  $^{15}\text{N}$  was not completely reallocated to the growing top of the *Sphagnum* plants. Vascular plants gained a bit of  $^{15}\text{N}$ , *V. oxycoccus* in particular (Fig. 6.1E). This  $^{15}\text{N}$  may be derived from 1997-litter that became overgrown by the mosses. The 1998-litter was hardly enriched in  $^{15}\text{N}$  (Fig. 6.1D).

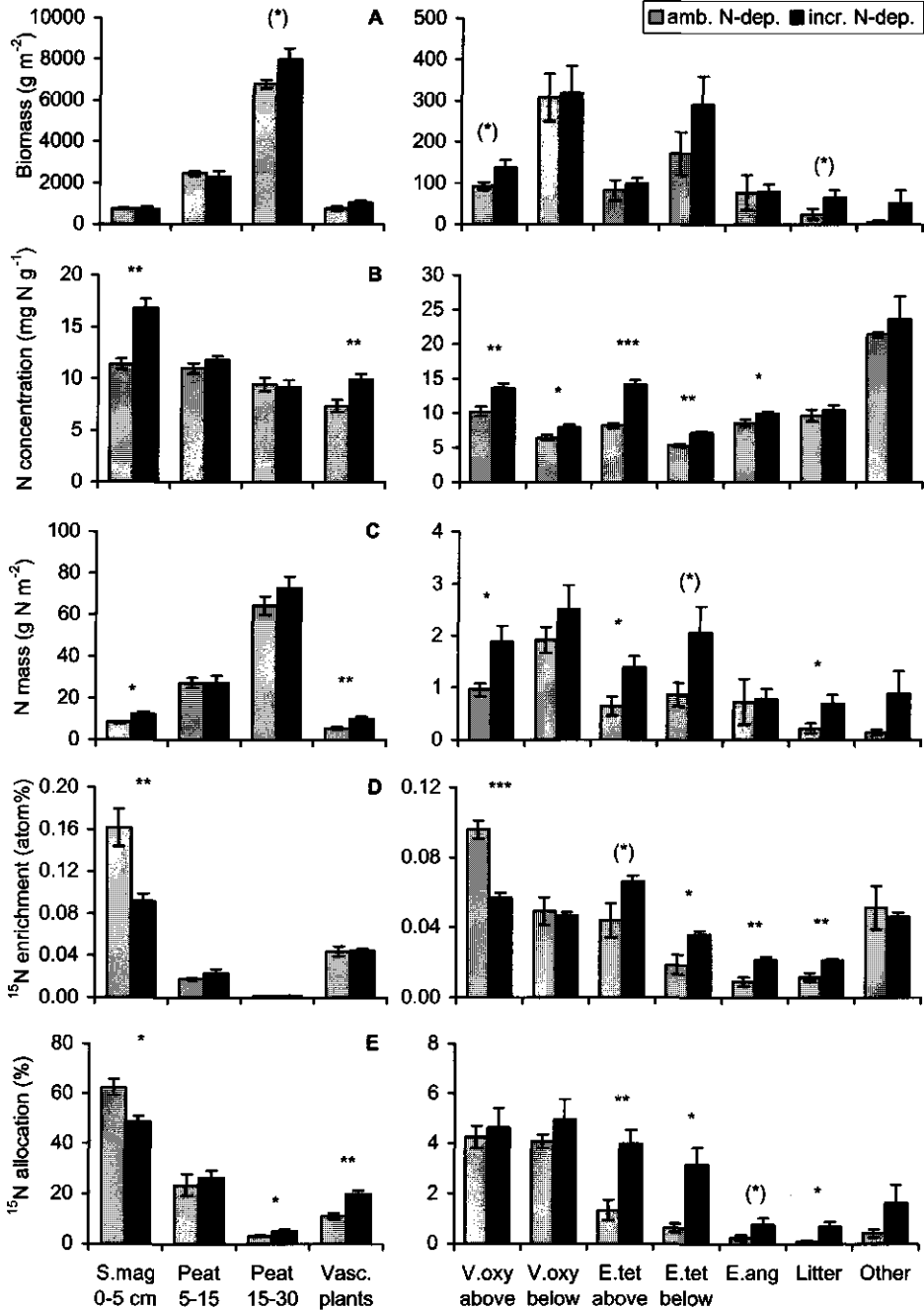
**Table 6.1** Recovery of the  $^{15}\text{N}$  tracer in the total ecosystem to a depth of 30 cm one week (May-97) and fifteen months (Aug-98 and N experiment) after addition, expressed as percentage of the amount added. Data are means  $\pm$  SE ( $n = 4-5$ ).

Field site		N experiment	
May-97	Aug-98	ambient N deposition	increased N deposition
43 $\pm$ 3	60 $\pm$ 5	93 $\pm$ 8	99 $\pm$ 6



**Figure 6.1** A) biomass, B) N concentration, C) N mass, D) <sup>15</sup>N enrichment (<sup>15</sup>N abundance minus <sup>15</sup>N abundance reference samples) and E) <sup>15</sup>N allocation (% of total amount of <sup>15</sup>N retrieved) for the compartments of the ecosystem at the field site one week and fifteen months after <sup>15</sup>N addition. Data are mean values ± SE (*n* = 4-5 plots). Significant differences between the dates (*t*-test) are indicated: (\*) *P* < 0.10, \* *P* < 0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.001.



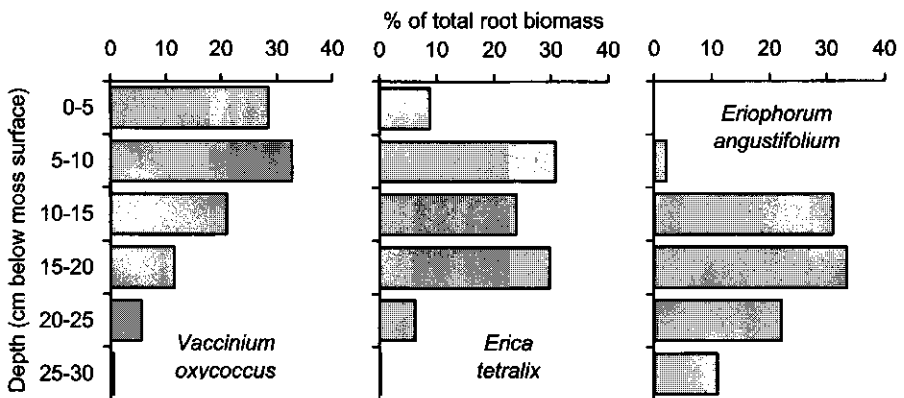


**Figure 6.2** A) biomass, B) N concentration, C) N mass, D)  $^{15}\text{N}$  enrichment ( $^{15}\text{N}$  abundance minus  $^{15}\text{N}$  abundance reference samples) and E)  $^{15}\text{N}$  allocation (% of total amount of  $^{15}\text{N}$  retrieved) for the compartments of the ecosystem in the N experiment (monoliths) after three growing seasons of N treatments (= fifteen months after  $^{15}\text{N}$  addition). Data are mean values  $\pm$  SE ( $n = 5$  plots). Significant differences between the N treatments ( $t$ -test) are indicated: (\*)  $P < 0.10$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

Distribution of  $^{15}\text{N}$  in the N experiment (Wageningen)

Total recovery of the  $^{15}\text{N}$  tracer, fifteen months after addition, was 96% on average without difference between the two treatments (Table 6.1). Under ambient N deposition, 63% of recovered  $^{15}\text{N}$  was still in the top 5 cm of *Sphagnum* fifteen months after addition (Fig. 6.2E).  $^{15}\text{N}$  retrieved in vascular plants was mainly in *V. oxycoccus*.  $^{15}\text{N}$  enrichment shows a clear gradient over depth: highest  $^{15}\text{N}$  enrichment in the top layer of *Sphagnum* and the shallow-rooting vascular plants and the lowest enrichment in the deep peat layer and deep-rooting plants (*E. angustifolium*) (Figs 6.2D and 6.3).

Increased N deposition changed the distribution of  $^{15}\text{N}$  between *Sphagnum* and vascular plants: significantly less  $^{15}\text{N}$  was found in *Sphagnum* and significantly more in the vascular plants (Fig. 6.2E). The proportion of  $^{15}\text{N}$  recovered in vascular plants increased from 8-14% in the N control treatment to 16-25% in the high N treatment. Particularly *E. tetralix* and *E. angustifolium* could capture more  $^{15}\text{N}$  when N deposition was doubled (Fig. 6.2E). All compartments were significantly  $^{15}\text{N}$ -enriched relative to the reference samples (*t*-test), even the deep peat layer of 15-30 cm depth in the N control treatment. The effect of N addition on  $^{15}\text{N}$  enrichment, fifteen months after addition, also followed a gradient over depth. The relatively deep-rooting species *E. angustifolium* and *E. tetralix* were significantly more  $^{15}\text{N}$ -enriched at high N deposition (Fig. 6.2D). In contrast,  $^{15}\text{N}$  enrichment was significantly reduced in high N treated *S. magellanicum* and *V. oxycoccus* compared with the N control. Although  $^{15}\text{N}$  enrichment in *V. oxycoccus* was reduced when N deposition was increased, the amount of  $^{15}\text{N}$  captured was not different between the two N treatments, suggesting that the  $^{15}\text{N}$  concentration was diluted by the large amounts of normal N this species captured in the two growing seasons after  $^{15}\text{N}$  addition.



**Figure 6.3** Distribution of root biomass over depth for *V. oxycoccus*, *E. tetralix* (including some roots of *Calluna vulgaris* and *Empetrum nigrum*) and *E. angustifolium*. Data are mean values ( $n = 10$  plots, 3 cores per plot) from the N experiment in Wageningen.

## Distribution of total N in the N experiment (Wageningen)

Large amounts of total N ( $^{14}\text{N} + ^{15}\text{N}$ ) are stored in the peat, vascular plants contained only 3-12% of the total N pool of the ecosystem to a depth of 30 cm. The inorganic N pool in soil water (to a depth of 30 cm) is not shown, but amounts only to 0.4-2.6% of the vascular plant pool.  $^{15}\text{N}$  enrichment was not measured in water samples. The excess N in the high N treatment accumulated in *Sphagnum* and vascular plants, mainly the shallow-rooted ones, and litter (Fig. 6.2C). The N pool in vascular plants almost doubled as a consequence of N addition (Fig. 6.2C). The accumulation was mainly the result of increased N concentration in the plant tissues (Fig. 6.2B). Only *V. oxycoccus* could benefit from the high N deposition by increasing its aboveground biomass (Fig. 6.2A). Even *E. angustifolium* showed increased N concentration in its tissues, but it had not responded in terms of biomass at the end of the experiment.

The difference in N mass between the two N treatments for *Sphagnum*, vascular plants and litter combined was  $8.5 \text{ g N m}^{-2}$ , i.e. 57% of the amount of  $15 \text{ g N m}^{-2}$  added during the three growing seasons. The peat layer of 15-30 cm in the high N treatment contained  $8.6 \text{ g N m}^{-2}$  more than that in the low N treatment. This difference in N mass between the two N treatments was not significant ( $P = 0.26$ ), as this peat layer contains large pools of N (Fig. 6.2C). Therefore, it is not certain if this difference in N mass can be explained by natural variation, and/or is the result of increased N supply (for example through increased belowground litter production).

## Discussion

### Initial distribution

*Sphagnum* is known to capture atmospheric N very effectively (Woodin and Lee 1987) and this is confirmed by our results. One week after  $^{15}\text{N}$  addition, 79% of the total amount of tracer retrieved was found in the living *Sphagnum*. In Wageningen, fifteen months after addition, still 63% of the amount recovered was in the living *Sphagnum* layer in the ambient N deposition treatment. It is not possible to say whether the applied N was assimilated by *Sphagnum* or was adsorbed to ion exchange sites. These results are in agreement with other  $^{15}\text{N}$ -labelling studies in *Sphagnum* dominated vegetation, where the proportion recovered in *Sphagnum* was at least 60% (Li and Vitt 1997; Francez and Loiseau 1999). This suggests that despite the high ambient N deposition in the Netherlands the capability of *Sphagnum* to capture atmospheric N deposition is still high.

The remarkably lower total recovery one week after addition compared to fifteen months later (Table 6.1) may be explained by the different treatment of the samples in the laboratory. In 1997, we rinsed the moss and vascular plant samples in tap water to facilitate separation of the fine roots of *Vaccinium* and the moss. Possibly,  $^{15}\text{N}$  adsorbed to the exterior of *Sphagnum* cells was washed off. The 1998 samples (both from Dwingeloo and Wageningen) had not been in contact with tap water. If some of the  $^{15}\text{N}$  was washed off, then 79% is an underestimation of the proportion captured by *Sphagnum*.

## Comparison field site – N experiment

Total recovery of  $^{15}\text{N}$  at the field site in Dwingeloo was lower than in the N experiment in Wageningen (monoliths). This difference may be explained by the weather conditions following  $^{15}\text{N}$  addition. In Dwingeloo,  $^{15}\text{N}$  was added during rainy weather: 6 mm of rain during that day and night. The next day,  $^{15}\text{N}$  was added in Wageningen followed by a 2.5 mm water addition to minimise uptake of  $^{15}\text{N}$  by the leaves of vascular plants, but the following day was sunny and warm. Secondly, the vegetation in Wageningen was kept in containers, while the plots in Dwingeloo had no physical borders. Water level in May is still relatively high and although the mire surface is rather flat at the field site, water may flow laterally to lower hollows. Thus, part of the  $^{15}\text{N}$  applied might have got lost during the rain event. In the containers water levels were not at overflow height the days after addition.

Total N pools and  $^{15}\text{N}$  partitioning were not significantly different between the field site and the N control treatment in Wageningen (*t*-test), which shows that the mesocosm experiment in Wageningen approaches field conditions quite well. Exceptions are the smaller N pools in the 0-5 cm *Sphagnum* layer and aboveground *V. oxycoccus*, and a shift in the proportion of  $^{15}\text{N}$  recovered from the 0-5 cm *Sphagnum* layer to the 5-15 cm layer at the field site. A possible explanation for these differences is that *Sphagnum* grew faster (in height) in Dwingeloo (not measured) than in Wageningen (3-5 cm). This may also explain the low biomass (bulk density) in the 0-5 cm layer, as we found negative correlations between bulk density and height growth of *Sphagnum* in our experiments. Faster *Sphagnum* height growth at the field site may also have resulted in a smaller proportion of *V. oxycoccus* biomass aboveground (total biomass or N mass was not different), as this species grows at the moss surface and a relatively large proportion of aboveground biomass might have become overgrown. In addition, the  $^{15}\text{N}$  added may have been captured at a lower depth within the 0-5 cm layer at the field site, because of the rainy weather conditions following addition, explaining the shift to the lower *Sphagnum* layer compared with the N control treatment in Wageningen.

## Effects of increased N deposition

Increased N deposition clearly changed the distribution of the  $^{15}\text{N}$  tracer among the compartments of the ecosystem. As expected, more  $^{15}\text{N}$  became available to the vascular plants, as shown by an significantly increased proportion of  $^{15}\text{N}$  recovered in vascular plants in the high N treatment (Fig. 6.2E). To our knowledge, this is the first study that clearly demonstrates that indeed more N from atmospheric deposition becomes available to vascular plants in bog vegetation at increased N deposition. The vegetation responded to three growing seasons of N addition by an increased vascular plant cover and reduced *Sphagnum* growth in the third growing season (Chapter 2).

It is not sure whether this negative response of *Sphagnum* was caused by the increased shading by vascular plants and/or by the large amounts of N accumulated in the *Sphagnum* shoots in the high N treatment. High N supply ( $10 \text{ g N m}^{-2} \text{ year}^{-1}$ ) has been shown to have negative effects on *Sphagnum* growth, also in the absence of shade or vascular plants (Press *et al.* 1986; Jauhiainen *et al.* 1994, 1998b). However, the negative relationship between vascular plant cover and *Sphagnum* growth we observed both in the N experiment and in a

$\text{CO}_2$  experiment (Chapter 2, Fig. 2.5), strongly suggests a negative effect of vascular plant cover on *Sphagnum* growth in the N experiment.

In addition, we showed that atmospherically deposited N reaches deeper layers at high N deposition, as shown by the significantly higher enrichment of the relatively deep-rooting species (*E. tetralix* and *E. angustifolium*) in the high N treatment (Fig. 6.2D). However, only the shallow-rooted *V. oxycoccus* increased its cover significantly after 3 growing seasons of increased N deposition (Chapter 2, Fig. 2.4). The deep-rooted *E. angustifolium* expanded in the high N treatment during the first two growing seasons, but its abundance declined in the third season, resulting in no net difference in biomass and N pool between the N treatments at the end of the experiment. The initial positive N effect on abundance of *E. angustifolium* is still reflected in litter biomass, which is mainly composed of dead *Eriophorum* shoots. Probably growth of *E. angustifolium* became limited by P during the experiment, as suggested by a high N:P ratio in its aboveground tissues (Chapter 2).

Adding  $15 \text{ g N m}^{-2}$  during 3 growing seasons resulted in significant accumulation of N in *Sphagnum*, *V. oxycoccus*, *E. tetralix* and litter (Fig. 6.2C). However, the amount accumulated in these compartments was only 57% of the amount added. It is not sure where the rest of the added N remained, as the N added cannot be distinguished from the N already present, in contrast to the  $^{15}\text{N}$  added. At the end of the experiment we measured increased inorganic N concentrations in water flown out the containers and in soil water at 30 and 60 cm depth in the high N treatment (data not shown). This suggests that the vegetation and peat were no longer holding all N supplied. Possibly the vegetation became saturated with N during the second or third growing season, but was not yet saturated at the time of  $^{15}\text{N}$  addition (start second growing season). The timing of  $^{15}\text{N}$  addition may also be very important. Francez and Loiseau (1999) found that the  $^{15}\text{N}$  recovery in the vegetation was higher when the  $^{15}\text{N}$  was added in June, when the plants were growing, compared to when added in August. Also Williams *et al.* (1999) found high recovery when the tracer was added in the summer months and attributed low recovery, as observed in October, to high water levels.

Concluding, even at high ambient N deposition in the Netherlands, the *Sphagnum* layer is still able to capture most of the atmospherically deposited N and leaves less than 10% to be taken up by vascular plants. Growth of *Sphagnum* is clearly not limited by N, but this does not prevent the *Sphagnum* layer from absorbing N supplied by atmospheric deposition. Possibly, part of the N captured is not assimilated by *Sphagnum*, but adsorbed to the exterior of *Sphagnum* cells or immobilised by microbes in the *Sphagnum* layer. Still, this N does not seem to be easily available for vascular plants.

At increased N deposition the *Sphagnum* layer is less able to capture or retain the deposited N, resulting in increased N availability for vascular plants, as shown by the almost doubled amount of  $^{15}\text{N}$  captured by vascular plants with doubled N deposition. As a result, vascular plants increased in cover and N concentrations in their tissues increased. The increased shading provided by the increased vascular plant cover, possibly combined with direct negative effects of high N deposition, reduced *Sphagnum* growth.

This reduced *Sphagnum* growth is expected to have serious consequences for peat accumulation in the long term. It will reduce the amounts of nutrients immobilised by the *Sphagnum* layer, thereby further increasing nutrient availability for vascular plants. In addition, the increased N concentrations in the plant tissues and the increased contribution

of vascular plant litter will increase decomposition and mineralisation rates, contributing to further expansion of vascular plants and possibly elimination of *Sphagnum*.

### **Acknowledgements**

Staatsbosbeheer, the National Forest Service in the Netherlands, is thanked for permission to take large peat monoliths from their terrain and to apply  $^{15}\text{N}$  at the field site. We thank Toos Lammers for the  $^{15}\text{N}$  analysis, and Wim Arp and Nico van Breemen for improving the text and helpful comments.

## General discussion

The results on the response of bog vegetation to elevated CO<sub>2</sub> and increased N deposition, obtained in the three experiments (outdoor, greenhouse and competition experiment) will be integrated and discussed in this chapter. Some results, not dealt with in the previous chapters, are added. The results on evapotranspiration for all three experiments were already discussed in Chapter 5. At the end the potential for carbon sequestration in bogs will be demonstrated and the implications of the observed vegetation responses for carbon sequestration and methane emission in the long term will be discussed.

### Vegetation response to elevated CO<sub>2</sub>

It was hypothesised that elevated CO<sub>2</sub> would change the competitive balance between mosses and vascular plants to the benefit of *Sphagnum*. Indeed, growth of *Sphagnum magellanicum* was affected by elevated CO<sub>2</sub> in all three experiments, but the nature of the response varied. Outdoors (Chapter 2), elevated CO<sub>2</sub> increased height increment in the second and third year (Fig. 2.1). Biomass growth was not significantly increased because of compensatory reductions in bulk density (mainly shoot dry mass per unit length) (Table 2.2). In the greenhouse experiment (Chapter 3) elevated CO<sub>2</sub> had visible deleterious effects. The response in terms of height increment was opposite to that observed outdoors (Fig. 3.1). CO<sub>2</sub>-induced increases in the number of shoots (counted as number of capitula) per unit area could not compensate for the dramatic reduction in height growth (Table 3.1). This negative CO<sub>2</sub> effect was attributed to an interaction with the raised temperature in the greenhouse, but in the competition experiment (Chapter 4), situated in the same climate conditions of the greenhouse, elevated CO<sub>2</sub> had again positive effects on *Sphagnum* growth (Fig. 4.1). *Sphagnum* green biomass was increased under elevated CO<sub>2</sub> mainly due to increases in capitulum dry mass and (non-significant) increases in height increment (Table 4.2).

The greenhouse did not offer a favourable environment for *Sphagnum* growth. All containers exposed to elevated CO<sub>2</sub> in the first greenhouse experiment contained bad-looking *Sphagnum*, having a dark-red colour (instead of a red-green colour when growing) and a fragile structure (capitula and branches easily broke off when touched). The same was seen for 12 (out of 40) containers in the competition experiment in the third growing season. One to three containers in each treatment (of five replicates) were classified as looking unhealthy. Chi-square tests revealed that there was no relation with CO<sub>2</sub>, N supply or competition treatment.

It remains unclear what caused the reduced vitality of *Sphagnum* in the greenhouse, but apparently *Sphagnum* in the greenhouse (at raised temperatures) was much more

vulnerable to disturbances (as diseases?) than outdoors. It is possible that in the first greenhouse experiment an infection in one container spread over all the other elevated CO<sub>2</sub> containers, as they all were near each other in the same compartment (actually pseudo-replication). However, in the competition experiment bad- and good-looking containers were located next to each other, without clusters of affected containers.

What caused the negative CO<sub>2</sub> effect in the greenhouse experiment? The positive effect in *Sphagnum* monocultures and mixtures with *Eriophorum angustifolium* in the competition experiment shows that it cannot be a combined effect of elevated CO<sub>2</sub> and raised temperature in general. However, the allelopathic substances theory (see Discussion Chapter 3) still provides a plausible explanation when these allelopathic substances were excreted by vascular plants other than *E. angustifolium*, but it is difficult to prove this theory. Another explanation is that there has been some contamination in the CO<sub>2</sub> source. If so, this contamination must have been present only in 1996, as in 1997 both the greenhouse and competition experiment were in the same compartments.

In addition to the direct positive CO<sub>2</sub> effect (as observed in the outdoor and competition experiment), the observed reductions in evapotranspiration in summer are expected to be beneficial for *Sphagnum* growth, by increasing moisture availability (Chapter 5). Although the reductions were small, in the dry summer months when *Sphagnum* is most prone to desiccation it might just make a difference. Several authors showed that growth of *Sphagnum* species is very sensitive to changes in water level or moisture conditions (Backéus 1988; Wallén *et al.* 1988; Li *et al.* 1992; Thormann and Bayley 1997; Schipperges and Rydin 1999; Jauhiainen *et al.* 1997). Morphological characteristics are also important the water uptake capacity of *Sphagnum*, as the main path of water transport in *Sphagnum* is external between leaves and between pendant branches and stems based on capillary rise (Hayward and Clymo 1982). In fact, *Sphagnum* shows morphological adjustments to experimentally varied water levels. It has been shown that length growth was reduced at lower water levels, but this was compensated by increased dry mass per unit length of stem (Jauhiainen *et al.* 1997) and thicker stems with thicker hyaline layers that may promote the abilities of holding and absorbing water (Li *et al.* 1992).

It seems that *Sphagnum* grows in height whenever it can afford it with respect to water availability. This was also seen in an experiment by Van der Welle in which *S. magellanicum* and *S. fallax* were grown in monocultures and mixtures on nutrient solution culture. In monoculture *S. fallax* grew faster in height than *S. magellanicum*, but when mixed *S. fallax* reduced its height increment relative to that in the monoculture (as extending above other shoots increases desiccation risk), without reducing biomass growth. The response of *S. magellanicum* to elevated CO<sub>2</sub> in the outdoor experiment (one of increased height growth) resembles the response to increasing water levels. Perhaps *Sphagnum* could afford it because of the reduction in evapotranspiration under elevated CO<sub>2</sub>. In the competition experiment overall height increment was rather low, but shoot density was high, suggesting relatively dry growing conditions. *S. magellanicum* responded to elevated CO<sub>2</sub> mainly by increasing capitulum biomass.

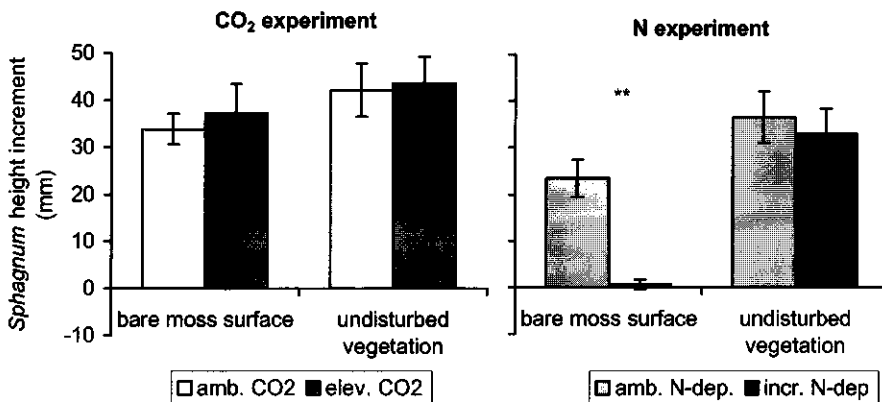
The only CO<sub>2</sub> experiments measuring growth of *Sphagnum* species so far, have been those of Jauhiainen *et al.* (1994, 1997, 1998). They performed several greenhouse experiments in which three different *Sphagnum* species were grown at several levels of



atmospheric CO<sub>2</sub> concentration (350-2000 ppmv) combined with N deposition (0-10 g N m<sup>-2</sup> year<sup>-1</sup>) or water level (0-30 cm below the capitulum level). The responses to elevated CO<sub>2</sub> varied between the species and even within one species the response was different between two experiments. Ombrotrophic *S. fuscum* showed a trend of increased height increment and production in one experiment, (although only at high water levels) (Jauhiainen *et al.* 1997), but reduced height increment (without affecting biomass production) in another experiment (Jauhiainen *et al.* 1994). Their experiments lasted at most four months and their conclusion that *Sphagnum* species respond only little to elevated CO<sub>2</sub>, may not hold when looking at longer time periods. The lack of CO<sub>2</sub> effect on biomass production they observed, is also partly caused by leaving out capitulum dry mass in their calculations of production, while capitulum dry mass increased with increased concentrations of CO<sub>2</sub>.

In our experiments *Sphagnum* hardly responded to elevated CO<sub>2</sub> in the first growing season. Backéus (1988) showed that variations in *Sphagnum* height growth determined over twenty years could be explained for 60% by weather (moisture) conditions in the previous growing season. Perhaps that also in our outdoor experiment the small height growth in 1997 (Fig 2.1) could be attributed to the dry spring and summer in 1996, whereas the large height growth measured in 1999 (Fig. 7.1) may have been the result of a wet 1998. This means that experiments lasting only one growing season are of limited use for describing the response of *Sphagnum* species.

The response of vascular plants to elevated CO<sub>2</sub> was less clear. In the experiments with intact bog vegetation we found no significant increases in production or biomass. In the outdoor experiment, there may have been small increases in biomass production, but these were compensated by a larger proportion of the aboveground biomass becoming overgrown by *Sphagnum*. Measurements on individual current-year shoots of *Vaccinium oxycoccus*, *Erica tetralix* and *E. angustifolium* from the outdoor experiment showed increases in shoot



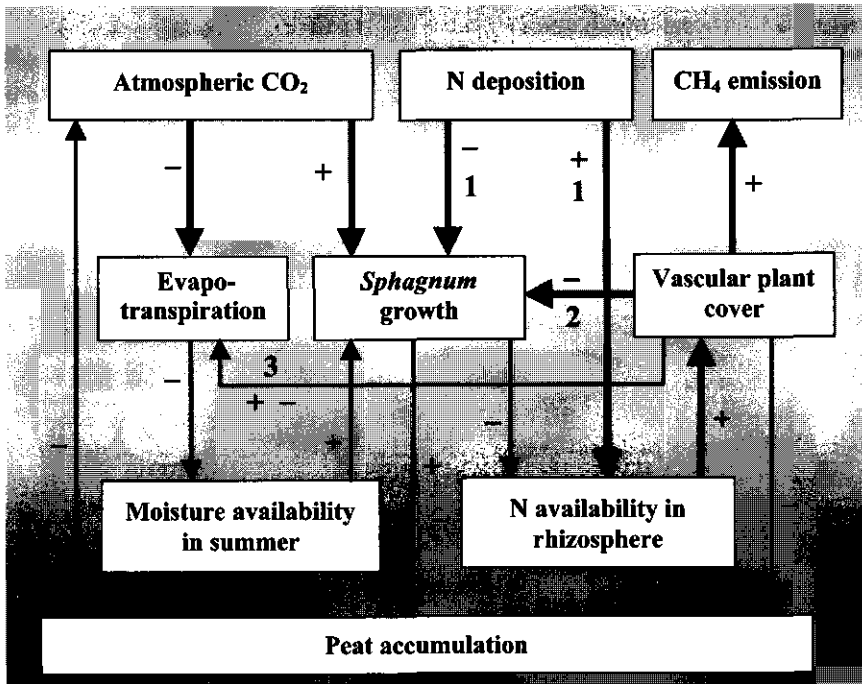
**Figure 7.1** *Sphagnum* height increment (mm) in the fourth growing season (May-October 1999), measured with cranked wires, two in the bare moss surface of the harvested pq-subplots and two in the undisturbed vegetation. Data are mean values  $\pm$  SE,  $n = 4$  plots (1 replicate plot of each treatment was used in September 1998 to fill holes, resulting from the final harvest, in the other four replicates). Level of significance: \*\*  $P < 0.01$  ( $t$ -test). CO<sub>2</sub> treatments were stopped in September 1998. N treatments were continued in 1999.

biomass for all three species, but these were only significant for *E. tetralix* (unpublished data). For the ericaceous species increases in shoot length were partly compensated by a reduced number of leaves per unit length of shoot, a response comparable to that of *S. magellanicum*. In the competition experiment, *E. angustifolium* increased biomass in response to elevated CO<sub>2</sub>, but this positive response was temporarily, probably due to nutrient limitation of growth in the relatively small containers. Thus, there may be small increases in production or biomass under elevated CO<sub>2</sub>, but the response is soon restricted by faster *Sphagnum* height growth and/or nutrient limitation. As long as growth of vascular plants is not severely nutrient limited, the relative abundance of both *Sphagnum* and vascular plant species may hardly change with elevated CO<sub>2</sub>. Among the vascular plants, small-statured species such as *Drosera rotundifolia* may show reduced abundance (see Fig. 2.3), but this species contributes very little to total vascular plant biomass.

*Sphagnum* can gain a competitive advantage over vascular plants at increasing atmospheric CO<sub>2</sub> concentrations, because their growth is less nutrient limited than that of co-occurring vascular plants (Sveinbjörnsson and Oechel 1992). Results from an experiment by Vermeulen (unpublished data) showed that *Sphagnum* species have a lower nutrient demand than co-occurring vascular plant species. Plants of two *Sphagnum* species and three vascular plant species were grown on nutrient solution culture at several levels of N, P and K. The two *Sphagnum* species (*S. magellanicum* and *S. fallax*) did not respond to reducing N, P or K supply, whereas growth of the vascular plants (*V. oxycoccus*, *E. angustifolium* and *Molinia caerulea*) was severely inhibited when N or P supply was reduced. Another reason for *Sphagnum* being less nutrient limited than vascular plants is that *Sphagnum* is very effective in intercepting the atmospheric supply of nutrients (mainly N). This has been reported in literature (Woodin and Lee 1987), Chapter 6, and by Van der Welle (unpublished data). *V. oxycoccus* growing mixed with *S. magellanicum* could only capture 5-20% of the amount of nutrients it captured when growing in monoculture, and its growth was dramatically reduced when competing *S. magellanicum* plants were present. Several fertilisation experiments in bogs have shown that growth of vascular plants in the current situation is limited by nutrients, which has been attributed to the interception of nutrients by the *Sphagnum* layer (Bartsch 1994; Lütke Twenhöven 1992; Thornmann and Bayley 1997; Maksimova and Yudina 1999).

*S. magellanicum* may be more sensitive to atmospheric CO<sub>2</sub> concentration than most other *Sphagnum* species because the chlorophyllous cells are enclosed completely by the water-filled hyaline cells (Daniels and Eddy 1985). This means a greater resistance to CO<sub>2</sub> uptake because of the longer diffusion path for CO<sub>2</sub> through water or cell wall from the surface of the leaf to the chloroplasts. Proctor *et al.* (1992) showed lower  $\delta^{13}\text{C}$  values for bryophyte species with photosynthetic cells enclosed by the hyaline cells (including *S. magellanicum*) than for other bryophyte species, implying greater diffusive limitation. The  $\delta^{13}\text{C}$  values also showed that most *Sphagnum* species (except *S. cuspidatum*) take up their CO<sub>2</sub> directly from the atmosphere (Proctor *et al.* 1992). It is well possible that growth of *S. magellanicum* is currently limited by atmospheric CO<sub>2</sub> concentration and thus may benefit from future increases.

Concluding, elevated CO<sub>2</sub> favours growth of *Sphagnum* both directly (because its growth is less nutrient limited than that of vascular plants) and indirectly through changes in evapotranspiration, but not necessarily at the cost of vascular plant growth (Fig. 7.2).



**Figure 7.2** Relational diagram of a bog ecosystem as affected by atmospheric CO<sub>2</sub> and N deposition. Thick arrows denote relations that were observed in our experiments; thin arrows denote relations derived from literature. The notes describe where non-linear relations were expected or observed.

Notes:

- ① N deposition must first reach a threshold value, necessary for saturating the *Sphagnum* layer, before N availability in the rhizosphere will increase and before it will negatively affect *Sphagnum* growth
- ② Only at high cover (possibly, more than 60%), vascular plants can reduce *Sphagnum* growth
- ③ At low vascular plant cover, increases in cover will reduce evapotranspiration as the evaporation from the *Sphagnum* surface is more reduced than vascular plant transpiration is increased. However at high vascular plant cover, increases in cover will increase evapotranspiration as the increase in vascular plant transpiration is larger than the reduction in evaporation.

## Vegetation response to increased N deposition

It was hypothesised that increased N deposition would result in increased vascular plant abundance, which would inhibit *Sphagnum* growth. In both experiments with intact bog vegetation vascular plants increased cover in response to N addition. The  $^{15}\text{N}$  measurements showed that even in the Netherlands at high ambient N deposition *Sphagnum* can still capture most of the aerially deposited N (at least the  $\text{NH}_4$  fraction) leaving only a small amount for vascular plants. As a result vascular plant growth in this type of bog vegetation is still partly limited by N. The responses to N treatments varied among the vascular plant species. At the beginning of the outdoor experiment, *E. angustifolium* appeared most responsive to N addition, as in the first two growing seasons its abundance increased most in the high N treatment (although not significantly, data not shown). This was part of the reason for choosing *E. angustifolium* as vascular plant species in the competition experiment. However, at the final harvest it proved to be the least responsive species (Fig. 2.3). At first it was thought that the added N could not reach the deep roots of *Eriophorum* as the N had to pass first the efficient filter of the *Sphagnum* cover and then the more shallow roots of *V. oxycoccus* and *E. tetralix*. However, both  $^{15}\text{N}$  measurements and competition experiment showed that aerially deposited N was available to *E. angustifolium*, but was not used for growth. Growth of *E. angustifolium* was apparently not limited by N.

In the greenhouse experiment *Rhynchospora alba* was most responsive to the N treatment (Table 3.2). This species occurred in only five plots in the outdoor experiment, making it impossible to assess its responsiveness in the outdoor experiment. *V. oxycoccus*, the most responsive species outdoors, also increased biomass (but not significantly) in the greenhouse experiment, which was visible as a larger amount of stems hanging over the edge of the high N containers. Perhaps the small area of moss surface limited the response of *V. oxycoccus* to N addition.

*Sphagnum* growth was inhibited by N addition in two of the three experiments. The growth inhibition in the outdoor experiment was probably an indirect effect through increased shading by the denser vascular plant cover, possibly combined with a direct negative N effect. The negative correlation between vascular plant cover and *Sphagnum* biomass growth, both in the N and in the  $\text{CO}_2$  experiment outdoors, provides evidence for this indirect effect of N. However, vascular plant cover must be high for inducing a negative effect on *Sphagnum* growth, as discussed in detail in Chapter 4. The presence of *E. angustifolium* did not affect growth of *S. magellanicum* in the competition experiment, probably because the cover was not high enough to provide severe shading. In the outdoor experiment vascular plant cover had increased to 60% after two seasons of high N treatment, and in the subsequent growing season *Sphagnum* growth was significantly reduced. In the greenhouse experiment vascular plant cover increased dramatically, but as this experiment was ended after two growing seasons, a possible *Sphagnum* response in the third growing season could not be determined.

The N treatments of the outdoor experiment were continued during the fourth growing season after the final harvest. The high N treatment now supported a dense cover of vascular plants below which *Sphagnum* was not always visible. Although height increment was hardly reduced (Fig. 7.1), *Sphagnum* shoot density (number of capitula per unit area)

had clearly declined (personal observation), which would have resulted in reduced biomass growth. The fourth growing season also gave evidence for the direct negative effect of N on *Sphagnum* growth. In the pq-subplot aboveground plant parts had been harvested, providing a bare *Sphagnum* surface. The high N treated *Sphagnum* without shading from vascular plants hardly grew in the fourth growing season, in contrast to the *Sphagnum* in the pq-subplots in all other treatments (Fig. 7.1). Although the high N *Sphagnum* was more disturbed than *Sphagnum* in the N control plots, as more vascular plants were clipped off at the final harvest, this could not explain the lack of recovery. Some plots in the CO<sub>2</sub> experiment were similarly disturbed (also had a high cover of vascular plants), but *Sphagnum* growth in these plots did recover. In addition, the bare *Sphagnum* surface had a less vital appearance in the high N treatment. Also in the competition experiment N addition had direct negative effects (in the monocultures), but these disappeared by the end of the experiment. Therefore, we have no evidence that *Sphagnum* growth is directly inhibited by an atmospheric N supply of 50 kg N ha<sup>-1</sup> year<sup>-1</sup> (corresponding with the high N treatment in the competition experiment) similar to the Dutch ambient N deposition.

Van der Welle (unpublished data) found no effect on growth of *S. magellanicum* grown on nutrient solution of N additions equivalent to 5, 25, 50, 75 and 100 kg N ha<sup>-1</sup> year<sup>-1</sup>, whereas almost similar Finnish experiments (Jauhiainen *et al.* 1994, 1998b) showed severe growth inhibition of *S. fuscum* and *S. magellanicum* at the same highest level of N supply. Perhaps, the Dutch *Sphagnum* has adapted to the increased levels of N deposition, whereas for Finnish *Sphagnum*, which has never experienced levels of more than 10 kg N ha<sup>-1</sup> year<sup>-1</sup>, an N supply of 100 kg N ha<sup>-1</sup> year<sup>-1</sup> must have been a shock. In an experiment by Baker and Boatman (1992) high concentrations of N in the culture solution (resulting in high N concentrations in plant tissue: 32-35 mg N g<sup>-1</sup>) did not inhibit growth of four *Sphagnum* species. They attributed this to the fact that the plants used in the experiments were grown from spores in culture solution containing high concentrations of nutrients (and balanced with high concentrations of other nutrients), so that the plants might have adapted. Apparently, our *Sphagnum* has found some way to deal with high N deposition. Perhaps, the absolute level of N deposition is less important for direct inhibition of *Sphagnum* growth, than the rate of change and the ratio with the supply of other nutrients.

Concluding, a further increase in N deposition will benefit vascular plant growth, which after reaching a cover of about 60%, reduces *Sphagnum* growth. In addition, high N deposition may directly inhibit *Sphagnum* growth, as shown by the reduced capacity to recover from disturbance. Once the cover of *Sphagnum* decreases, the situation will get worse due to positive feedback (follow arrows between *Sphagnum* growth, N availability, vascular plant cover and back to *Sphagnum* growth in Fig 7.2), but it is not clear what level of N deposition or what rate of change will trigger this feedback loop.

## Vegetation response to elevated CO<sub>2</sub> and increased N deposition

It is difficult to predict what will happen if both levels of atmospheric CO<sub>2</sub> concentration and N deposition will increase simultaneously. In the outdoor experiment, the separate CO<sub>2</sub> and N experiments showed opposite effects on *Sphagnum* growth and N concentration in plant tissue (Chapter 2). Will these opposite effects compensate for each other when both levels of CO<sub>2</sub> and N deposition rise, or is the effect of one aspect of global change

dominant over the effect of the other factor? In both greenhouse experiments using a factorial design of CO<sub>2</sub> and N treatments, we found no statistical interactions between CO<sub>2</sub> and N treatments. However, N deposition was not increased relative to field conditions, as the high N treatment in the greenhouse (5 g N m<sup>-2</sup> year<sup>-1</sup>) corresponds with the ambient N deposition in the Netherlands. In both greenhouse experiments, *Sphagnum* growth responded mainly to CO<sub>2</sub> treatment. Vascular plants responded to N treatment (greenhouse experiment) or to CO<sub>2</sub> treatment (competition experiment) with only a tendency of CO<sub>2</sub> by N treatment interaction in the greenhouse experiment. Also Jauhiainen *et al.* (1994, 1998a) found no clear interactions between the CO<sub>2</sub> and N treatments on *Sphagnum* growth.

The CO<sub>2</sub> and N effects were not opposite for vascular plant growth and increases in vascular plant cover can be expected in response to elevated CO<sub>2</sub> combined with increased N deposition. The indirect negative effect through increased shading may then be dominant over the positive CO<sub>2</sub> effect on *Sphagnum* growth, resulting in similar effects as observed for increased N deposition alone.

### Interactions between *Sphagnum* and vascular plants

*S. magellanicum* seems not much influenced by vascular plants, except at the extreme ends. In the absence of vascular plants *Sphagnum* is more prone to desiccation, as shown by the increased evapotranspiration in *Sphagnum* monocultures and in containers with a low cover of vascular plants in the outdoor experiment. However, a dense cover of vascular plants can inhibit *Sphagnum* growth by shading and by increasing evapotranspiration. The absence of a *Sphagnum* surface clearly benefits vascular plant growth as shown in removal experiments in tundra (Hobbie *et al.* 1999), the competition experiment of Van der Welle and even in our competition experiment. However, without a *Sphagnum* layer the associated typical bog species will disappear as well, as they are the only species that can cope with the harsh conditions created by the *Sphagnum* layer (even though this means they cannot grow at their maximum rate in the current situation). Without the *Sphagnum* layer other species with faster growth rates will outcompete the specialised bog species.

The relationship between *Sphagnum* and vascular plants seems balanced within certain limits. *Sphagnum* has an opportunistic growth strategy and can adjust to changed conditions. In the competition experiment *Sphagnum* showed an elongation effect in the presence of vascular plants. Small increases in vascular plant cover may favour *Sphagnum* growth by reducing evaporation rates. Large height growth of *Sphagnum* will cause a negative feedback reaction when it means an increased exposure to wind and solar radiation and thus increased evaporation. For both *Sphagnum* and vascular plants the optimal strategy is to grow at approximately the same rate. Vascular plants can adjust to increased *Sphagnum* height growth. For example, a significant part of *V. oxycoccus* became overgrown by *Sphagnum* each summer-autumn, but within the three growing seasons of the outdoor experiment, it easily re-established on top of *Sphagnum* each spring. Only *D. rotundifolia* seems at risk when *Sphagnum* height growth is increased.

Concluding, under normal conditions the (positive) interactions between *Sphagnum* and vascular plants result in a stable equilibrium, but when the vegetation is severely disturbed (for example by adding 5 g N m<sup>-2</sup> year<sup>-1</sup> during three growing seasons in surplus to the already high ambient N deposition) negative interactions start to play a role which

will result in a positive feedback loop, soon leading to the disappearance of the whole vegetation type.

## Can these results be extrapolated to other bog sites?

The BERI project was set up as a regionally replicated experiment. The other sites (in Finland, Sweden, England and Switzerland) did not show the same vegetation responses as the Dutch site. *Sphagnum* growth was not affected by elevated CO<sub>2</sub> at the other sites. Only at the Swiss site N addition also had a negative effect on *Sphagnum* growth as a result of increased cover of the taller moss species *Polytrichum strictum* (Berendse *et al.* submitted). It is difficult to compare the sites, as they are different in many aspects, not only in climate and ambient N deposition, but also in species composition, probably related to differences in hydrology and nutrient status. Some differences between the sites that may explain the differential response are:

- 1) The Dutch site experiences a high ambient N deposition. However, also at the other sites N was not limiting growth of *Sphagnum* as it did not respond positively to N addition. N limitation cannot be blamed for the lack of CO<sub>2</sub> response in *Sphagnum* growth at the other sites. However, the high ambient N deposition must be important in determining the response to further increases in N deposition. The Swiss site, which also showed a response to N addition, had the second highest ambient N deposition. The experimental increases in N deposition have probably saturated the *Sphagnum* layer at the Dutch and Swiss site, resulting in increased N availability for other species, which increased their cover at the cost of *Sphagnum* growth. At the other sites the *Sphagnum* layer still accumulated N and had not become saturated after three growing seasons of N addition (Berendse *et al.* submitted).
- 2) *Sphagnum* experienced favourable growing conditions at the Dutch site: a relatively long growing season, mild temperatures and limited fluctuations in water level, making a CO<sub>2</sub> response possible.
- 3) At the Dutch site the moss layer is dominated by *S. magellanicum*, which might be more responsive to CO<sub>2</sub> enrichment than other species. *S. magellanicum* occurred also at the Swedish site, but not as dominant as in our site.

Although, the response to the CO<sub>2</sub> and N treatments at the other BERI sites was not similar to that at the Dutch site, this does not mean that our results are not valid for bog vegetation in general. The other sites may respond more slowly to elevated CO<sub>2</sub>, due to temperature, nutrient or soil moisture constraints, and show a delayed response to increased N deposition, because the *Sphagnum* layer is not yet saturated with N. Thus, responses similar to those observed at the Dutch site may become apparent at a longer term.

The bog vegetation at our field site apparently does not suffer from the ambient high N deposition. Also Barkman (1992) and Everts and De Vries (1984) noticed that the bog vegetation in the heath pools in the north of the Netherlands had not changed since 1960, despite large increases in N deposition. Does this mean that other bog sites do not need to fear N deposition loads of 40-50 kg N ha<sup>-1</sup> year<sup>-1</sup>? There are reasons that may explain why the bog vegetation of the heath pools in the State Forestry of Dwingeloo does not suffer from the high ambient N deposition:

- 1) The total amount of N deposited on the bog vegetation is lower than the total N deposition given for the region, because of the location of the heath pools in a forest. Bulk N deposition measured at Reigersplas (similar heath pool located in the same forest; data from Limpens) was similar to that at Witteveen (which is used for the determination of total N deposition in the region), but dry N deposition on the bog vegetation may be lower because of interception by the surrounding trees. In addition, the sheltered location in a forest may create favourable humid conditions.
- 2) The high N deposition is balanced by relatively high input of other nutrients from blown-in sand, pollen and leaves from surrounding trees, influx of mineral-enriched water, and influence by the mineral subsoil, as the heath pools have only thin peat layers. This may enable a continuing high rate of *Sphagnum* growth (and capture of atmospheric deposition) and prevent it from suffering from high N supply directly (if this negative effect is caused by a nutrient imbalance as suggested by Jauhiainen *et al.* (1998b)).

These special characteristics of the heath pools may be responsible for the occurrence of typical ombrotrophic bog vegetation, despite the high ambient N deposition. Outside the heath pools, hardly any living bog vegetation can be found in the Netherlands (Chapter 1). It is well possible that the heath pools have a higher threshold value for N deposition for saturating the *Sphagnum* layer than other bog sites.

## Implications for carbon sequestration and methane emission

The bog vegetation as dealt with in our experiments has the potential to sequester large amounts of carbon, as illustrated by the following calculations (Table 7.1). Carbon sequestration is determined by the difference between production (yearly C uptake) and decomposition (yearly C release) of plant material. Production is determined as the sum of *Sphagnum* production (Table 2.2), aboveground vascular plant production (Table 2.3), belowground biomass of graminoid species (Table 2.4; assuming that belowground biomass of these species is fully current-year produced) and half of the fine root biomass of ericaceous species. Decomposition is more difficult to calculate. Limpens measured 8% mass loss in brown *Sphagnum* material from our N control containers after one year of being buried in a litterbag in the Reigersplas (unpublished data). This value is used for an estimation of decomposition. It is assumed that older material in deeper layers decomposes at slower rates and that decomposition in the peat below 20 cm depth is negligible. Biomass values from the ambient CO<sub>2</sub> and ambient N deposition containers ( $n = 10$ ) and carbon concentration of *Sphagnum*, vascular plants, litter and different peat layers (including belowground vascular plant litter, mainly dead stems of ericaceous species) are used for calculating CO<sub>2</sub> uptake and CO<sub>2</sub> release (Table 7.1).

The result of the calculations is an estimated carbon uptake of 300 g C m<sup>-2</sup> year<sup>-1</sup> and carbon release of 100 g C m<sup>-2</sup> year<sup>-1</sup>, leaving 200 g C m<sup>-2</sup> year<sup>-1</sup> for carbon sequestration in the bog vegetation at the experimental site. For comparison, a carbon sequestration of 65 g C m<sup>-2</sup> year<sup>-1</sup> over the past 80 years has been measured in a Dutch pine forest, by comparing carbon pools in a chronosequence (De Kovel *et al.* 2000). However, CO<sub>2</sub> flux measurements, also in a Dutch coniferous forest, showed a C sequestration of 210 g C m<sup>-2</sup> year<sup>-1</sup> in 1997 (Valentini *et al.* 2000). Gorham (1991) has calculated a long-term carbon sequestration of 29 g C m<sup>-2</sup> year<sup>-1</sup> for boreal and subarctic peatlands, corresponding with a



height increment of 0.5 mm year<sup>-1</sup>. Our bog vegetation is relatively young (due to peat cutting) and relatively fast growing. The moss surface relative to the container edge increased 36 mm on average between May 1996 and August 1998. This value is lower than the height increment measured by cranked wires, because there has been some compaction in the peat, but clearly demonstrates that peat has accumulated.

**Table 7.1** Estimation of carbon sequestration in ombrotrophic bog vegetation. Values for biomass and C concentration are mean values from ambient CO<sub>2</sub> and ambient N deposition treatments ( $n = 10$ ) determined at the final harvest (September 1998). See text for further explanation.

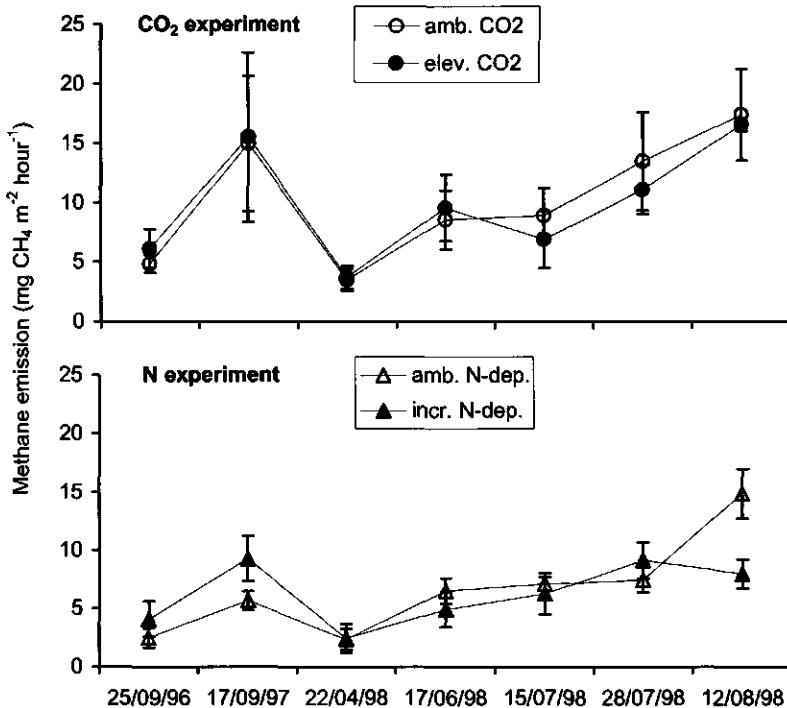
Component	Biomass g m <sup>-2</sup>	Carbon %	C mass g C m <sup>-2</sup>	Assumptions	C uptake g C m <sup>-2</sup> yr <sup>-1</sup>	C release g C m <sup>-2</sup> yr <sup>-1</sup>
<i>Sphagnum</i> production	316	46	145		145	
Aboveground production vascular plants	175	53	92		92	
Belowground graminoid species	67	48	32	fully current-year produced	32	
Roots ericaceous species	131	52	68	half current-year produced	34	
Peat 5-10 cm	907	44	401	8% loss year <sup>-1</sup>		32
Peat 10-15 cm	1577	49	776	4% loss year <sup>-1</sup>		31
Peat 15-20 cm	2504	46	1153	2% loss year <sup>-1</sup>		23
Aboveground litter	34	50	17	fully decomposed		17
Sum					304	103

Elevated CO<sub>2</sub> has the potential to increase growth rates of both *Sphagnum* and vascular plants without much changing the relative abundance of both, and if there are changes they will be in favour of *Sphagnum*. An increase in *Sphagnum* growth would increase carbon sequestration (follow arrows between *Sphagnum* growth, N availability, vascular plant cover, *Sphagnum* growth and peat accumulation in Fig. 7.2), when decomposition of *Sphagnum* material is not severely affected by elevated CO<sub>2</sub>. Preliminary results from a *Sphagnum* decomposition experiment by Limpens (unpublished data) show no difference in mass loss of brown *Sphagnum* between material from elevated CO<sub>2</sub> containers and from controls after one year of decomposition in litterbags buried in the *Sphagnum* layer in the field. However, the decomposition took place under ambient CO<sub>2</sub> conditions. Elevated CO<sub>2</sub> may increase decomposition rates when microbial activity increases as a result of increased exudation of easily degradable carbon compounds by plant roots. This effect may be counteracted by changes in tissue chemistry (higher C:N ratio, more phenolics), which are supposed to slow down decomposition. So far, very little is known about decomposition under elevated CO<sub>2</sub> at field conditions. From what we know at this moment, it can be concluded that elevated CO<sub>2</sub> has the potential to increase carbon sequestration in bog vegetation.

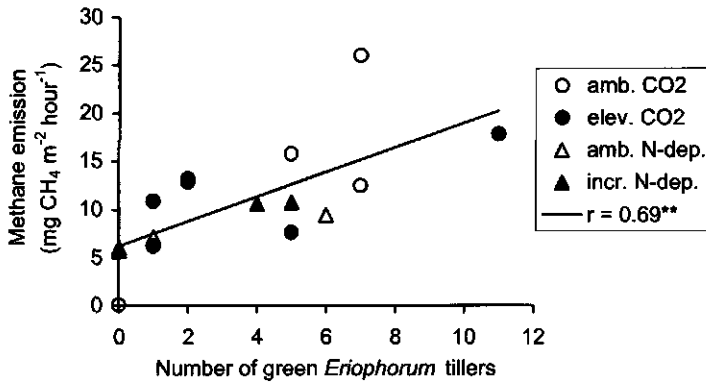
In contrast, increased N deposition is expected to reduce carbon sequestration, both by changing the relative abundance of species and by changing N concentration in the plant

tissues. Also brown *Sphagnum* and brown Eriophorum leaves showed increased N concentrations after three growing seasons of increased N deposition (data not shown). Preliminary results from Limpens (unpublished data) show greater mass loss in brown *Sphagnum* from the high N containers relative to material from the N control treatment. Also Coulson and Butterfield (1978) showed increased decomposition rates of *Sphagnum* tissues enriched in N. In addition an increased proportion of vascular plants in litter will also speed up decomposition rates, as they are generally decomposed at faster rates than *Sphagnum* (Coulson and Butterfield 1978; Hobbie 1996).

Increased N deposition is expected to increase methane emission through the increase in vascular plant cover. N deposition (and CO<sub>2</sub>) by itself seems to have little effect on CH<sub>4</sub> emission in mires (Saarnio *et al.*, 2000), which is confirmed by our own data (Fig. 7.3). However, vascular plants play an important role in methane emission (Joabsson *et al.* 1999), as they deliver the substrate for methane-producing bacteria. Methane emission from a bare *Sphagnum* surface has been found to be negligible (Saarnio *et al.*, 1998; Frenzel and Rudolph, 1998). Our own results demonstrate that particularly *E. angustifolium* strongly enhances methane emission (Fig. 7.4). This deep-rooted species has aerenchymae (air channels) throughout the plant that facilitate transport of oxygen to the roots (located in



**Figure 7.3** Methane emission, measured by closed chamber method in subplots of 19 cm diameter, at several dates in the CO<sub>2</sub> experiment (upper graph) and the N experiment (lower graph) outdoors. Data are means  $\pm$  SE ( $n = 2-5$ ). Measurements not showing a linear increase in CH<sub>4</sub> concentration were omitted. Only dates with more than 15 successful measurements are shown. Repeated measures analysis revealed no significant treatment effects.



**Figure 7.4** Relation between methane emission and tiller density of *Eriophorum angustifolium*, measured on 28/07/98 in the outdoor experiment. The same relation was observed on 15/07/98. \*\*  $P < 0.01$

the permanently waterlogged peat). In the reverse direction methane is transported from the soil, via roots and stems, to the air, bypassing methane-oxidising bacteria in the top peat layer.

Although increased N deposition is often thought to increase carbon sequestration by stimulating plant (particularly tree) growth (Table 1.1), this is not necessarily the case. The consequences of the effects of global changes on the species composition of communities are often overlooked, neglecting important feedbacks. In our case, increased N deposition will likely reduce carbon sequestration because of favouring relatively high-turnover species with higher N demands. In the most recent review of global change research in terrestrial ecosystems (Mooney *et al.*, 1999) it is now recognised that changes in relative abundance of species and community composition may be more important for ecosystem functioning and carbon sequestration than changes in plant physiology or biomass.

Most models however do not take the dynamics species composition into account and the algorithms in those models are derived from earlier global change experiments with single species (Körner, 1995). However, results obtained in laboratory or growth chamber studies are often in disagreement with findings from field studies in undisturbed vegetation (Körner 1995). For example, it is generally believed that C<sub>3</sub> plants will be more responsive to elevated CO<sub>2</sub> than C<sub>4</sub> plants, but a field experiment showed even opposite trends (Owensby *et al.* 1999). The same applies to the theory that fast-growing species are more responsive to elevated CO<sub>2</sub> than slow-growing species: when investigated in a competitive setting in their natural environment, no advantage for fast growing species was found (Mooney *et al.* 1999).

The BERI experiments were intended to last for two growing seasons, but fortunately the outdoor experiment could be extended with one more growing season using financial reserves from our own group. After one growing season, the outdoor experiment showed no changes at all; after the second growing season some individual species showed a response and after the third growing season we could see the result of interactions between species. A slow response was also observed in other nutrient-poor ecosystems with long-lived plant species (Warwick *et al.* 1998; Leadley *et al.* 1999), because growth of these species is at

least partially determined by nutrient uptake in the previous seasons (Chapin 1980; Jonasson and Shaver 1999). Three growing seasons is still rather short for studying vegetation response. Chapin *et al.* (1995) showed that the tundra vegetation response to experimental treatments after nine years was different from that observed after three years.

To obtain a deeper insight in the vegetation response to global change, we need more and particularly longer experiments in intact vegetation under natural conditions. Only then interactions between species and feedbacks through nutrient and moisture availability can develop, which will ultimately determine the vegetation response. The insights obtained from such experiments can be used to improve models that are necessary for predicting long-term carbon sequestration in terrestrial ecosystems, but which now lack species interactions. Mire vegetation deserves more attention, as changes in these ecosystems can have important repercussions on the concentrations of the greenhouse gases CO<sub>2</sub> and CH<sub>4</sub> in the atmosphere. An assessment of the response to elevated CO<sub>2</sub> combined with raised temperatures is urgently needed (see Chapter 3), as the elevated atmospheric CO<sub>2</sub> concentrations will likely be followed by increases in temperature.

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## Summary

As a result of human activities all over the world, atmospheric carbon dioxide (CO<sub>2</sub>) concentration and nitrogen (N) deposition have been increasing. These two components of global change will certainly affect ecosystems, because carbon and nitrogen are essential elements to all organisms. However, the nature and magnitude of such effect are still largely unknown. Terrestrial ecosystems are not only affected by global change, but also influence global change in turn. By absorbing significant amounts of CO<sub>2</sub>, terrestrial ecosystems can slow down the build up of CO<sub>2</sub> in the atmosphere and consequently reduce the rate of climate change. Mire ecosystems are potentially important in this regard, because by forming peat, they constitute a long-term sink for atmospheric CO<sub>2</sub>. Within mires, *Sphagnum*-dominated ombrotrophic bogs have the greatest peat accumulation potential, due to extremely low rates of decomposition. Remarkably, so far, global change research has hardly paid any attention to peat bogs.

Typical bog plant communities are comprised of peat mosses (*Sphagnum*) and associated vascular plants. The *Sphagnum* species are the main peat-formers. Compared to *Sphagnum*, vascular plants contribute less to peat accumulation and, moreover, contribute more to methane emission. Therefore, global warming due to greenhouse gases should be alleviated whenever *Sphagnum* expands at the expense of vascular plants, and vice versa. Ombrotrophic bogs must be very sensitive to changes in atmospheric composition as they depend on the atmospheric supply of nutrients. *Sphagnum* is very effective in intercepting the atmospheric deposition and thereby reduces this supply of nutrients to vascular plants.

We hypothesised that elevated CO<sub>2</sub> will change the competitive balance between mosses and vascular plants to the benefit of *Sphagnum*, because it is less nutrient limited than vascular plants. Increased N deposition was expected to result in opposite effects, *i.e.* a competitive advantage for vascular plants. *Sphagnum* may no longer capture all N from atmospheric deposition when N deposition is increased. Consequently more N may reach the rhizosphere, which increases growth rates of vascular plants. The resulting shading by an increased vascular plant cover may depress *Sphagnum* growth.

To test these hypotheses I investigated the effects of elevated atmospheric CO<sub>2</sub> and increased N deposition on bog vegetation in the Netherlands. I conducted three experiments in which the vegetation response to experimentally varied levels of atmospheric CO<sub>2</sub> and N deposition was followed during two to three growing seasons. Special attention was paid to the relationship between peat mosses and vascular plants. For all three experiments peat monoliths were used, cut from a heath pool located in the State Forestry of Dwingeloo. The vegetation of all monoliths was dominated by *Sphagnum magellanicum*, with *Vaccinium oxycoccus*, *Erica tetralix*, *Eriophorum angustifolium* and *Drosera rotundifolia* as co-occurring species. I measured *Sphagnum* growth, relative abundance of plant species, vascular plant biomass, chemical composition of plant tissues (C, N, P, K), water use of the vegetation and methane emission.

In the first experiment ("outdoor experiment", Chapter 2), I used large peat monoliths (110 cm diameter, 60 cm deep) with intact bog vegetation, kept outdoors in large containers. The vegetation was exposed to elevated CO<sub>2</sub> or increased N deposition for three

growing seasons. Elevated CO<sub>2</sub> conditions (target concentration 560 ppmv) were created using MiniFACE technology. N deposition was increased by 5 g N m<sup>-2</sup> year<sup>-1</sup> by adding dissolved NH<sub>4</sub>NO<sub>3</sub> at three-week intervals during the growing season. Three seasons of elevated atmospheric CO<sub>2</sub> resulted in increased height growth of *Sphagnum magellanicum*. Most vascular plants were not significantly affected by elevated CO<sub>2</sub>, but the low-statured *Drosera rotundifolia* was negatively influenced by the increased *Sphagnum* height growth. Adding N increased vascular plant cover, with *Vaccinium oxycoccus* being most responsive. *Sphagnum* growth was significantly reduced by N addition in the third growing season, presumably by the increased shading by vascular plants, given the observed negative relation between vascular plant cover and *Sphagnum* growth.

Because CO<sub>2</sub> and N are likely to interact, I studied the effects of elevated CO<sub>2</sub> in combination with nitrogen supply on the growth of *Sphagnum* mosses and vascular plants in two greenhouse experiments. In the first greenhouse experiment ("greenhouse experiment", Chapter 3), peat monoliths (34 cm diameter, 40 cm deep) with intact bog vegetation were exposed to ambient (350 ppmv) or elevated (560 ppmv) atmospheric CO<sub>2</sub> combined with low (no N addition) or high (5 g N m<sup>-2</sup> yr<sup>-1</sup> added) N deposition during two growing seasons. In this experiment, elevated atmospheric CO<sub>2</sub> had unexpected deleterious effects on growth of *Sphagnum magellanicum*. The negative CO<sub>2</sub> effect was strongest in the warmest months, suggesting a combined effect of elevated CO<sub>2</sub> and the raised temperatures in the greenhouse. High N deposition favoured *Rhynchospora alba*. Only at high N supply, elevated CO<sub>2</sub> tended to increase total aboveground vascular plant biomass.

In a second greenhouse experiment ("competition experiment", Chapter 4), peat monoliths (24 cm diameter, 30 cm deep) were used to create three competition modes: *Sphagnum* monoculture, *Eriophorum* monoculture and *Sphagnum* + *Eriophorum* mixture. The plants were subjected to the same CO<sub>2</sub> and N treatments as in the greenhouse experiment for three growing seasons. The addition of *Eriophorum* tillers in the mixture did not affect *Sphagnum* biomass, because *Eriophorum* density did not become high enough to cause severe shading of the moss surface. The *Sphagnum* layer had a negative effect on flowering of *Eriophorum*, resulting in a reduced biomass. Elevated CO<sub>2</sub> and/or increased N deposition did not change these competitive relationships between *Sphagnum* and *Eriophorum*, but had independent effects on the two species. Elevated CO<sub>2</sub> had a positive effect both on *Sphagnum* and *Eriophorum* biomass, but the effect on *Eriophorum* was transient, probably because of P limitation. N addition had a direct negative effect on *Sphagnum* height growth in the first growing season, but *Sphagnum* biomass at the end of the experiment was not affected. Surprisingly, *Eriophorum* biomass did not respond to N addition.

Water loss by evapotranspiration in July-August was determined in all three experiments (Chapter 5). Elevated CO<sub>2</sub> reduced water use of the bog vegetation in all three experiments, but vascular plant biomass and exposure of the moss surface to wind also affected evapotranspiration. In the outdoor experiment evapotranspiration was largely determined by evaporation from the *Sphagnum* moss surface (as affected by exposure to wind) and less by vascular plant transpiration. Still, elevated CO<sub>2</sub> significantly reduced evapotranspiration by 9-10%. Vascular plants reduced evapotranspiration in the outdoor experiment, but increased water use in the greenhouse experiment.

To study the fate of the deposited N, a small amount of  $^{15}\text{N}$  tracer was applied to the experimental plots of the N experiment outdoors and to additional plots in the untreated field site (Chapter 6). The distribution of the  $^{15}\text{N}$  tracer among *Sphagnum*, vascular plants and peat was determined at the final harvest. At the field site, one week after addition 79% of the total amount of  $^{15}\text{N}$  retrieved was found in the living *Sphagnum* layer, with 8% being captured by vascular plants. Increased N deposition significantly increased the availability of N to vascular plants, as shown by the reduced proportion of  $^{15}\text{N}$  in *Sphagnum* and the increased amount of  $^{15}\text{N}$  in vascular plants.

In conclusion, elevated  $\text{CO}_2$  appears to benefit growth of *Sphagnum*, but not necessarily at the cost of vascular plant growth. Non-significant increases in vascular plant biomass were observed, but the response appears restricted by faster *Sphagnum* height growth and/or nutrient limitation. *Sphagnum* can take advantage of elevated  $\text{CO}_2$  because its growth is less nutrient limited than that of vascular plants. In addition to the direct positive  $\text{CO}_2$  effect, the observed reductions in evapotranspiration in summer are expected to be beneficial for *Sphagnum*, as its growth is very sensitive to changes in moisture availability.

Increased N deposition favours growth of vascular plants, which, after reaching a certain cover (possibly 60%), reduces *Sphagnum* growth through increased shading. Three seasons of N addition caused the *Sphagnum* layer to become saturated with N, increasing N availability for vascular plants. In addition, high N deposition may directly inhibit *Sphagnum* growth, but the only indication for this, was a reduced capacity to recover from disturbance.

As expected, the vegetation responses to elevated  $\text{CO}_2$  and increased N deposition were opposite in terms of the competitive relationship between peat mosses and vascular plants. If both levels of atmospheric  $\text{CO}_2$  and N deposition would increase simultaneously, the vegetation response may well resemble that observed for increased N deposition alone, but our experiments provided at best weak evidence for this.

In outdoor experiment, it was estimated that the bog vegetation sequestered carbon at a high rate of  $200 \text{ g C m}^{-2} \text{ year}^{-1}$ . As elevated  $\text{CO}_2$  benefits *Sphagnum* growth, it is expected that carbon sequestration in bogs increases with increasing levels of atmospheric  $\text{CO}_2$ . In contrast, increased N deposition will likely reduce carbon sequestration, both by increasing the relative abundance of vascular plants and by increasing N concentrations in dead plant material. In addition, increased N deposition is expected to increase methane emission through the increase in vascular plant cover.

For predicting the implications for carbon sequestration of changes in terrestrial ecosystem (as induced by global change), we need to know the vegetation response in terms of relative abundance of different species. Long-term experiments in intact vegetation under natural conditions are needed, as only then interactions between species and feedbacks through nutrient and moisture availability can develop, which will ultimately determine the vegetation response. This thesis describes such responses to elevated  $\text{CO}_2$  and increased N deposition for ombrotrophic bog vegetation in the Netherlands for a three-year period. It must be considered as a first step to a deeper insight in the bog vegetation response to global change.

## Samenvatting

Door toedoen van menselijk handelen wereldwijd, nemen de kooldioxide (CO<sub>2</sub>) concentratie in de lucht en de stikstof (N) depositie toe. Deze twee onderdelen van 'Global Change' zullen zeker hun invloed hebben op ecosystemen, omdat koolstof en stikstof onmisbare elementen zijn voor al het leven op aarde. Hoe sterk en hoe deze effecten precies zullen zijn, is nog grotendeels onbekend. Ecosystemen zijn niet alleen slachtoffer van *Global Change*, maar kunnen de *Global Change* ook beïnvloeden. Zo nemen terrestrische ecosystemen grote hoeveelheden CO<sub>2</sub> op, waardoor de toename van het broeikasgas CO<sub>2</sub> in de lucht afgeremd wordt en daarmee ook de snelheid van klimaatverandering. Moerasesystemen kunnen wat dat betreft een belangrijke rol spelen, omdat door veenvorming CO<sub>2</sub> voor lange tijd wordt vastgelegd. Dit geldt vooral voor hoogvenen, omdat daar dood plantenmateriaal extreem langzaam wordt afgebroken, wat bepalend is voor de veenvorming. Des te opmerkelijke is het dat hoogvenen tot nog toe nauwelijks aandacht hebben gekregen in het *Global Change* onderzoek.

Karakteristieke hoogveenvegetaties bestaan uit veenmossen (*Sphagnum*) en bijbehorende vaatplanten. De *Sphagnum*-soorten zijn de belangrijkste veenvormers. Vergeleken met de veenmossen, dragen vaatplanten veel minder bij aan veengroei en dragen ze ook nog bij aan de emissie van methaan (ander belangrijk broeikasgas). Dus, het broeikas effect zou kunnen verminderen wanneer de veenmossen zich uitbreiden ten koste van de vaatplanten. De hoogveenplanten zijn voor hun voeding geheel afhankelijk van wat er uit de lucht valt (regen en droge depositie) en moeten dus wel gevoelig zijn voor veranderingen in de samenstelling van de lucht. *Sphagnum* kan die atmosferische depositie goed opvangen en vasthouden, waardoor slechts weinig voedingsstoffen (nutriënten) uit de lucht hun weg vinden naar de wortels van de vaatplanten.

Onze hypothese was dat bij een hogere CO<sub>2</sub> concentratie in de lucht (hoog CO<sub>2</sub>) de concurrentieverhouding tussen *Sphagnum* en vaatplanten verschuift in het voordeel van *Sphagnum*. Dit werd verwacht omdat *Sphagnum* minder last zou hebben van nutriëntengebrek dan vaatplanten en zich daarom kan uitbreiden bij meer CO<sub>2</sub>. Verder verwachten wij dat een toename in N-depositie (extra N-depositie) tegenovergestelde gevolgen zou hebben, dus een concurrentievoordeel voor vaatplanten. Bij een nog hogere N-depositie zou *Sphagnum* de extra N niet meer kunnen vasthouden, waardoor meer N beschikbaar komt voor de vaatplanten en zij zich kunnen uitbreiden. De toenemende beschaduwning van het mosoppervlak kan dan de groei van *Sphagnum* verminderen.

Om deze hypothesen te testen, heb ik onderzoek gedaan naar de gevolgen van hogere CO<sub>2</sub> concentraties en N-depositie op hoogveen in Nederland. Ik heb drie experimenten gedaan, waarbij de veranderingen in de vegetatie, als gevolg van CO<sub>2</sub>- en N-toediening, werden bijgehouden. De concurrentieverhouding tussen *Sphagnum* en de vaatplanten heeft hierbij speciale aandacht gehad. Voor alle drie de experimenten hebben we stukken hoogveen uit een vennetje in Boswachterij Dwingeloo gehaald. De moslaag van die stukken hoogveen bestond vrijwel geheel uit *Sphagnum magellanicum* (Hoogveenveenmos); belangrijke vaatplantensoorten waren *Vaccinium oxycoccus* (Kleine veenbes), *Erica tetralix* (Gewone dophei), *Eriophorum angustifolium* (Veenpluis) en *Drosera rotundifolia* (Ronde zonnedaauw). Gedurende twee of drie groeiseizoenen werden metingen gedaan aan de groei van *Sphagnum*, bedekking van de verschillende plantensoorten,



biomassa van de vaatplanten, nutriëntenconcentraties in het plantmateriaal (C, N, P en K), waterverbruik van de vegetatie en methaanemissie.

Het eerste experiment ("buitenexperiment", hoofdstuk 2) deed ik met grote stukken veen (110 cm doorsnee, 60 cm diep), compleet met de vegetatie, die in grote bakken waren ingegraven in de proeftuin. De hoogveenvegetatie werd onderworpen aan een hoog CO<sub>2</sub> en extra N-depositie behandeling gedurende drie groeiseizoenen. Hoge CO<sub>2</sub> concentraties (560 ppmv) werden verkregen met behulp van zogenaamde MiniFACE-ringen. De N-depositie werd verhoogd met 5 g N m<sup>-2</sup> jaar<sup>-1</sup> door drie groeiseizoenen lang elke drie weken een ammoniumnitraat oplossing uit te gieten over de vegetatie. Drie groeiseizoenen hoog CO<sub>2</sub> hadden een snellere groei van *Sphagnum magellanicum* tot gevolg. De meeste vaatplantensoorten reageerden niet noemenswaardig op de hoog CO<sub>2</sub> behandeling, maar de kleine zonnedauw werd overgroeid door het sneller groeiende *Sphagnum*. N-toediening had tot gevolg dat de vaatplanten zich uitbreidden (met name veenbes). De groei van *Sphagnum* was significant minder in het derde groeiseizoen, zeer waarschijnlijk door de toegenomen beschaduwing door de vaatplanten. We vonden een negatieve relatie tussen vaatplantenbedekking en *Sphagnum*-groei.

Omdat de invloed van CO<sub>2</sub> en van N van elkaar kunnen afhangen, heb ik de gevolgen van hoog CO<sub>2</sub> gecombineerd met extra N-depositie op de groei van *Sphagnum* en vaatplanten onderzocht in twee kasexperimenten. In het eerste kasexperiment ("kasexperiment", hoofdstuk 3), werden stukken veen (34 cm doorsnee en 40 cm diep), compleet met vegetatie, aan twee CO<sub>2</sub> behandelingen (laag: 350 ppmv en hoog: 560 ppmv) gecombineerd met twee N-behandelingen (laag: geen N-toediening en hoog: 5 g N m<sup>-2</sup> jaar<sup>-1</sup> toediening) onderworpen voor twee groeiseizoenen. In dit experiment had hoog CO<sub>2</sub> onverwachte dramatische gevolgen voor de groei van *Sphagnum*. Het negatieve CO<sub>2</sub>-effect was het sterkst in de warmste maanden, waardoor het erop lijkt dat de combinatie van hoog CO<sub>2</sub> met de hogere kastemperaturen fatale gevolgen heeft voor *Sphagnum*. Extra N-depositie bevoordeelde *Rhynchospora alba* (Witte snavelbies). De vaatplanten breidden zich het meest uit bij de combinatie hoog CO<sub>2</sub> met hoog N, maar statistisch was dit niet significant.

In het tweede kasexperiment ("concurrentie-experiment", hoofdstuk 4) werden weer stukken veen gebruikt (24 cm doorsnee en 30 cm diep) om drie verschillende vegetaties te maken: *Sphagnum* monocultuur, *Eriophorum* monocultuur en *Sphagnum* + *Eriophorum* samen (mix). De zelfde combinaties van CO<sub>2</sub>- en N-behandelingen als in het kasexperiment werden uitgevoerd, maar nu voor drie groeiseizoenen. De toevoeging van *Eriophorum* planten, zoals in de mix, had geen invloed op de groei van *Sphagnum*, waarschijnlijk omdat de *Eriophorum* zich niet genoeg uitbreidde om voor hinderlijke beschaduwing te zorgen. *Sphagnum* had wel invloed *Eriophorum*: met een *Sphagnum*-toplaag bloeiden veel minder planten, waardoor ook de biomassa van *Eriophorum* minder was in de mix. De CO<sub>2</sub> en N-behandelingen veranderden deze verhouding tussen *Sphagnum* en *Eriophorum* niet, maar ze hadden wel invloed op de twee soorten afzonderlijk. Hoog CO<sub>2</sub> had een gunstige invloed op zowel *Sphagnum* als *Eriophorum*, hoewel het positieve effect op *Eriophorum* tijdelijk was, waarschijnlijk doordat op het eind van het experiment de groei door een gebrek aan nutriënten gehinderd werd. N-toevoeging had een ongunstig effect op de groei van *Sphagnum* in het eerste seizoen, maar aan het eind van het experiment was er geen verschil

meer met de controle-behandeling. Verrassend was dat *Eriophorum* niet reageerde op de N-behandelingen.

In alle drie de experimenten heb ik het waterverbruik van de vegetatie (of evapotranspiratie) in juli-augustus gemeten (hoofdstuk 5). Hoog CO<sub>2</sub> verminderde de evapotranspiratie in alle drie de experimenten, maar vaatplanten en blootstelling van het mosoppervlak aan de wind waren ook belangrijk voor het waterverbruik. In het buitenexperiment bestond de evapotranspiratie voornamelijk uit verdamping (evaporatie) van de moslaag en niet zozeer uit het watergebruik van de vaatplanten (transpiratie), waar CO<sub>2</sub> invloed op heeft. Toch kon hoog CO<sub>2</sub> de evapotranspiratie nog met 9-10% verminderen. Een toename in vaatplantenbiomassa verminderde het waterverbruik in het buitenexperiment, maar vergrootte de evapotranspiratie in het kasexperiment.

Om na te gaan waar de N-depositie terecht komt, heb ik een kleine hoeveelheid gelabeld N (<sup>15</sup>N) toegediend aan de bakken met N-behandeling in het buitenexperiment, en ook aan de ongestoorde hoogveenvegetatie in het vennetje waar de stukken veen vandaan komen (hoofdstuk 6). De verdeling van de <sup>15</sup>N over *Sphagnum*, vaatplanten en veen werd bepaald tijdens de eindogst. In het veld werd een week na toediening 79% van de totale hoeveelheid <sup>15</sup>N die teruggevonden werd, aangetroffen in het *Sphagnum* en slechts 8% in de vaatplanten. Bij een hogere N-depositie neemt de N-beschikbaarheid voor vaatplanten toe. Dit werd aangetoond door het kleinere aandeel <sup>15</sup>N in *Sphagnum* en de grotere hoeveelheid <sup>15</sup>N in vaatplanten in de bakken waar extra N depositie was toegediend.

Concluderend, hoog CO<sub>2</sub> bevordert de groei van *Sphagnum*, hoewel dat niet ten koste lijkt te gaan van de groei van vaatplanten. Sommige vaatplanten leken zich zelfs enigszins uit te breiden, maar het CO<sub>2</sub>-effect was uiteindelijk toch beperkt door de snellere hoogtegroeï van *Sphagnum* en door nutriëntengebrek. *Sphagnum* verkrijgt het concurrentievoordeel doordat het minder gauw last zal hebben van nutriëntengebrek dan vaatplanten. Daar komt nog bij dat de waargenomen verminderingen in het waterverbruik 's zomers bij hoog CO<sub>2</sub> gunstig zijn voor de groei van *Sphagnum*, omdat het erg gevoelig is voor te lage waterstanden.

Extra N-depositie bevoordeelt de groei van vaatplanten, die, nadat ze zich tot een bedekkingspercentage van ongeveer 60% hebben uitgebreid, de groei van *Sphagnum* belemmeren door beschaduwning. Tijdens de drie seizoenen met N-toediening is de *Sphagnum*-laag verzadigd geraakt met N, waardoor de N-beschikbaarheid voor de vaatplanten toeneemt. Daar komt bij dat N ook een direct negatief effect op *Sphagnum* lijkt te hebben, zo kon het *Sphagnum* in de hoog N bakken zich nauwelijks herstellen na de oogst.

Zoals verwacht veranderde de vegetatie, ten aanzien van de concurrentieverhouding tussen *Sphagnum* en vaatplanten, in tegenovergestelde richtingen onder invloed van hoog CO<sub>2</sub> en extra N-depositie. Als zowel CO<sub>2</sub> als N-depositie toenemen, dan verwacht ik dat de vegetatie op dezelfde manier verandert als we gezien hebben voor extra N-depositie alleen, maar de experimenten geven hier maar een paar aanwijzingen voor.

Voor het buitenexperiment heb ik berekend dat de hoogveenvegetatie maar liefst zo'n 200 g C m<sup>-2</sup> jaar<sup>-1</sup> heeft vastgelegd in 1998. Bij hogere CO<sub>2</sub> concentraties in de lucht kan de koolstofvastlegging in hoogveen nog toenemen, omdat de groei van *Sphagnum* wordt bevordert. Bij hogere N-depositie, echter, zal de koolstofvastlegging afnemen, omdat het aandeel van sneller-afbrekbare vaatplanten in de vegetatie toeneemt en omdat de

N-concentraties in het plantenmateriaal toenemen, wat ook de afbreekbaarheid bevordert. Daar bovenop komt nog dat de grotere vaatplantenbiomassa zal zorgen voor een toename in de methaanemissie.

Om te voorspellen wat de gevolgen voor de koolstofvastlegging van veranderingen in ecosystemen (bijvoorbeeld als gevolg van klimaatverandering) zijn, is het nodig om te weten hoe de soortensamenstelling van de vegetatie verandert. Langlopende experimenten in natuurlijke vegetatie zijn nodig, omdat alleen dan allerlei relaties tussen soorten en terugkoppelingen via nutriënten- en waterbeschikbaarheid zich kunnen ontwikkelen, die uiteindelijk bepalen hoe de vegetatie verandert. Dit proefschrift beschrijft hoe de vegetatie in Nederlands hoogveen kan veranderen als gevolg van hogere CO<sub>2</sub> concentraties en N-depositie. De experimenten duurden drie groeiseizoenen en ze moeten worden als een eerste stap op weg naar beter inzicht in het functioneren van het hoogveenecosysteem onder invloed van *Global Change*.

## *Curriculum vitae*

Monique Maria Petronella Dorothea Heijmans werd geboren op 5 februari 1970 te Nederweert. In 1988 behaalde zij het VWO-diploma aan de Philips van Horne scholengemeenschap te Weert. In september 1988 begon zij aan de studie Milieuhygiëne aan de Landbouwniversiteit Wageningen. Na de propaedeuse *cum laude* te hebben afgerond, koos zij in de doctoraalfase voor de afstudeerrichting Waterkwaliteitsbeheer, met een afstudeervak Waterkwaliteitsbeheer en een stage Aquatische ecologie bij de afdeling Water van de provincie Gelderland te Arnhem. Vervolgens deed zij extra afstudeervakken Natuurbeheer en Ecohydrologie, respectievelijk bij de vakgroep Natuurbeheer en bij het Instituut voor Bos- en Natuurbeheer (IBN-DLO, thans Alterra) te Wageningen.

Na in augustus 1994 te zijn afgestudeerd, deed zij in 1995 vrijwilligerswerk bij de stichting SBW Advies & Onderzoek, waarna een korte aanstelling volgde als projectuitvoerder, gedetacheerd bij de afdeling Rivieren van het Rijksinstituut voor Zoetwaterbeheer and Afvalwaterbehandeling (RIZA) te Arnhem. Aansluitend volgde een tijdelijke aanstelling als onderzoeker bij het IBN-DLO, waarna zij in maart 1996 kon beginnen als Assistent in Opleiding bij de vakgroep Terrestrische Oecologie en Natuurbeheer van de Landbouwniversiteit Wageningen. Het onderzoek over de gevolgen van 'Global Change' voor de vegetatie van hoogvenen maakte deel uit van een Europees onderzoeksproject (BERI). De resultaten van de Nederlandse experimenten, onder leiding van de promotoren Prof. Dr. F. Berendse en Prof. Dr. N. van Breemen, zijn vastgelegd in dit proefschrift. Tot juli 2001 zal zij werkzaam zijn als postdoc bij de leerstoelgroep Natuurbeheer en Plantenecologie van de Wageningen Universiteit om de effecten van klimaatverandering op de lange-termijn vegetatie-ontwikkeling in hoogvenen te modelleren.