Revival of Dutch *Sphagnum* bogs: a reasonable perspective?

een wetenschappelijke proeve op het gebied
van de Natuurwetenschappen, Wiskunde en Informatica

Proefschrift

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door

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geboren op 7 juni 1971
te Wehl

A'j goed vennewater wollen hemmen, mos ie 'n gat stekken en doar 't water in loaten kommen. Met vennewater ko'j alles genezen. A'j oe esnee hadden of in 't been ehakt, dan heul ie det in 't vennewater. Det zuuveren, 't heulp mirakel. Zwearn ko'j der ok met genezen, det mos ie wè steeds herhalen. Wa'j bie 'n dokter nie kloar kreengen, doar heulp 't vennewater wè bie. Dan gung ie noar 't venne en halen vennewater.

Bron: J.H. Alferink, Nijverdal

Aan mijn ouders
Voor Thijmen

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General introduction

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Peatlands or mires are peat-forming ecosystems which have developed on wet sites in the landscape where organic material can accumulate. Mire formation was initiated or stimulated as a result of climate changes after the Late Glacial period (which ended ca. 8,000 BC). On a global scale, peatlands are widespread and extensive, occurring in almost all regions of the world. A fundamental subdivision of peatlands is that into *fens* and *bogs* (Gore 1983; Wheeler & Proctor 2000). Fens (equivalent to Dutch *laagveen*) are minerotrophic peatlands fed by groundwater and/or surface water in addition to precipitation. In climatic regions characterised by relatively high precipitation levels combined with relatively low rates of evapotranspiration, accumulation of peat can continue above the original ground level and water table. These peatlands are more or less exclusively fed by precipitation and are known as *ombrotrophic bogs* or *raised bogs* (equivalent to Dutch *hoogveen*).

Figure 1.1: Typical ombrotrophic bog vegetation dominated by bog mosses (*Sphagnum magellanicum, S. papillosum* and *S. rubellum*), *Vaccinium oxycoccus, Drosera rotundifolia* and *Eriophorum angustifolium.* [Reprinted in colour on page 188]

The raised bogs which developed in the Netherlands were of the plateau type or lens type, and had a characteristic slightly domed relief. Hydrologically, raised bogs can be regarded as two-layered (*diplotelmic*) systems comprised of an uppermost 'active layer' (*acrotelm*) and a lower so-called 'inert layer' (*catotelm*) which is permanently water-saturated (e.g., Ingram 1978; Clymo 1987). Up to the late Subboreal, the peatlands kept expanding, reaching their maximum extent about 3,000 BC. At that time, half of the present-day area of the Netherlands was covered by peatlands: approximately 1,000,000 hectares of bogs and 500,000 hectares of fens (Joosten 1994). Half of the bogs were situated in the coastal plain and half in the eastern and southern pleistocene areas (Pons 1992). Typical plant species of bogs (see Figure 1.1) in Western Europe include bog mosses (*Sphagnum* species), sedges (*Carex* species and *Eriophorum* species) and heathers (*Calluna, Erica* and *Andromeda*) (characterising the vegetation classes Oxycocco-Sphagnetea and Scheuchzerietea).

Decline of the Dutch bogs

After 3,000 BC, the peatlands in the low parts of the Netherlands started to decline as a result of anthropogenic deforestation in the catchment areas of the rivers, leading to increased and irregular river discharges and sediment loads (Pons 1992). Since the discovery of peat as an alternative to wood for fuel, large-scale peat extraction and subsequent transition into arable land have destroyed most of the remaining Dutch bogs. At the beginning of the seventeenth century, areas totalling 180,000 hectares were still covered by raised bogs (Neijenhuijs 1973). Around 1900, the area had been further reduced to approximately 90,000 hectares and nowadays less than 3,600 hectares are covered by bogs. These bog relics, however, have lost the specific characteristics of raised bog systems due to peat cutting, drainage and burning. The total area of undisturbed, peat-forming raised bog vegetation in the Netherlands has been estimated at only 5 hectares (Barkman 1992).

Restoration of cut-over bogs by rewetting

The present-day scarcity of typical bog vegetation has made the restoration of cut-over bogs an important issue (Joosten 1995; Wheeler & Shaw 1995; Gorham & Rochefort 2003). In addition, bogs are important terrestrial carbon sinks or sources and have a potential influence on global carbon cycles (Gorham 1991). Restoration and conservation of the bog relics in the Netherlands started with the acquisition by the National Forest Service (*Staatsbosbeheer*) of a relatively intact part (the 'Meerstalblok') of the former Bourtanger Moor in the north-eastern part of the country, in 1968 (Schouten *et al.* 1998). The aim of restoration management is to restore cut-over bogs into regenerating, self-sustaining ecosystems with the

appearance and composition of 'natural' bogs (Wheeler & Shaw 1995). The restoration measures taken in bog remnants mostly entail rewetting of the desiccated cut-over bog by blocking drainage ditches and constructing bunds to retain precipitation (Wheeler & Shaw 1995; Price *et al.* 2003). However, the recovery of *Sphagnum*-dominated vegetation has been found to be a complex issue, partly because little is known about the basic processes involved (Joosten 1995; Money 1995; Wheeler & Shaw 1995).

One of the common strategies is to inundate extensive areas, as this allows large parts of the surface to remain water-saturated throughout the year. As a result of such rewetting measures, the remaining peat layers have often become deeply inundated over vast areas (Figure 1.2). Under such conditions, the development of *Sphagnum*-dominated vegetation depends on a rapid expansion of submerged *Sphagnum cuspidatum* (see, e.g., Lamers *et al.* 1999; Smolders *et al.* 2003). However, the growth of submerged *Sphagnum cuspidatum* has frequently failed to materialise on deeply inundated locations due to limited availability of dissolved carbon dioxide (CO_2) and light in the water layer (Baker & Boatman 1990; Paffen & Roelofs 1991; Wheeler & Shaw 1995; Smolders *et al.* 2003).

Figure 1.2: In the 'Amsterdamse Veld' (part of the Bargerveen reserve), deep inundation has led to the development of large water bodies with little or no *Sphagnum* growth.

Under these conditions, *Sphagnum* growth can be promoted if the remaining peat layers become buoyant. These floating rafts can then be colonised by *Sphagnum cuspidatum* and *Sphagnum fallax* (Lamers *et al.* 1999), while other *Sphagnum* species and vascular plants may become established later (Money 1995). However, deeply inundated cut-over bogs frequently lack floating peat (Figure 1.2; Meade 1992; Wheeler & Shaw 1995; Lamers *et al.* 1999).

Bog restoration by development of floating rafts

The development of floating rafts is often observed at locations where the surface layer of the peat, including its vegetation, (usually referred to by the German term *bunkerde*) has been replaced after peat harvesting. After inundation, this poorly humified *bunkerde* becomes buoyant, thereby providing a substrate for *Sphagnum* colonisation. These areas are now characterised by well-developed bog vegetation. In the Netherlands, however, the remaining peat layers frequently consist only of highly decomposed catotelm peat, which remains submerged after inundation. The buoyancy of such floating rafts appears to depend on the accumulation of methane (CH_4) bubbles in the peat, which are produced by anaerobic decomposition processes (Scott *et al.* 1999; Lamers *et al.* 1999). High CH_4 production rates seem essential for the raft's buoyancy and it is important to know which biogeochemical processes are crucial for this. The presence of slightly buffered water or groundwater, for instance, may stimulate the development of floating rafts by enhancing the decomposition of organic matter (Lamers *et al.* 1999). One of the topics of the research project described in the present thesis was to identify the mechanisms influencing the development of floating rafts by means of field observations and laboratory experiments, and to assess how the development of floating rafts can be stimulated in restoration projects.

Effect of high nitrogen deposition rates

As bogs are ombrotrophic, both their restoration and their conservation may be strongly influenced by changes in airborne nitrogen (N) fluxes. The emissions of reduced (NH_x) and oxidised (NO_y) nitrogen compounds have strongly increased due to human activities (Krupa 2003). Nitrogen oxides originate mainly from the combustion of fossil fuels, whereas ammonia is volatilised from intensive animal husbandry and agricultural systems (e.g., Bobbink & Lamers 2002; Krupa 2003). Increased concentrations of oxidised and reduced N in the atmosphere result in higher N deposition rates in ecosystems. The total N deposition rate that an ecosystem is subject to depends on the canopy structure, as a larger leaf area and a rougher deposition surface enhance the amount of atmospheric N captured by the

vegetation (Van Breemen *et al.* 1982; Heil *et al.* 1988). In non-forest ecosystems in central and western Europe, present N deposition rates can amount to 2 - 6 g N m^{-2} year^{-1} (Bobbink & Heil 1993), compared to the estimated background inputs of 0.1 - 0.3 g N m^{-2} year^{-1} in the early 1900s (e.g., Galloway 1995). Increased atmospheric N inputs appear to have significant effects on the vegetation composition in various natural and semi-natural ecosystems (e.g., Bobbink *et al.* 1998; Bobbink & Lamers 2002). As most temperate ecosystems have developed under N-limited conditions, increased availability of N forms a serious threat to these ecosystems. Numerous N addition experiments have shown that in ecosystems where N limits the productivity, the competitive balance between plant species is strongly affected by N (e.g., Heil & Diemont 1983; Berendse & Aerts 1984; Bobbink *et al.* 1988).

Ombrotrophic bogs are probably among the systems that are most sensitive to N enrichment, and the empirical critical N load for ombrotrophic bogs has been estimated at 0.5 - 1 g N m^{-2} year^{-1} (Bobbink & Roelofs 1995; Risager 1998; Bobbink *et al.* 2003). In the Netherlands, characteristic *Sphagnum* species have been replaced by more nitrophilous *Sphagnum* species due to N enrichment (Greven 1992). Numerous studies have shown the effects of increased N availability on *Sphagnum* species (e.g., Press *et al.* 1986; Baxter *et al.* 1992; Jauhiainen *et al.* 1994; Gunnarsson & Rydin 2000), although findings have not been consistent across studies. The actual background N deposition rate significantly determines the response of *Sphagnum* to the increased availability of N (Aerts *et al.* 1992; Gunnarsson & Rydin 2000).

Figure 1.3: Invasion of the Bargerveen reserve by Purple moor grass (*Molinia caerulea*) and Birch trees (*Betula* sp.). [Reprinted in colour on page 188]

At relatively low atmospheric input (< 1 g N m^{-2} year^{-1}), *Sphagnum* has been found to respond to increased N deposition levels by stimulated growth, indicating N limitation (Malmer 1990). At intermediate N loads (1-2 g N m^{-2} year^{-1}), N no longer limits growth but the *Sphagnum* layer does not reach its maximum (organic) N concentration (Pitcairn *et al.* 1995; Lamers *et al.* 2000; Berendse *et al.* 2001). Above 2 g N m^{-2} year^{-1}, the *Sphagnum* layer reaches its maximum N concentration and *Sphagnum* growth is negatively affected (Lamers *et al.* 2000; Gunnarsson & Rydin 2000). In this situation, N leaches through the *Sphagnum* layer to the roots of vascular plants (Lee & Woodin 1988; Aerts *et al.* 1992; Lamers *et al.* 2000), and the competition between *Sphagnum* mosses and vascular plant species seems to be affected. In areas with high atmospheric N loads, invasions of more N-demanding grass species (e.g., *Molinia caerulea*) and trees (*Betula pubescens*) have been observed, together with a decline of ombrotrophic species (Figure 1.3; Barkman 1992; Aaby 1994).

In addition to high atmospheric N loads, cut-over bogs often suffer from the effects of falling water tables, which may also stimulate nutrient availability by stimulating aerobic mineralisation processes. Hayward and Clymo (1983) suggested that increased nutrient availability in a situation of desiccation is not only the result of increased mineralisation rates, but is probably also caused by reduced growth of *Sphagnum* mosses at low water tables. Under wet conditions, *Sphagnum* shows high N uptake rates, resulting in low N concentrations in the peat water (e.g., Lee & Woodin 1988; Jauhiainen *et al.* 1998). Vascular plants depend on N mobilised by mineralisation processes in the underlying peat (Malmer 1993; Malmer *et al.* 1994). In addition, the productivity of *Sphagnum* mosses may be much more reduced than that of vascular plants during periods of water deficiency at the surface (Malmer *et al.* 1994). Saplings of *Betula*, for instance, are less rapidly overgrown by *Sphagnum*. Since experimental evidence was lacking, it remained unclear whether the invasion of Dutch bogs by Birch trees (*Betula*) and Purple moor grass (*Molinia caerulea*) has to be attributed to desiccation, high N deposition levels or a combination of both. The studies presented in this thesis tried to elucidate the main cause of the vegetation changes in Dutch bogs.

Aim and outline of the thesis

As discussed above, the restoration and conservation of cut-over bogs are frustrated by various problems. Until recently, many expensive restoration measures were taken in Dutch bogs, but with limited success (Schouwenaars *et al.* 1997). In order to elucidate the key factors determining successful restoration of cut-over bogs, the Dutch Ministry of Agriculture, Nature and Food Quality has funded a research programme as part of a national nature action plan ('*Overlevingsplan Bos en Natuur*'). Various research institutes have joined forces in this multi-focussed programme,

addressing hydrology and hydrochemistry, effects of high levels of N deposition and nutrient cycling (Limpens 2003), and on bog-related fauna (Van Duinen *et al.* 2003). The work presented in this thesis includes research on the role of hydrochemistry in rewetting cut-over bogs and on the effects of high atmospheric N deposition on the composition of bog vegetation. The main questions raised and hypotheses proposed are listed in Table 1.1 and the corresponding answers are discussed in the General discussion (chapter 8).

The key processes involved in the development of floating rafts in bog restoration were unravelled by field observations, field experiments and laboratory experiments (chapters 2, 3 & 4). Chapter 2 discusses the importance of physico-chemical peat characteristics for the development of floating rafts by comparing inundated and buoyant peat substrates. Two different experiments were carried out to simulate the roles of peat quality and water quality (chapters 3 & 4). Chapter 3 describes the results of a greenhouse experiment testing the effects of groundwater chemistry (bicarbonate-buffered and/or sulphate-rich) and peat quality on the development of floating rafts. A similar experiment was conducted outdoors on a larger scale, although a simpler method of mixing in lime with the different peat substrates was applied instead of buffering by bicarbonate (chapter 4).

The role of high N deposition rates in the observed changes in the vegetation composition of Dutch bogs was determined in two studies (chapters 5 & 6). The question whether the invasion of *Molinia caerulea* and *Betula pubescens* is triggered by desiccation or by high atmospheric N loads was addressed in a field experiment in Ireland, where desiccation had not led to invasions of these species in bogs. The effects of high N loads were investigated there in a 3-year N addition field experiment (chapter 5). This was supplemented by a laboratory experiment in which the effects of high N deposition rates were determined under non-desiccated conditions. The chosen range of N addition rates was suitable for estimating the critical atmospheric N load for ombrotrophic bog vegetation (chapter 6). In addition to N addition experiments, the impact of 'natural' addition of nutrients by bird droppings is described in chapter 7. Eutrophication by bird droppings has led to local changes in the vegetation composition of Irish bogs, similar to those observed in the Netherlands as a result of increased N deposition.

Chapter 8 summarises and evaluates the main results reported the preceding chapters. The various findings are translated into recommendations for the conservation of ombrotrophic vegetation and successful restoration of cut-over bogs.

Table 1.1: Main questions and hypotheses of the thesis.

Question	*Hypothesis*
What are the key processes involved in the development of floating rafts after rewetting of cut-over bogs?	Buoyancy of the residual peat layers depends on sufficient methane production rates; hence, the peat has to contain relatively large amounts of easily decomposable organic matter (chapters 2, 3 & 4).
What is the influence of buffered groundwater on the development of floating peat?	The increased pH resulting from the presence of buffered groundwater in residual peat layers stimulates methane production rates, and affects the buoyancy of the peat, provided that the peat contains sufficient easily decomposable organic matter (chapters 3 & 4).
Can the presence of poorly humified peat stimulate the development of floating rafts?	High methane production rates in poorly humified peat result in buoyancy of this peat, so the presence or introduction of poorly humified peat can stimulate the development of floating rafts (chapter 4).
Is the invasion of bogs by nitrophilous vascular plants, particularly *Betula* sp. and *Molinia caerulea*, the result of high N deposition rates?	At high N deposition rates, *Betula* and *Molinia* are still limited by N and are able to profit from increased availability of N (chapters 5 & 6).
What is the impact of nutrients derived from bird droppings on the vegetation composition and structure of bogs?	Increased availability of bird-derived nutrients (P, N and K) stimulates the growth of non-characteristic species on ombrotrophic bogs (chapter 7).
Is the revival of Dutch *Sphagnum* bogs a reasonable perspective?	Physico-chemical characteristics of the peat relics and high N deposition rates frustrate *Sphagnum* bog development (chapters 2 - 7).

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Development of floating rafts after rewetting of cut-over bogs: the importance of peat quality

Floating peat developed in the Mariapeel nature reserve after taking rewetting measures

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Abstract

The usual method of restoring cut-over bogs is to rewet the peat surface, but this often leads to the remaining peat layers being deeply inundated. For *Sphagnum*-dominated vegetation to develop at deeply inundated locations, it is important for floating rafts of buoyant residual peat to develop. In this study, the chemical and physical characteristics of buoyant and inundated peat collected from rewetted cut-over bog were compared. In general, buoyant peat was poorly humified; high methane (CH_4) production rates (≥ 2 µmol g^{-1} DW d^{-1}) were important to ensure buoyancy. Although the peat water CH_4 concentrations increased with depth, the CH_4 production rates were higher in the uppermost peat layers. High CH_4 production rates were related positively with P concentrations and negatively with lignin concentrations. The pH to bulk density ratio (≥ 0.05) also appeared to be a good indicator of CH_4 production rates, providing an easy and cheap to measure parameter for restoration practitioners. Our results indicated that analysing certain simple characteristics of the residual peat can greatly improve the success of the rewetting measures taken in cut-over bogs. If the analysis reveals that the residual peat is unsuitable for floating raft formation, deep inundation is inappropriate unless suitable peat from other locations can be introduced.

Introduction

Raised bogs are unique ecosystems that support distinctive plant and animal communities. World-wide, the area of raised bogs has been drastically reduced by human activities, such as peat cutting. At the beginning of the 17th century there were still 180,000 hectares of raised bogs in the Netherlands (Neijenhuijs 1973), but few small relics remain. These are typically cut-over bogs that suffer the effects of water draw-down and are largely dominated by *Molinia caerulea* (Barkman 1992; Money 1995; Lamers *et al.* 2000). With so few natural bogs, the restoration of cut-over bogs has become important (Joosten 1995; Wheeler & Shaw 1995). The aim of restoration management is to restore the cut-over bogs to a regenerating, self-sustaining ecosystem with the appearance and composition of a 'natural' system (Wheeler & Shaw 1995). However, restoration is complicated, partly because little is known about the way *Sphagnum*-dominated vegetation recovers at anthropogenically disturbed sites (Joosten 1995; Money 1995; Wheeler & Shaw 1995). Successful

restoration relies on the development of a dense *Sphagnum*-dominated vegetation and the formation of a new functional acrotelm (Joosten 1995; Wheeler & Shaw 1995; Money & Wheeler 1999). The spongy acrotelm has a strong self-regulating effect on the depth of the water table as a result of its high hydraulic conductivity and the ability to retain water in dry periods (Ingram 1978; Proctor 1995). To enable successful growth of *Sphagnum*, it is crucial to create stable, permanently wet, ombrotrophic conditions at the bog surface (Wheeler & Shaw 1995).

Restoration entails raising the water table by blocking drainage ditches and constructing bunds to retain precipitation (Wheeler & Shaw 1995). Often this inundates the remaining peat layers. Shallow inundation, i.e. the year-round water-saturated conditions close to the ground surface (conditions that *Sphagnum* requires for optimal growth) (Wheeler & Shaw 1995; Grosvernier *et al.* 1997), is very difficult or impossible to achieve. Deep inundation (> 0.3 m) is acceptable if the submerged *Sphagnum cuspidatum* expands rapidly, or if the residual peat floats and provides appropriate conditions for *Sphagnum* growth. But submerged *Sphagnum cuspidatum* normally grows vigorously with shallow inundation (< 0.3 m) (Joosten 1995; Money 1995; Wheeler & Shaw 1995; Money & Wheeler 1999; Smolders *et al.* 2002a) where there is sufficient dissolved carbon dioxide (CO_2) and light (Baker & Boatman 1990; Paffen & Roelofs 1991; Wheeler & Shaw 1995; Smolders *et al.* 2002a).

Inundation can lead to the development of floating rafts of poorly humified peat that offer favourable conditions for *Sphagnum* growth (Money 1995; Joosten 1995; Lamers *et al.* 1999; Smolders *et al.* 2002b). Their buoyancy depends on methane (CH_4) bubbles generated by anaerobic decomposition becoming trapped in the peat (Scott *et al.* 1999; Lamers *et al.* 1999; Smolders *et al.* 2002b). Floating rafts are colonised initially by *Sphagnum cuspidatum* and *Sphagnum fallax* (Lamers *et al.* 1999) and later by other *Sphagnum* species and vascular plants (Money 1995). Deeply inundated cut-over bogs frequently lack floating peat (Meade 1992; Wheeler & Shaw 1995; Lamers *et al.* 1999; Smolders *et al.* 2002b).

Since buoyancy depends on high CH_4 production rates, it is important to know major factors limiting CH_4 production. Several studies have shown that CH_4 production is limited by peat quality (Updegraff *et al.* 1995; Yavitt *et al.* 1997; Bergman *et al.* 1998, 2000; Smolders *et al.* 2002b), temperature (Williams & Crawford 1984; Dunfield *et al.* 1993; Schulz *et al.* 1997; Bergman *et al.* 2000) and pH (Williams & Crawford 1984; Dunfield *et al.* 1993; Bergman *et al.* 1998; Segers 1998; Smolders *et al.* 2002b). Though Segers (1998) reports that the optimal pH for most methanogenic bacteria is around neutral, Williams & Crawford (1985) found that bacteria isolated from acidic *Sphagnum* peat substrates were able to produce CH_4 at pH 3.1, although they were not able to detect bacterial growth below pH 5.3. Decades of exposure to aerobic conditions, coupled with a lack of buffered groundwater frequently cause the

remaining surface layers in cut-over bogs to become acidified, with pH values below 4 (Lamers *et al.* 1999; Smolders *et al.* 2002b), thereby hampering methanogenesis.

In this study we examined the mechanisms influencing the development of floating rafts by performing field observations and laboratory experiments. We also studied the chemical characteristics of buoyant and inundated peat in relation to CH_4 production rates. We hypothesised that to be buoyant, peat must be poorly humified and have a high potential CH_4 production rate. We used our results to try to predict the buoyancy of peat substrates and to develop effective rewetting measures for cut-over bogs.

Materials and methods

Experimental sites

We used several cut-over peatlands in the Netherlands where rewetting measures had been carried out: Amsterdamse veld (52°41′50″N; 7°01′50″E), Dwingelerveld (52°49′30″N; 6°26′30″E), Haaksbergerveen (52°07′50″N; 6°46′20″E), Hatertse vennen (51°47′10″N; 5°47′30″E), Korenburgerveen (51°59′15″N; 6°39′15″E), Mariapeel (51°24′90″N; 5°54′90″E), Meerstalblok (52°41′35″N; 7°01′50″E), Pikmeeuwenwater (51°30′90″N; 6°9′90″E), Tuspeel (51°11′85″N; 5°53′55″E) and Zwart Water (51°23′50″N; 6°11′30″E). For detailed descriptions of these cut-over bogs see Schouwenaars *et al.* (2002). In order to make comparisons with intact raised bogs, we included three bogs in the midlands of Ireland in this study: Clara bog (53°20′N; 7°38′E), Raheenmore bog (53°20′N; 7°20′E) and Scragh bog (53°35′N; 7°22′E).

Seasonal floating raft

Floating rafts developed spontaneously in the Haaksbergerveen nature reserve after rewetting measures had been taken. Most of these rafts are permanently buoyant, but some only float in summer. In order to reveal whether CH_4 production in summer was responsible for the observed buoyancy, methane concentrations were measured between November 1998 and June 2000 (at 0.2 and 0.5 m depth) in one of these seasonally floating rafts (for methods used, see depth profiles).

Depth profiles

At nine locations in the Netherlands (Haaksbergerveen, Korenburgerveen (*n* = 2), Zwart Water and Pikmeeuwenwater) and Ireland (Clara bog, Raheenmore bog (*n* = 2) and Scragh bog) water and peat samples were taken at three depths: 0.1 m, 0.5 m and 1.0 - 3.0 m (the depth depended on the thickness of the peat profile). The peat

samples were stored in airtight polyethylene bags at 4 °C until the potential decomposition rates were measured. Anaerobic peat water samples were taken using ceramic cups (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands), connected to 100% vacuum PVC syringes (50 ml). The first 25 ml collected was discarded to exclude internal stagnant sampler water. For CH_4 analysis, samples were taken by connecting vacuum infusion flasks (30 ml) to the ceramic cups and then the CH_4 concentrations were measured in the headspace. The pH, alkalinity, and CO_2 concentrations in the peat water were measured within one day (see chemical analysis), after which the samples were stored at -20 °C until further analysis.

Chemical characteristics of the peat and methane production rate

Between April and May 2000, 30 peat samples were taken at eight locations in the Netherlands that had been subjected to inundation for at least two years: Haaksbergerveen ($n = 5$), Zwart Water ($n = 2$), Mariapeel ($n = 7$), Hatertse vennen ($n = 4$), Amsterdamse veld ($n = 2$), Dwingelerveld ($n = 3$), Meerstalblok ($n = 5$) and Tuspeel ($n = 2$). At 13 sites, samples were taken from floating rafts that had developed after taking rewetting measures, a further 17 samples were taken from inundated peat at sites where no floating rafts had developed. The peat samples were stored in airtight polyethylene bags at 4 °C. The next day, the pH was determined in water samples squeezed from the peat. Peat water samples were taken by placing two Rhizon soil moisture samplers (10 cm; Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) in the peat, both connected to a 100 ml anaerobic vacuum infusion flask. The samples were analysed for nutrients and major ions (see chemical analysis). The bulk density (g DW l^{-1} FW) of the peat was determined by drying a volume of 1 l of fresh peat at 70 °C for 48 hours and then measuring the dry weight. Size fractions (< 1 mm, 1 - 5 mm and > 5 mm) were determined by wet-sieving homogenised peat samples (50 g) over 1 mm and 5 mm mesh sieves.

Potential decomposition rates (CH_4 and CO_2 production) were measured by incubating 150 g of fresh peat anaerobically in 250 ml infusion flasks sealed with airtight stoppers. For each sample incubations were carried out in triplicate. After the flasks had been filled, the gases were evacuated and then flushed with pure nitrogen gas to remove all CH_4, CO_2 and O_2 from the substrate and headspace. The evacuation and flushing were repeated several times. The flasks were kept in the dark at 20 °C, and the concentrations of CH_4 and CO_2 in the headspace were measured weekly, over a period of 28 days. The CH_4 and CO_2 production rates were calculated by linear regression of the measurements and expressed on a dry weight basis.

Chemical analysis

The pH was determined with a combination pH electrode with an Ag/AgCl internal reference (Orion Research, Beverly, USA). The CH_4 was measured with ethane as an internal standard, using a Pye Unicam gas chromatograph (Unicam Cambridge, UK) equipped with a flame-photometric detector and a Porapak Q (80/100 mesh) column (Waters Chromatography, Etten-Leur, the Netherlands). The CO_2 measurements were carried out using an infrared carbon analyser (model PIR-2000, Horiba Instruments, Irvine, USA). Extinction at 450 nm was measured (Shimadzu spectrophotometer UV-120-01) for colorimetric background correction and as an estimate of humic substance concentration (Smolders *et al.*, 2003), after citric acid had been added to a concentration of 0.6 mg l^{-1} (to prevent precipitation of metal ions). The samples were stored in iodated polyethylene bottles (50 ml) at -20 °C until further analysis.

To analyse nutrient concentrations in the peat, dried samples (48 hours at 70 °C) were ground up in liquid nitrogen. Nitrogen and carbon concentrations were measured in dried samples with a CNS analyser (type NA1500; Carlo Erba Instruments, Milan, Italy). Two-hundred milligrams of the dried material was digested in sealed Teflon vessels in a Milestone microwave oven (type mls 1200 Mega, Sorisole, Italy) after addition of 4 ml HNO_3 (65%) and 1 ml H_2O_2 (30%) (Kingston & Haswell 1997). After dilution, the digestates were kept at 4 °C until analysis. Different organic matter fractions (lignin, hemicellulose, cellulose + cutine, and soluble constituents) of freeze dried peat samples were determined according to Goering & Van Soest (1970). The peat organic matter content was determined by weight loss after ignition (550 °C for 4 hours).

The concentration of *o*-phosphate was measured colorimetrically with a Technicon AA II system, using ammonium molybdate (Henriksen 1965). Nitrate and ammonium were measured colorimetrically with a Traacs 800+ auto-analyser, using hydrazine sulphate (Technicon 1969) and salicylate (Grasshoff & Johannsen 1977) respectively. Potassium was measured by flame photometry (FLM3 Flame Photometer, Radiometer, Copenhagen, Denmark). Sulphur and phosphorus were determined by inductively-coupled plasma emission spectrophotometry (type FLAMEVML2-9032034, Spectro Analytical Instruments, Kleve, Germany).
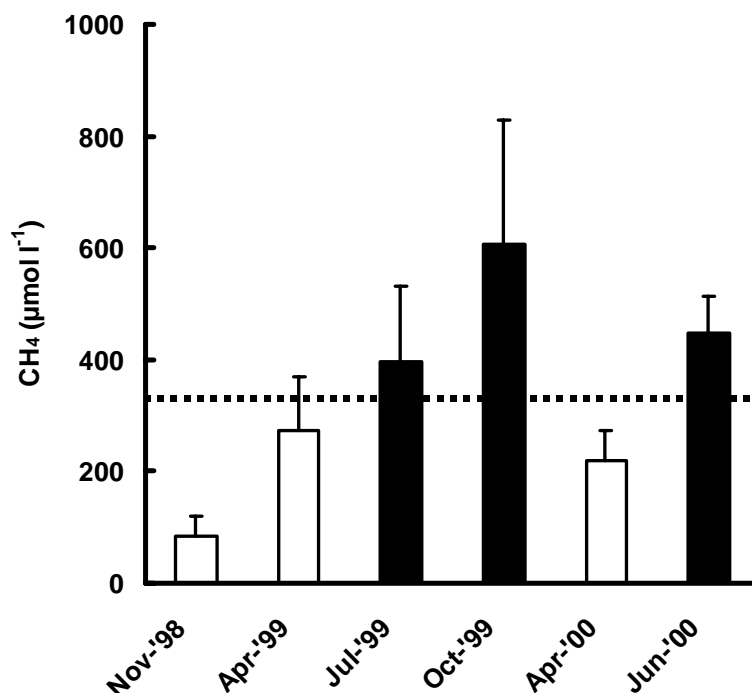
Data analysis

Prior to statistical analysis, data were log-transformed to make the variance less dependent on the means and to fit a normal distribution. All statistical analyses were carried out using the SPSS for Windows software package (version 10.0.7; SPSS Inc., Chicago, USA). Differences in chemical characteristics between buoyant and inundated peat were tested with independent samples *t*-test. Correlation between

CH$_4$ concentrations and CH$_4$ production rate was analysed with a Pearson correlation, and described by an exponential regression analysis. Correlations between chemical characteristics of the peat and potential decomposition rate were analysed with a Pearson correlation, and significant correlations were described by a power regression analysis. For clarity of presentation, the means and standard errors (SE) presented in the figures represent the non-transformed data.

Results

Floating raft development and methane production rate

The floating raft in the Haaksbergerveen reserve was buoyant when the CH$_4$ concentrations exceeded 350 - 400 µmol l^{-1} (Figure 2.1). During winter the CH$_4$ concentrations were significantly lower and the raft was inundated. Peat from the Dutch and Irish bogs revealed the highest potential CH$_4$ production rates in the top layer of the peat (Figure 2.2). The CH$_4$ concentrations and CH$_4$ production rates were inversely correlated (Pearson correlation: R^2 = 0.608; *P* = 0.000) in these sites.

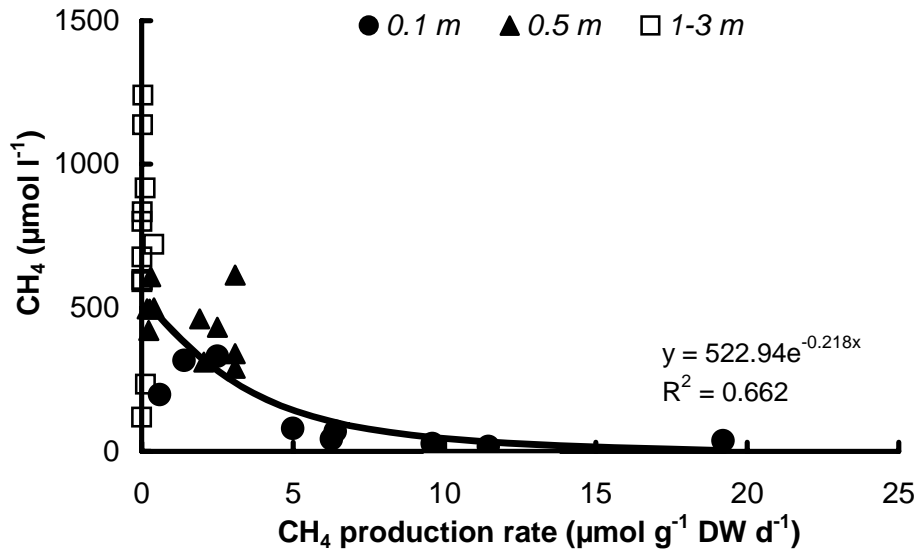**Figure 2.1:** Peat water methane concentrations (means ± 1 SE; *n* = 2) in a floating raft showing a seasonal buoyancy pattern in the Haaksbergerveen nature reserve, the Netherlands, between November 1998 and June 2000 at 0.2 - 0.5 m depth. Dark bars indicate buoyancy of the raft. (*t*-test: *P* = 0.005).

Table 2.1: Physical and chemical characteristics (means ± 1 SE) of buoyant (*n* = 13) and inundated peat (*n* = 17) from various locations in the Netherlands. Characteristics that differ significantly are in bold (*t*-test). ns *P* > 0.05, * *P* ≤ 0.05, ** *P* ≤ 0.01, *** *P* ≤ 0.001.

	Inundated peat	*Buoyant peat*	
pH	4.3 ± 0.2	4.7 ± 0.2	ns
SO_4^{2-} (µmol l^{-1})	124 ± 31	87 ± 19	ns
Bulk density (g DW l^{-1} FW)	**105 ± 14**	**52 ± 5**	***
Fraction < 1 mm	**0.55 ± 0.05**	**0.37 ± 0.04**	*
Fraction > 5 mm	**0.36 ± 0.04**	**0.50 ± 0.03**	*
Water fraction	**0.90 ± 0.01**	**0.94 ± 0.01**	**
C (mg g^{-1} DW)	453 ± 14	458 ± 4	ns
N (µmol g^{-1} DW)	1076 ± 71	1261 ± 111	ns
K (µmol g^{-1} DW)	4.2 ± 0.8	4.6 ± 0.7	ns
P (µmol g^{-1} DW)	**6.5 ± 1.0**	**10.0 ± 1.2**	*
C:N ratio (g g^{-1})	40 ± 3	34 ± 3	ns
C:P ratio (g g^{-1})	**3276 ± 513**	**1839 ± 320**	*
C:K ratio (g g^{-1})	4306 ± 750	3469 ± 591	ns
N:P ratio (g g^{-1})	**98 ± 12**	**63 ± 6**	*
CH_4 production rate (µmol g^{-1} DW d^{-1})	**0.8 ± 0.2**	**4.4 ± 0.7**	***
CO_2 production rate (µmol g^{-1} DW d^{-1})	**1.0 ± 0.2**	**2.6 ± 0.3**	**
Total C production rate (µmol g^{-1} DW d^{-1})	**1.8 ± 0.4**	**7.0 ± 1.0**	***
Cell soluble fraction (mg g^{-1} DW)	347 ± 30	293 ± 20	ns
Hemicellulose (mg g^{-1} DW)	**226 ± 41**	**351 ± 33**	**
Cellulose + cutine (mg g^{-1} DW)	110 ± 16	109 ± 19	ns
Lignin (mg g^{-1} DW)	318 ± 29	247 ± 18	ns
Lignin:P ratio (g g^{-1})	**2590 ± 561**	**1062 ± 276**	*
Lignin:N ratio (g g^{-1})	**23 ± 3**	**15 ± 2**	*
Lignin:K ratio (g g^{-1})	3341 ± 759	2070 ± 486	ns
CH_4 production rate (µmol l^{-1} FW d^{-1})	**63 ± 16**	**203 ± 28**	***
pH:(bulk density) ratio	**0.05 ± 0.01**	**0.10 ± 0.01**	***

Buoyant peat was characterised by significantly higher CO_2 and CH_4 production rates, and P and hemicellulose concentrations compared to inundated peat (Table 2.1). The C:P, N:P, lignin:P and lignin:N ratios were significantly lower in buoyant peat. Several other chemical characteristics, such as pH and N, K and lignin concentrations did not differ significantly between buoyant and inundated peat (Table 2.1). There also were several physical characteristics that differed between buoyant and inundated peat (Table 2.1). Since the CH_4, produced by decomposition processes provides buoyancy, we focussed on identifying the chemical and physical characteristics of the peat substrates determining decomposition rates.
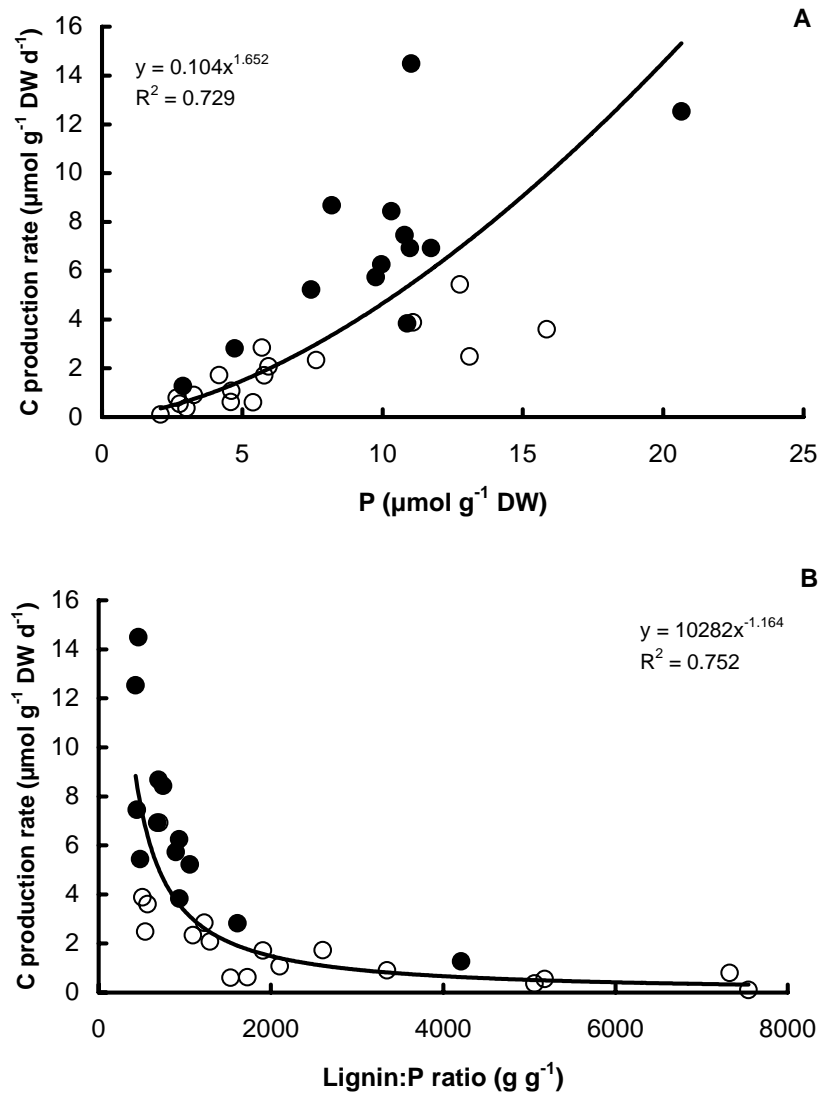
Figure 2.2: Methane production rates and methane concentrations measured in the peat from nine bogs in the Netherlands and Ireland at various depths (0.1, 0.5 and 1 - 3 m). The relation between methane production rates and methane concentrations has been described by an exponential regression analysis ($P = 0.000$).

Table 2.2: Some chemical and physical characteristics of the peat that significantly affect the potential decomposition rate (CO_2 + CH_4 production) of the peat. Relations between characteristics and potential decomposition rate are described by a power regression analysis (C production rate = $a \cdot X^b$). * $P \le 0.05$, ** $P \le 0.01$, *** $P \le 0.001$.

X	a	b	R^2	
pH	0.002	4.738	0.452	***
SO_4^{2-} (µmol l⁻¹)	68.29	-0.758	0.210	*
K (µmol g⁻¹ DW)	0.516	1.212	0.427	***
N (µmol g⁻¹ DW)	4.431	1.887	0.232	**
P (µmol g⁻¹ DW)	0.104	1.652	0.729	***
N:P ratio (g g⁻¹)	14109	-2.104	0.604	***
C:N ratio (g g⁻¹)	2393	-1.930	0.321	***
C:P ratio (g g⁻¹)	235466	-1.495	0.680	***
C:K ratio (g g⁻¹)	18102	-1.104	0.404	***
N:P ratio (g g⁻¹)	16200	-2.044	0.652	***
Hemicellulose (mg g⁻¹ DW)	0.569	0.696	0.215	**
Lignin (mg g⁻¹ DW)	3090824	-2.506	0.512	***
Lignin:N ratio (g g⁻¹)	486.8	-1.849	0.610	***
Lignin:K ratio (g g⁻¹)	3245	-0.951	0.530	***
Lignin:P ratio (g g⁻¹)	10282	-1.164	0.752	***
Fraction < 1 mm	0.763	-1.353	0.374	***
Fraction > 5 mm	14.36	1.828	0.594	***
Water fraction	9.684	15.766	0.545	***
pH:(bulk density) ratio	209.78	1.603	0.734	***
Bulk density (g DW l⁻¹ FW)	4460	-1.763	0.638	***

Methane production rate and peat chemistry

In the buoyant and inundated peat substrates studied the CH_4 and CO_2 production rates were significantly correlated (Pearson's correlation: $R^2 = 0.84$; $P < 0.001$; data not shown). We focussed on the correlations between the potential decomposition rate ($CO_2 + CH_4$ production rate, henceforth called the C production rate) of the peat during anaerobic incubation and on several physical and chemical characteristics of the peat. The significant correlations were best described by a power function (Table 2.2).

Figure 2.3: C production rates ($CH_4 + CO_2$), measured by anaerobic incubations, and A) P concentrations ($P = 0.000$) and B) lignin:P ratios ($P = 0.000$) in the peat. Each dot represents one of the peat samples that had become buoyant ($\bullet$; $n = 13$) or remained inundated ($\circ$; $n = 17$) after rewetting of the peat surface. The relations between the C production rates and P concentrations and lignin:P ratios have been described by a power regression analysis.

Figure 2.4: C production rates (CH_4 + CO_2) and the pH of water squeezed from the peat. Each marker represents one of the peat samples that had become buoyant (n = 13) or remained inundated (n = 17) after rewetting of the peat surface. The peat samples were divided into four categories based on their lignin:P ratios (g g^{-1}).

The C production rates were significantly correlated with P concentrations and lignin:P ratios of the peat (Figure 2.3). High P, N and K concentrations in the peat were related positively with high C production rates (Figure 2.3a & Table 2.2), and as a result high C:P, C:N, C:K and N:P ratios were related negatively with the C production rates. High lignin concentrations were correlated with low C production rates, but high lignin to nutr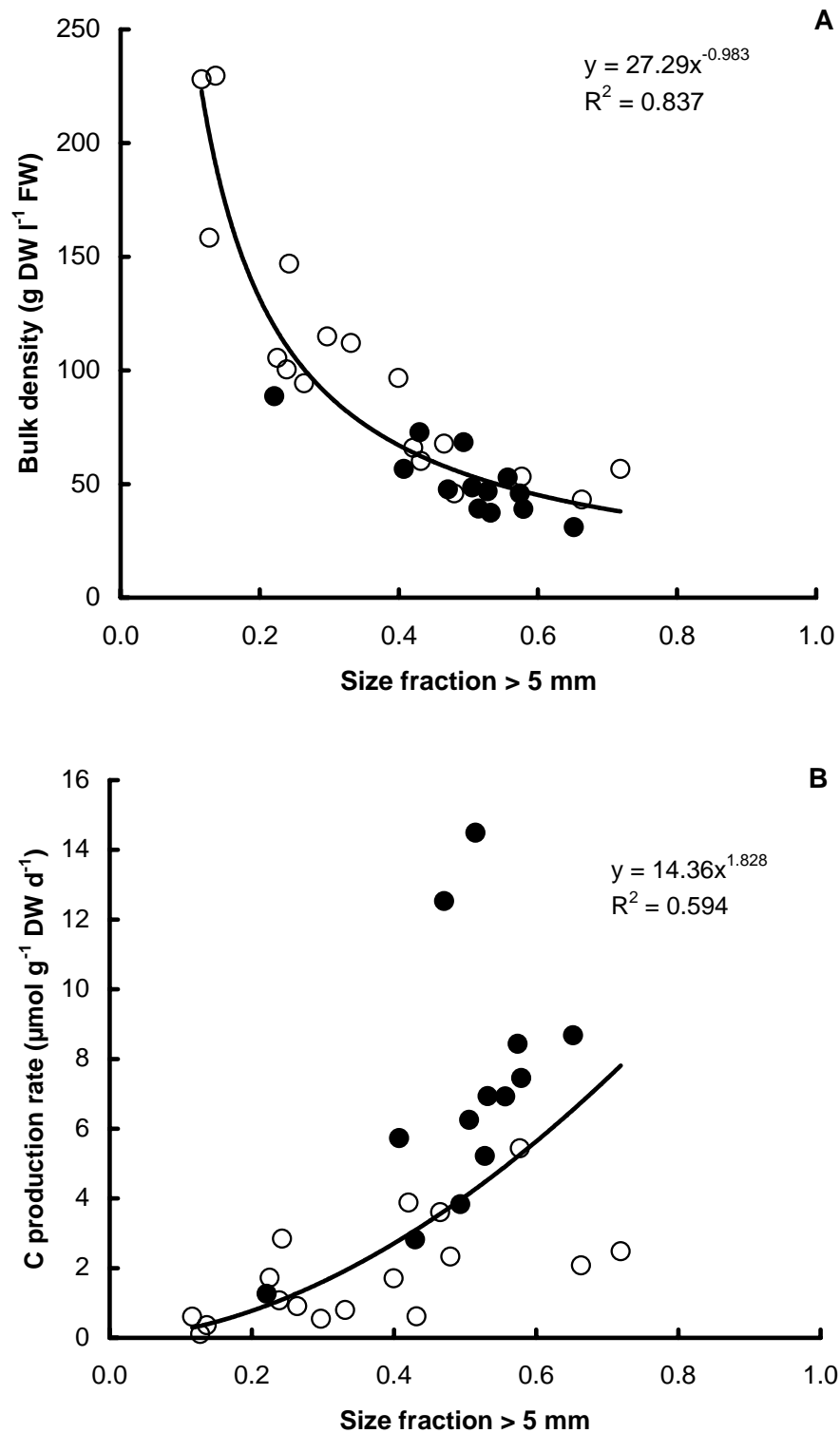ient ratios were particularly related to low C production rates (Table 2.2 & Figure 2.3b). High hemicellulose concentrations correlated with high C production rates (Table 2.2), whereas the concentrations of cell soluble matter and cellulose + cutine did not correlate significantly with C production rates (data not shown). High concentrations of sulphate in the peat water indicated lower C production rates (Table 2.2).

High C production rates were related with high pore water pH values (Figure 2.4 & Table 2.2) and low peat bulk densities (Figure 2.5a). The peat's pH to bulk density ratio, showed an even stronger correlation with C production rates than pH or bulk density alone (Figure 2.5b). Substrates having a low bulk density were characterised by a substantial fraction of large peat particles (Figure 2.6a). The fraction of large peat particles (> 5 mm) was related positively with high C production rates, whereas the fraction of small peat particles (< 1 mm) was related negatively with the C production rates (Table 2.2 & Figure 2.6b).

Figure 2.5: C production rates (CH_4 + CO_2) and A) bulk density (P = 0.000) and B) ratios between pH and bulk density (adapted from Smolders *et al.* 2002a; P = 0.000) of the peat. Each dot represents one of the peat samples that had become buoyant (•; n = 13) or remained inundated (○; n = 17) after rewetting of the peat surface. The relations between the C production rates and bulk density and the pH:(bulk density) ratio have been described by a power regression analysis.

Figure 2.6: Bulk density (A; $P = 0.000$) and C ($CH_4 + CO_2$) production rates (B; $P = 0.000$) of the peat and the fraction of peat particles larger than 5 mm. Each dot represents one of the peat samples that had become buoyant (●; $n = 13$) or remained inundated (○; $n = 17$) after rewetting of the peat surface. The relations between the particle size and the C production rates and bulk density have been described by a power regression analysis.

Discussion

Floating raft formation and methane production rate

Our finding that CH_4 concentrations in peat water were much higher when the raft was floating than when it was inundated agrees with other studies (Scott *et al.* 1999; Lamers *et al.* 1999; Smolders *et al.* 2002b). The optimum temperature for CH_4 production in peat soils is between 25 and 30 °C (Williams & Crawford 1984; Dunfield *et al.* 1993; Bergman *et al.* 1998, 2000). However, temperature is not the only factor that accounts for summer buoyancy. The availability of easily degradable compounds (e.g. root exudates), which are important substrates for the methanogenic bacteria also peaks in summer. Together these two factors explain most of the seasonal variation in CH_4 production rates (Bergman *et al.* 2000).

Most floating rafts are permanently buoyant, and their CH_4 concentrations remain high throughout the year (Smolders *et al.* 2002b). High CH_4 concentrations depend not only on high production rates, but also on the peat's capacity to retain the CH_4 bubbles produced. The mass of CH_4 stored in gas bubbles is estimated to be as much as 3 times the mass of dissolved CH_4, depending on the time of the year, and is frequently large enough to serve as a buffer between microbial production of CH_4 and the release of CH_4 to the atmosphere (Fechner-Levy & Hemond 1996). Presumably the seasonal floating raft in the Haaksbergerveen did not have the appropriate structure to retain sufficient CH_4 bubbles for buoyancy during periods with low CH_4 production rates.

Though the CH_4 concentrations in the peat profiles increased with depth (Figure 2.2), potential CH_4 production rates decreased with depth, indicating that superficial, relatively young, poorly humified peat supports the greatest CH_4 production rates. Other studies have also reported this decline in methanogenesis with depth (Williams & Crawford 1984; Yavitt *et al.* 1987, 2000). The surface layers have a high gas conductivity so that the CH_4 produced is readily vented to the atmosphere or oxidised by methanotrophic bacteria, and relatively little is retained within the peat (Segers 1998). In the deeper layers CH_4 is retained due to the low gas conductivity, and a substantial amount of CH_4 is stored (Brown *et al.* 1989). The CH_4 must be entrapped in the gaseous phase, since even at the pressures present in deep peat layers it is poorly soluble in water.

Methane production rates and peat chemistry

The poor substrate quality of highly decomposed peat limits both CO_2 and CH_4 production rates, even though 95% of the peat consists of organic matter (Bridgham & Richardson 1992). Carbon mineralisation rates are usually highest in recently

formed relatively coarse, light organic fractions (Hassink 1995; Van den Pol - Van Dasselaar & Oenema 1999; Bozkurt *et al.* 2001). Since decomposition processes break down larger organic particles into smaller ones, an increase in the decomposition extent will result in the peat having a higher bulk density (Figure 2.6a; Damman 1988; Wheeler & Shaw 1995; Van den Pol - Van Dasselaar & Oenema 1999; Bozkurt *et al.* 2001). Therefore, peat with a lower bulk density is usually less decomposed and tends to have a higher potential decomposition rate than heavier peat (Bozkurt *et al.* 2001). This agrees with our study, where substrates having a low bulk density, a limited fraction of peat particles smaller than 1 mm and a substantial fraction larger than 5 mm, had relatively high C production rates (Table 2.2).

According to Grumpelt (1991), the dry bulk density of slightly decomposed peat varies between 40 and 80 g l^{-1} and increases with continuing decomposition to 70 - 150 g l^{-1} in moderately decomposed peat, and to 120 - 250 g l^{-1} in highly decomposed peat. Based on this classification the average bulk density of the inundated peat was moderately decomposed (105 g l^{-1}) and that of buoyant peat was slightly decomposed (52 g l^{-1}). As heavier peat does not float as easily as lighter peat, a higher bulk density will also negatively affect peat buoyancy.

In our study, potential C production rates showed positive relationships with concentrations of P, N and K in the peat (Table 2.2 & Figure 2.3a). Coulson and Butterfield (1978) reported a strong correlation between the microbial decomposition of plant substrates and the N and P concentrations of the substrates. Given that nutrient availability can limit the activity of decomposing organisms, it seems likely that decomposition processes and CH_4 production rates will be hampered when C:N, C:P or C:K ratios are high, and when N, P or K concentrations are low (Swift *et al.* 1979; Updegraff *et al.* 1995; Beltman *et al.* 1996; Smolders *et al.* 2002b). Aerts *et al.* (2001) also have suggested that the potential decay rates of *Sphagnum* litter are controlled by N and P availability and are most strongly determined by P-related litter chemistry variables.

Our finding that peat with high lignin concentrations had low C production rates indicates that lignin increases with humification and may retard the activity of decomposing organisms (Swift *et al.* 1979; Bozkurt *et al.* 2001), resulting in a slower breakdown of organic litter (Yavitt *et al.* 1997; Aerts & Chapin 2000). Selective removal of the more easily metabolised carbon compounds by decomposer organisms results in larger proportions of resistant organic compounds, such as lignin, as decomposition proceeds (Bozkurt *et al.* 2001). Lignins from *Sphagnum* mosses are particularly rich in *p*-hydroxyphenols, which are the most stable phenolic compounds in surface peat (Yavitt *et al.* 2000).

Increased pH is known to enhance CH_4 production by stimulating the activity of methanogenic bacteria (Williams & Crawford 1984; Dunfield *et al.* 1993; Segers 1998) and by increasing the hydrolysis of organic substrates (Kok & Van de Laar 1991). The

enhanced hydrolysis, in turn, results in an increased availability of substrates for methanogenic bacteria such as acetate or H_2 (Lamers *et al.* 1999; Smolders *et al.* 2002b). Yet, *Sphagnum* peat often has pH below 4 and therefore even in poorly humified peat CH_4 production rates may still be low. In our study, high C production rates occurred only at pH values above 4 (Figure 2.4). As Lamers *et al.* (1999) and Smolders *et al.* (2002b) have demonstrated, buffered groundwater may increase pH of the peat water, thereby enhancing microbial decomposition of the peat and CH_4 production. The influence of buffered groundwater in the peat base led to a rapid development of floating rafts in the Haaksbergerveen reserve (the Netherlands) after rewetting measures had been taken (Lamers *et al.* 1999).

In large parts of the Netherlands groundwater is strongly enriched with sulphate (Lamers *et al.* 1998). Sulphate can hamper methanogenesis because sulphate-reducing and methanogenic bacteria compete for substrates (Bhattacharya *et al.* 1996; Lamers *et al.* 1999; Smolders *et al.* 2002b). Although not very strong, a significant negative correlation was found between sulphate concentrations and potential C production rate (Table 2.2). This in accordance with the results of Lamers *et al.* (1999) and Smolders *et al.* (2002b) who showed that the presence of sulphate-enriched groundwater hampers the formation of floating rafts. Sulphate enriched (ground)water should therefore never be used to inundate bogs.

Stimulated methane production rates and the carbon balance of bogs

Bogs are important terrestrial sinks or sources of carbon and may have a potential influence on global carbon cycling (Gorham 1991; Dunfield *et al.* 1993). Rewetting measures can affect the carbon balance of peatlands and change these areas from carbon sinks to sources of carbon emissions to the atmosphere. Scott *et al.* (1999) found that the CH_4 fluxes from floating rafts were much larger than the fluxes from the pre-flood and the post-flood (submerged) peat surfaces. Since the upper peat layer of floating rafts is still anaerobic, there is less oxidation of CH_4 by methanotrophic bacteria, and the fluxes of CH_4 to the atmosphere are larger (Buttler *et al.* 1994). The temperature of buoyant peat can be much higher than that of inundated peat, and Crill *et al.* (1988) reported that tripling the soil temperature boosted the CH_4 flow by a factor of 74.

Since the cut-over bogs have been completely altered by human activities they have lost most of their function as a carbon sink. Measurements in a typical former Dutch raised bog with a shallow peat layer and vegetation dominated by *Molinia caerulea* revealed a net release of 97 g C m^{-2} yr^{-1} to the atmosphere (Nieveen *et al.* 1998). One reason that rewetting can increase carbon losses is because it stimulates anaerobic decomposition. However, if rewetting achieves its aim of increasing *Sphagnum* growth, it seems probable that ultimately these cut-over bogs will recover their

function as C sink. Scott *et al.* (1999) noted that the extremely high fluxes associated with newly lifted peat may decrease as the floating rafts age. In time the bare floating rafts will be colonised by *Sphagnum* species that can retain large amounts of C. The increased anaerobic decomposition rates enhance the CO_2 concentrations and fluxes, and the CO_2 emitted by the peat provides a source of C for the growing *Sphagnum* (Smolders *et al.* 2001). Turetsky and Wieder (1999) have proved empirically that the refixation of C may be an important pathway for C cycling within peatlands, potentially capturing significant proportions of peat-produced CO_2 before it escapes to the atmosphere. In addition, the CH_4 produced can be oxidised to CO_2 by methanotrophic bacteria, thereby reducing CH_4 emission rates (Frenzel & Karofeld 2000). We therefore hypothesise that rewetting measures will increase C losses only temporarily. Ultimately, when the system has been restored to a peat accumulating system, there will be much more C fixation than there was in the unrestored cut-over bog.

Table 2.3: Physical and chemical prerequisites for peat able to form floating rafts after deep inundation of cut-over bogs. Data are based on the analysis of buoyant peat collected from six locations in the Netherlands ($n = 13$).

Chemical characteristics	*Buoyant peat*
pH	≥ 4.0
Bulk density (g DW l^{-1} FW)	≤ 75
Fraction < 1 mm	≤ 0.50
Fraction > 5 mm	≥ 0.40
P (μmol g^{-1} DW)	≥ 10
C:P ratio (g g^{-1})	≤ 3000
N:P ratio (g g^{-1})	≤ 75
CH_4 production rate (μmol g^{-1} DW d^{-1})	≥ 2
Total C production rate (μmol g^{-1} DW d^{-1})	≥ 3
Hemicellulose (mg g^{-1} DW)	≥ 220
Lignin (mg g^{-1} DW)	≤ 300
Lignin:P ratio (g g^{-1})	≤ 1000
Lignin:N ratio (g g^{-1})	≤ 20
CH_4 production rate (μmol l^{-1} FW d^{-1})	≥ 150
pH:(bulk density) ratio	≥ 0.05

Prospects for the restoration of Sphagnum-dominated vegetation by floating raft formation

Our study has shown that if floating rafts are to develop after deep inundation of cut-over bogs, poorly humified peat must be present. Table 2.3 summarises some of the physical and chemical characteristics of peat that predispose peat to becoming buoyant after deep inundation. The ratio of pore water pH (squeezed from the peat) to peat bulk density appears to be a simple and reliable indicator of whether the peat

is suitable for the formation of floating rafts. From our peat samples we conclude that peat that is suitable for floating raft formation has a pH above 4.0 and a bulk density below 75 g l^{-1}, resulting in a pH:(bulk density) ratio above 0.05. This pH to bulk density ratio is easy for nature managers to measure. Among the other appropriate peat characteristics for determining buoyancy are the C:P and lignin:P ratio, and the size of the peat particles (Table 2.3).

In most Dutch cut-over bogs, however, the residual peat is often inadequate for floating raft formation, since it is mostly the strongly humified catotelm peat which is left after peat harvesting. This strongly humified peat does not become buoyant after inundation (Smolders *et al.* 2002b; Tomassen *et al.* 2003). In some areas, the surface layer of the peat including its vegetation (usually referred to by the German term *bunkerde*), has been returned after peat harvesting. After inundation, this poorly humified *bunkerde* became buoyant, providing a substrate for *Sphagnum* colonisation. In these locations, floating rafts subsequently developed, even though the residual peat was strongly humified. If no floating rafts develop and it is impossible to achieve waterlogged conditions or shallow inundation, a good option is to introduce poorly humified substrates. This measure is similar to returning the *bunkerde*. A feasibility study (Tomassen *et al.* 2003) has recently been carried out successfully. Poorly humified substrates derived from sod-cutting in wet heathlands and from peat cutting activities in bogs all appeared to become buoyant if pore water pH was higher than 4.5 (Tomassen *et al.* 2003). If the substrate was too acidic, incorporation of small amounts of lime was necessary to raise its pH and to stimulate CH_4 production and so buoyancy of the substrate (Smolders *et al.* 2003; Tomassen *et al.* 2003). Re-vegetation of the bare substrate will be important as fresh, recently produced and thus easily decomposable organic matter has to provide sufficient CH_4 to warrant buoyancy on the long term (Smolders *et al.* 2003).

Based on the above reported effect of several physical and chemical characteristics of the peat on C production rates, we conclude that it is possible to determine if peat has the appropriate composition for the development of floating rafts. If the remaining peat layers in a cut-over bog are very decomposed, deep inundation (> 0.5 m) is not advisable unless poorly humified peat can be introduced.

Acknowledgements

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Peat bog restoration by floating raft formation: the effects of groundwater and peat quality

Equipment for methane emission measurements (picture taken by Frank Spikmans)

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Peat bog restoration by floating raft formation: the effects of groundwater and peat quality

Abstract

1. A prerequisite for the restoration of desiccated bog remnants is rewetting the peat surface. Frequently in Europe, extensive areas are flooded in order to maximise water retention, and growth of peat mosses is often observed in the shallow zones. In deeper waters, regeneration appears to depend on whether residual peat will become buoyant and form floating rafts.

2. In order to study the initial stages of peat bog regeneration, conditions required for peat buoyancy were studied on peat monoliths collected from three cut-over bog remnants in the Netherlands. The effects of different peat quality and water chemistry on buoyancy of the monoliths, as well as growth of *Sphagnum cuspidatum* and nutrient availability, were followed in a glasshouse experiment.

3. Both groundwater and peat quality affected the buoyancy of the monoliths and the growth of *S. cuspidatum*. When groundwater containing bicarbonate (1 mmol l^{-1} HCO_3^-, pH 6.0) was applied, the pH of peat monoliths increased from *c.* 3.5 to *c.* 4.5 due to acid buffering. As a result, two of the peat types became more buoyant and the concentration, production and emissions of methane (CH_4) increased. It was concluded that the increase in CH_4 production, induced by the increased pH, was responsible for the buoyancy.

4. When groundwater contained both HCO_3^- (1 mmol l^{-1}) and sulphate (1 mmol l^{-1}), pH was further increased to approximately pH 5.0 due to alkalinity generated by the SO_4^{2-} reduction process. CH_4 production, however, decreased because of interference from the SO_4^{2-}, as confirmed in additional incubation experiments. Phosphate concentrations, however, greatly increased in the HCO_3^-/SO_4^{2-} addition treatment due to the interaction between sulphide and iron phosphate precipitates.

5. In one of the peat types, treatments did not influence CH_4 production and buoyancy, most probably because of its low decomposability. The chemical characteristics of the peat, notably the concentrations of lignin and soluble phenolics as well as C:N, C:P and C:K ratios, were all higher than in the other two peat types.

6. The increase of *S. cuspidatum* biomass during the experiment appeared to be strongly related to the N:P ratios of the capitula, which differed considerably among the three peat types.

7. We conclude that when bog remnants are inundated the prospects for bog regeneration are largely determined by peat quality and water chemistry. Peat mats with low concentration of lignin and phenolics and low C:N ratios are most likely to become buoyant in water with a higher pH, so providing suitable environments for *Sphagnum* species. When peat quality is inadequate, either shallow inundation or the addition of suitable peat from elsewhere is indicated.

Introduction

Peat bogs may have a potential influence on global carbon cycling as important terrestrial sinks or sources of carbon (Gorham 1991; Yavitt *et al.* 1997). Also, they form unique ecosystems supporting specific plant and animal communities. The conservation and restoration of these highly threatened systems has become an important topic in recent decades (Joosten 1995; Wheeler & Shaw 1995; Schouten *et al.* 1998). However, the restoration of cut-over peat remnants is complicated, due to the lack of knowledge of the basic processes involved in the regeneration of peat bog vegetation in human-disturbed sites (Joosten 1995; Money 1995; Wheeler & Shaw 1995; Schouten *et al.* 1998).

In the Netherlands peat bogs have practically disappeared, although more than 250,000 hectares were once covered with raised bogs. At present, only very small relics are left and most of these can be characterised as desiccated cut-over peat remnants, largely dominated by *Molinia caerulea* (L.) Moench (Money 1995; Schouten *et al.* 1998; Lamers *et al.* 2000). As typical peat bog vegetation has become very rare, major efforts are dedicated to the rehabilitation of these systems. In cut-over peatlands, successful restoration relies on the development of a new functional acrotelm, which, by its capacity for hydrological self-regulation, will be able to stabilise and maintain a high water level (Hayward & Clymo 1982; Schouten *et al.* 1998). Successful recolonisation by *Sphagnum* species, however, can only be expected when water-saturated conditions close to the surface can be maintained throughout the entire year (Hayward & Clymo 1982; Wheeler & Shaw 1995; Grosvernier *et al.* 1997; Price *et al.* 1998). Therefore a prerequisite for peat bog restoration consists of rewetting the desiccated peat surface.

Hydrological measures to create wet conditions usually aim to retain rainwater within areas surrounded by dams (Meade 1992; Wheeler & Shaw 1995). In cut-over peat remnants in Europe, one of the usual strategies is to inundate extensive areas, as this allows large parts of the surface to remain water-saturated throughout the year. In such a scenario, however, peat bog regeneration is often a slow process as it starts from early succession stages (Meade 1992; Wheeler & Shaw 1995). Frequently, the regeneration of a *Sphagnum* vegetation is only observed in the shallow parts of the flooded areas, where the growth of *Sphagnum cuspidatum* Hoffm. can lead to the

formation of loose floating rafts. In deeply flooded parts (> 50 cm), however, the growth of most aquatic *Sphagnum* species seems to be strongly limited by the availability of dissolved carbon and light (Baker & Boatman 1990; Paffen & Roelofs 1991; Wheeler & Shaw 1995; Smolders *et al.* 2001).

For the development of *Sphagnum*-dominated vegetation on deeper waters, the presence or formation of floating rafts seems to be essential (Money 1995; Lamers *et al.* 1999). In inundated peat remnants, floating rafts develop when residual peat from the flooded soil becomes buoyant. Methane bubbles trapped in the peat are involved in the buoyancy of peat substrates (Lamers *et al.* 1999; Scott *et al.* 1999). As the floating peat mats stay in contact with the fluctuating water table, they provide an ideal (permanently water-saturated) substrate for *Sphagnum* colonisation (Money 1995). Initially, *S. cuspidatum* and *Sphagnum fallax* Klinggr. are the main colonisers of these rafts (Lamers *et al.* 1999). In later stages of succession, other *Sphagnum* species and higher plants may invade (Money 1995). However, a frequently encountered problem in flooded peatlands is the lack of floating peat (Meade 1992; Wheeler & Shaw 1995; Schouten *et al.* 1998; Lamers *et al.* 1999). In most cases the surface layers of cut-over peat remnants have been exposed to aerobic conditions for decades, and the peat is generally very acidic with pH values below 4 (Lamers *et al.* 1999). From a microbiological point of view such acidic conditions are unfavourable for methane production (Segers 1998) and therefore inundation could fail to result in floating raft formation in those areas.

Lamers *et al.* (1999) investigated the floating ability of peat from a location where rewetting by inundation in the 1980s was successful. The development of floating rafts resulted in the formation of *Sphagnum* carpets, now dominated by *Sphagnum magellanicum* Brid. Methane production and buoyancy of the peat floating rafts at this study site appeared to have been enhanced by the high pH of the peat, resulting from the local influence of calcareous groundwater in the peat base. Thus, the influence of buffered groundwater from the (direct) surroundings could be advantageous by decreasing the acidity of the peat remnant and thereby stimulating microbial processes including methane production. In contrast with natural peat bogs that have become more or less hydrologically isolated from minerotrophic groundwater, peat remnants consist of rather thin layers of residual peat that are more easily subject to groundwater influence when hydrological conditions are appropriate (Schouten *et al.* 1998; Lamers *et al.* 1999). A current problem in the Netherlands, however, is that sulphate levels in groundwater are frequently high, probably as a result of high atmospheric sulphate deposition in the past and the oxidation of pyrite in the subsoil due to desiccation (Stuyfzand 1993; Lamers *et al.* 1998). High sulphate levels might impair methane production (Lovley & Klug 1983) and the influence of sulphate-enriched groundwater could therefore interfere with floating raft formation.

The study of Lamers *et al.* (1999) focused on the relationship between groundwater quality and peat buoyancy and neglected the role of peat quality. It is very likely, however, that even if groundwater quality is optimal, the decomposability of the peat will play an important role in potential methane production (Swift *et al.* 1979; Updegraff *et al.* 1995; Yavitt *et al.* 1997; Segers 1998). For instance, in many inundated cut-over bog remnants, only small patches of peat (with a surface area of a few m²) become buoyant after inundation (Figure 3.1). These patches consist of poorly humified *Sphagnum* peat that has formed in recent decades in depressions of the cut-aways. These observations confirm that peat quality plays a very important role in floating raft formation.

Figure 3.1: Small floating raft in an inundated part of Mariapeel (the Netherlands). After inundation of large areas only small patches of peat became buoyant. These patches consisted of young, poorly humified, *Sphagnum* peat that was still present in former depressions. In the major part of the area, however, only strongly humified peat was present and no floating raft formation was observed.

We investigated the interactions between peat characteristics and groundwater quality on the development of floating rafts in cut-over bog remnants, in order to determine the conditions necessary for restoration. The influence of artificial groundwater containing bicarbonate with or without sulphate was simulated in an experimental set-up in a glasshouse to reveal its effects on buoyancy of peat monoliths from three different cut-over peatlands in the Netherlands. The monoliths were collected on sites where flooding of large surface areas was under consideration

as a restoration measure. Peat biogeochemistry and the biomass increase of introduced *S. cuspidatum* were recorded. Additional incubation experiments on the effects of pH and sulphate on methane production were carried out with one of the peat substrates, in order to confirm the results from the glasshouse experiment.
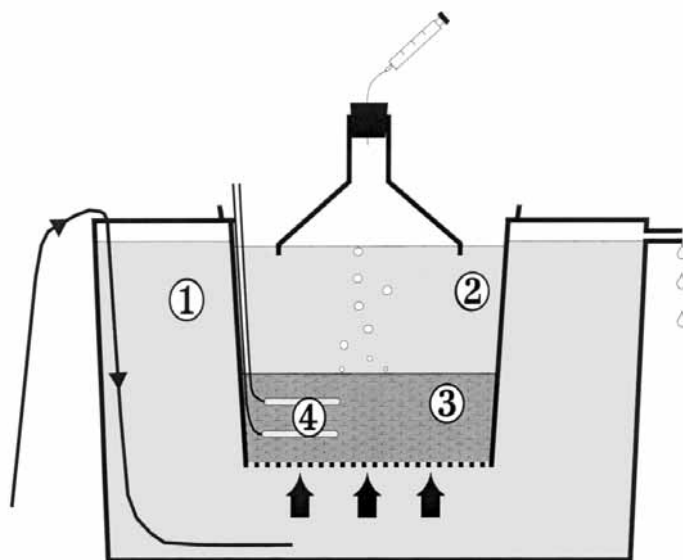
Materials and methods

Collection of monoliths

In March 1998, peat monoliths (12 on each location) were collected from three cut-over bog remnants in the Netherlands in non-waterlogged parts of Haaksbergerveen (HV; 52°07′50″N; 6°46′20″E), Mariapeel (MP; 51°24′90″N; 5°54′90″E) and Amsterdamse Veld (AV; 52°41′50″N; 6°55′50″E). In HV, peat was collected close to the location where rewetting had resulted in successful development of floating rafts, whereas in MP and AV it was collected at locations that were to be rewetted in the summer of 1998. The monoliths (depth 30 cm; diameter 50 cm), were cut carefully and transported undisturbed to the glasshouse in airtight plastic bags. From each location four samples from the upper 10 cm of the peat were taken, well mixed, dried (48 hours at 70 °C) and preserved for further analysis. At all locations the peat layer consisted of (strongly) humified peat, which is the main residual peat type in most cut-over peatlands in the Netherlands. The depth of the remaining peat layer in the sample locations varied between 10 and 40 cm.

Experimental set-up

In the glasshouse the monoliths were reduced in size to a depth of 10 cm and diameter of 35 cm and placed in plastic containers (height 27 cm, diameter 35 cm) with perforated bottoms (diameter of holes 1 cm) (Figure 3.2). Each container was placed in a larger container (50 × 40 cm) with a depth of 35 cm (Figure 3.2). This outer compartment was filled with water of differing chemical composition and covered with black plastic to reduce evaporation. The water in the outer compartment was continuously refreshed by a peristaltic pump (5 l day^{-1}), each compartment receiving water from a different 60 l stock container. Three different water types were applied (Table 3.1): a control medium (pH 3.6), a medium containing 1 mmol l^{-1} bicarbonate (pH 6.0), and a medium containing 1 mmol l^{-1} bicarbonate and 1 mmol l^{-1} sulphate (pH 6.0). Initially, all inner containers were filled with the water type used for the control treatment (pH 3.6; Table 3.1). Every day 100 ml of water was collected from the inner compartment to create an upward water flow from the outer compartment into the inner one. This 100 ml included evaporation that was measured in a control procedure using a non-perforated

container without monoliths. There were four replicates for each treatment. The experimental duration was 6 months (March - September 1998). Buoyancy of the monoliths was determined by means of a thin hook by which the position of the underside of the monolith, relative to the perforated bottom of the inner compartment, was measured. In each inner compartment 100 g of fresh *S. cuspidatum* was introduced in the water layer (spread evenly) at the onset of the experiment. In each peat monolith, four soil water samplers (Rhizon SMS - 10 cm; Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) collected peat water at two different depths (Figure 3.2). Samples were collected every 2 weeks by connecting the samplers to nitrogen pre-flushed vacuumed infusion flasks. All samplers from the same monolith were connected to one flask (pooled during sampling). Methane was measured in the headspace of infusion flasks. In the flasks that were meant for methane analysis peat water was collected until the flasks were half-full.

Figure 3.2: Experimental set-up with funnel for methane emission measurement. 1, outer compartment; 2, inner compartment; 3, peat monolith; 4, peat water samplers (see explanation in text).

Table 3.1: Chemical composition of the solutions used in the experiment. All concentrations are in μmol l^{-1}.

	Control	*HCO$_3^-$*	*HCO$_3^-$/SO$_4^{2-}$*
pH	3.6	6.0	6.0
NaHCO$_3$	-	1000	1000
Na$_2$SO$_4$	-	-	1000
KCl	30	30	30
MgCl$_2$	30	30	30
CaCl$_2$	150	150	150

Methane production and emission rates

In the first week of August and the first week of September, methane (CH_4) emission rates were measured by means of a funnel (diameter 22.5 cm) closed with a rubber stopper, which was placed upside-down onto the water surface of the inner compartment (Figure 3.2). Accumulation of CH_4 under the funnel was measured every 12 hours over a period of 3 days by carefully taking air samples with syringes. As CH_4 concentration showed a linear increase, methane emission could be calculated by simple linear regression. CH_4 production was also measured at the end of the experiment by incubating 100 g of peat from each monolith used in the experiment in 200 ml infusion flasks, sealed with an airtight rubber stopper. For each monolith incubations were carried out in duplicate. After filling, the flasks were repeatedly vacuumed and flushed with oxygen-free nitrogen gas to remove all CH_4 from the substrate and the headspace. The flasks were then kept in the dark at 20 °C, and over a period of 2 weeks CH_4 concentrations in the headspace were measured every 2 days. CH_4 production was calculated by linear regression of the measurements. At the end of the experiment, subsamples were dried for 48 hours at 70 °C. Similar incubations were carried out with peat samples from the control treatments in which, after an initial period of 30 days, 500 µmol of sodium acetate were added as a substrate for the methanogenic bacteria.

Effects of pH and sulphate on methane production in incubations

To reveal the effects of pH and sulphate levels on the potential CH_4 production in peat substrates, additional incubation experiments were carried out in the laboratory. Peat samples collected from HV were incubated at different pH values or sulphate concentrations. To adjust the peat to the desired pH or sulphate concentration, 100 g of peat, freshly collected in the field, was mixed with 50 ml bi-distillated nitrogen-flushed water. For the pH treatment, pH values of 3.6, 4.8, 5.8 and 6.6 were achieved by adding sodium hydroxide every 8 hours until the pH became stable. A maximum of six additions was needed before all treatments were equilibrated at the desired pH. For the sulphate treatment, different amounts of sodium sulphate were added to obtain concentrations in the peat water of 0, 200, 500, 1000, 1500 and 2000 µmol l^{-1}. CH_4 and CO_2 concentrations in the headspace were measured every 2 days. As the data showed a linear increase with time, net CH_4 and CO_2 production could both be calculated by linear regression. All incubations were carried out at 20 °C in the dark and with four replicates for each concentration.
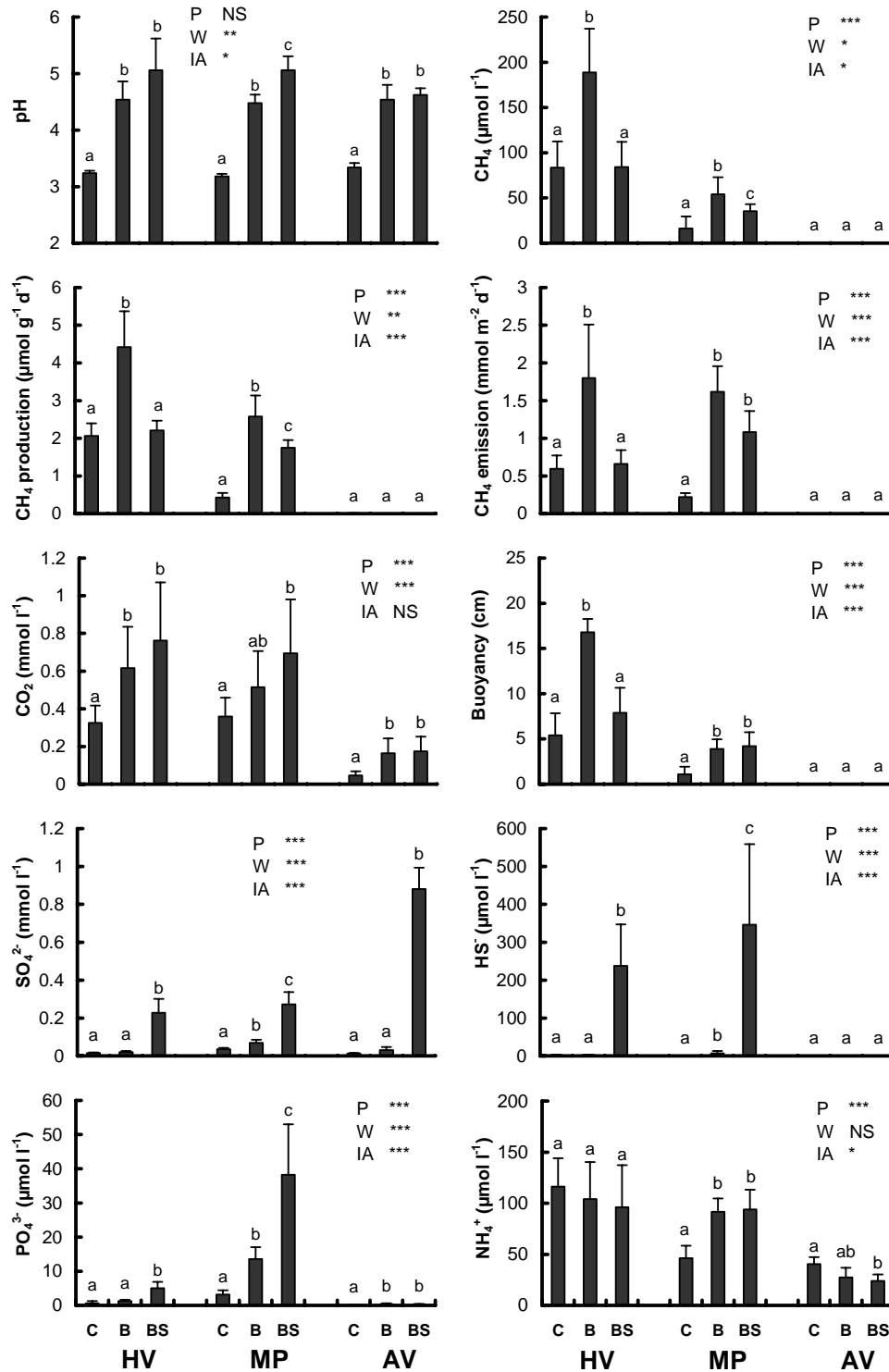
Chemical analyses

Digestates of ground-dried peat and *S. cuspidatum* samples were prepared with the aid of a Milestone microwave (type mls 1200 Mega, Milestone Inc., Sorisole, Italy),

using nitric acid and hydrogen peroxide. After dilution the digestates were kept at −20 °C until analysis (see below). Carbon and nitrogen contents were analysed in dried peat and *Sphagnum* samples with the aid of a CNS analyser (type NA 1500; Carlo Erba Instruments, Milan, Italy). The lignin and hemicellulose plus soluble constituent fractions of dried peat samples were determined according to Goering & Van Soest (1970). The methanol soluble phenolics content of the peat was determined using the method developed by Hagerman & Butler, as described in Waterman & Mole (1994). Sulphide was analysed in subsamples fixed immediately after collection of peat water with an equivalent amount of sulphide antioxidant buffer (SAOB), and measured as described by Lamers *et al.* (1999). Sulphate was measured according to Technicon Autoanalyzer Methodology (1981), orthophosphate according to Henriksen (1965) and ammonium according to Grasshoff & Johannsen (1977). Digestates were analysed for P, Fe, Mn, Ca and Mg, using an inductively coupled plasma emission spectrophotometer (ICP; Spectroflame, Spectro Analytical Instuments, Kleve, Germany). Na and K were measured using a FLM3 Flame Photometer (Radiometer, Copenhagen, Denmark). CH_4 was measured with ethane as an internal standard on a Pye Unicam gas chromatograph (Unicam, Cambridge, UK) equipped with a flame photometric detector and a Porapak Q (80/100 mesh) column (Waters Chromatography, Etten-Leur, the Netherlands). CO_2 analyses were carried out using an infrared carbon analyser (model PIR-2000; Horiba Instruments, Irvine, USA).

Statistical analysis

All statistical analyses were carried out using SPSS 7.5 (SPSS Inc. 1996). The results from the glasshouse experiment were analysed using the GLM procedure. For each experimental unit we calculated the mean values of the data obtained in the final 2 months of the experiment (four sample dates in August–September). These mean values were log-transformed to reduce heterogeneity of variances and analysed using a two-way ANOVA test. A Tukey's post-hoc test was performed to locate differences between treatments. The data from the pH incubation experiment were log-transformed (to reduce heterogeneity of variances) and analysed with a one-way ANOVA and a Tukey's post-hoc test to identify differences between the means of the different treatments. The results from the sulphate incubation experiment were log-transformed and analysed with a one-way ANOVA for repeated measures.

Figure 3.3: Peat water characteristics and buoyancy at the final stage of the glasshouse experiment. Means (*n* = 4) are given with their standard deviation. C, control treatment; B, bicarbonate treatment; BS, combined bicarbonate/sulphate treatment. HV, Haaksbergerveen; MP, Mariapeel; AV, Amsterdamse Veld: field sites where peat was collected. Results of two-way ANOVA on log-transformed data are given in the graphs. P, peat (location); W, water (treatment); IA, the interaction peat × water. NS, not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Different characters above the bars indicate significant differences ($P < 0.05$) between the treatments (C, B, BS) for monoliths from the same field site (one-way ANOVA with Tukey's posteriori test, *d.f.* = 2).
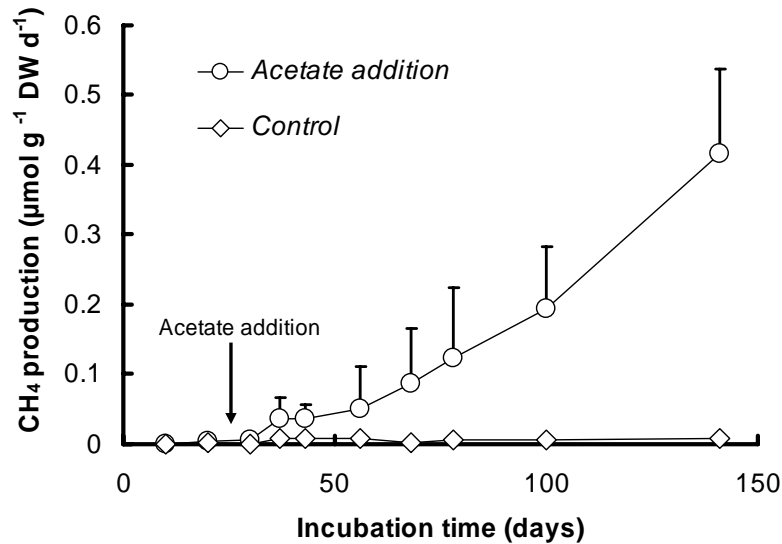
Results

The characteristics of the different peat substrates used in the glasshouse experiment are presented in Table 3.2. The peat of the AV was characterised by low N, P and K concentrations and high C:N, C:P and C:K values. Furthermore, this peat contained a relatively high fraction of lignin and a low fraction of hemicellulose plus soluble compounds. The concentration of methanol soluble phenolics was also considerably higher than for the two other peat substrates.

Table 3.2: Characteristics of the peat substrates that were used in the experiments. All values present the mean of four replicates.
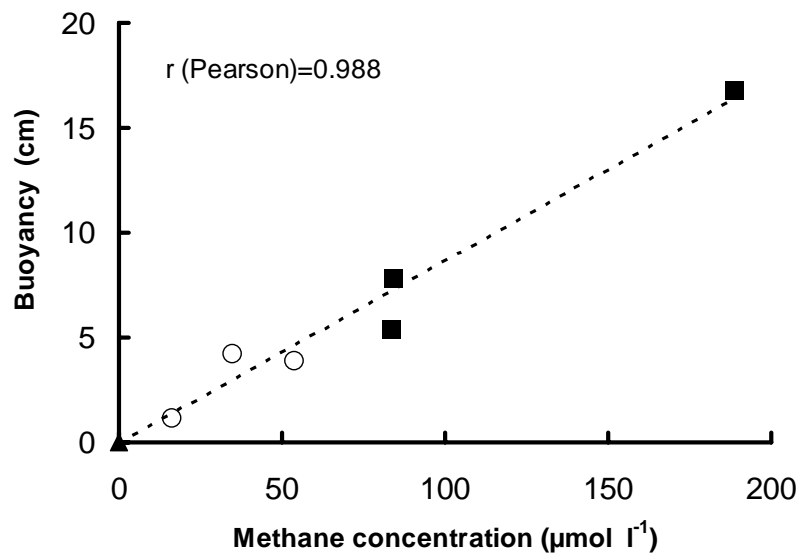
	Haaksbergerveen	*Mariapeel*	*Amsterdamse veld*
N (μmol g^{-1} DW)	989	1089	672
P (μmol g^{-1} DW)	15.7	17.4	5.2
K (μmol g^{-1} DW)	18.8	11.2	6.2
C:N ratio (g g^{-1})	26.3	29.7	49.7
C:P ratio (g g^{-1})	740	814	2851
C:K ratio (g g^{-1})	488	1002	1882
Lignin (g g^{-1} DW)	0.179	0.204	0.349
Hemicellulose plus soluble fraction (g g^{-1} DW)	0.501	0.542	0.214
Phenolics (methanol soluble) (g g^{-1} DW)	1.8	2.7	8.3
Lignin:N ratio (g g^{-1})	12.9	13.4	37.1
Ca (μmol g^{-1} DW)	56	81	55
Mg (μmol g^{-1} DW)	16	25	44
S (μmol g^{-1} DW)	167	109	84
Fe (μmol g^{-1} DW)	26.4	47.3	4.5
Mn (μmol g^{-1} DW)	0.3	1.1	0.2
Na (μmol g^{-1} DW)	6.8	11.4	6.2

The results of the glasshouse experiment are summarised as the mean values of the four analyses carried out in the fifth and sixth months of the experiment (Figure 3.3). In the bicarbonate treatment, the pH of the peat water increased by more than one unit compared with the control treatment, from values lower than 3.5 to values around 4.5, for all locations (Figure 3.3). In the combined bicarbonate/sulphate treatment, pH increased to values of *c.* 5.0 in the HV and MP monoliths, while in the AV monoliths no additional increase (compared with the bicarbonate treatment) was observed. Methane (CH_4) concentrations, emissions and production rates were significantly higher in the bicarbonate treatment compared with the control treatment for the HV and MP monoliths (Figure 3.3). In the HV monoliths, the combined bicarbonate and sulphate treatment resulted in concentrations, production and emission rates of CH_4 equal to the control values, while in the MP monolith values were found that were intermediate between the control and bicarbonate treatments. In the AV monoliths, values for CH_4-related parameters were very low in

all treatments and no significant differences were obtained between the treatments (Figure 3.3). The addition of sodium acetate to the incubation of AV peat from the control treatment resulted in an increase in CH_4 production (Figure 3.4). After acetate addition, CH_4 production increased gradually during the incubation period, indicating a (slow) gradual growth of the poorly developed population of methanogenic bacteria.

Figure 3.4: Methane production in incubations of Amsterdamse Veld (AV) peat obtained from the control treatment at the end of the glasshouse experiment. After 30 days 500 µmol sodium acetate was added to the incubation. Means ($n = 4$) are given; bars indicate SD. DW, dry weight.

Figure 3.5: The relationship between the methane concentrations in the peat moisture and the buoyancy of the monoliths. HV, Haaksbergerveen (■); MP, Mariapeel (o); AV, Amsterdamse Veld (▲).

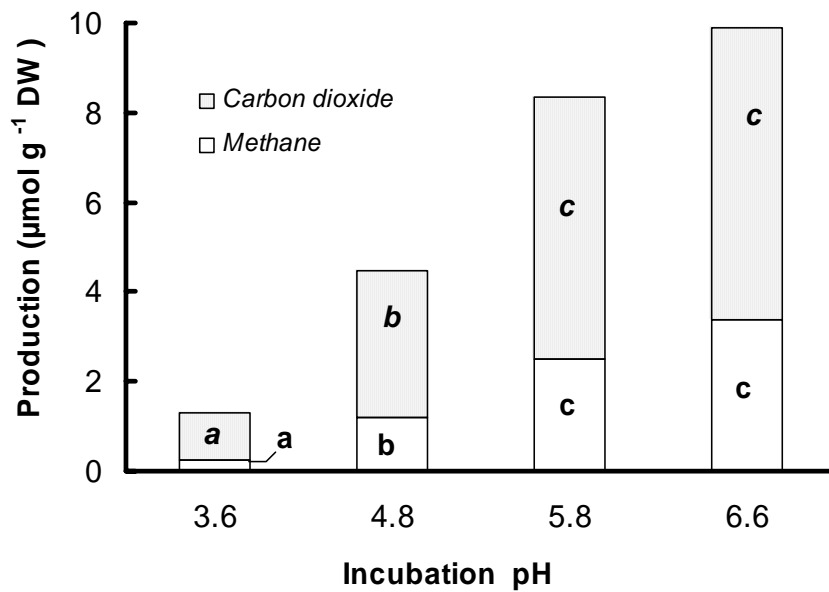Buoyancy of the peat monoliths was strongly correlated with the measured CH_4 concentrations in the peat water (Figure 3.5). Almost maximum buoyancy (*c.* 17 cm) was observed in the bicarbonate treatment for the HV monoliths (Figure 3.3). In general, buoyancy was highest in the HV monoliths, while those from AV showed no buoyancy in any of the treatments.
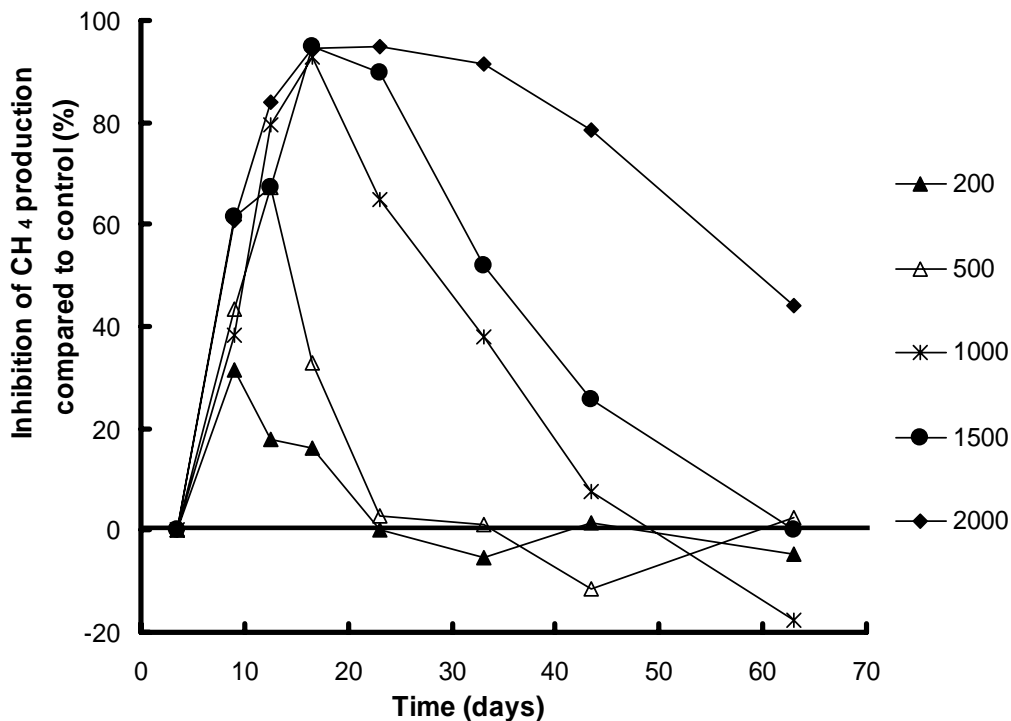

Figure 3.6: The biomass increase of *Sphagnum cuspidatum* as a percentage of the original introduced fresh weight (FW) plotted against the N:P (g g^{-1}) ratio in the capitula at the end of the experiment. B, bicarbonate treatment; BS, combined bicarbonate sulphate treatment; C, control treatment. HV, Haaksbergerveen (■); MP, Mariapeel (○); AV, Amsterdamse Veld (▲).

Carbon dioxide (CO_2) levels in the monoliths increased in the bicarbonate treatment and the combined bicarbonate/sulphate treatments. In the AV monoliths CO_2 levels were much lower than in the monoliths from the other locations. Sulphate levels were increased in the combined bicarbonate/sulphate treatment in all locations but much more in the monoliths from AV compared with those from HV and MP. Sulphide levels were high in the bicarbonate/ sulphate treatment for the monoliths from HV and MP, indicating sulphate reduction, but remained very low in the AV monoliths (Figure 3.3). Phosphate and ammonium concentrations were low in the AV monoliths independent of the treatment applied. In the bicarbonate and bicarbonate/sulphate treatments of the MP monoliths and the bicarbonate/sulphate treatment of the HV monoliths, however, phosphate concentrations were significantly higher compared with the control treatment. In all treatments MP monoliths showed the highest phosphate concentrations. The increase of the *Sphagnum* biomass during the experimental period was negatively correlated with the N:P ratio of the capitula and was highest for the moss growing on the MP monoliths (Figure 3.6), independent of the applied treatment.

Figure 3.7: The effects of incubation pH on the production of methane and carbon dioxide in peat from a cut-over bog remnant (Haaksbergerveen; see Table 3.2 for characteristics). Different characters in the bars indicate significant differences ($P < 0.05$; according to Tukey's post-hoc test) between the treatments. Results of one-way ANOVA: CO_2 production ($F_{3,15} = 107.65$, $P < 0.001$); CH_4 production ($F_{3,15} = 85.08$, $P < 0.001$).

Figure 3.8: The inhibiting effect of initial sulphate concentrations (μmol l^{-1}) in peat water on the methane production of incubated peat ($n = 4$) from a cut-over bog remnant (Haaksbergerveen; see Table 3.2 for characteristics). Results of ANOVAs for repeated measures are as follows: date ($F_{7,105} = 87.35$, $P < 0.001$); sulphate concentration ($F_{4,15} = 107.84$, $P < 0.001$); interaction ($F_{7,105} = 13.97$, $P < 0.001$).

The incubation experiments at different pH values and sulphate levels confirmed the results obtained in the glasshouse experiments. In the incubations, the potential CH_4 production appeared to be influenced significantly by pH (Figure 3.7) and sulphate concentration of the peat water (Figure 3.8). Higher pH levels strongly increased the production of CH_4 and CO_2. The higher the sulphate concentration, the stronger the inhibition of CH_4 production and the longer the duration of the inhibition.

Discussion

Methanogenesis

In the glasshouse experiment, bicarbonate buffering led to increased CH_4 production rates and, as a result, increased CH_4 concentrations in the peat monoliths from HV and MP, but not in those from AV. An increase in the production of CH_4 and CO_2 at higher pH was also observed in the incubation experiment (Figure 3.7). Increased pH is known to enhance methane production by stimulating the activity of methanogenic bacteria (Williams & Crawford 1984; Dunfield *et al.* 1993; Segers 1998). Increasing the hydrolysis of organic substrates (Kok & Van de Laar 1991) can also result in an increased availability of substrates for methanogenic bacteria (such as acetate or H_2) (Lamers *et al.* 1999). In the peat water of the HV and MP monoliths, the combined addition of bicarbonate and sulphate resulted in higher pH values compared with the addition of only bicarbonate. In addition, sulphate was reduced to sulphide yielding additional bicarbonate (Giblin *et al.* 1990; Lamers *et al.* 1998) This additional pH increase did not lead to extra CH_4 production, however, because of the competition for substrates (mainly acetate) between sulphate-reducing and methanogenic bacteria (Lovley & Klug 1983; Bhattacharya *et al.* 1996; Lamers *et al.* 1999). The sulphate-reducing bacteria reduced the availability of substrate for methanogens, thus impairing further CH_4 production: an effect confirmed in the incubation experiments (Figure 3.8).

Nutrient availability

The influence of alkaline and sulphate-enriched water not only affects net CH_4 production but also nutrient availability. Increased mineralisation of peat leads to increased CH_4 concentrations and increased phosphate levels in the peat water (Lamers *et al.* 1999). Sulphide accumulation may greatly increase phosphate availability as it interacts with iron phosphates to form iron sulphide. As a result phosphate is released from iron phosphate complexes, leading to the net mobilisation of phosphate (Smolders *et al.* 1995; Lamers *et al.* 1998). In this study, in the sulphate treatment, high levels of sulphide accumulated in the peat water in the HV and MP

monoliths, and phosphate levels were strongly increased. Phosphate concentrations reached high values in peat water, especially in the MP monoliths. The high iron content of the MP peat (Table 3.2) may indicate that in this location a relatively large part of the P is present as iron phosphates, from which P is easily mobilised by sulphide. The influence of sulphate-rich groundwater will thus not only hamper net methane production but may also lead to 'internal eutrophication' (ultimately stimulating algal growth) and sulphide toxicity in flooded peat remnants.

Buoyancy

The accumulation of CH_4 was clearly involved in the observed buoyancy of the monoliths (Figure 3.5; Fechner-Levy & Hemond 1996; Lamers *et al.* 1999; Scott *et al.* 1999). In the bicarbonate treatment of the HV monoliths, CH_4 levels increased to mean values of 189 μmol l^{-1}, which coincided with maximal buoyancy. These values are comparable with the lowest concentrations measured in well-developed floating rafts in the field at different locations in Europe (Table 3.3). CH_4 production and the resulting buoyancy may be very poor under the acidic conditions that normally prevail in rainwater flooded peat. Peat quality, however, can differ importantly between locations. In our experiment, the AV monoliths in particular seemed to be extremely inert, as indicated by the low CH_4 production rates and the low carbon dioxide and CH_4 concentrations in the peat water samples. The sulphate and sulphide analyses indicated that sulphate reduction rates were also very low in the bicarbonate/sulphate treatment. Although bicarbonate buffering did increase the pH of the peat in the bicarbonate treatment, no concomitant stimulation of methane production could be observed. The addition of acetate, however, did lead to increased methane production rates in the AV monoliths (Figure 3.4). This confirms the hypothesis that the low availability of appropriate organic substrate for methanogens caused the lack of methane production in the AV monoliths in the glasshouse experiment.

Table 3.3: Methane concentrations in floating rafts with a well-developed *Sphagnum* vegetation (from different European peat bog areas; A.J.P. Smolders & H.B.M. Tomassen, unpublished data). All concentrations are in μmol l^{-1}.

Locations	Mean	Minimum	Maximum	
Haaksbergerveen monoliths (experiment)	189	158	261	($n = 4$)
Hautes Fagnes (Belgium)	729	473	1138	($n = 5$)
Clara Bog (Ireland)	534	328	689	($n = 7$)
Haaksbergerveen (the Netherlands)	772	264	1317	($n = 6$)
Tuspeel (the Netherlands)	370	246	567	($n = 4$)
Fochteloërveen (the Netherlands)	811	474	1088	($n = 4$)

Peat quality

Nutrient availability usually limits the activity of decomposing organisms. Therefore, decomposition processes and methane production may be hampered when C:N ratios and/or C:P ratios are high and/or when N, P or K concentrations are low (Brinsson 1977; Swift *et al.* 1979; Updegraff *et al.* 1995; Beltman *et al.* 1996; Szumigalski & Bayley 1996; Aerts & De Caluwe 1999). C:N, C:P and C:K ratios were high in all three peat substrates but particularly in the AV substrate. Lignin and phenolics contents were also noticeably higher in the AV peat, while the fraction of more easily decomposable material (soluble plus hemicellulose fraction) was much lower (Table 3.2) compared with the other peat substrates. Lignin and phenolics may retard the activity of decomposing organisms (Swift *et al.* 1979), resulting in a slower breakdown of organic litter (Yavitt *et al.* 1997; Aerts & Chapin 2000). The unfavourable chemistry probably explains the observed lack of methane production/emission and, as a consequence, the lack of buoyancy of the AV monoliths, independently of the treatment applied.

Sphagnum growth

This study demonstrates that peat characteristics also affect the growth of *S. cuspidatum* in shallowly inundated zones to a considerable degree. Growth of *S. cuspidatum* was strongest on the MP monoliths, which was related to lower N:P ratios in the *Sphagnum* tissue. *Sphagnum* growth is known to be optimal at tissue N:P ratios between 10 and 14 (Aerts *et al.* 1992). At N:P ratios well above 16, growth is limited by P (Koerselman & Verhoeven 1992; Koerselman & Meuleman 1996; Aerts & Chapin 2000). Accordingly, *Sphagnum* growth was P limited on the HV and AV monoliths (Figure 3.6). In general, *Sphagnum* is assumed to be P limited in the industrialised world due to the strongly increased atmospheric N deposition during the last few decades (Aerts *et al.* 1992; Aerts & Chapin 2000). P availability was sufficiently high to warrant optimal growth only on the MP monoliths. The high P levels due to sulphate reduction in the MP peat indicate that more loosely bound P (probably iron bound) was present in this peat substrate. On the AV monoliths, biomass increase was lower than expected when related to the N:P ratios. However, the very low CO_2 levels indicate that carbon dioxide was probably (co)limiting the growth of *Sphagnum* on these monoliths.

Implications for restoration

We conclude that, in inundated bog remnants, floating raft formation usually does not occur because of the lack of methane production in very acidic and strongly humified peat substrates. As strongly humified peat is relatively inert, acidic conditions result in a (very) low decomposition rate and therefore a low availability

of organic substances for methanogens and other micro-organisms (Bridgham & Richardson 1992; Boon & Mitchell 1995). The upwelling of groundwater enriched in bicarbonate but poor in sulphate through residual peat can stimulate the buoyancy of peat due to its effect on methane production. This, however, will only occur if the peat quality is adequate. It appears that peat with relatively low lignin and phenolic content and with a low C:N, C:P and C:K ratio has a higher potential for methane production. The combination of peat quality and hydrochemistry is likely to determine floating raft development and vegetation succession. Once a well-developed acrotelm has developed on the floating peat, methane production in the freshly formed, poorly humified, *Sphagnum* peat will provide sufficient methane to reinforce the buoyancy process.

If peat quality is inadequate, floating raft formation is unlikely to occur and shallow inundation or waterlogging will very probably provide the best conditions for *Sphagnum* development (Meade 1992; Wheeler & Shaw 1995). The introduction of peat with a high potential methane production might also be considered. In some successful regeneration projects (for instance at Meerstalblok, the Netherlands), the poorly humified top layer of recently exploited peat bog ('white' peat) was introduced into relatively deep pools (> 1 m). In contrast to the strongly humified material that usually characterises cut-over peat remnants, this material started floating rapidly and a *Sphagnum*-dominated vegetation developed within a few years.

Acknowledgements

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Restoration of cut-over bogs by floating raft formation: an experimental feasibility study

Experimental set-up of the floating raft feasibility study

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Restoration of cut-over bogs by floating raft formation: an experimental feasibility study

Abstract

Stimulation of floating peat by the introduction of poorly humified peat from four cut-over bogs in the Netherlands was studied in an one-year outdoor experiment. The pH of the various peat substrates was increased by adding different amounts of lime: $0 – 2 – 4 – 8$ g $CaCO_3$ kg^{-1} fresh peat. Both peat type and lime addition were found to affect the buoyancy of the peat substrates. Low nutrient concentrations in the peat, together with a high bulk density, proved to be unfavourable for creating floating peat. Three of the peat types proved too acidic (pH < 4.5) to produce sufficient methane (approx. 400 - 600 μmol CH_4 l^{-1}), and buoyancy was only achieved if lime was mixed in with the peat. The smallest amount of lime added (2 g kg^{-1} fresh peat) was sufficient to maintain buoyancy for at least one year. Lime addition did not stimulate nutrient mobilisation by mineralisation, although P, N and K concentrations in the peat water were relatively high. It is concluded that floating peat can be initiated by the introduction of poorly humified peat. If the peat substrates are too acidic, lime can be added to stimulate buoyancy of the peat. Results are discussed in relation to restoration prospects.

Introduction

Worldwide, the area of raised bogs has been drastically reduced by human activities such as peat extraction, agriculture and forestry. Joosten (2000) states that about 60% of pristine European peatlands have been altered, and that the Netherlands has lost the complete resource (15,000 km^2) during two millennia of human impact. The restoration of cut-over bogs has attracted great interest in recent decades (Joosten 1995; Wheeler & Shaw 1995; Gorham & Rochefort 2003). The fundamental problem in bog restoration is a lack of water caused by drainage, compounded by the instability of water storage during the growing season caused by rainfall and evapotranspiration from the residual peat with a low storage capacity (Price *et al.* 2003). Rewetting measures taken in bog remnants involves retaining incoming precipitation by blocking drainage ditches and constructing bunds (Wheeler & Shaw 1995; Price *et al.* 2003). Successful restoration depends on the development of a dense *Sphagnum* vegetation which forms a new functional acrotelm (Joosten 1995; Wheeler & Shaw 1995; Money & Wheeler 1999; McNeil & Waddington 2003). The spongy acrotelm has a strong self-regulating effect on the depth of the water table, as a result

of its high hydraulic conductivity and the capacity to retain water in dry periods (Ingram 1978; Proctor 1995). To enable successful growth of *Sphagnum,* it is crucial to create stable, permanently wet, ombrotrophic conditions at the bog surface (Wheeler & Shaw 1995; McNeil & Waddington 2003; Price *et al.* 2003).

Although rewetted cut-over peatlands in northern Europe have been successfully returned to a functional state close to that of pristine peatlands (Vasander *et al.* 2003), cut-over peatlands are often difficult to restore to their former state (Joosten 1995; Money 1995; Wheeler & Shaw 1995; Gorham & Rochefort 2003). Shallow inundation, i.e. the year-round water-saturated conditions close to the ground surface, that *Sphagnum* requires for optimal growth (Wheeler & Shaw 1995; Grosvernier *et al.* 1997; McNeil & Waddington 2003), is very difficult or impossible to achieve. Deep inundation (> 0.5 m) is acceptable if submerged *Sphagnum cuspidatum* expands rapidly, or if the residual peat floats and provides appropriate conditions for *Sphagnum* growth. But submerged *Sphagnum cuspidatum* normally only grows vigorously in the more shallowly inundated parts (< 0.5 m) (Joosten 1995; Money 1995; Wheeler & Shaw 1995; Money & Wheeler 1999; Smolders *et al.* 2002a) where there is sufficient dissolved carbon dioxide (CO_2) and light (Baker & Boatman 1990; Paffen & Roelofs 1991; Wheeler & Shaw 1995; Smolders *et al.* 2002a).

Sphagnum growth can be promoted by the residual peat becoming buoyant, as floating peat is permanently waterlogged but never flooded (Money 1995; Joosten 1995; Lamers *et al.* 1999; Smolders *et al.* 2002b). However, deeply inundated cut-over bogs frequently lack floating peat (Meade 1992; Wheeler & Shaw 1995; Lamers *et al.* 1999; Smolders *et al.* 2002b). Floating raft development has been observed in locations where poorly humified peat and/or slightly buffered water are present (Lamers *et al.* 1999; Smolders *et al.* 2002b). Their buoyancy depends on the accumulation of methane (CH_4) bubbles in the peat generated by anaerobic decomposition processes (Scott *et al.* 1999; Lamers *et al.* 1999; Smolders *et al.* 2002b). High CH_4 production rates are essential for the rafts' buoyancy and several studies mention that peat quality (Updegraff *et al.* 1995; Yavitt *et al.* 1997; Bergman *et al.* 1998, 2000; Smolders *et al.* 2002b) and peat pH (Williams & Crawford 1984; Dunfield *et al.* 1993; Bergman *et al.* 1998; Segers 1998) are important for methanogenesis. In the Netherlands, decades of exposure to aerobic conditions, coupled with the lack of buffered groundwater frequently cause the remaining surface layers in cut-over bogs to become acidified, with pH values below 4 (Lamers *et al.* 1999). Methanogenesis in these acid residual peat layers may be quite low, even in poorly humified peat, and they will remain submerged after inundation (Smolders *et al.* 2002b).

In some areas, the surface layer of the peat including its vegetation (usually referred to by the German term *bunkerde*) has been returned after peat harvesting. After inundation, this poorly humified *bunkerde* became buoyant, providing a substrate for *Sphagnum* colonisation (Lamers *et al.* 1999; Smolders *et al.* 2002b). These areas are now

characterised by well-developed bog vegetation. At deeply inundated locations where no floating rafts develop and shallow inundation cannot be achieved, the introduction of poorly humified substrate could be a useful restoration measure, which is comparable with the restitution of *bunkerde* in the past (Smolders *et al.* 2002b). If the substrate is too acidic, mixing in small amounts of lime with the peat to raise the pH might stimulate CH_4 production rates and buoyancy.

We studied the feasibility of stimulating floating rafts by chemically manipulating poorly humified peats in an outdoor enclosure experiment. Buoyancy of peat substrates from four cut-over bogs was examined. The pH of the peat was increased by mixing in various amounts of lime. Peat biogeochemistry and buoyancy of the peat were followed for one year. Additional incubation experiments were carried out to determine the potential CH_4 production rates of the peat substrates used. We hypothesised that only if the introduced peat was poorly humified, with a pH high enough to produce sufficient CH_4, floating peat can develop. The results are discussed in relation to restoration prospects.

Methods
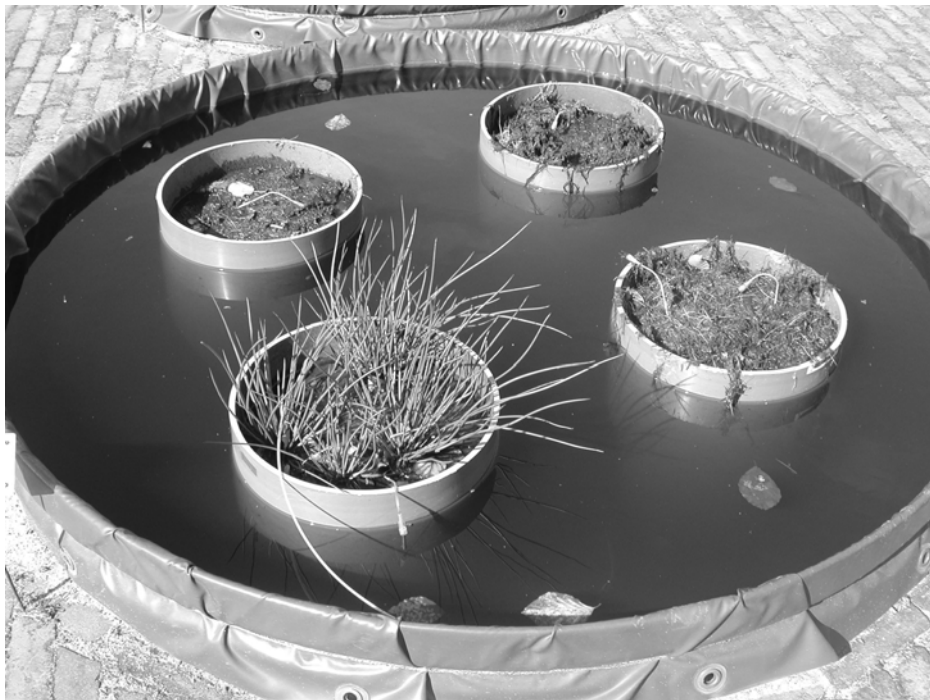
Experimental set-up

In September 1999, 16 artificial ponds at the Botanical gardens of the University of Nijmegen were filled with a 20 cm layer of highly decomposed catotelm peat from the Bargerveen reserve, which had not yet become buoyant in March 2001 (Table 4.1 for chemical characteristics of the peat). In each artificial pond (diameter 1.8 m, depth 0.85 m; Figure 4.1), four small PVC enclosures (diameter 0.4 m, length 0.8 m) were placed, which had previously been soaked in a large, rainwater-filled basin for two days to remove possible chemical softeners. In February and March 2001, peat was collected from four cut-over bogs in the Netherlands: Bargerveen (52°41'50"N; 6°55'50"E), Haaksbergerveen (52°07'50"N; 6°46'20"E), Mariapeel (51°24'90"N; 5°54'90"E) and Tuspeel (51°11'85'N; 5°53'55"E). The substrates were obtained as follows: at the Bargerveen reserve (BV) from sod-cutting activities in a wet heathland, at the Haaksbergerveen reserve (HV) from a waterlogged site where relatively poorly humified acrotelm peat was still present, at the Mariapeel (MP) and the Tuspeel (TP) reserves from desiccated locations with moderately humified catotelm peat.

The peat substrates (25 kg fresh peat) were introduced into the enclosures in March 2001. The pH of the peat substrates was increased by mixing in lime (dolokal®: $CaCO_3$ with 5% MgO; grain diameter < 0.1 mm) with the substrates at four concentrations: 0, 2, 4 and 8 g kg^{-1} fresh peat (0, 400, 800 and 1600 g lime m^{-2}). A 15

cm layer of freshly collected and limed substrate was introduced into each enclosure and covered with 5 cm of unlimed substrate to prevent the lime from dissolving in the water layer. In the control treatment, a 20 cm layer of unlimed substrate was introduced. The amount of peat introduced corresponded to 200 kg fresh peat m^{-2}. The experiment had a full-factorial design (peat type * lime) with each treatment consisting of four replicates, randomly distributed over the ponds. The temperature of the water layer was measured in three enclosures, three times a week (Figure 4.2).
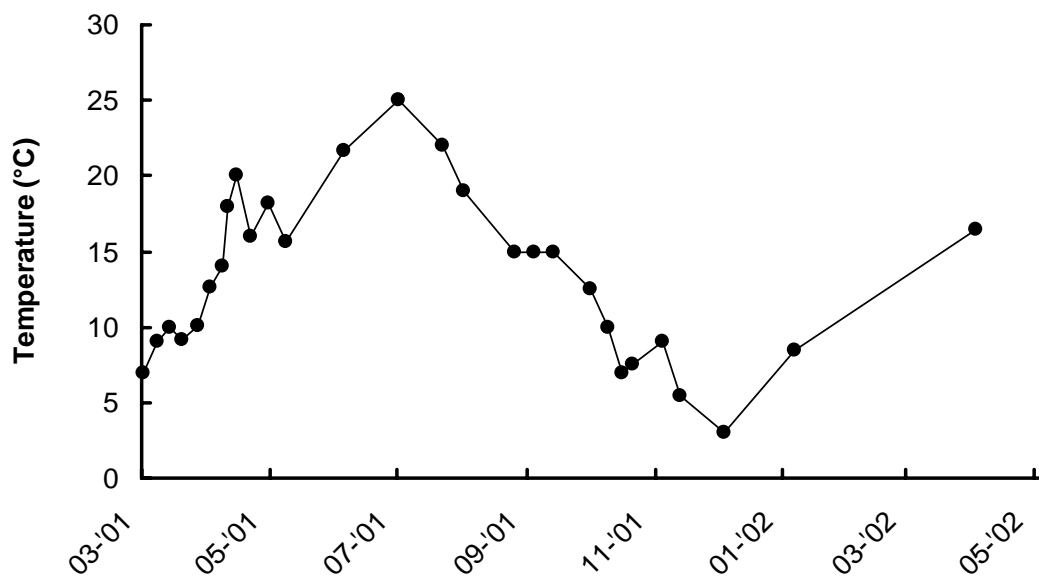
Soil moisture samplers (Rhizon SMS – 10 cm; Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) were placed in the introduced peat, at depths of 0 - 10 cm, to allow the collection of peat water samples. Peat water samples were collected monthly with the help of vacuum infusion flasks (100 ml) and were analysed for pH, CH_4, inorganic carbon (CO_2 and HCO_3^-), *o*-phosphate, nitrate, ammonium, potassium, calcium and magnesium concentrations. In the period between March and May 2001, no floating peat had developed yet, and surface water samples were taken in dark polyethylene bottles (500 ml) to determine the effects of peat introduction and lime addition on the water layer chemistry. The buoyancy of the substrates was checked regularly and substrates were classified as buoyant, partly buoyant or inundated based on the position of the substrate surface in relation to the bottom of the enclosure. In May 2002, the vegetation which had established on the newly-developed floating peat was described.

Figure 4.1: Photograph of an artificial pond with four enclosures, in which floating peat had developed after the introduction of peat substrates. The vegetation of the Bargerveen (BV) substrate is dominated by *Juncus effusus*. The other peat substrates remained almost without vegetation.

Table 4.1: Concentrations and ratios of the major nutrients (means ± SE; strongly humified peat: $n = 2$ and other peat types $n = 4$) of the peat types used in the experiment, at the start of the experiment (March 2001). Different letters indicate significant differences ($P < 0.05$) between the poorly humified peat types (one-way ANOVA).

Peat type	N $\mu mol\ g^{-1}DW$	P $\mu mol\ g^{-1}DW$	K $\mu mol\ g^{-1}DW$	C:N ratio $g\ g^{-1}$	C:P ratio $g\ g^{-1}$	C:K ratio $g\ g^{-1}$	N:P ratio $g\ g^{-1}$
Strongly humified peat	578 ± 12	5.8 ± 0.1	4.5 ± 0.0	61 ± 1	2770 ± 36	2809 ± 3	45 ± 2
Bargerveen (BV)	1118 ± 41[a]	23.7 ± 1.1[a]	24.6 ± 1.2	30 ± 1[a]	636 ± 27[a]	486 ± 25	22 ± 1[a]
Haaksbergerveen (HV)	1288 ± 11[a]	22.7 ± 0.1[a]	8.2 ± 2.7	25 ± 0[a]	651 ± 11[a]	1892 ± 534	26 ± 0[ab]
Mariapeel (MP)	585 ± 71[b]	11.7 ± 2.0[b]	35.7 ± 28.6	59 ± 7[b]	1393 ± 243[b]	1305 ± 431	23 ± 2[a]
Tuspeel (TP)	611 ± 21[b]	9.0 ± 0.2[b]	5.6 ± 0.5	49 ± 1[b]	1508 ± 32[b]	1959 ± 158	31 ± 1[b]

Figure 4.2: Temperature of the water layer during the experiment (March 2001 – May 2002).

Methane production rate

Potential methane production rates of each substrate were measured by incubating 50 g of fresh peat anaerobically in 250 ml infusion flasks sealed with airtight stoppers. Incubations were carried out in triplicate for the five lime concentrations (0 – 2 – 4 – 8 – 25 g lime kg⁻¹ fresh peat). After the flasks had been filled, the gases were evacuated and then flushed with pure nitrogen gas to remove all CH_4, CO_2 and O_2 from the substrate and headspace. The evacuation and flushing were repeated several times. The flasks were kept in the dark at 20 °C, and CH_4 concentrations in the headspace were measured weekly, over a period of three months. The CH_4 production rates were calculated by linear regression of the measurements and expressed on a dry weight basis.

Chemical analysis

The pH was determined with a combination pH electrode with an Ag/AgCl internal reference (Orion Research, Beverly, USA). The methane was measured with ethane as an internal standard, using a Pye Unicam gas chromatograph (Unicam, Cambridge, UK) equipped with a flame-photometric detector and a Porapak Q (80/100 mesh) column (Waters Chromatography, Etten-Leur, the Netherlands). The total inorganic carbon concentrations were measured using an infrared carbon analyser (model PIR-2000, Horiba Instruments, Irvine, USA). Extinction at 450 nm was measured (Shimadzu spectrophotometer UV-120-01) for colorimetric background correction and as an estimate of humic substance concentrations, after citric acid had been added to a concentration of 0.6 mg l^{-1} (to prevent precipitation of metal ions). Surface water samples were filtered through a Whatman GF/C filter (pore size 1.2 µm), after which the water samples were stored (for a maximum of 6 weeks) in iodated polyethylene bottles (50 ml) at -20 °C until further analysis.

The bulk density (g DW l^{-1} fresh peat) of the peat was determined by drying a volume of 1 l of fresh peat at 70 °C for 48 hours and then measuring the dry weight. Size fractions (< 1 mm, 1-5 mm & > 5 mm) were determined by wet-sieving homogenised peat samples (50 g) over 1 mm and 5 mm mesh sieves. To analyse nutrient concentrations in the peat, dried samples (48 hours at 70 °C) were ground up in liquid N_2. Nitrogen and carbon concentrations were measured with a CNS analyser (type NA1500; Carlo Erba Instruments, Milan, Italy). 200 mg of the dried material was digested in sealed Teflon vessels in a Milestone microwave oven (type mls 1200 Mega, Sorisole, Italy) after addition of 4 ml HNO_3 (65%) and 1 ml H_2O_2 (30%). After dilution, the digests were kept at 4 °C until analysis.

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

Data analysis

Prior to statistical analysis, data were log-transformed to make the variance less dependent on the means and to fit a normal distribution. All statistical analyses were carried out using the SPSS for Windows software package (version 10.0.7; SPSS Inc., Chicago, USA). Differences between treatments (peat type * lime addition) were tested with a two-way ANOVA at the 0.05 confidence limit. Tukey's Student range
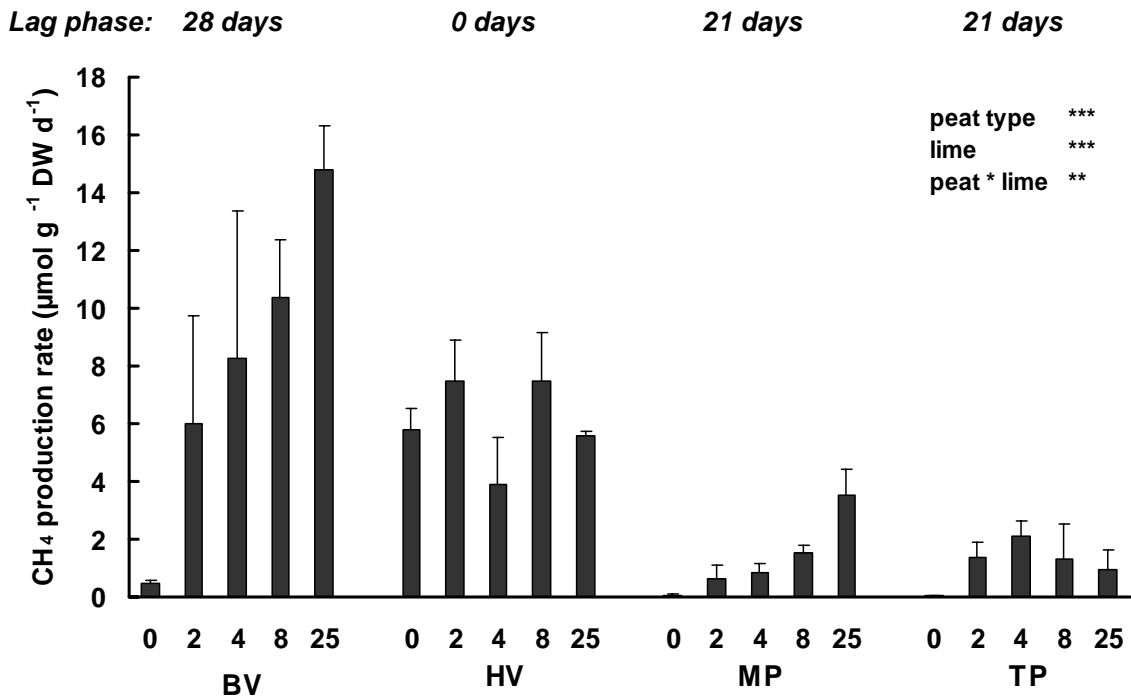
tests were used to identify differences between treatments. Differences in peat water and surface water concentrations during the experiment were tested with a two-way analysis of variance for repeated measures (General Linear Model). Differences in characteristics of the peat types introduced and in the cover of *Juncus effusus* on the BV substrate were identified with an one-way ANOVA. For clarity of presentation, the means and standard errors (SE) presented in the figures represent the non-transformed data.

Results

The chemical and morphological characteristics of the various peat substrates used in the experiment are presented in Tables 4.1 and 4.2. The peat from Mariapeel (MP) and Tuspeel (TP) had significantly lower N and P concentrations, and higher C:N ratios and C:P ratios than the Bargerveen (BV) and Haaksbergerveen (HV) substrates (Table 4.1). The peat from BV had a large fraction of small peat particles (< 1 mm) and hence a very high bulk density (Table 4.2). The peat from HV and TP contained a substantial fraction of peat particles larger than 5 mm and had the lowest bulk density (Table 4.2). The potential CH_4 production rates of the peat substrates, as determined by the anaerobic incubations, were significantly influenced by the type of substrate and the amount of lime mixed in with the peat (Figure 4.3). The CH_4 production in HV peat started immediately (no lag phase) and addition of lime had limited effects on the production rate. In the other substrates, the CH_4 production rates were very low when no lime was mixed in with the peat (Figure 4.3). For BV and MP substrates, the CH_4 production rates increased with increasing amounts of lime mixed in. The highest CH_4 production rates were measured in peat from BV with 25 g lime kg^{-1} mixed in (Figure 4.3). The CH_4 production rates in MP and TP substrates were inferior to those in BV and HV substrates.

Table 4.2: Some physical characteristics of the peat types used in the experiment, as determined at the start of the experiment (March 2001).

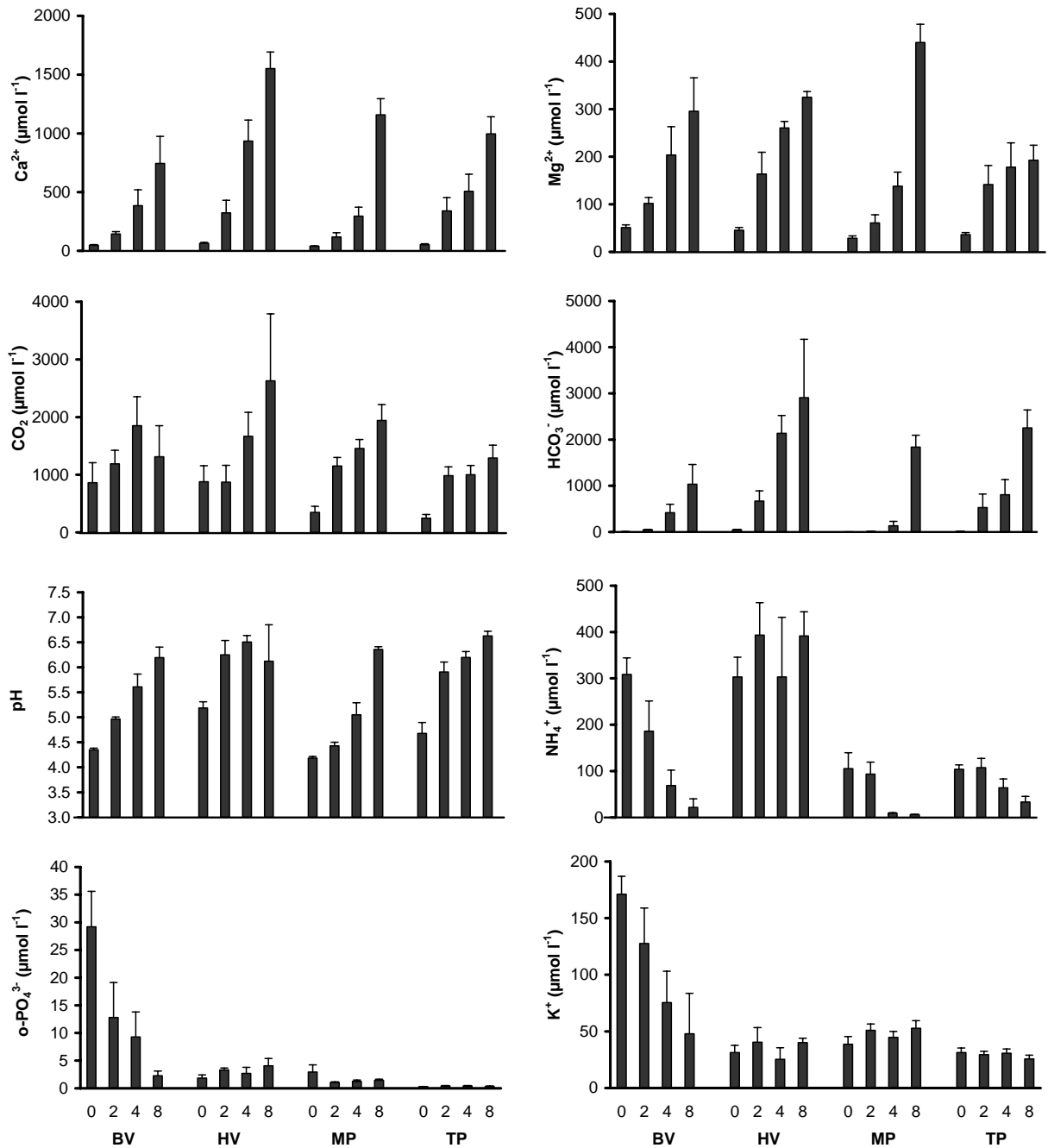| Peat type	< 1 mm	1-5 mm	> 5 mm	Bulk density
	%	%	%	g DW l⁻¹ fresh peat
Bargerveen (BV)	70.2	4.7	25.1	204
Haaksbergerveen (HV)	39.8	15.7	44.5	99
Mariapeel (MP)	60.4	16.9	22.7	128
Tuspeel (TP)	37.4	4.7	57.9	82

Figure 4.3: Potential methane production rate (means + 1 SE; *n* = 3) as measured in peat from four locations and mixed with various amounts of lime (0, 2, 4, 8 and 25 g lime kg^{-1} fresh peat) after 3 months of anaerobic incubation. The length of the lag phase is indicated at the top. Statistics shown in the figure relate to peat type, lime and the interaction between peat type and lime (peat * lime). * $P ≤$ 0.01, ** $P ≤$ 0.001; peat types: BV = Bargerveen, HV = Haaksbergerveen, MP = Mariapeel, TP = Tuspeel.
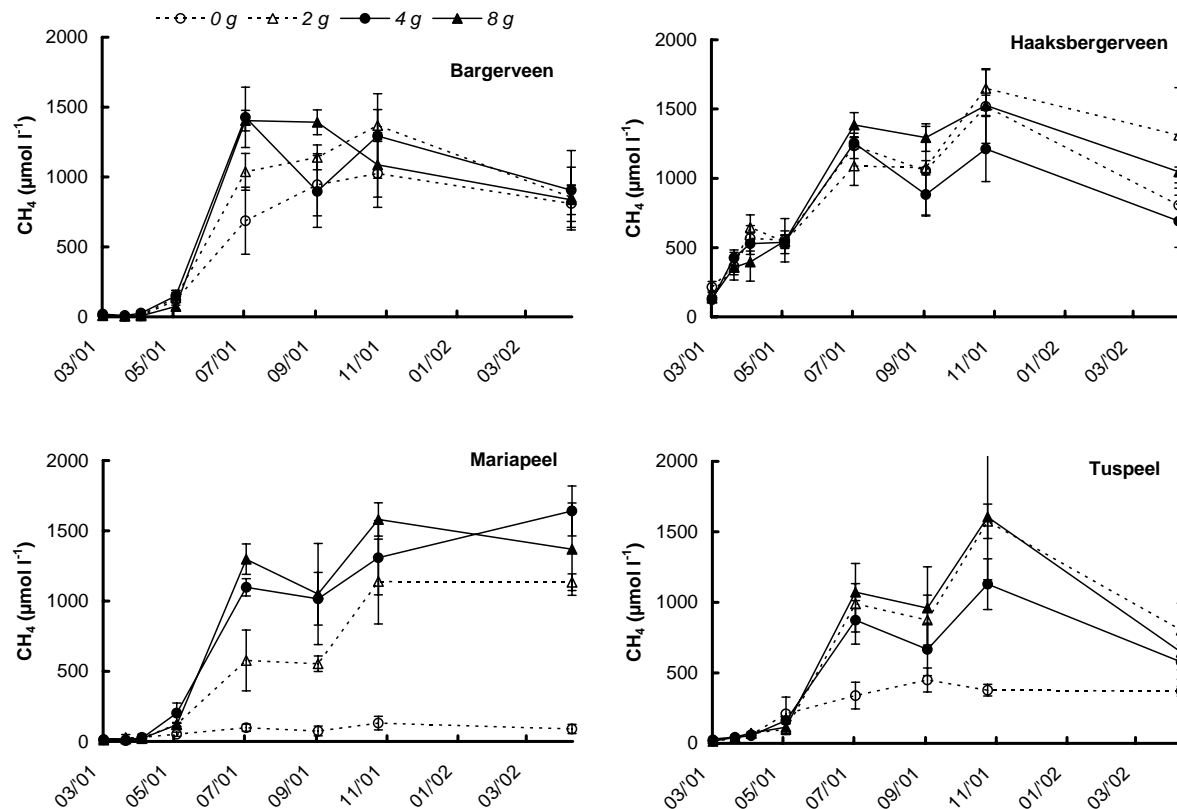
Table 4.3: Within- and between-subject effects in two-way repeated measures analysis of variance (General Linear Models) of the effects of peat type and lime addition on the concentrations of several nutrients in the peat water between March 2001 and May 2002. *F*-values are given, with their level of significance: * $P ≤$ 0.05, ** $P ≤$ 0.01, *** $P ≤$ 0.001.

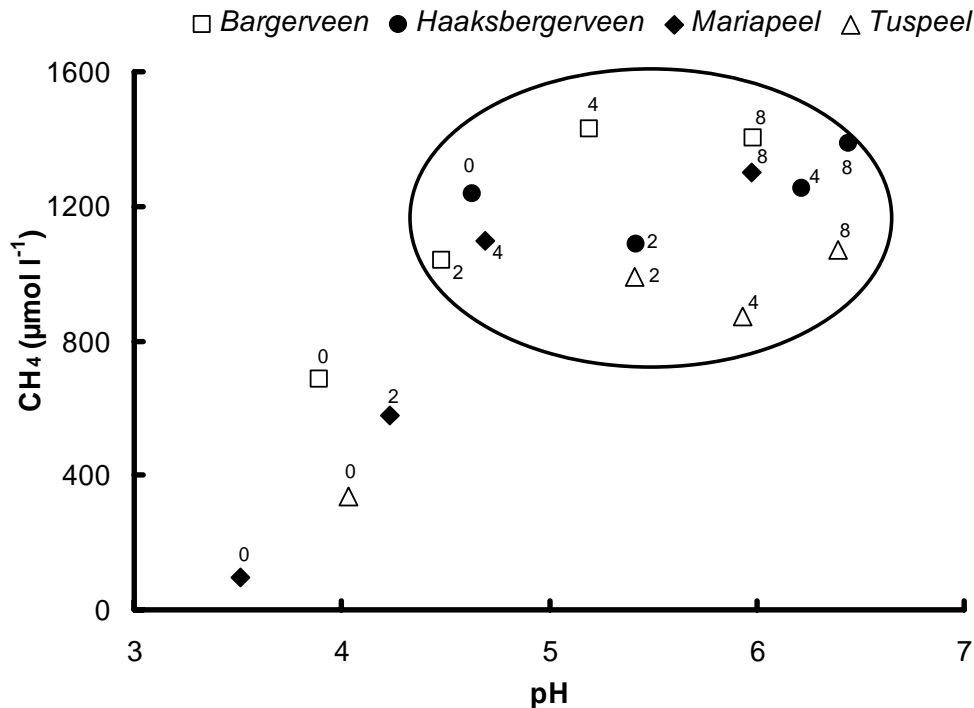|	pH	Ca^{2+}	CO$_2$	HCO$_3^-$	Mg^{2+}	CH$_4$	o-PO$_4^{3-}$	NH$_4^+$	K$^+$
Within-subject effects									
Time (*d.f.* = 7)	16.67***	20.70***	100***	12.33***	13.24***	461***	14.48***	27.77***	43.75***
Time*peat type (*d.f.* = 21)	2.53***	4.06***	3.63***	3.25***	1.12	25.24***	6.75***	15.73***	6.60***
Time*lime (*d.f.* = 21)	2.36***	2.73***	2.61***	1.20	1.94	3.13***	3.33***	7.35***	2.35**
Time*peat type*lime (*d.f.* = 63)	1.30	1.58**	1.80***	1.20	0.80	1.35	1.62**	2.90***	1.33
Between-subject effects									
Peat type (*d.f.* = 3)	29.28***	0.39	30.11***	10.73***	4.38**	119***	259***	97***	257***
Lime (*d.f.* = 3)	129***	123***	69.09***	134***	77.5***	15.85***	7.30***	5.69**	2.94*
Peat type*lime (*d.f.* = 9)	2.58**	0.79	4.07***	3.16**	1.18	6.94***	4.07***	9.83***	1.31

Figure 4.4: Peat water characteristics (means + 1 SE; *n* = 4) of peat from four locations mixed with various amounts of lime (0 – 8 g lime kg^{-1} fresh peat) in May 2002, 14 months after the start of the experiment. Peat types: BV = Bargerveen, HV = Haaksbergerveen, MP = Mariapeel, TP = Tuspeel.

Figure 4.5: Methane concentrations in peat water (means ± 1 SE; n = 4) of peat from four locations and mixed with various amounts of lime (0 – 8 g lime kg^{-1} fresh peat) between March 2001 and May 2002. Results of two-way repeated measures ANOVA for methane are given in Table 4.3.

Addition of lime significantly increased pH values and calcium, magnesium and bicarbonate concentrations in the peat water (Figure 4.4 & Table 4.3). The concentrations fluctuated in the course of the experiment (Table 4.3). Only the concentrations measured in May 2002 are shown in Figure 4.3. Increasing the buffering of the peat substrates had significant effects on the CH$_4$ concentrations in the peat water (Figure 4.5; Table 4.3). During the first months of the experiment, the CH$_4$ concentrations remained very low in the peat water, regardless of the liming level (Figure 4.5). Only in the HV peat the CH$_4$ concentrations were relatively high right from the start of the experiment (Figure 4.5). In the other three peat substrates, the CH$_4$ concentrations started to increase from June 2001, probably as a result of the higher temperatures (Figure 4.2). The CH$_4$ concentrations in the MP and TP substrates were very low in the control treatment; its concentrations increased with the addition of larger amounts of lime (Figure 4.5). In July 2001, buoyancy of the substrates was determined by the pH and CH$_4$ concentrations in the peat water (Figure 4.6). The buoyant substrates showed both high peat water pH and high CH$_4$ concentrations. As a result of the high CH$_4$ concentrations, all HV substrates became buoyant within a few months, regardless of the amount of lime added (Figure 4.6 & 4.7). For the other substrates, buoyancy was first observed in the limed enclosures

(Figure 4.7). For buoyancy of the substrates, a minimum concentration of 400 – 600 μmol l^{-1} CH$_4$ was needed. The addition of unlimed substrates from BV, MP and TP resulted in only partially buoyant peat (Figure 4.6 & 4.7). These substrates also showed some seasonal variation in buoyancy (Figure 4.7).
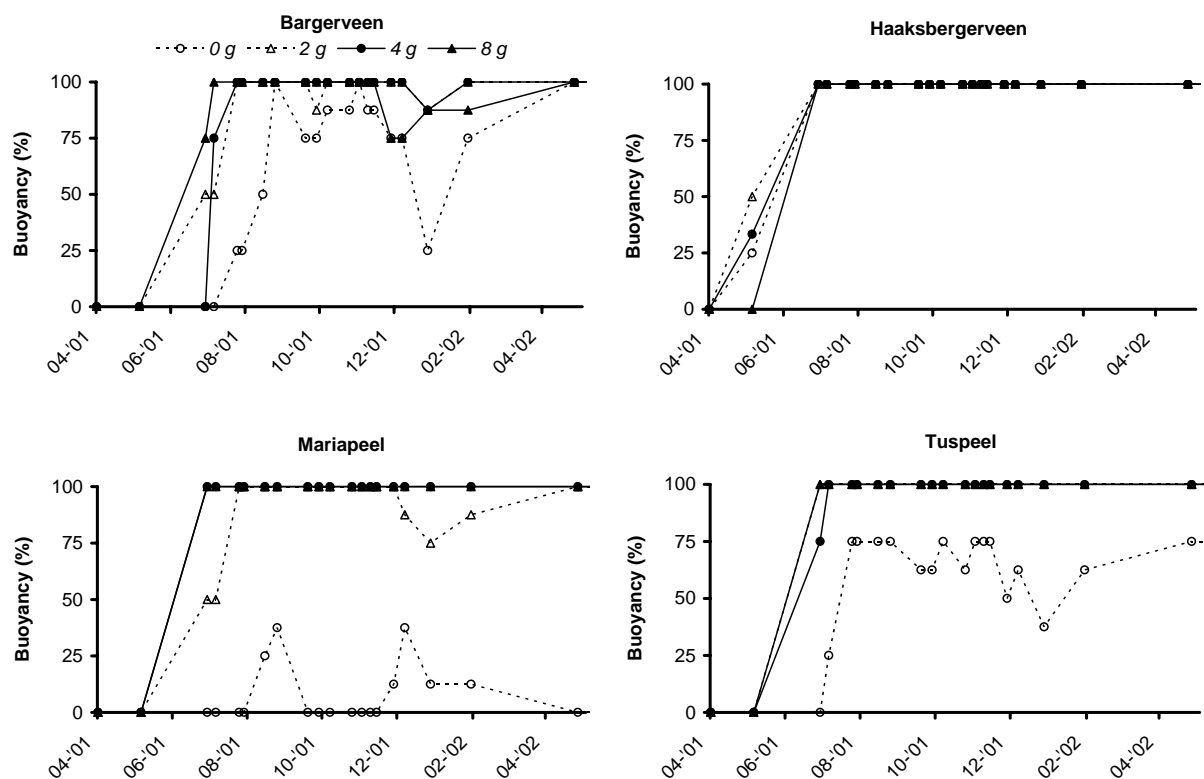

Figure 4.6 : Peat water pH and methane concentrations ($n = 4$) in peat from four locations and mixed with various amounts of lime in July 2001. The substrates within the circle were buoyant, while those outside the circle were completely or partly inundated. The numbers indicate the amount of lime added to the peat (0 – 8 g lime kg^{-1} fresh peat).

The concentrations of the major nutrients (N, P and K) changed significantly in the course of the experiment and were affected by both the type of substrate introduced and the addition of lime (Table 4.3). In the BV substrate, nutrient concentrations were relatively high and addition of lime reduced the peat water nutrient concentrations (Figure 4.4). The HV peat had very high NH$_4^+$ concentrations (300 – 400 μmol l^{-1}), regardless of liming levels, and o-PO$_4^{3-}$ and K$^+$ concentrations were relatively low (Figure 4.4). The major nutrient concentrations were lowest in the MP and TP substrates, and lime addition did not lead to additional mobilisation of nutrients (Figure 4.4).

In July 2002, the vegetation of the floating peat substrates was still poorly developed. Colonisation by *Sphagnum* species proved to be difficult for all substrates. On the BV substrates a dense vegetation dominated by *Juncus effusus* had developed (Figure

4.1). The *J. effusus* cover was significantly greater on the peat mixed with 8 g lime kg⁻¹ than on the unlimed BV peat (Table 4.4).

Figure 4.7: Buoyancy of peat substrates from four locations mixed with various amounts of lime (0 – 8 g lime kg⁻¹ fresh peat) between April 2001 and May 2002. The figures show the percentage of replicates that have become buoyant.

Table 4.4: Estimated cover of *Juncus effusus* (means ± 1 SE; $n = 4$) growing on floating Bargerveen peat mixed with various amounts of lime (0 – 8 g lime kg⁻¹ fresh peat), in July 2002. Different letters indicate significant differences ($P \leq 0.05$) between lime treatments (one-way ANOVA).

Lime treatment	*Estimated cover*
g lime kg⁻¹ fresh peat	*%*
0	30 ± 10[a]
2	40 ± 4[ab]
4	53 ± 6[ab]
8	68 ± 7[b]

Discussion

The role of peat type in floating peat development

In the enclosures which were filled with highly decomposed catotelm peat in September 1999, no floating peat was observed before the introduction of poorly or moderately decomposed peat. It is known that the buoyancy of floating peat mats is provided by CH_4 bubbles accumulating in the peat (Scott *et al.* 1999; Lamers *et al.* 1999; Smolders *et al.* 2002b). The poor substrate quality of highly decomposed, humified peat limits both CH_4 and CO_2 production rates, even though 95% of the peat consists of organic matter (Bridgham & Richardson 1992). The highly decomposed peat used in the experiment had a very low pH (September 1999: pH 3.6 – 3.8), hampering the activity of methanogenic bacteria (Williams & Crawford 1984; Dunfield *et al.* 1993; Segers 1998; Smolders *et al.* 2002b). In addition to the unfavourable pH of the highly decomposed peat, the nutrient concentrations were relatively low (Table 4.1) and CH_4 production may also have been reduced by the low N, P or K concentrations (Swift *et al.* 1979; Updegraff *et al.* 1995; Beltman *et al.* 1996; Smolders *et al.* 2002b).

Introduction of poorly humified peat from four locations in March 2001 led to the development of floating peat within some months (Figure 4.7). The activity of methanogenic micro-organisms is stimulated at higher temperatures (Dunfield *et al.* 1993; Bergman *et al.* 1998, 2000) and the optimum temperatures for CH_4 production in peat soils are between 25 and 30 °C (Williams & Crawford 1984; Dunfield *et al.* 1993). At the start of the experiment, the temperature of the water layer was still relatively low (Figure 4.2), so CH_4 production rates were probably also low. As temperatures increased, methanogenesis was stimulated, so that CH_4 accumulated in the peat (> 400 – 600 µmol l^{-1}), leading to buoyancy of these substrates (Figure 4.7).

Anaerobic incubation of the peat substrates at 20 °C revealed that the lag time for CH_4 production also differed between the various substrates (Figure 4.3). In the HV substrate, CH_4 production started immediately and the peat probably already contained a large population of methanogenic bacteria, since it had a favourable pH and had been collected from a waterlogged, anaerobic location. The other peat substrates were collected from desiccated, acidic sites and probably contained a poorly-developed population of methanogenic bacteria. We conclude that if the peat introduced originates from locations with poor conditions for methanogenic bacteria, it will take longer before floating peat develops.

The time for floating peat to develop differed between the peat types and the liming levels (Figure 4.7). It was particularly the peat collected in HV which rapidly became buoyant. This peat was characterised by a relatively high pH (> 5.0; Figure 4.4), which is favourable for methanogenesis. Increased pH is known to enhance CH_4

production not only by stimulating the activity of methanogenic bacteria but also by increasing the hydrolysis of organic substrates (Kok & Van de Laar 1991). The hydrolysis in turn results in an increased availability of substrates for methanogenic bacteria such as acetate or H_2 (Lamers *et al.* 1999; Smolders *et al.* 2002b). Mobilisation of substrates for methanogenic bacteria depends on the peat chemistry, which was also favourable in the HV substrate, in view of the concentrations and ratios of major nutrients (Table 4.1). Even if the decomposability of the substrate is potentially adequate for methanogenesis, CH_4 production rates may still be low if the peat is very acidic, as was the case with the other peat types. As noted in the Introduction, the pH values of already acidic *Sphagnum* peat layers are nowadays often below 4 (Lamers *et al.* 1999). Development of floating peat proved to be difficult without liming for the acidic BV, MP and TP substrates (Figure 4.7). Anaerobic incubations confirmed the low potential CH_4 production rates in these substrates when no lime was added (Figure 4.3).

The development of floating peat is also influenced by physical characteristics of the peat. Decomposition breaks down larger organic particles into smaller ones, resulting in the peat having a higher bulk density (Wheeler & Shaw 1995; Van Den Pol - Van Dasselaar & Oenema 1999; Bozkurt *et al.* 2001). According to the bulk density classification of Grumpelt (1991), the peat substrates from HV, MP and TP were moderately decomposed and that from BV highly decomposed (Table 4.2). CH_4 production rates are usually highest in the relatively coarse and light organic fractions, which can also easily trap gas bubbles (Hassink 1995; Van Den Pol - Van Dasselaar & Oenema 1999; Bozkurt *et al.* 2001; Smolders *et al.* 2002a). Due to the combination of higher CH_4 production rates and the relatively low bulk density, poorly humified peat floats more easily than strongly humified peat. The BV substrate, however, had a high CH_4 production rate, which caused good buoyancy, despite a high bulk density. This substrate was obtained from sod-cutting activities in a wet heathland vegetation and probably contained a large amount of easily degradable compounds such as root exudates and fresh litter (Bergman *et al.* 2000).

Addition of lime and floating peat development

Mixing in lime with the peat substrates significantly increased the CH_4 production rates and led to increased CH_4 concentrations in the peat water. CH_4 production in the HV substrate was not stimulated by the addition of lime, since the substrate already had a relatively high pH and high CH_4 production rates. CH_4 production rates in the other peat types were stimulated by the addition of lime. Liming increased the pH and bicarbonate buffering of the substrates, which is known to stimulate CH_4 production rates, provided that the availability of organic substrates is not rate-limiting. Addition of 2 g of lime proved to stimulate CH_4 production sufficiently to create and maintain floating peat for at least one year using the acidic

peat substrates collected in BV, MP and TP (Figure 4.7). Lime addition levels should not be higher than necessary, as there is evidence that the combination of a high pH and high Ca^{2+} concentrations is lethal to many *Sphagnum* species (Clymo 1973). In Estonia, precipitation of calcium-rich alkaline fly ash increased peat water pH and calcium concentrations, leading to the disappearance of *Sphagnum* mosses (Karofeld 1996). The addition of 4 and 8 g lime increased Ca^{2+} concentrations to levels above 500 μmol l^{-1} (at depths of 0 – 10 cm), which are toxic to *Sphagnum* (Money 1995; Sliva & Pfadenhauer 1999), and could hamper future *Sphagnum* development on the newly created floating peat. The presence of an unlimed top layer probably meant that the pH and Ca^{2+} concentrations in the top layer of the floating peat were much lower. The unlimed top layer, also left pH values and calcium, magnesium and bicarbonate concentrations in the water layer unaffected by lime addition (data not shown).

High pH values raise not only CH_4 but also CO_2 production rates (e.g. Yavitt *et al.* 1997). In addition, the added calcium carbonate will dissolve, resulting in CO_2 release. Increased benthic CO_2 concentrations may be beneficial, as they are known to stimulate the growth of *Sphagnum* species establishing on newly formed floating rafts (Lamers *et al.* 1999; Smolders *et al.* 2001). Turetsky and Wieder (1999) have proved empirically that the refixation of C may be an important pathway for C cycling within peatlands, potentially capturing significant proportions of peat-produced CO_2 before it escapes to the atmosphere.

Lime addition and eutrophication

The addition of large amounts of lime might lead to eutrophication due to excessive decomposition of the peat (Lamers *et al.* 1999; Smolders *et al.* 2002b). We found no evidence for this, even in the final month of our experiment, there was still no stimulated nutrient mobilisation by lime addition (Figure 4.4) In the BV substrate, the NH_4^+, o-PO_4^{3-} and K^+ concentrations even decreased with increasing amounts of lime mixed in with the peat. Binding of o-PO_4^{3-} to calcium probably resulted in the precipitation of calcium-phosphate complexes (apatite and hydroxyapatite) and in lower o-PO_4^{3-} concentrations in the peat water of the BV substrate (Stumm & Morgan 1981). Uptake of nutrients by the dense vegetation on the BV substrates probably also lowered nutrient concentrations, since the vegetation cover was significantly increased by the addition of lime (Table 4.4). At increased pH buffering of the peat, the reduced availability of H^+ ions will result in an increased cation adsorption capacity of the peat and probably in increased adsorption of NH_4^+ and K^+ (e.g. Stumm & Morgan 1981).

N and P concentrations in the peat water were relatively high for all peat types. While the NH_4^+ concentrations in the unlimed peat substrates ranged from 100 to 300 μmol l^{-1}, field values for *Sphagnum*-dominated locations vary between 10 and 50 μmol

l^{-1} in the Netherlands (0 - 10 cm depth; Tomassen *et al.* 2002). *O*-PO$_4^{3-}$ concentrations as high as 30 μmol l^{-1} were measured in the BV substrate, while concentrations of 3 μmol l^{-1} are relatively high for Dutch bogs (Tomassen *et al.* 2002). These high nutrient concentrations could have negative effects on the development of a *Sphagnum*-dominated vegetation in the future. *Sphagnum* mosses immobilise most of the nutrients supplied from the atmosphere and thereby restrict vascular plants to nutrients available from mineralisation processes in the peat layer (e.g. Malmer *et al.* 1994). High nutrient availability in the peat water increases the growth of vascular plants, which may hamper *Sphagnum* growth due to shading (e.g. Hayward & Clymo 1983). On the substrates from BV, high P, N and K concentrations led to the development of a dense *Juncus effusus* vegetation, which may complicate colonisation and growth of *Sphagnum* species by shading.

The colonisation of the newly created floating peat by *Sphagnum* species proved to be very slow. After one year, *Sphagnum* establishment was rarely observed. The introduced peat contained no viable diaspores of *Sphagnum* species and colonisation by *Sphagnum* from nearby locations was not possible in our experiment. The growth of *Sphagnum* species and peat accumulation, however, are essential to maintain high CH$_4$ production rates in the long term, by providing new organic matter and ensuring long-term development of the rafts.

Perspectives for bog restoration by floating peat development

Our experiment showed that the development of floating peat can indeed be successfully stimulated by adding relatively poorly humified peat. If the peat is too acidic (pH < 4.5), mixing in small amounts of lime with the peat (2 g lime kg^{-1} fresh peat) is sufficient to increase CH$_4$ production rates. Since anaerobic decomposition processes and CH$_4$ production are acid-consuming processes (see, e.g., Lamers *et al.* 1999), liming is only necessary to initiate methanogenesis. The results obtained in this outdoor experiment provide a good indication of the feasibility for the creation of floating rafts on deeply inundated locations in cut-over bogs. On a deeply inundated location in the Mariapeel reserve, introduction of poorly humified limed peat in large enclosures also led to buoyant peat (Smolders & Tomassen unpublished).

In countries in which poorly humified peat is relatively scarce, the introduction of substrate is only feasible on a relatively small scale. In our experiment, we used the equivalent of 2000 tonnes of peat per hectare, while an additional 4000 kg of lime ha^{-1} was required for acidic peat substrates. Even if the availability of donor peat is not a problem, this type of restoration measures is rather expensive and time-consuming. Therefore, the introduction of peat should probably be restricted to situations where only strongly humified peat is left and shallow inundation of the cut-over bog is not possible (see also Smolders *et al.* 2003).

Nevertheless, we think that the introduction of peat could be a useful restoration method in these deeply inundated bogs which lack other ways for successful restoration. In order to obtain sufficient donor peat, the top layer of the peat could be removed on sites which are still severely drained despite hydrological measures to reduce water shortage. This removed peat could then be introduced on deeply inundated sites provided that this peat has the appropriate chemical and physical characteristics for buoyancy. At the same time wetter conditions will be created on the donor site.

Acknowledgements

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Chapter 4

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Expansion of invasive species on ombrotrophic bogs: desiccation or high N deposition?

Experimental site of the nitrogen addition experiment at Clara bog, Ireland

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Expansion of invasive species on ombrotrophic bogs: desiccation or high N deposition?

Abstract

1. In many ombrotrophic bog areas the invasion of grass (e.g. *Molinia caerulea*) and tree (e.g. *Betula pubescens*) species, has become a major problem. We investigated whether the invasion of such species is due to high atmospheric nitrogen (N) deposition by conducting a fertilisation experiment.

2. The effects of experimentally increased N input on *Molinia, Betula* and *Eriophorum vaginatum* were studied in desiccated bog vegetation in Ireland where there is relatively low background N deposition. Four different N treatments were applied for 3 years: 0 (control), 2, 4 and 8 g m^{-2} year^{-1}.

3. Ammonium and nitrate concentrations in the peat moisture increased at high N addition rates, leading to significantly higher carbon:nitrogen (C:N) and nitrogen:phosphorus (N:P) ratios in the top layer of the peat. The potential CO_2 production rate of the peat was not stimulated at high N addition rates due to severe acidification of the peat.

4. Despite high tissue N:P ratios (above 40), above-ground biomass production by *Molinia* was stimulated at high N addition rates, and foliar nutrient concentrations were unaffected. In contrast to *Molinia, Betula* and *Eriophorum* were unable to increase their above-ground biomass, probably due to P limitation. Regrowth of the lichen *Cladonia portentosa* was suppressed at high N addition rates.

5. *Synthesis and applications.* We conclude that the invasion of bogs by *Molinia* and *Betula* is likely to be less affected by desiccation than by increased N availability. Apparently, *Molinia* is well adapted to P-limiting conditions, which may explain its success in regions with increased N deposition levels. The high availability of P in many Dutch bogs compared with Irish bogs, together with prolonged high N deposition levels, may explain the strong increase in both *Molinia* and *Betula* observed in the Netherlands. As long as N and P availabilities in Dutch bogs are too high to prevent invasion of *Betula* and/or *Molinia*, management measures stimulating growth of *Sphagnum* mosses could probably reduce the negative effects of high N deposition levels.

Introduction

Levels of atmospheric nitrogen (N) deposition have greatly increased over large parts of north-west Europe during the past century, reaching levels of 1.5 – 6 g N m^{-2} year^{-1} (Bobbink & Heil 1993). There is clear evidence that this has forced ecological changes in oligotrophic N-limited ecosystems (Roelofs 1986; Aerts *et al.* 1992; Bobbink *et al.* 1998). For dwarfshrub heathlands, increases in the long-term rate of N supply can cause a shift in the composition of the vegetation towards more competitive species (Heil & Diemont 1983; Berendse & Aerts 1984; Roelofs 1986; Aerts & Berendse 1988), a process accompanied by the loss of more sensitive species (Press *et al.* 1986; Pitcairn *et al.* 1995). At the end of the 1960s, a rapid loss of the lichen and bryophyte flora was observed in the Netherlands, due to high atmospheric N levels (Van Dobben *et al.* 1983; De Smidt & Van Ree 1991; Greven 1992). Among the ecosystems that are most sensitive to N enrichment are ombrotrophic bogs, which receive most of their nutrients from the atmosphere (Bobbink *et al.* 1998). The critical load for ombrotrophic bogs (the maximum amount of deposition that does not lead to changes in the composition of the vegetation) has been estimated to be between 0.5 and 1 g N m^{-2} year^{-1} (Bobbink & Roelofs 1995; Risager 1998; Tomassen *et al.* 2003).

Ombrotrophic bogs are unique wet ecosystems, supporting characteristic plant and animal communities. World-wide, the area of bogs has been drastically reduced by human activities such as peat cutting, and bogs often suffer the effects of drainage. As a response to the present-day rarity of typical bog vegetation, the conservation and restoration of bogs has become important (Joosten 1995). In areas with increased N deposition levels the invasion of ombrotrophic bogs by certain nitrophilous grass (e.g. *Molinia caerulea*) and tree (*Betula pubescens*) species has occured, together with a decline of ombrotrophic species (Barkman 1992; Aaby 1994; Hogg *et al.* 1995; Risager 1998). Under wet conditions *Sphagnum* has high N uptake rates, resulting in low N concentrations in the peat moisture (e.g. Lee & Woodin 1988; Jauhiainen *et al.* 1998; Lamers *et al.* 2000), with vascular plants depending on N mobilised by mineralisation processes in the underlying peat (Malmer *et al.* 1994). At high N deposition rates (> 2 g N m^{-2} year^{-1}), however, the *Sphagnum* layer reaches its maximum N content and N leaches from the *Sphagnum* layer to the roots of vascular plants (Lee & Woodin 1988; Aerts *et al.* 1992; Lamers *et al.* 2000).

Several authors, however, have ascribed the invasion of nitrophilous species to increased mineralisation rates as a result of desiccation (e.g. Eigner 1995) rather than to increased inputs of air-borne N. Hayward & Clymo (1983) suggested that increased nutrient availability in a situation of desiccation is not only the result of increased mineralisation rates, but is probably also caused by reduced growth of *Sphagnum* mosses at low water tables. In desiccated *Sphagnum*, gas exchange is suppressed, resulting in reduced photosynthetic rates and growth (Schipperges & Rydin 1998), reduced immobilisation of nutrients by the *Sphagnum* layer (Aldous

2002) and increased availability of nutrients for vascular plants. In addition, during periods of surface water stress, the productivity of *Sphagnum* mosses may be much lower than that of vascular plants (Malmer *et al.* 1994). Saplings of *Betula*, for instance, are less rapidly overgrown by *Sphagnum* suffering from water stress.

In the Irish midlands no invasion of *Molinia* and *Betula* has yet occured, even on (non-excavated) severely desiccated bog areas (H.B.M. Tomassen, personal observations). As N deposition levels in the Netherlands are much higher than in the Irish midlands, differences in peat moisture N availability could be responsible for the vegetation changes observed in the Netherlands. This idea is supported by the observed invasion of *Molinia* and *Betula* in ombrotrophic vegetation on floating rafts that are permanently wet but are subjected to high N loads (Lamers *et al.* 2000). Because experimental evidence is lacking, it remains unclear whether the invasion of Dutch bogs by *Betula* and *Molinia* is due to desiccation, high N deposition levels or a combination of the two.

We hypothesised that the observed vegetation changes in the Netherlands are mainly the result of high N deposition levels, leading to high N concentrations in the peat moisture, thereby promoting the growth of nitrophilous species such as *Molinia*, *Betula* and *Eriophorum vaginatum*. To test this hypothesis, the growth of *Molinia*, *Betula* and *E. vaginatum* was investigated in a 3-year N fertilisation experiment on a desiccated location in an Irish raised bog. Four different N addition rates were used, ranging from 0 to 8 g m^{-2} year^{-1}. In addition, differences in the nutritional status of Dutch and Irish bogs were determined by comparing nutrient concentrations in peat moisture samples.

Materials and methods

Field site

We studied the effects of high N deposition levels in a field experiment in Ireland (Clara bog) to avoid the high current background N input of 4 g m^{-2} year^{-1} in the Netherlands. The Irish bogs resemble the former Dutch bogs closely, but background N deposition levels are considerably lower. Clara bog (County Offaly; 53°19.504'N; 7°36.544'W) is situated in a depression adjacent to a complex of esker ridges, in the midlands of Ireland. Clara bog (665 ha) is 4.24 km long and 1.20 km wide and divided by a road into a western and an eastern part. In 1983, the eastern part of Clara bog was drained in preparation for peat extraction. Although the drains were blocked in 1989 and 1995-1996, large parts of Clara East are still severely desiccated due to drainage and small-scale peat extraction. The experimental site was located at the edge of a cut-away, where the peat was severely desiccated. *Sphagnum* has

completely disappeared from this site, which is currently dominated by *Calluna vulgaris* (L.) Hull (0.5 – 0.75 m height), *Cladonia portentosa* (Dufour) Coem. and some *Eriophorum angustifolium* Honck.

Background deposition level

Between March 2000 and March 2001, background deposition levels of nutrients were measured at the experimental site by collecting bulk precipitation. Each of the rainwater collectors (*n* = 3) consisted of a black polyethylene bottle (2 l) connected to a funnel (74.5 cm²), containing 2 ml of a 200 mg l^{-1} $HgCl_2$ solution to inhibit microbial activity. A plastic filter was placed in the funnel to avoid contamination of the rainwater by organic matter and insects. The collector was placed in a PVC pipe dug into the peat, and rainwater was collected 20 cm above ground level. Six times a year, samples were collected and their volume measured in the field. pH was determined within 4 hours of collection and the samples were stored at –20 °C until further analysis.

Experimental design

In July 1998, 16 plots measuring 150 x 150 cm were set out on the eastern part of Clara bog. Before the target species were introduced, the above-ground biomass of the standing vegetation was removed to avoid shading by the dense *Calluna* vegetation. In each plot, three *Betula pubescens* Ehrh. saplings, one *Molinia caerulea* (L.) Moench tussock and one *Eriophorum vaginatum* L. tussock were introduced, all of which had been collected from a nearby cut-away.

Three soil moisture samplers (Rhizon, 10 cm; Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands), were placed in each of the plots, to a depth of 0 - 10 cm, to allow extraction of the peat moisture for the purpose of analysing its chemical composition. Four treatments were applied, differing in N concentrations and leading to N loads of 0, 2, 4 and 8 g N m^{-2} $year^{-1}$ (0 – 0.57 mol N m^{-2} $year^{-1}$). Each treatment consisted of four replicates, randomly assigned to the plots. N was partially supplied as ammonium sulphate, since co-deposition of sulphate and ammonium has frequently been observed in the Netherlands (Bobbink & Heil 1993). N was added as ammonium (70%) and nitrate (30%) using NH_4NO_3 and $(NH_4)_2SO_4$, based on the current ratios in the Netherlands (1994 situation; Lamers 1995), which resulted in additional sulphur (S) loads of 0, 0.9, 1.8 and 3.7 g S m^{-2} $year^{-1}$ (0 – 0.11 mol S m^{-2} $year^{-1}$). N was dissolved in local bog water (5 l) and sprayed over the plots with a watering can. We were careful to add the nutrients during rainy weather, or the nutrients were applied in a smaller volume of bog water after which the remainder was used to wash away the nutrients and to avoid damage to the leaves. The experiment lasted from July 1998 to September 2001, N being supplied in 12 monthly applications during the first year. In the subsequent 2 years, due to practical

limitations N was added in six applications during the growing season. The controls received only bog water in the same amount used in the N treatments.

Sampling and measurements

Before every application of N, peat moisture was collected by connecting 50 ml syringes to each sampler. The three subsamples were pooled and pH was measured. Citric acid was added to a final concentration of 0.6 mmol l^{-1} to prevent metal precipitation; samples were then stored (for a maximum of 6 weeks) in iodated polyethylene bottles (50 ml) at –20 °C until further analysis.

Growth of *Betula*, *Molinia* and *E. vaginatum* was estimated non-destructively twice a year. Growth of *Betula* was estimated from the height and the number of leaves, that of *Molinia* from the number and length of leaves and inflorescences, that of *E. vaginatum* from the diameter, number of inflorescences and height. At final harvest, the above-ground biomass of *Betula*, *Molinia* and *E. vaginatum* was carefully cut and sorted into leaves, stems and inflorescences. Regrowth of *Calluna* and *C. portentosa* was measured by estimating the cover. Next, samples were taken from *Betula*, *Molinia*, *E. vaginatum*, *Calluna* and *C. portentosa* for foliar nutrient analysis. Peat samples were taken (5 subsamples per plot; 0 – 5 cm depth) and stored at 4 °C in airtight polyethylene bags until further analysis.

Nutritional status of Dutch bogs versus Irish bogs

Between 1998 and 2001, nutrient concentrations in the peat moisture were measured at various locations in the Netherlands [n_{total} = 12: Bargerveen (n = 3), Dwingelerveld (n = 3), Fochteloërveen (n = 1), Haaksbergerveen (n = 2), Korenburgerveen (n = 2) and Tuspeel (n = 1)] and in Ireland [n_{total} = 7; Clara bog (n = 3), Raheenmore bog (n = 2) and Sharavogue bog (n = 2)]. The vegetation at the experimental sites was dominated by *Sphagnum* species. Peat moisture was collected four times a year at a depth of 0 - 10 cm using ceramic soil moisture samplers (Eijkelkamp Agrisearch Equipment) connected to 50 ml syringes. Samples were stored in iodated polyethylene bottles (50 ml) at –20 °C until further analysis.

Chemical analysis

Peat moisture pH was determined with a combination pH electrode with an Ag/AgCl internal reference (Orion Research, Beverly, CA). Peat samples were thoroughly mixed and large roots were removed. To analyse nutrient concentrations in plant tissue and peat, dried samples (48 hours at 70 °C) were ground in liquid N. N and carbon (C) concentrations were measured with a CNS analyser (type NA1500; Carlo Erba Instruments, Milan, Italy). Phosphorus (P), potassium (K) and S

concentrations were determined by digesting 200 mg of dried material in sealed Teflon vessels in a Milestone microwave oven (type mls 1200 Mega, Milestone Inc., Sorisole, Italy) after addition of 4 ml HNO_3 (65%) and 1 ml H_2O_2 (30%). After dilution, the digests were kept at 4 °C until analysis.

Water-extractable and exchangeable nutrients and minerals were determined by adding 200 ml bi-distilled water or 0.2 mol l^{-1} NaCl, respectively, to 40 g of thoroughly mixed peat. The mixtures were shaken for 1 hour (100 movements min^{-1}; VKS 75 Shaker; Edmund Bühler; Tübingen, Germany), after which the pH of the solution was measured. The suspension was then centrifuged (12000 r.p.m. for 20 min; 23,500 g) and the supernatant stored in 50 ml polyethylene bottles at -20 °C. Potential C mineralisation rates (CO_2 production) were measured by aerobic incubation of 100 g peat in 250 ml infusion flasks, sealed with an airtight stopper. For each plot incubations were carried out in duplicate. The flasks were kept in the dark at 20 °C, and CO_2 concentrations were measured in the headspace, using an infrared carbon analyser (model PIR-2000, Horiba Instruments, Irvine, CA, USA), for 6 weeks. CO_2 production rates were calculated by linear regression and expressed on a dry weight (DW) basis.

The concentration of ortho-phosphate was measured colorimetrically with a Technicon AA (Auto Analyser) II system (Technicon Instruments Corp., Tarrytown, NY, USA), using ammonium molybdate (Henriksen 1965). Nitrate and ammonium were measured colorimetrically with a Traacs 800+ auto-analyser, using hydrazine sulphate (Technicon 1969) and salicylate (Grasshoff & Johannsen 1977) respectively. K was measured by flame photometry (FLM3 Flame Photometer, Radiometer, Copenhagen, Denmark). S and P were determined by inductively coupled plasma emission spectrophotometry (type FLAMEVML2-9032034; Spectro Analytical Instruments, Kleve, Germany).

Statistical analysis

Prior to statistical analysis, data were log-transformed to make the variance less dependent on the means and to fit a normal distribution. All statistical analyses were carried out using the SPSS for Windows software package (version 10.0.7; SPSS Inc., Chicago, IL). Differences between treatments were tested with a one-way ANOVA at the 0.05 confidence limit. Tukey post-hoc tests were used to identify differences between treatments. Differences in peat moisture concentrations during the experiment were tested with the general linear model (GLM) procedure for repeated measures. One experimental plot of the control treatment was found to be situated in a small depression and remained significantly wetter during the experiment. This wet plot produced more *Molinia* biomass; this replicate was therefore excluded from the data set (Dixon's test: $P \leq 0.01$; Sokal & Rohlf 1981). Differences in growth

parameters of *Molinia* between treatments were identified using a univariate GLM procedure with a Tukey post-hoc test. Possible correlations between growth parameters and peat moisture N concentrations, and between peat characteristics and potential CO_2 production rates, were assessed with a Pearson correlation. Differences in nutrient concentrations between Dutch and Irish bogs were tested with an independent samples *t*-test. For clarity of presentation, the means and standard errors (SE) presented in the figures represent the non-transformed data.

Results

Background deposition levels on Clara bog

The bulk N deposition level at Clara bog was 0.46 g N m⁻² year⁻¹, comprising of 30% oxidised N (NO_x) and 70% reduced N (NH_x) (Table 5.1). The deposition level of P was 0.01 g P m⁻² year⁻¹ and the bulk deposition level of S was 0.38 g S m⁻² year⁻¹. At the experimental site, N applications constituted an extra rainfall of 13 mm year⁻¹. The amount of N supplied by the bog water used was negligible (Table 5.1).

Table 5.1: Amount of precipitation and bulk deposition levels of N (NH_4 and NO_3), P, K and S on Clara bog (mean ± 1 SE; *n* = 3; March 2000 – March 2001) and in the 'Pikmeeuwenwater' (the Netherlands; 1994-95; L.P.M. Lamers & H.B.M. Tomassen, unpublished data), and the flux of nutrients deposited by added bog water (in 2001).
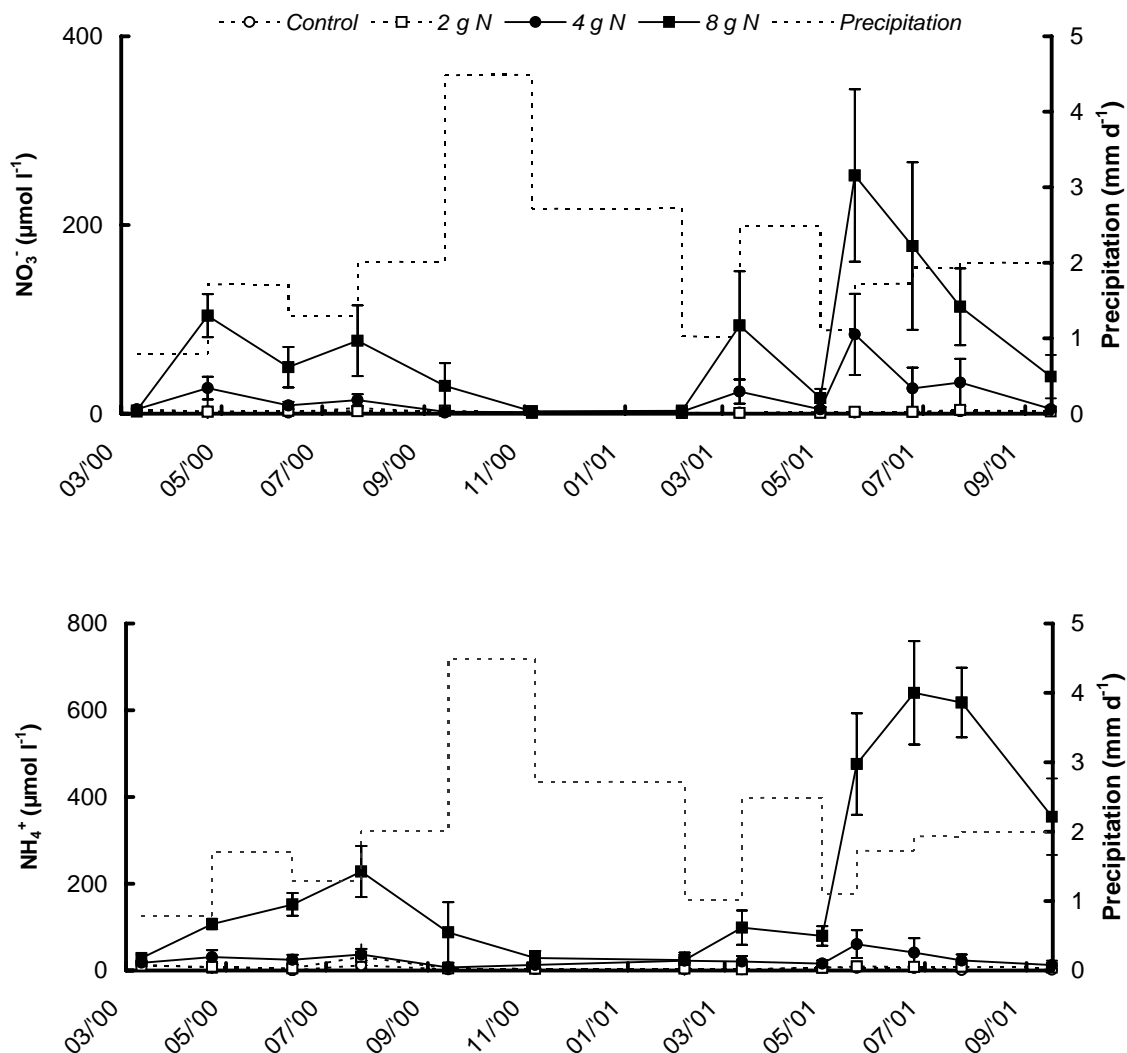
	Precipitation	*N-NH₄⁺*	*N-NO₃⁻*	*N*	*P-PO₄³⁻*	*K*	*S*
	mm	*g m⁻² year⁻¹*	*g m⁻² year⁻¹*	*g m⁻² year⁻¹*	*g m⁻² year⁻¹*	*g m⁻² year⁻¹*	*g m⁻² year⁻¹*
Bulk deposition level Clara bog	1003 ± 36	0.32 ± 0.05	0.13 ± 0.00	0.46 ± 0.05	0.01 ± 0.00	0.25 ± 0.06	0.38 ± 0.02
Bulk deposition level Pikmeeuwenwater	874	1.26	0.70	1.96	0.03	0.32	0.99
Bog water	13	0.001	0.000	0.002	0.001	0.009	0.017

Water chemistry

Additions of N led to a significant increase in peat moisture ammonium and nitrate concentrations ($P \leq 0.001$; Figure 5.1). The N concentrations showed a seasonal pattern with high concentrations in summer and low concentrations in winter. Concentrations measured during the summer of 2001 were higher than those measured in the summer of 2000, especially in the highest N treatment (Figure 5.1). Ammonium and nitrate concentrations remained low in the control and 2 g N m⁻² year⁻¹ treatments. Addition of 4 and 8 g N m⁻² year⁻¹ increased ammonium and nitrate concentrations significantly. Sulphate (measured as total S) concentrations

were significantly increased at higher addition rates ($P \leq 0.001$; GLM for repeated measures; data not shown), as ammonium was partly added as ammonium sulphate. Peat moisture sulphate concentrations showed a similar seasonal pattern as the ammonium and nitrate concentrations, with high concentrations in summer.

The pH in the peat moisture was very low during the experiment and ranged from 4.0 to 2.8, being significantly lower at the higher N addition rates ($P \leq 0.05$; GLM for repeated measures; data not shown). The pH decreased during the summer and increased again in winter. For all N treatments, phosphate and K concentrations were below 1 µmol l^{-1} and 20 µmol l^{-1}, respectively, and did not differ significantly between the treatments (data not shown).

Figure 5.1: Peat moisture nitrate and ammonium concentrations (mean ± 1 SE; $n = 4$) from March 2000 until September 2001 at various experimental N addition rates: control (0), 2, 4 and 8 g N m^{-2} $year^{-1}$. Amount of precipitation is indicated by the dotted line. N effect: $P \leq 0.001$ (GLM for repeated measures).
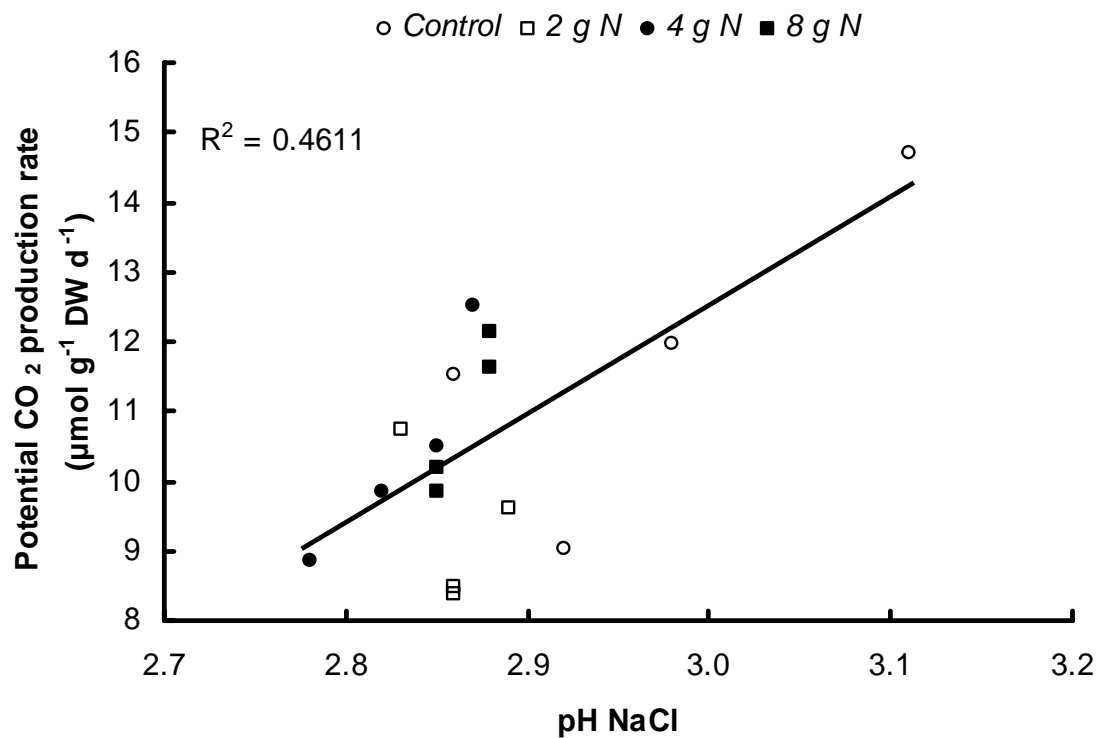
Peat chemistry

After 3 years of experimental N addition, C:N, C:P and N:P ratios of the peat were significantly affected (Table 5.2). C:N ratios were significantly lower ($P \leq 0.01$), and C:P ratios were significantly higher ($P \leq 0.05$), than in the control treatment at an addition rate of 8 N g m^{-2} year^{-1}. N:P ratios at addition rates of 4 g N m^{-2} year^{-1} and higher were significantly increased compared with the control treatment ($P \leq 0.001$; Table 5.2). There was no correlation between potential CO_2 production rates of the peat and the various nutrient ratios, but higher pH_{NaCl} of the peat significantly increased potential CO_2 production rates ($P \leq 0.01$; Figure 5.2). Water-extractable ammonium and sulphate concentrations were increased at extra N loads of 4 and 8 g m^{-2} year^{-1}, respectively ($P_{ammonium} \leq 0.001$ and $P_{sulphate} \leq 0.05$; Table 5.3). Water-extractable ammonium concentrations did not differ between the control and 2 g m^{-2} year^{-1} treatments. Doubling the addition rate from 2 to 4 g N m^{-2} year^{-1} increased water-extractable ammonium concentrations threefold, and doubling input rates from 4 to 8 g N m^{-2} year^{-1} led to a fivefold increase. Ammonium concentrations measured in sodium extractions were two to six times as high as those in water extractions (Table 5.3).

Table 5.2: C:N, C:P and N:P ratios and potential CO_2 production rates of the peat (means $\pm$ 1 SE; $n = 4$) at various N addition rates, after 3 years. Different letters indicate significant differences ($P \leq 0.05$) between N treatments (one-way ANOVA).
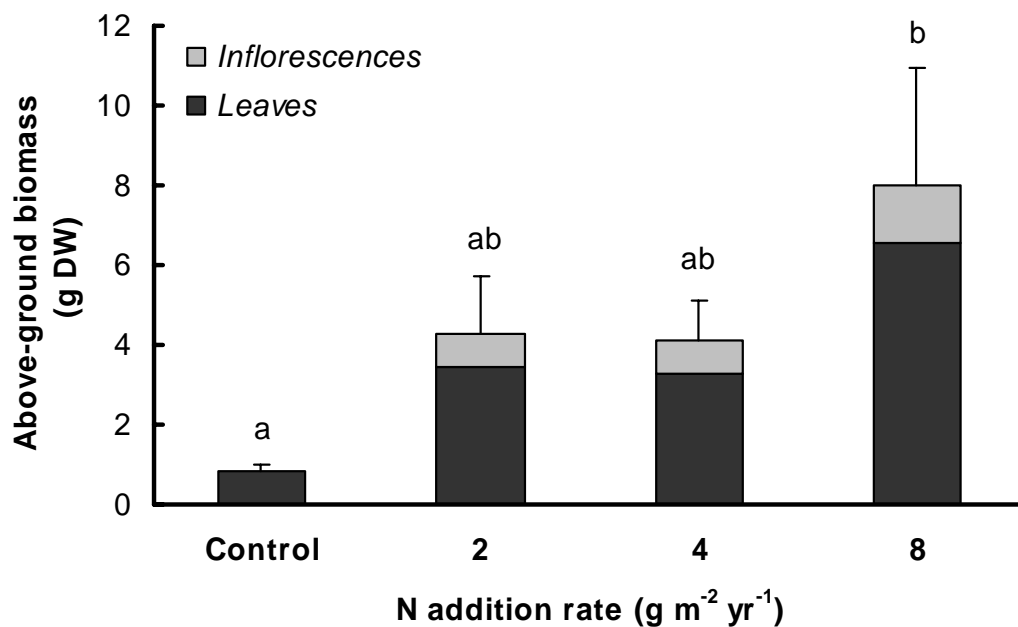
N addition rate	C:N ratio	C:P ratio	N:P ratio	Potential CO₂ production rate
g m⁻² year⁻¹	g g⁻¹	g g⁻¹	g g⁻¹	μmol g⁻¹ DW d⁻¹
Control (0)	37 ± 1[a]	1409 ± 56[a]	38 ± 1[a]	11.81 ± 1.17
2	38 ± 1[a]	1561 ± 24[ab]	41 ± 0[ab]	9.30 ± 0.55
4	35 ± 0[ab]	1552 ± 23[ab]	44 ± 1[bc]	10.43 ± 0.77
8	34 ± 0[b]	1618 ± 40[b]	47 ± 1[c]	10.94 ± 0.55

Table 5.3: Concentrations of water-extractable nitrate, ammonium, phosphate and sulphate and exchangeable ammonium in the top layer of the peat (μmol kg⁻¹ DW; mean $\pm$ 1 SE; $n = 4$) after 3 years at various N addition rates. Different letters indicate significant differences ($P \leq 0.05$) between N treatments (one-way ANOVA).

N addition rate	NO₃⁻ (H₂O)	NH₄⁺ (H₂O)	PO₄³⁻ (H₂O)	SO₄²⁻ (H₂O)	NH₄⁺ (NaCl)
g m⁻² year⁻¹	μmol kg⁻¹ DW	μmol kg⁻¹ DW	μmol kg⁻¹ DW	μmol kg⁻¹ DW	μmol kg⁻¹ DW
Control (0)	45 ± 9	140 ± 21[a]	69 ± 9	791 ± 96[a]	257 ± 41[a]
2	74 ± 11	129 ± 16[a]	48 ± 8	758 ± 56[a]	459 ± 216[a]
4	74 ± 25	423 ± 42[b]	64 ± 21	997 ± 115[ab]	1706 ± 482[b]
8	143 ± 19	2261 ± 264[c]	71 ± 11	1360 ± 165[b]	13189 ± 1832[c]

Figure 5.2: Potential CO_2 production rate and pH_{NaCl} of the peat after 3 years of exposure to various experimental N addition rates. Pearson correlation: $P \leq 0.01$.
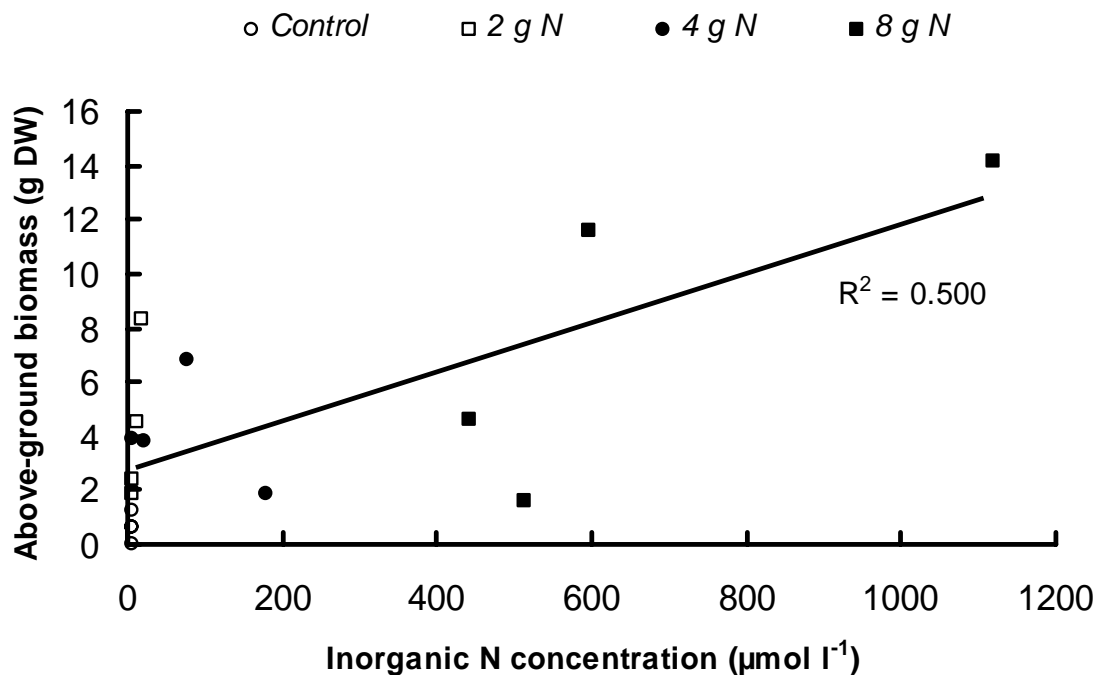

Figure 5.3: Above-ground biomass of *Molinia caerulea* after 3 years of exposure to various rates of experimental N addition (mean + 1 SE; $n = 4$). Different letters indicate significant differences ($P \leq 0.05$) between N treatments (univariate GLM).

Biomass and nutrient concentrations of the vegetation

Above-ground biomass production of *Molinia* was significantly stimulated by increased N addition rates over the 3-year period ($P \leq 0.05$; Figure 5.3). At high N addition rates, numbers of leaves and inflorescences as well as height were slightly increased (data not shown). After 3 years of N addition, peat moisture N concentrations and above-ground biomass of *Molinia* were significantly correlated ($P \leq 0.01$; Figure 5.4). Foliar nutrient concentrations of *Molinia* did not differ between the various N treatments, and N:P ratios ranged from 41 to 52 (Table 5.4).

N addition had no significant effects on the growth of *Betula* and *E. vaginatum* (data not shown), nor on the foliar nutrient concentrations of *E. vaginatum* (Table 5.4). N:P ratios were above 18 for all treatments, suggesting that the growth of *E. vaginatum* was limited by P rather than N (Koerselman & Meuleman 1996). Growth conditions seemed unsuitable for the *Betula* saplings, and some of them even died, regardless of the N treatment level. Foliar N concentrations and N:P ratios in *Betula* increased significantly at addition rates of 8 g N m^{-2} year^{-1} ($P_N \leq 0.05$ and $P_{N:P\ ratio} \leq 0.001$; Table 5.4). N:P ratios in *Betula* leaves ranged from 20 to 76, suggesting growth limitation by P (Koerselman & Meuleman 1996).
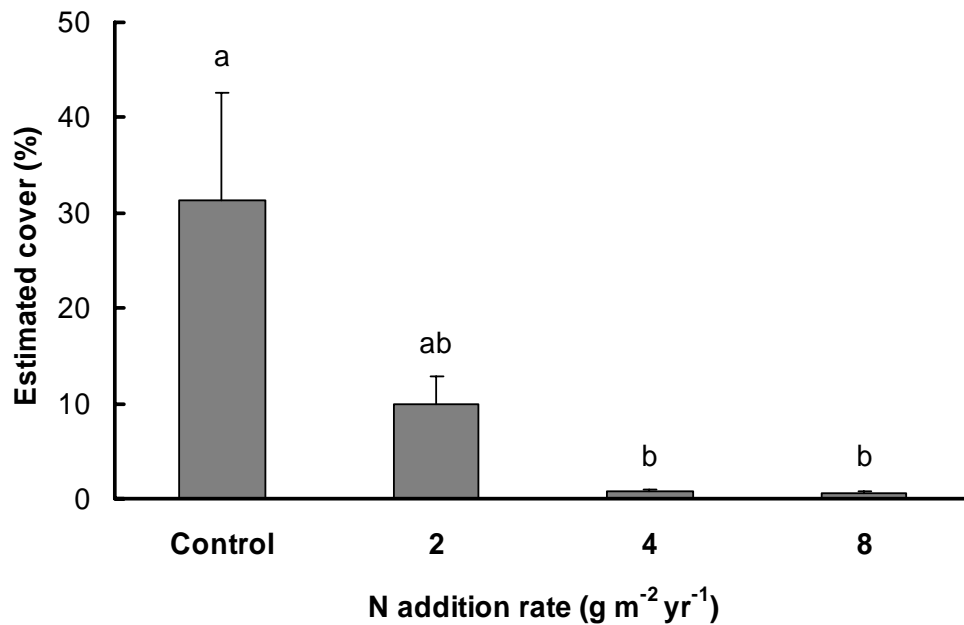

Figure 5.4: Correlation between above-ground biomass of *Molinia caerulea* and total inorganic N concentrations in the peat moisture (average concentrations during the 2001 growing season) at various rates of experimental N addition. Pearson correlation: $P \leq 0.01$.

Table 5.4: Major nutrient and S concentrations, and N:P ratios (mean ± 1 SE; *n* = 4) in *Molinia caerulea*, *Betula pubescens*, *Eriophorum vaginatum*, *Calluna vulgaris* and *Cladonia portentosa* after 3 years at various N addition rates. Different letters indicate significant differences (*P* ≤ 0.05) between N treatments (one-way ANOVA).

	N deposition rate	N	P	K	S	N:P ratio
	g m^{-2} year^{-1}	µmol g^{-1} DW	µmol g^{-1} DW	µmol g^{-1} DW	µmol g^{-1} DW	g g^{-1}
Molinia	Control (0)	1227 ± 92	12.3 ± 0.9	204 ± 12.7	49 ± 4	46 ± 5
	2	1340 ± 63	14.7 ± 0.6	205 ± 14.4	57 ± 4	41 ± 2
	4	1285 ± 124	13.1 ± 1.8	220 ± 14.4	64 ± 8	46 ± 5
	8	1452 ± 66	12.8 ± 0.8	179 ± 12.5	65 ± 2	52 ± 4
Betula	Control (0)	948 ± 123[a]	19.9 ± 4.7	98 ± 9	32 ± 3	24 ± 5[ab]
	2	1325 ± 182[ab]	32.4 ± 8.0	202 ± 48	67 ± 44	20 ± 2[a]
	4	1488 ± 172[ab]	18.4 ± 2.2	123 ± 27	42 ± 3	37 ± 4[b]
	8	1958 ± 235[b]	12.9 ± 2.6	125 ± 6	39 ± 13	76 ± 14[c]
E. vaginatum	Control (0)	671 ± 65	17.0 ± 2.1	132 ± 4	31 ± 1	18 ± 1
	2	681 ± 32	16.5 ± 2.5	140 ± 11	28 ± 1	20 ± 1
	4	660 ± 46	15.0 ± 1.6	133 ± 14	30 ± 1	20 ± 1
	8	740 ± 47	13.9 ± 3.1	111 ± 19	31 ± 1	27 ± 4
Calluna	Control (0)	761 ± 28[a]	11.9 ± 0.3	82 ± 8	39 ± 1[a]	29 ± 1[a]
	2	808 ± 20[a]	11.5 ± 0.3	89 ± 9	40 ± 1[a]	32 ± 1[a]
	4	1009 ± 86[b]	11.3 ± 0.8	76 ± 8	45 ± 3[ab]	41 ± 1[b]
	8	1404 ± 31[c]	11.9 ± 0.7	77 ± 7	52 ± 1[b]	54 ± 5[c]
Cladonia	Control (0)	431 ± 27[a]	8.5 ± 1.1	24 ± 1	21 ± 2[a]	24 ± 2[a]
	2	627 ± 14[b]	7.8 ± 0.1	23 ± 1	30 ± 1[b]	36 ± 1[b]
	4	692 ± 40[b]	8.3 ± 0.8	28 ± 3	34 ± 2[b]	38 ± 2[b]
	8	983 ± 3[c]	7.6 ± 0.2	27 ± 1	46 ± 1[c]	58 ± 2[c]

At the start of the experiment, the above-ground biomass of the natural vegetation was removed. During the 3 years of experimental N additions, these species produced new biomass. At the control treatment, 31% of the plot was covered with the lichen *Cladonia portentosa*. N addition suppressed this regrowth dramatically (*P* ≤ 0.001): in the plots treated with extra loads of 4 and 8 g N m^{-2} year^{-1}, less than 1% of the surface was covered by *C. portentosa* (Figure 5.5). N and S concentrations in *C. portentosa* were significantly increased at addition rates of 2 g N m^{-2} year^{-1} or higher (Table 5.4). During the first year, regrowth of *Calluna* was slightly greater at higher N loads, but differences were not significant after 3 years (data not shown). Foliar N and S concentrations of *Calluna* were significantly higher at N loads of 4 and 8 g m^{-2} year^{-1} (*P* ≤ 0.001; Table 5.4). N:P ratios in *Calluna* were far above 16, suggesting its growth was greatly limited by P (Koerselman & Meuleman 1996).

Figure 5.5: Treatment effects on regrowth of *Cladonia portentosa* after 3 years (mean + 1 SE; *n* = 4). Different letters indicate significant differences ($P \leq 0.05$) between N treatments (one-way ANOVA).

Discussion

Background deposition levels of nutrients

Our data on both the quantity and composition of the bulk deposition (Table 5.1) differed from measurements at two nearby rainfall monitoring stations (Jordan 1997). Annual bulk deposition levels measured at the Kinnitty and Birr monitoring stations (at distances of 41 and 49 km, respectively) were two to three times lower than our measurements at Clara bog. The amount of oxidised N was comparable with the deposition levels that we measured, but the amount of reduced N was four- to fivefold higher at Clara bog. The elevated atmospheric ammonia deposition (NH_x) levels we observed may have resulted from the extensive agricultural activities taking place in the immediate surroundings of Clara bog (Buijsman *et al.* 1987). Compared with the bulk deposition levels of N in the Netherlands (2 g m^{-2} year^{-1}; Table 5.1), levels in Ireland were four times lower. High N deposition levels in the Netherlands also resulted in significantly higher ammonium concentrations in the peat moisture compared with those measured in Irish bogs (Figure 5.6).

In areas with relatively low concentrations of atmospheric pollutants, bulk precipitation on *Calluna*-dominated vegetation amount to approximately 60 - 75% of

the total atmospheric input (personal communication, R. Bobbink). The calculated total atmospheric input on Clara bog must have been around 0.6 - 0.8 g N m^{-2} year^{-1}, which is within the range of the empirically estimated critical load for ombrotrophic bogs (0.5 – 1.0 g N m^{-2} year^{-1}; Bobbink & Roelofs 1995). This was as expected since no expansion of nitrophilous species in ombrotrophic vegetation has so far been observed. The total input levels of N on the experimental plots, including the current background deposition level, were approximately 0.7 (control), 2.7, 4.7 and 8.7 g N m^{-2} year^{-1}. It must be noted that the annual N load was added in six applications, which is common in fertilisation experiments, and this could have led to an imbalance between the supply and demand of nutrients in the vegetation. A laboratory experiment with weekly N applications, however, revealed comparable effects of high N addition rates on the growth of *Molinia* and *Betula* (Tomassen *et al.* 2003). Therefore we assume that the results of this experiment approached reality.

Effect of N on rhizosphere chemistry

As N was applied only during the growing season, peat water N concentrations were higher during summer (Figure 5.1). Although the vegetation takes up available N during this season, the applied N was completely taken up only at an addition rate of 2 g N m^{-2} year^{-1}. At higher N addition rates, N was not completely taken up by the vegetation, partly because of the absence of a living *Sphagnum* layer. Incomplete N uptake by the vegetation was also indicated by the relatively higher fractions of ammonium adsorbed to the peat (Table 5.3) at addition rates of 4 g N m^{-2} year^{-1} or higher. In a similar experiment carried out in the same period at a location on Clara Bog dominated by *Sphagnum* mosses, addition of 4 g N m^{-2} year^{-1} for 3 years resulted in much lower peat moisture N concentrations (Limpens 2003). *Sphagnum* has a high N uptake rate, resulting in low N concentrations in the peat moisture (e.g. Lee & Woodin 1988; Jauhiainen *et al.* 1998). In the Netherlands, with a total N deposition level of 4 g m^{-2} year^{-1}, ammonium concentrations of up to 50 µmol l^{-1} have been measured. In our experiment, the availability of ammonium in the rhizosphere during winter was comparable with the concentrations measured in the Netherlands, but during the summer the availability of ammonium was much higher due to the N applications (Figure 5.1) and the absence of a living *Sphagnum* layer. During the winter, when no nutrients were applied, the large amounts of precipitation (Figure 5.1) probably flushed ammonium, nitrate and sulphate from the peat, resulting in lower concentrations.

The observed decrease in pH during the summer is probably due to increased (bio)geochemical oxidation processes in the dry peat. Oxidation processes generate protons and lead to acidification of the peat (Drever 1997). The addition of N further decreased the pH in the peat moisture. Uptake of ammonium by the vegetation (Raven 1985) and mobilisation of protons from the peat cation exchange complex at

high ammonium concentrations (e.g. Clymo & Hayward 1982), may have been responsible for the observed treatment effect.

As we expected, N addition significantly affected C:N and N:P ratios of the peat (Table 5.2). Peat C:P ratios, however, were also influenced by N addition. Increased N availability probably stimulated uptake of P, resulting in slightly lower peat P concentrations and significantly higher C:P ratios. The differences in C:N, C:P and N:P ratios of the peat had no significant effects on the mineralisation rate (i.e., potential CO_2 production). Several studies have found increased mineralisation rates at low C:N and C:P ratios (Aerts & Chapin 2000) due to increased nutrient availability for micro-organisms. As the low pH of the peat probably caused the lack of stimulation of the mineralisation rate by high N inputs, the potential CO_2 production rate was significantly influenced by the pH_{NaCl} of the peat (Figure 5.2). An increased pH is known to enhance mineralisation by stimulating microbial activity (Smolders *et al.* 2002). From the above we conclude that desiccation of the peat does not necessary lead to increased mineralisation rates, because acidification of the peat as a result of oxidation processes, ammonium uptake by plants and proton exchange processes can inhibit mineralisation.

Effect of N addition on vascular plants

The above-ground biomass of *Molinia* increased with the N addition rate (Figure 5.3), indicating limitation by N, as has also been found in earlier studies of heathlands (e.g. Berendse & Aerts 1984; Roelofs 1986; Heil & Bruggink 1987; Aerts & Berendse 1988). Growth limitation by N was also indicated by the constant foliar N concentrations in *Molinia* (Table 5.4), showing that extra available N was used for biomass production. However, foliar N:P ratios ranged from 41 to 52, suggesting that growth was severely limited by P (Koerselman & Meuleman 1996). Kirkham (2001) reported average N concentrations and N:P ratios of 1320 µmol g^{-1} DW and 21, respectively, in *Molinia* from upland regions in England and Wales. Foliar N concentrations of *Molinia* in our experiment were comparable to those measured by Kirkham (2001), but P concentrations were much lower, resulting in higher N:P ratios (Table 5.4). Despite these very high N:P ratios, the growth of *Molinia* was still stimulated, indicating that *Molinia* is a species well adapted to low P availability, as has been found elsewhere (Kirkham 2001; Tomassen *et al.* 2003).

In contrast, we found no N effect on *Betula* and *E. vaginatum*. It is likely that nutrients other than N were limiting to their growth. On drained mires in northern Finland, NPK and PK fertilisation has been found to stimulate the growth of *Betula pubescens*, while N alone had no effect (Penttila & Moilanen 1997). N addition in our experiment significantly increased the foliar N concentration in *Betula* (Table 5.4), suggesting that its growth was not limited by N. The N:P ratios show that the growth of *Betula* was
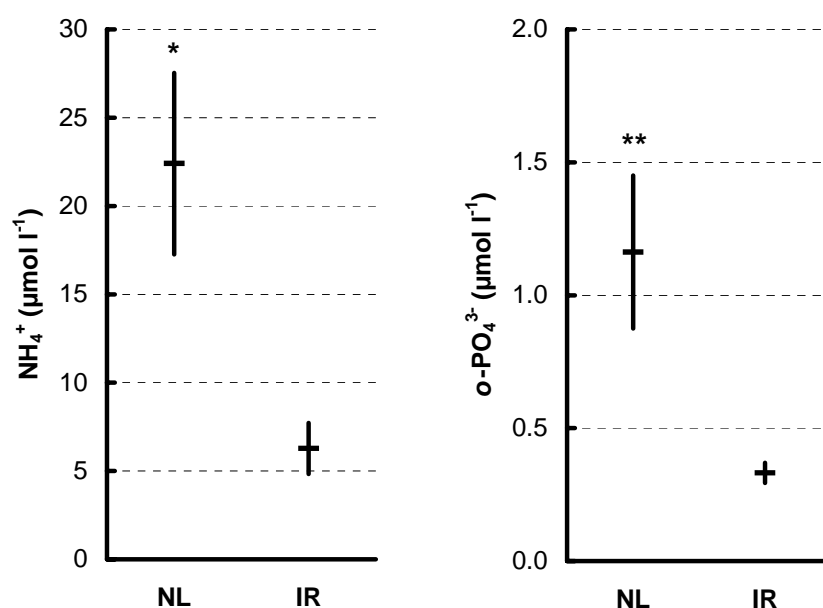
limited by P in all N treatments (Table 5.4; see also Koerselman & Meuleman 1996). Optimum nutrition levels for *Betula pendula* are known to be achieved at N:P ratios between 10 and 12 (Ericsson & Ingestad 1988). Hytönen & Kaunisto (1999) found growth limitation by P at foliar P concentrations below 65 μmol g^{-1} DW. This indicates that the growth of *Betula* in our experiment was probably constrained by P. In a N addition experiment in the laboratory, growth of *Betula* in a wet *Sphagnum fallax* vegetation also seemed to be limited by P (Tomassen *et al.* 2003).

In our experiment, the growth of *E. vaginatum* was not stimulated by N addition. Redbo-Torstensson (1994), however, found increased densities of *E. vaginatum* at addition rates of 1 g N m^{-2} year^{-1} or more in central south Sweden, after 4 years. In contrast, fertilisation experiments on drained peat sites in southern Norway and Sweden showed stimulated growth of *E. vaginatum* after NPK and PK fertilisation, indicating P or K limitation (Finér & Brække 1991). As in the cases of *Molinia* and *Betula*, the N:P ratios suggested that *E. vaginatum* was limited by P (Table 5.4). P limitation was also observed in a study by Leith *et al.* (1999), in which tissue N concentrations of *E. vaginatum* increased at higher N input levels and were twice those measured in our experiment (1360 N μmol g^{-1} DW), while P concentrations decreased at high N deposition levels and were comparable with the concentrations we measured (17 μmol P g^{-1} DW; Leith *et al.* 1999).

In our experiment, regrowth of *Calluna* was not significantly stimulated by N addition after 3 years, which has been found for *Calluna* growing in heathland vegetation (Roem *et al.* 2002). Foliar N concentrations of *Calluna* increased upon N addition (Table 5.4), and were in the same range as those measured in the U.K., where N concentrations in shoots reached levels of 640 μmol g^{-1} DW at additional N loads of 1.5 g m^{-2} year^{-1} (Power *et al.* 1995; Uren *et al.* 1997). In a large-scale study by Pitcairn *et al.* (1995) tissue N concentrations in mature *Calluna* growing on heathland soil were significantly correlated with the total deposition level of N, whereas Risager (1998) found no effect of N deposition levels on tissue N concentrations in *Calluna* growing on ombrotrophic bogs. This discrepancy could be explained by suppression of the growth by water shortage, which can only occur in heathland soils, but also by the presence of a *Sphagnum* layer on bogs, which limits the availability of N for *Calluna* (Risager 1998). The correlation between N deposition and foliar N concentrations of *Calluna* in our experiment is probably due to both the absence of a *Sphagnum* layer and the extremely high N inputs. In our experiment, tissue N:P ratios in *Calluna* indicated P limitation (29 - 54), which has been found for *Calluna* in heathlands (Roem *et al.* 2002).

Peat moisture P concentrations in the rhizosphere of Irish bogs were very low compared to those measured in Dutch bogs (Figure 5.6). Beltman *et al.* (1996) also found P shortage in Irish blanket bogs. *Molinia* and *Betula* were both able to grow around wooden poles on Clara bog, where the availability of both N and P was high

as a result of increased input by bird droppings (H.B.M. Tomassen & A.J.P. Smolders, unpublished data).The high P concentrations in Dutch bogs probably enabled the expansion of *Molinia, Betula* and *E. vaginatum* at high N deposition levels. Why most of the Dutch bogs are rich in P remains unclear. There is not much information about the input of atmospheric P, but it probably ranges from 0.01 to 0.1 g P m^{-2} year^{-1}, the main sources being fine soil particles, pollen, and the burning of plant material, coal and oil (Newman 1995). We did indeed measure higher P deposition levels in the Netherlands than in Ireland (Table 5.1). Increased P availability in Dutch bogs often results from former agricultural use, such as buckwheat fire cultivation (Joosten 1995) or from the inflow of nutrients from surrounding pastures, which are extensively fertilised (Koerselman *et al.* 1990). Several bogs are located in former heathland pools, which could have been enriched with P in the past by large numbers of waterfowl, as bird droppings contains high concentrations of P (H.B.M. Tomassen & A.J.P. Smolders, unpublished data).

Figure 5.6: Concentrations (mean ± 1 SE) of ammonium and phosphate at a depth of 0 - 10 cm in bogs in the Netherlands (NL; *n* = 12) and Ireland (IR; *n* = 7). * *P* ≤ 0.05; ** *P* ≤ 0.001 (*t*-test).

Effect of N on regrowth of lichens

Lichens are very sensitive to atmospheric pollution and have almost completely disappeared from ombrotrophic bogs in the Netherlands (H.B.M. Tomassen, personal observations) and *C. portentosa* has declined under Dutch forest canopies since 1960 (Van Dobben *et al.* 1983). In our experiment, regrowth of *Cladonia*

portentosa was suppressed at high N addition rates (Figure 5.5). We cannot exclude that the low application frequency could have stimulated the detrimental effects of N on *C. portentosa*, although the disappearance of lichens at high nutrient availability has been confirmed by various other studies (e.g. Press *et al.* 1998; Gordon *et al.* 2001). Discoloration and die-back of *C. portentosa* in Danish heathlands has been associated with atmospheric N pollution (Søchting & Johnsen 1987). Vagts & Kinder (1999) found that NPK fertilisation stimulated the growth of *C. portentosa*, but P or N alone had a negative effect. In addition to the effects of high N deposition levels, S pollution may also have had deleterious effects (Farmer *et al.* 1992). Tissue N and S concentrations in *C. portentosa* were significantly elevated at addition rates above 2 g N m^{-2} year^{-1} (Table 5.4). Several other studies have found an effect of N deposition levels on the tissue N concentrations in *C. portentosa* (Søchting 1990; Hyvärinen & Crittenden 1998a). *C. portentosa* transplanted to sites subjected to high N deposition levels had increased N concentrations in both apices and bases due to retarded growth at these locations (Hyvärinen & Crittenden 1998b). Because lichens are very sensitive to S as well as N pollution (Poikolainen *et al.* 1998), we think that both the high S and high N loads hampered the regrowth of *Cladonia* in our experiment. Fortunately, this seems to be a reversible process as a systematic mapping of epiphytic lichens on conifers in Finland in 1985-86 and 1995 showed an increase in lichen abundance due to decreasing S deposition levels (Poikolainen *et al.* 1998). The coverage of *Cladonia* in our experiment, however, had not yet changed a year after the experimental N additions had ended (data not shown).

Invasion of bogs: desiccation or N effects?

Is the invasion of bogs in the Netherlands by species such as *Betula* and *Molinia* a result of desiccation or long-term high atmospheric nitrogen loads? The results of our experiment show that experimental elevation of N deposition to Dutch levels did indeed stimulate the growth of *Molinia* under desiccated conditions. However, this was not the case for *Betula* and *E. vaginatum*, most probably because their growth was limited by P instead of N. Foliar N:P ratios indicated that the growth of *Molinia*, *Betula* and *E. vaginatum* was limited by P. Despite this presumed P limitation, however, *Molinia* was able to increase its biomass at increased N additions rates, although *Betula* and *Eriophorum* clearly were not. The N:P ratio tool developed by Koerselman & Meuleman (1996) is based on results obtained at the vegetation level and not for individual plant species. Our results indicate that the N:P ratio is not a suitable tool for detecting the absence of N limitation in *Molinia*. This is in agreement with findings by Güsewell & Koerselman (2002) who found that the N:P ratios cannot predict the responses of individual plant species to fertilisation.

The effects of desiccation on the invasion of bogs by *Molinia* and *Betula* are likely to be smaller than those of increased N availability. Experimental N fertilisation of

Molinia and *Betula* in a wet vegetation dominated by *S. fallax* revealed consistent effects of high N addition rates (Tomassen *et al.* 2003). Under wet conditions, growth of *Molinia* was also stimulated by N (4 g m^{-2} year^{-1}), whereas that of *Betula* was probably limited by P instead of N. It is plausible that the drastic changes in vegetation composition in Dutch bogs can be ascribed to the combined effects of high N deposition levels and relatively high P availability. We propose that invasion of *Betula* in bogs can only take place in conditions of simultaneously high N and relatively high P availability. If N deposition levels increase while P availability remains extremely low, only *Molinia* is likely to be able to outcompete other species.

Prospects for ombrotrophic bogs at high N loads

As long as the critical N loads for ombrotrophic bogs are greatly exceeded in the Netherlands, invasion of bogs by species such as *Molinia* and *Betula* is likely to remain a problem. The effects of high N loads, however, will be more pronounced under desiccated conditions due to reduced growth or even absence of *Sphagnum* mosses. A living *Sphagnum* layer can immobilise large amounts of N and make it unavailable for vascular plants (e.g. Lamers *et al.* 2000; Limpens 2003; Tomassen *et al.* 2003). Additional management measures to optimise growing conditions for *Sphagnum,* such as rewetting and mowing (reduction of shading), could therefore probably decline the negative effects of high atmospheric N input.

Acknowledgements

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

Experimental set-up of the laboratory experiment

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

Abstract

1. In order to test whether the observed invasion of ombrotrophic bogs in the Netherlands by *Molinia caerulea* and *Betula pubescens* is the result of long-term high nitrogen (N) loads, we conducted a 3-year fertilisation experiment with *Sphagnum fallax* turfs. Six different N treatments were applied ranging from 0 (control) to 4 g N m^{-2} year^{-1}.

2. During the experimental period, ammonium concentrations in the peat moisture remained very low due to high uptake rates by *Sphagnum.* Tissue N concentrations in *S. fallax* showed a linear response to the experimental N addition. Excess N was accumulated as N-rich free amino acids such as arginine, asparagine and glutamine, especially at N addition rates of 0.25 g m^{-2} year^{-1} or higher, indicating N-saturation.

3. Despite the high tissue N:P ratio (above 35), above-ground biomass production by *Molinia* was still stimulated at N addition rates of 4 g m^{-2} year^{-1}, and foliar nutrient concentrations were unaffected compared to the control. In contrast to *Molinia*, *Betula* was unable to increase its above-ground biomass. Foliar N concentrations in *Betula* were significantly higher at N addition rates of 4 g m^{-2} year^{-1} and excess N was stored in foliar arginine, making up 27% of the total N concentration. Evapotranspiration was increased at higher N addition rates due to stimulated total above-ground biomass production of the vegetation.

4. N addition at the actual Dutch deposition rate of 4 g m^{-2} year^{-1} stimulated the growth of *Molinia* in this experiment, providing evidence that the observed dominance of *Molinia* on ombrotrophic bogs in the Netherlands is caused by high N deposition levels. Based on the observed changes in biomass production and tissue nutrient concentrations, we assume that a long-term deposition of 0.5 g N m^{-2} year^{-1}, or higher, leads to undesirable changes in species composition and increased risk of desiccation.

Introduction

Ombrotrophic bogs are traditionally regarded as nitrogen (N)-limited. In areas with increased N deposition levels, however, productivity of *Sphagnum* may change from being N-limited to phosphorus (P)-limited (Aerts *et al.* 1992). In non-forest

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

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

Although an effect of increased N availability on the growth of *Sphagnum* has been observed in other experiments (e.g. Ferguson & Lee 1983; Aerts *et al.* 1992), findings have not been consistent across the various studies. The actual background deposition has a significant effect on the response of *Sphagnum* to increased availability of N (Aerts *et al.* 1992; Gunnarsson & Rydin 2000). At relatively low atmospheric input (< 1 g N m^{-2} year^{-1}), *Sphagnum* has been found to respond to increased N deposition levels by increased growth, indicating N-limitation (Malmer 1990). At higher N loads (1 - 2 g N m^{-2} year^{-1}), N no longer limits growth but the *Sphagnum* layer does not reach its maximum organic N content (Pitcairn *et al.* 1995; Lamers *et al.* 2000; Berendse *et al.* 2001). Above 2 g N m^{-2} year^{-1}, the *Sphagnum* layer reaches its maximum N content and *Sphagnum* growth is affected (Lamers *et al.* 2000; Gunnarsson & Rydin 2000). In this situation, N leaches from the *Sphagnum* layer to the roots of vascular plants (Lee & Woodin 1988; Aerts *et al.* 1992; Lamers *et al.* 2000).
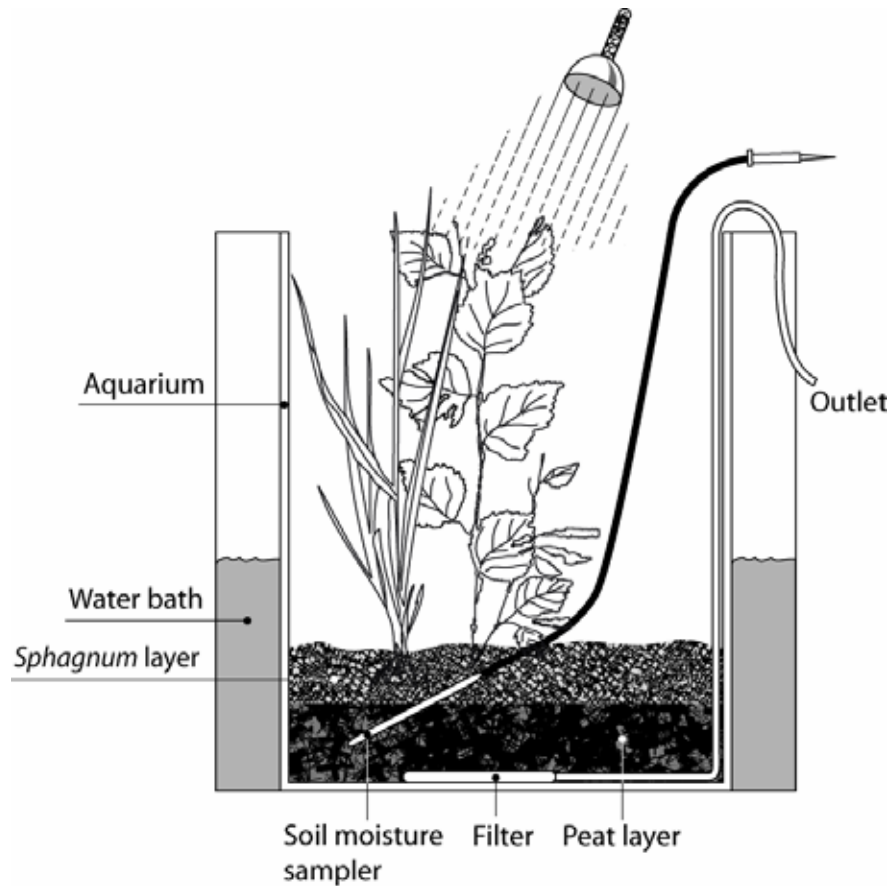
Various N addition experiments have also found changes in the species composition of the *Sphagnum* layer (e.g. Press *et al.* 1986; Lütke Twenhöven 1992; Risager 1998). Under N-limiting conditions, complete N immobilisation by the *Sphagnum* layer causes vascular plants to depend on N mobilised by mineralisation processes in the underlying peat (Malmer 1993; Malmer *et al.* 1994). Increased availability of nutrients in the rhizosphere leads to an increased cover of vascular plants and a reduction in *Sphagnum* growth due to shading (Hayward & Clymo 1983; Heijmans *et al.* 2001; Berendse *et al.* 2001). Hogg *et al.* (1995) found that cutting back *Molinia* reduced the competition for light and stimulated the growth of *Sphagnum*. Thus, increased N deposition levels also causes changes in the competition between *Sphagnum* and vascular plants.

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

Materials and methods

Experimental set-up

Turfs were collected from an ombrotrophic floating raft (4 ha) in the 'De Hamert' nature reserve in the Netherlands (51°32'N, 6°10'E). The upper 10 cm were used in the experiment and the vegetation consisted mainly of *Sphagnum fallax* (klinggr.) Klinggr. (synonymous with *Sphagnum recurvum* P. Beauv. Var. *mucronatum* (Russ.) Warnst.; 95 - 100% cover) along with some *Vaccinium oxycoccus* L. and *Drosera rotundifolia* L. The turfs (n = 24) were cut and were placed in glass containers (24 x 24 x 32 cm) on the same day. All containers were placed in a temperature-regulated water bath in a climate control room with a light intensity of 100 µmol m^{-2} s^{-1} at the vegetation level (Figure 6.1). Summer and winter were simulated by gradually increasing or decreasing the temperature and photoperiod (between 15 °C, 16 hours and 3 °C, 8 hours). The length of the winter period differed slightly between the various years due to technical problems. This variation, however, stayed within the natural range. Concentrations of atmospheric CO_2 at the vegetation level were ambient (approx. 370 µmol CO_2 mol^{-1}). Three soil moisture samplers (Rhizon SMS - 10 cm; Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) were placed in each turf, (at depths of 0 - 10 cm) to allow the chemical composition of the peat moisture to be analysed.

Figure 6.1: Experimental set-up for one turf including introduced *Betula pubescens* and *Molinia caerulea.*

Six treatments were applied, differing in N concentrations and leading to N loads of 0, 0.25, 0.5, 1, 2 and 4 g N m^{-2} year^{-1} (0 - 0.29 mol N m^{-2} year^{-1}). N was added as ammonium (65%) and nitrate (35%) using NH_4NO_3 and NH_4Cl based on the actual ratios in the Netherlands (situation 1994; Lamers 1995). The background deposition level of N in the climate control room was negligible (< 0.05 g m^{-2} year^{-1}, data not shown). Artificial rainwater was sprayed directly on the turfs three times a week, at a rate equivalent to a rainfall of 750 mm (the mean annual rainfall in the Netherlands). Besides the various N concentrations, the solution contained 5 mg l^{-1} sea salt ('Marine mix + Bio-elements', Wiegandt GmbH, FRG), 30 µmol L^{-1} KCl, 10 µmol L^{-1} $CaCl_2$, 10 µmol l^{-1} Fe-EDTA, 10 µmol l^{-1} KH_2PO_4, 0.7 µmol l^{-1} $ZnSO_4$, 0.8 µmol l^{-1} $MnCl_2$, 0.2 µmol l^{-1} $CuSO_4$, 0.8 µmol l^{-1} H_3BO_3 and 0.008 µmol l^{-1} $(NH_4)_6Mo_7O_{24}$. The surplus water was removed via an overflow system in order to keep the water level at 4 cm below the capitula (maximum fluctuation 5 mm). Each treatment consisted of four replicates, randomly distributed over the water bath. After a pre-treatment period of 4 months (rainwater without N), the concentration of ammonium in the peat moisture had dropped from 50 - 60 µmol l^{-1} to < 10 µmol l^{-1} (data not shown). Six saplings of *Betula pubescens* Ehrh. (collected in a nearby heathland; 4.25 ± 0.59 cm high; total fresh weight 6.14 ± 0.91 g) and five vegetative shoots of *Molinia caerulea* (L.) Moench (collected at 'De Hamert'; total fresh weight 3.10 ± 0.27 g) were then

planted in each container. Growth of *Betula* and *Molinia* was measured non-destructively every three months. Growth of *M. caerulea* was determined by counting the number of living shoots and that of *Betula* by measuring the length and number of leaves. The total N concentrations of the capitula (upper 2 cm) and stems (2 - 4 cm) of *S. fallax* were determined twice a year. After 3 years of N addition, the total biomass of *Betula* and *Molinia* plants was determined.

Sampling

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

Chemical analysis

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

CO_2 and CH_4 production rates were measured by incubating 200 g of fresh peat in 500 ml infusion flasks, sealed with an airtight rubber stopper. Incubations were carried out in duplicate for each turf. After filling, the flasks were repeatedly vacuumed and flushed with oxygen-free nitrogen gas to remove all CO_2 and CH_4

from the peat and the headspace. The flasks were kept in the dark at 20 °C, and CO_2 and CH_4 concentrations were measured weekly over a period of 4 weeks. CO_2 and CH_4 production rates were calculated by linear regression of the measurements, and expressed on a dry weight basis.

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

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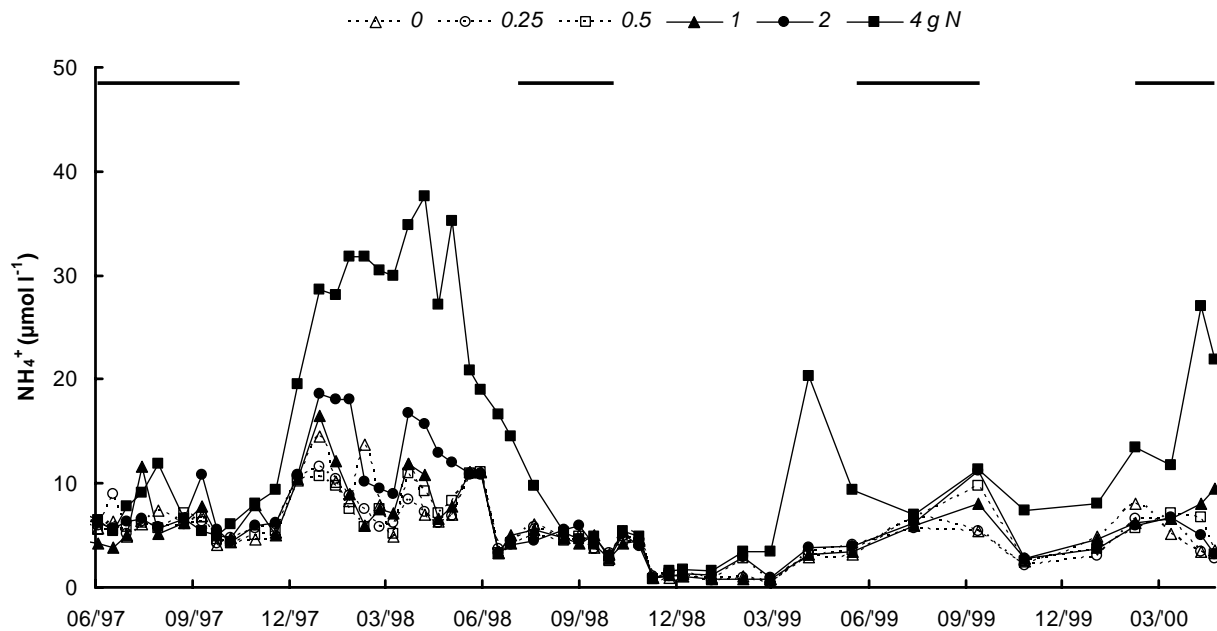
Statistical analysis

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

Results

Water chemistry

The ammonium concentrations in the peat moisture showed a seasonal pattern and were significantly influenced by time ($P < 0.001$; Figure 6.2). N addition significantly raised the concentration of ammonium in the peat moisture ($P < 0.01$). During the first growing season (6 months) the concentrations of ammonium in the peat moisture remained very low (Figure 6.2). Lowering the temperature led to a strong increase in peat moisture ammonium concentration at the highest N treatment (4 g m^{-2} year^{-1}) during the first winter period. From the start of the second growing season, the ammonium concentrations gradually dropped again to concentrations comparable with those in the other treatments. During the second and third winter periods, no clear ammonium peak was measured. At the end of the experiment, peat moisture ammonium concentrations at the highest N addition rate were significantly elevated compared to those in the treatments with N addition rates of 1 g m^{-2} year^{-1} or less (Figure 6.2; $P < 0.05$). Nitrate concentrations were low throughout the experiment (≤ 6 μmol l^{-1}; Table 6.1).

Figure 6.2: Peat moisture ammonium concentrations between June 1997 and May 2000 at different experimental N addition rates (*n* = 4). Summer periods (15 °C and photoperiod of 16 hours) are indicated by horizontal lines.

Table 6.1: Peat moisture pH and carbon dioxide, nitrate, phosphate and potassium concentrations (μmol l^{-1}) during the third growing season (March until May 2000) at different experimental N addition rates (means ± 1 SE; *n* = 12). Different letters indicate significant differences (*P* < 0.05) between N treatments (one-way ANOVA).

N addition rate g m^{-2} year^{-1}	pH	CO$_2$ μmol l^{-1}	NO$_3^-$ μmol l^{-1}	PO$_4^{3-}$ μmol l^{-1}	K$^+$ μmol l^{-1}
0	3.91 ± 0.04[a]	61 ± 6	2.2 ± 0.9[a]	0.38 ± 0.18	30 ± 5[a]
0.25	3.83 ± 0.03[ab]	49 ± 8	1.7 ± 0.3[ab]	0.19 ± 0.04	17 ± 5[ab]
0.5	3.81 ± 0.04[ab]	56 ± 6	5.6 ± 1.2[ab]	0.40 ± 0.14	15 ± 6[ab]
1	3.73 ± 0.04[bc]	59 ± 7	4.5 ± 0.8[ab]	0.54 ± 0.16	23 ± 5[ab]
2	3.61 ± 0.04[c]	49 ± 5	3.2 ± 0.6[ab]	0.48 ± 0.18	7 ± 2[b]
4	3.44 ± 0.04[d]	64 ± 8	6.1 ± 1.5[b]	0.43 ± 0.10	9 ± 2[b]

Peat moisture pH fluctuated between 3.0 and 4.0 during the experimental period, and from spring 1999 onwards, pH was lower at higher N addition rates (*P* < 0.001). During the third growing season, the addition of 4 g N m^{-2} year^{-1} led to pH 3.4, vs. 3.9 for the control (Table 6.1). The average phosphate concentration in the peat moisture remained low during the entire experiment: 0 - 0.5 μmol l^{-1} (Table 6.1). However, one of the 4 g N m^{-2} year^{-1} replicates contained very high phosphate concentrations at the start of the experiment. The concentration dropped from 129 μmol l^{-1} at the start of the experiment to 0.5 μmol l^{-1} at the end. Potassium (K) concentrations at the start of the experiment ranged from 55 to 75 μmol l^{-1}. During the pre-treatment period, K concentrations dropped below 20 μmol l^{-1} and stabilised around 10 μmol l^{-1}. At the end of the experiment, K concentrations in the peat moisture increased slightly, with the highest concentrations found in the control treatment (30 μmol l^{-1}; Table 6.1).
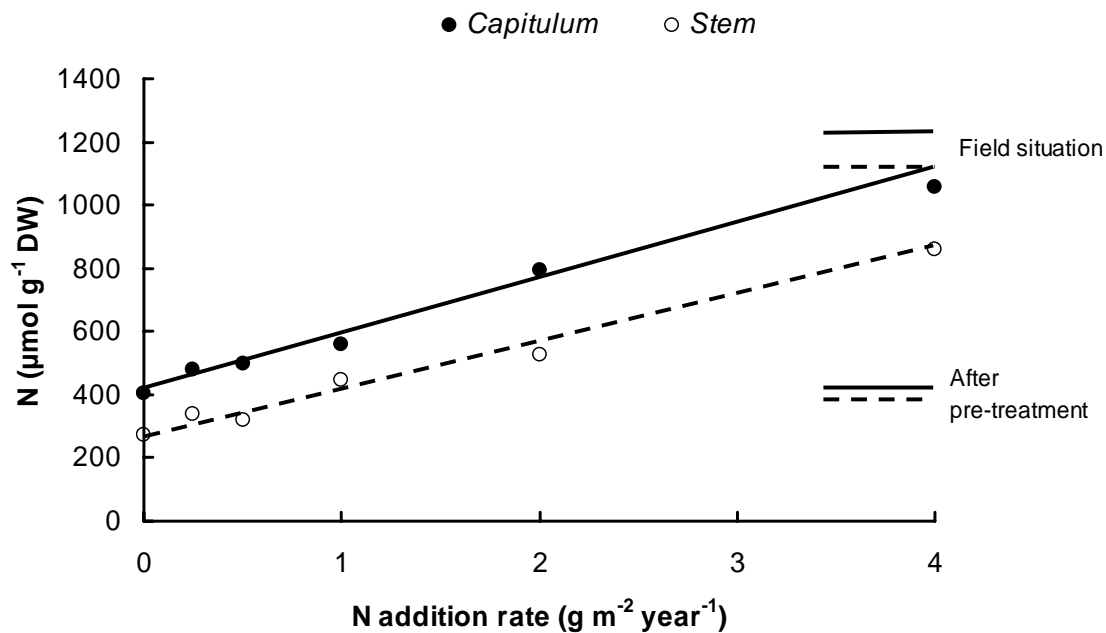
Sphagnum layer

At the start of the experiment (field measurement, winter 1996-1997), the N concentrations in the capitula and stems of *S. fallax* were 1214 and 1115 μmol N g^{-1} DW, respectively (Figure 6.3). During the 4 months of the pre-treatment period (no N addition) the N concentrations in the capitula and stems of *S. fallax* dropped to 409 and 365 μmol g^{-1} DW, respectively, at the start of the N addition (Figure 6.3). As a result of higher N addition rates, the N concentrations in *S. fallax* were significantly higher after 3 years (*P* < 0.001; Figure 6.3). Concentrations were consistently slightly higher in capitula than in stems. The relationship between N addition rates and the tissue N concentrations in capitula and stems of *S. fallax* was linear. Capitulum N concentrations in *S. fallax* receiving no N were comparable with those measured directly after the pre-treatment period, whereas stem concentrations in *S. fallax*, receiving 0.5 g N m^{-2} year^{-1} or less, decreased (Figure 6.3). Compared to the N concentrations measured at the field location, both capitulum and stem concentrations were lower for all N treatments.

Table 6.2: Concentrations (μmol g⁻¹ DW) of phosphorus and potassium, and C:N and N:P ratios (g g⁻¹) in capitula and stems of *Sphagnum fallax* subjected to different experimental N addition rates (means ± 1 SE; n = 4). Different letters indicate significant differences ($P < 0.05$) between N treatments (one-way ANOVA).

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

Figure 6.3: N concentrations in capitula and stems of *Sphagnum fallax* after 3 years at different rates of experimental N addition (linear regression: $R^2_{capitula}$ = 0.988 and R^2_{stems} = 0.985). Solid and dashed horizontal lines indicate N concentrations in capitula and stems, respectively, measured in the field and after the pre-treatment period.

P concentrations in the capitulum tissue of *Sphagnum* were significantly lower than those in the controls at an addition of 2 g N m⁻² year⁻¹ ($P < 0.05$; Table 6.2). For an addition rate of 4 g N m⁻² year⁻¹ the effect was not significant due to high variance.
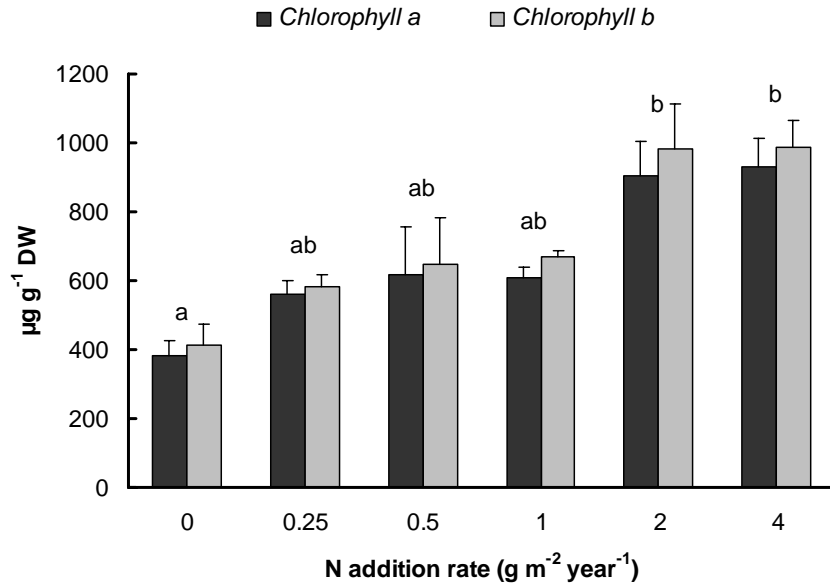
Stem tissue concentrations in different treatments were all comparable, though lower than capitulum concentrations. K concentrations in capitula and stems were significantly lower at higher N addition rates ($P < 0.05$; Table 6.2). Due to increasing N concentrations and slightly decreasing carbon concentrations (data not shown), C:N ratios in capitula and stems were significantly lower at higher N loads ($P < 0.001$; Table 6.2). C:N ratios in stem tissue were higher than those in capitulum tissue. N:P ratios in capitula and stems were significantly increased at higher N addition rates ($P < 0.001$; Table 6.2). N:P ratios at N addition rates of 2 and 4 g m^{-2} year^{-1} were over 16, suggesting P limitation (Koerselman & Meuleman 1996)

Table 6.3: Concentrations (μmol g^{-1} DW) of arginine (ARG), asparagine (ASN), glutamine (GLN), aspartic acid (ASP), glutamic acid (GLU) and serine (SER) in *Sphagnum fallax*, *Betula pubescens* and *Molinia caerulea* subjected to different experimental N addition rates (means ± 1 SE; n = 4). Different letters indicate significant differences ($P < 0.05$) between N treatments (one-way ANOVA).

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

The concentrations of the leaf pigments chlorophyll a and b in the capitula of *S. fallax* increased as a result of N addition ($P < 0.001$; Figure 6.4). The capitulum concentrations of the free amino acids arginine, asparagine, glutamine and glutamic acid increased significantly with higher N addition rates (Table 6.3; $P < 0.001$, $P < 0.001$, $P < 0.01$ and $P < 0.01$, respectively). Compared to the control (no N addition) arginine and asparagine concentrations were significantly higher at N loads of 0.25 g m^{-2} year^{-1}. Free amino acids started to accumulate even after only 1 year of N addition (Tomassen *et al.* 2000). The fraction of N stored in amino acids significantly

increased at a N load of 1 g m^{-2} year^{-1} or higher (Table 6.4). After 3 years of addition at 4 g N m^{-2} year^{-1}, approximately 18% of the total N concentration was stored in N-rich free amino acids.

Figure 6.4: Concentrations of chlorophyll a and b in capitula of *Sphagnum fallax* after 3 years at different rates of experimental N addition (means + 1 SE; *n* = 4). Different letters indicate significant differences (*P* < 0.05) between N treatments (one-way ANOVA).

Biomass and nutrient concentrations in Molinia

N addition significantly increased the above-ground biomass of *Molinia* (*P* < 0.05; Figure 6.5). Individual above-ground biomass of *Molinia* increased by more than three fold, from 0.2 to 0.7 g DW, with increasing N addition rates. Root to shoot ratio of *Molinia* varied between 1.6 and 2.0 and did not differ significantly between treatments. Litter production after 3 years was significantly higher upon addition of 4 g N m^{-2} year^{-1} compared to the other N treatments (*P* < 0.01; data not shown). N addition stimulated inflorescence production (*P* < 0.05). The total mean number of inflorescences per aquarium at the end of the experiment varied from 0 (0.25 g N m^{-2} year^{-1}) to 3.7 (4 g N m^{-2} year^{-1}). The concentration of N in the green leaves of *Molinia* ranged between 900 and 1230 µmol g^{-1} DW However, there were no significant differences (Table 6.5), nor for the concentrations of P and K in the leaves (Table 6.5). N:P ratios in *Molinia* leaves ranged between 33 and 44 (no significant differences), suggesting growth limitation by P (Koerselman & Meuleman 1996). The concentrations of all free amino acids measured were very low (Tables 6.3 & 6.4) and made up only a small fraction of the total N concentration.

Table 6.4: Concentrations of amino acid N, and fractions of amino acid N of total tissue N in *Sphagnum fallax*, *Betula pubescens* and *Molinia caerulea* subjected to 3 years of different experimental N addition rates (means ± 1 SE; $n = 4$). Different letters indicate significant differences ($P < 0.05$) between N treatments (one-way ANOVA).

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

Table 6.5: Foliar concentrations ($\mu mol\ g^{-1}$ DW) of nitrogen, phosphorus and potassium, and N:P ratios ($g\ g^{-1}$) in *Betula pubescens* and *Molinia caerulea* subjected to different experimental N addition rates (means ± 1 SE; $n = 4$). Different letters indicate significant differences ($P < 0.05$) between N treatments (one-way ANOVA).
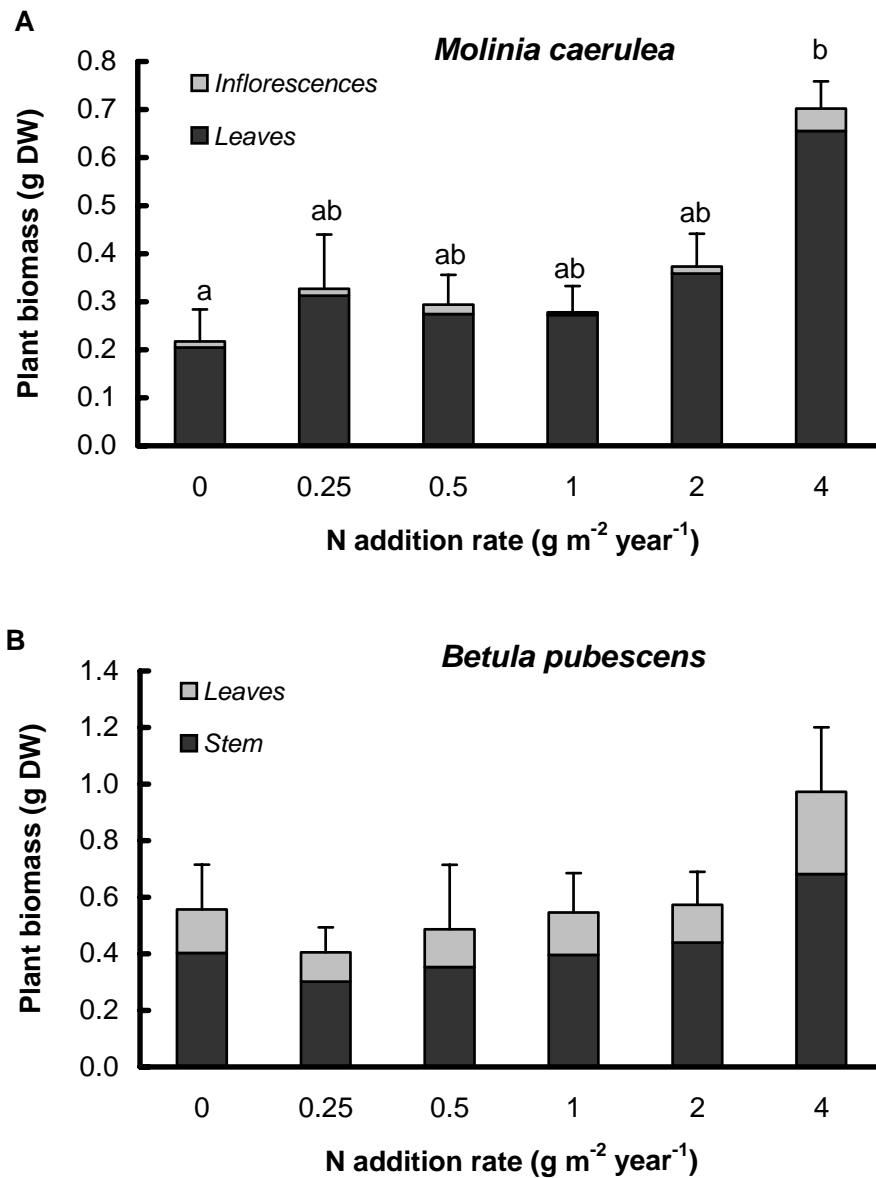
	N addition rate	N	P	K	N:P ratio
	$(g\ m^{-2}\ year^{-1})$	$\mu mol\ g^{-1}\ DW$	$\mu mol\ g^{-1}\ DW$	$\mu mol\ g^{-1}\ DW$	$g\ g^{-1}$
Betula	0	608 ± 48[a]	25.3 ± 5.9	228 ± 16	14 ± 5[a]
	0.25	594 ± 59[a]	16.1 ± 3.0	193 ± 30	18 ± 2[ab]
	0.5	676 ± 96[a]	19.0 ± 4.7	209 ± 27	18 ± 3[ab]
	1	561 ± 62[a]	16.8 ± 4.0	205 ± 30	16 ± 2[ab]
	2	733 ± 101[a]	13.4 ± 1.1	232 ± 14	25 ± 3[ab]
	4	1403 ± 80[b]	23.0 ± 5.9	241 ± 24	32 ± 6[b]
Molinia	0	904 ± 99	11.4 ± 1.5	391 ± 38	38 ± 6
	0.25	928 ± 59	11.2 ± 0.3	325 ± 33	38 ± 3
	0.5	1059 ± 79	14.9 ± 3.8	372 ± 12	38 ± 9
	1	930 ± 31	12.4 ± 2.2	400 ± 28	38 ± 7
	2	1094 ± 30	11.6 ± 1.0	394 ± 19	44 ± 5
	4	1233 ± 108	26.4 ± 12.3	297 ± 74	33 ± 10

Biomass and nutrient concentrations in Betula

Above-ground biomass of *Betula* was approximately 0.5 g DW at a load of 2 g N m^{-2} year^{-1} or lower (Figure 6.5). Although addition of 4 g N m^{-2} year^{-1} almost doubled the biomass of *Betula* to 1.0 g DW, no significant effects could be detected on the above-ground biomass of *Betula*. Root to shoot ratio in *Betula* varied between 0.8 and 1.1 and was not affected by N addition. Litter production was significantly stimulated after 3 years of N addition ($P < 0.01$; data not shown).
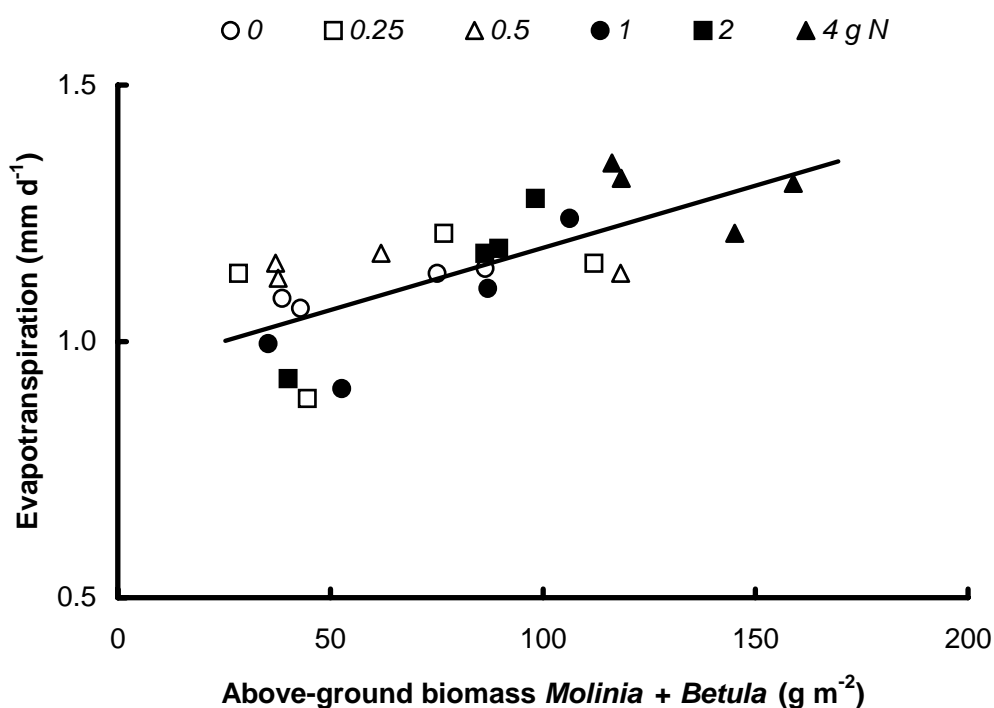

Figure 6.5: Individual above-ground biomass of *Molinia caerulea* (A) and *Betula pubescens* (B) after 3 years at different rates of experimental N addition (means + 1 SE; $n = 4$). Different letters indicate significant differences ($P < 0.05$) between N treatments (one-way ANOVA).

Foliar N concentration in *Betula* increased from 560 - 730 μmol g^{-1} DW at additions of 2 g N m^{-2} year^{-1} and lower, to 1400 μmol g^{-1} DW at the highest N treatment (Table 6.5; $P < 0.01$). The rather constant N concentration at lower N addition rates may indicate that *Betula* growth was limited by N. The concentrations of P and K in the leaves showed no significant differences (Table 6.5). N:P ratios in leaf tissue of *Betula* at loads of 0.25 g N m^{-2} year^{-1} and higher were above 16, suggesting growth limitation by P (Koerselman & Meuleman 1996). However, only at an addition rate of 4 g N m^{-2} year^{-1} was N accumulated as arginine (Table 6.3; $P < 0.001$). The concentrations of the other amino acids measured remained very low. Addition of 4 g N m^{-2} year^{-1} resulted in the storage of 378 μmol g^{-1} N in amino acids, which was 27% of the total N concentration (Table 6.4).

Evapotranspiration

Evapotranspiration was positively correlated with the above-ground biomass of *Molinia* and *Betula* (Figure 6.6). As indicated, the total above-ground biomass of both species was stimulated by N addition.

Figure 6.6: Relationship between above-ground biomass of *Betula pubescens* plus *Molinia caerulea* (g m^{-2}) and evapotranspiration (mm day^{-1}) during the final growing season of the experiment. Different N treatments are indicated by different symbols (linear regression: R^2 = 0.485; $P < 0.001$).

Discussion

Experimental design

We investigated the possible effects of elevated N deposition levels on the growth of *Betula* and *Molinia* in *Sphagnum fallax* turfs in a laboratory experiment that enabled us to eliminate the high background level of 4 g N m^{-2} year^{-1} in the field. In contrast to most earlier fertilisation experiments, in which N was added only six times a year, N was added three times a week in the present study. A low application frequency leads to an imbalance between the supply and demand of nutrients in the vegetation. It is not only the net rate, but also the regime of N deposition which influences its long-term effects. Many fertilisation experiments have been conducted over short periods and several of these studies mention the discrepancy between short-term (≤ 1 to 2 years) and long-term (≥ 3 to 4 years) responses (e.g. Rochefort *et al.* 1990; Gunnarsson & Rydin 2000; Aerts *et al.* 2001). This is why we conducted a 3-year experiment.

Rhizosphere chemistry

Addition of 2 g N m^{-2} year^{-1} or less had hardly any effect on the concentrations of free ammonium in the peat moisture of *S. fallax* turfs (Figure 6.2). During the first winter, an increase in free ammonium was only observed at an addition rate of 4 g N m^{-2} year^{-1}. At the other addition rates, the N added was completely taken up by the vegetation (especially *S. fallax*). Jauhiainen *et al.* (1998) also found high N uptake rates in *Sphagnum fallax*. *Sphagnum* species lack cuticles and, their leaves being only one cell layer thick, they are able to efficiently capture and utilise the atmospheric supply, thus making it unavailable for the roots of vascular plants (Woodin & Lee 1987; Lee & Woodin 1988; Malmer *et al.* 1994). In this situation, with low N availability in the rhizosphere, hardly any N was available for vascular plants like *Betula* and *Molinia*.

In the second and third years, there was still no major increase in N concentrations in the peat moisture, as the further development of the vegetation took up all added N. Only the addition of 4 g N m^{-2} year^{-1} led to increased ammonium concentrations during the final months of the experiment (Figure 6.2). However, these concentrations were still considerably lower than those measured in the surface layer of the peat at the site of origin (50 µmol l^{-1}), where a strong increase in abundance of *Molinia* and *Betula* has been observed in recent decades (personal observations).

Peat moisture pH at the end of the experiment was significantly lower at higher N addition rates due to cation exchange by *S. fallax.* Uptake of ammonium by *Sphagnum* is compensated for by excretion of protons, resulting in lower peat moisture pH (Clymo 1987). Carbon dioxide concentrations were very low compared to those measured in the top layer of peat in other Dutch peat bogs (500 - 1500 µmol l^{-1};

Tomassen & Smolders unpublished data). Smolders *et al.* (2001) found that *S. magellanicum* growing in a terrestrial situation not only depends on atmospheric CO_2 but also on high CO_2 concentrations in the peat moisture. Therefore, the growth of *S. fallax* in this experiment may have been limited by carbon, especially at the higher N addition rates.

Despite relatively high phosphate concentrations in the artificial rainwater, peat moisture phosphate concentrations were below 0.5 µmol l^{-1} (Table 6.1) due to high *Sphagnum* uptake rates, and probably limited optimal *Betula* and *Molinia* growth.

Nutrient supply and Sphagnum

The high uptake of N by *S. fallax* led to a change in colour due to increased chlorophyll a and b concentrations (Figure 6.4). *Sphagnum* which received 0.5 g N m^{-2} year^{-1} or more contained elevated concentrations of chlorophyll a + b compared to those measured in *S. fallax* from Northern Italy (1.2 µmol g^{-1} DW; Gerdol *et al.* 1996). It has been found for *Sphagnum cuspidatum* that mosses from a high-N site contained higher chlorophyll concentrations than those from a low-N site (Baxter *et al.* 1992). An increase in tissue chlorophyll concentrations can be the result of decreased growth dilution when P becomes limiting (Marschner 1986). In addition, if growth of *Sphagnum* is limited by CO_2, increased production of chlorophyll can enhance CO_2 fixation (Rice 1994; Smolders *et al.* 2001).

After 3 years of N addition, only the N concentrations in the capitula of *S. fallax* in the 4 g N m^{-2} year^{-1} treatment were in the same range of those measured at the field location (Figure 6.3). This was to be expected, as the *S. fallax* turfs were collected from a site with a long-term total deposition of approx. 3.5 g N m^{-2} year^{-1}. At lower N addition rates, the added N was insufficient to maintain constant tissue N concentrations, due to dilution by growth. The low capitulum N concentration of 400 µmol g^{-1} DW in *S. fallax* receiving no N appears to be the lower limit, since N concentrations after 3 years remained at the range of those measured directly after the pre-treatment. This is consistent with data from Malmer (1990) showing 410 µmol g^{-1} DW as the lowest N concentration measured in the apical part of *Sphagnum*.

N concentrations in *S. fallax* showed a strong linear correlation with the amount of N added (Figure 6.3). Increased N concentrations due to elevated N deposition rates have also been found for other *Sphagnum* species, including *S. fuscum, S. magellanicum, S. palustre, S. angustifolium* and *S. papillosum* (Pitcairn *et al.* 1995; Williams & Silcock 1997; Jauhiainen *et al.* 1998). *Sphagnum* species can therefore be used as biological indicators to estimate N deposition levels based on their tissue N concentrations (e.g. Risager 1998; Lamers *et al.* 2000; Gunnarsson & Rydin 2000). A maximum N concentration (Lamers *et al.* 2000; Berendse *et al.* 2001) was not reached in our experiment. Van der Heijden *et al.* (2000) propose a capitulum N concentration

of 15 mg g^{-1} DW (= 1071 µmol g^{-1} DW) as an indication of N pollution stress in *S. fallax*. In our experiment, the N concentrations in the capitula of *Sphagnum* receiving 4 g N m^{-2} year^{-1} equalled this critical value.

Based on the N:P ratio, *Sphagnum* growth appeared to be limited by N at loads of 1 g N m^{-2} year^{-1} and lower (Table 6.2; Koerselman & Meuleman 1996). Higher N loads resulted in P limitation (N:P ratio > 16). To prevent ammonium toxicity, many plants respond by synthesizing specific amino acids and amines, particularly those with a low C:N ratio (Marschner 1986). The concentrations of free amino acids in *S. fallax* strongly increased above an addition rate of 0.5 - 1 g N m^{-2} year^{-1}, corresponding to P-limiting conditions according to the N:P ratio (Tables 6.2 & 6.3). Arginine (C:N ratio 1.5) and asparagine (C:N ratio 2.0) concentrations in particular were elevated, due to nutrient imbalance in *Sphagnum* at increased ammonium availability. Several other studies have mentioned the production of free amino acids including arginine, asparagine and glutamine for different *Sphagnum* species at high N loads (Thönes & Rudolph 1983; Baxter *et al.* 1992; Nordin & Gunnarsson 2000; Smolders *et al.* 2001; Limpens & Berendse 2003). The present experiment allows the conclusion that the concentrations of N-rich free amino acids, which are produced as a detoxification mechanism, can be used as a good indication of future N saturation. Based on the concentrations of N-rich amino acids, we propose that N loads above 0.25 - 0.5 g m^{-2} year^{-1} lead to N saturation.

Nutrient supply and growth of Molinia and Betula

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

N addition had no effect on the above-ground biomass production by *Molinia* in the first experimental year, probably due to low N availability because of immobilisation by *Sphagnum*. After one year, N effects became more obvious and addition of 4 g N m^{-2} year^{-1} had a significant effect on above-ground biomass production by *Molinia* compared to the control treatment (Figure 6.5). Various experiments in other ecotypes have also shown stimulation of the growth of *M. caerulea* by N (e.g. Roelofs 1986; Heil & Bruggink 1987; Aerts & Berendse 1988). In addition, N addition had a significant stimulating effect on the production of inflorescences by *Molinia.* In a few of the turfs receiving high N loads, *Molinia* expanded by producing seedlings.

Growth and architecture of the *Betula* saplings (above-ground biomass, length, number of branches and leaves, and leave surface) was not significantly influenced by N addition within 3 years (Figure 6.5). High N addition rates did, however, lead to higher litter production, which may have negative effects on nutrient cycling and *Sphagnum* growth by shading (Heijmans *et al.* 2001). On the long-term, however, growth of *Betula* in our study might significantly be stimulated. If the uptake of nutrients by *Sphagnum* is hampered by shading, and if nutrient mobilisation from litter is stimulated, nutrient availability will increase for *Betula*.

Nutritional status of Molinia and Betula

Increased N availability had no effect on foliar N concentrations in *Molinia*, indicating that all N was used for biomass production and N was limiting growth. However, the N:P ratio was above 33 for all treatments (Table 6.5), suggesting that *Molinia* was strongly limited by P (Koerselman & Meuleman 1996). Güsewell *et al.* (1998) proposed that the N:P ratio could be a appropriate tool to predict short-term effects of nutrient enrichment at the level of individual species. The observed growth response at high N:P ratio supports the idea that *Molinia* is a species adapted to low P availability, as has also been found in earlier studies (Kirkham 2001). High N deposition levels have changed a substantial proportion of *Calluna*-dominated uplands in England and Wales from N-limited ecosystems into P-limited ones, favouring species like *Molinia* that are better adapted to P limitation (Kirkham 2001). Despite high N:P ratios, the growth of *Molinia* in our experiment was still limited by N. This is in agreement with Thornton (1991), who found an absence of growth response to P supply at low N availability, indicating growth limitation of *Molinia* by N. Based on our results, therefore the N:P ratio is not a suitable tool for detecting the absence of N limitation in *Molinia*. The N:P tool developed by Koerselman & Meuleman (1996) was based on results obtained at the vegetation level and not for individual plant species.

Based on the N:P ratio, the growth of *Betula* was limited by P above a load of 0.25 g N m^{-2} year^{-1} (Table 6.5; see also Koerselman & Meuleman 1996). Optimum nutrition for *Betula pendula* growth are known to be achieved at N:P ratios between 10 and 12, although a higher relative P requirement has been observed under nutritional stress conditions (Ericsson & Ingestad 1988). Foliar nutrient concentrations in our experiment were relatively low (Table 6.5). At P concentrations below 65 μmol g^{-1} DW, growth of *Betula* is limited by P, and normal concentrations have been reported to be 65 – 130 μmol g^{-1} DW (Hytönen & Kaunisto 1999). Fertilisation experiments on drained mires in northern Finland showed a positive effect of NPK and PK fertilisation on the growth of *Betula pubescens* (Penttila & Moilanen 1997), and no effect of N fertilisation. In contrast to *Molinia*, the *Betula* saplings in our experiment were not able to use the added N at high loads, due to P shortage. Peat moisture P

concentrations in the rhizosphere of Dutch bogs are relatively high (0.5 – 2.5 µmol o-PO$_4^{3-}$ l^{-1}) compared to those measured in Ireland and Norway (< 0.5 µmol o-PO$_4^{3-}$ l^{-1}) (Tomassen & Smolders unpublished data). The high P concentrations in Dutch bogs probably enable expansion of *Betula* at high levels of N deposition.

Just like *Sphagnum*, trees can respond to N saturation by detoxifying the excess ammonium to N-rich free amino acids, especially arginine (e.g. Van Dijk & Roelofs 1988). In our experiment, *Betula* accumulated foliar arginine upon addition of 4 g N m^{-2} year^{-1}, but unlike what we found in *S. fallax*, none of the other N-rich free amino acids were formed (Table 6.3). The fraction of amino acid N was 27% of the total N concentration, indicating a strong nutrient imbalance in the *Betula* saplings (Table 6.4). Näsholm & McDonald (1990) studied *Betula pendula* seedlings and found higher concentrations of total amino acid N in root and shoot at greater N supply. In their study, higher concentrations of amino acid N were mainly due to high concentrations of citrulline, glutamine, γ-aminobutyric acid and arginine, but amino acid N made up only 3 - 4% of the total foliar N concentration.

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

Interactions between Sphagnum and vascular plants

Sphagnum growth can be inhibited by high N availability (e.g. Jauhiainen *et al.* 1998). As N deposition levels increases, the growth of *Sphagnum* may decrease, and with it its function as a 'sink' for atmospheric elements (Lee & Woodin 1988). The resulting enhanced availability of N in the rhizosphere stimulates the growth of higher plants. *Sphagnum* peat produced under high N loads probably has a lower C:N ratio and is therefore more easily decomposed by bacteria (Aerts & Chapin 2000). However, mineralisation rates of peat from the present experiment showed no relationship with the N addition rates (data not shown). Despite the effect of N on the tissue N

concentration in *Sphagnum*, no differences in peat N concentrations were found between the various treatments. The added N was completely reabsorbed from dying *Sphagnum* parts, leading to similar mineralisation rates and no extra N source for vascular plants. Several other studies have found that P had a stronger effect on the mineralisation than N (Hogg *et al.* 1994; Aerts & Chapin 2000; Aerts *et al.* 2001).

Increased growth of vascular plants in bog systems at higher N deposition rates has been observed in various studies (e.g. Heijmans *et al.* 2001; Berendse *et al.* 2001; Limpens *et al.* 2003). Such plants include the shallowly rooted species *Vaccinium oxycoccus* (e.g. Lütke Twenhöven 1992; Heijmans *et al.* 2001), *Andromeda polifolia* and *Eriophorum vaginatum* (Redbo-Torstensson 1994). Previous studies have suggested that shading by vascular plants may reduce *Sphagnum* growth (Hayward & Clymo 1983; Heijmans *et al.* 2001). In the present experiment, an increase in the above-ground biomass of vascular plants, especially *Vaccinium oxycoccus,* was observed at the expense of *Sphagnum* growth (data not shown).

Increased total cover by *Betula* and *Molinia* had a stimulating effect on the evapotranspiration (Figure 6.6). Takagi *et al.* (1999) found similar increased evapotranspiration rates due to the invasion of vascular plants. The stimulated growth of vascular plants in bog vegetation also increases the vegetation structure and thereby the interception of water and input of dry deposition (e.g. Heil *et al.* 1988). The concomitant desiccation and N eutrophication hampers the growth of *Sphagnum* species and may ultimately enhance decomposition processes and stimulate the growth of N-dependent vascular plants. In the Netherlands, the dominance of *Betula* and *Molinia* is a common feature in the bog relicts, probably resulting from prolonged high N deposition levels combined with relatively high P availability.

Vegetation changes in N-polluted bogs

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

Acknowledgements

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How bird droppings can affect the vegetation composition of ombrotrophic bogs

Large densities of waterfowl influences the nutrient input of bog pools at Clara bog, Ireland
(Picture taken by Emiel Brouwer)

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Submitted

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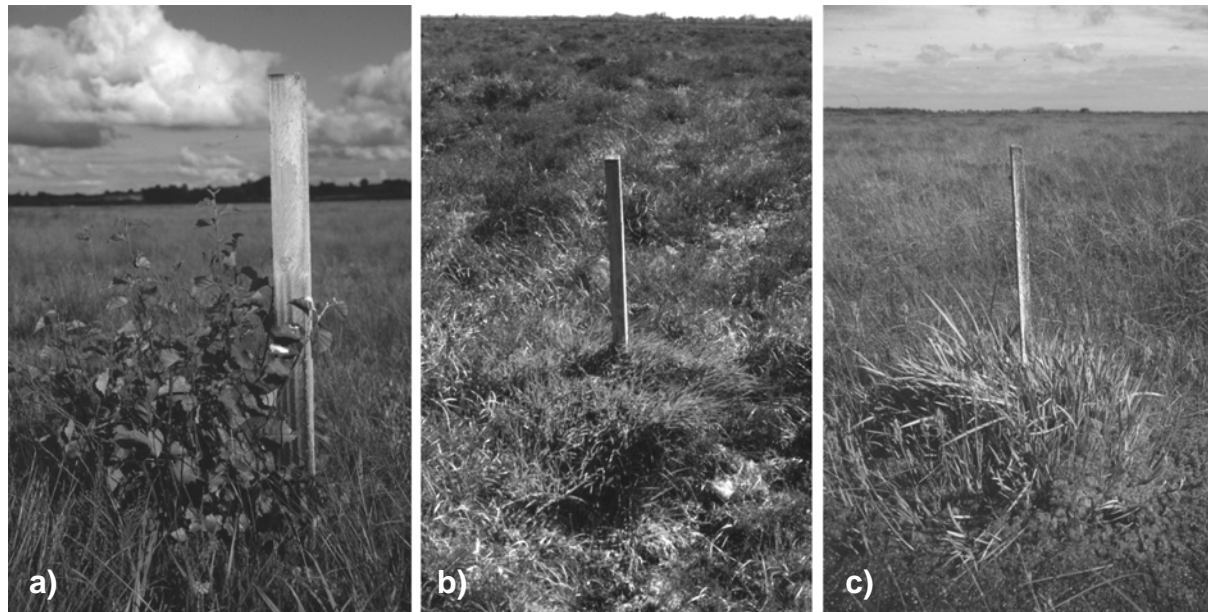
Abstract

Ombrotrophic bogs depend on nutrient input from the atmosphere, so bird droppings may be a significant nutrient source. We studied the influx of N, P and K by bird droppings, and their effects on the vegetation composition and development of Irish ombrotrophic bogs. Bird droppings significantly increased the influx of N, K and particularly P. Concentrations of N, P and K in the peat and vegetation were much higher at sites with bird droppings than at reference sites without bird droppings. *Pinus sylvestris* trees were able to grow much better at the sites with bird droppings and growth was severely limited by P and K at the reference sites. Sites with bird droppings allowed atypical species to grow on ombrotrophic bogs. These self-enhancing changes in vegetation composition may have important effects on the vegetation structure, atmospheric nutrient input and interception of precipitation.

Introduction

Ombrotrophic bogs are unique ecosystems characterised by vegetation adapted to low nutrient availability. In pristine areas, production is generally limited by nitrogen (N), but in areas with increased N influx *Sphagnum* productivity may change from being N-limited to being phosphorus (P)-limited (Aerts *et al.* 1992). Increased atmospheric nutrient inputs (especially N) can have important effects on the vegetation composition in various natural and semi-natural ecosystems (e.g., Bobbink *et al.* 1998; Bobbink & Lamers 2002) including ombrotrophic bogs (Tomassen *et al.* 2003; Limpens *et al.* 2003). As a result of high atmospheric nutrient influx, ombrotrophic bogs have been invaded by more nutrient-demanding species such as *Molinia caerulea* and *Betula* sp., a development which has been accompanied by a decline of ombrotrophic species (Barkman 1992; Aaby 1994; Hogg *et al.* 1995; Risager 1998).

At low N deposition rates, N concentrations in the peat water are low as a result of high N uptake rates by *Sphagnum* (e.g. Lee & Woodin 1988; Jauhiainen *et al.* 1998), so vascular plants depend on N mobilised by mineralisation processes in the underlying peat (Malmer 1993; Malmer *et al.* 1994). At high N deposition rates, some N leaches through the *Sphagnum* layer to the roots of vascular plants (Lee & Woodin 1988; Aerts *et al.* 1992; Lamers *et al.* 2000). Increased availability of nutrients in the rhizosphere leads to an increased cover of vascular plants and a reduction in

Sphagnum growth due to shading (Hayward & Clymo 1983; Heijmans *et al.* 2001; Berendse *et al.* 2001).

Figure 7.1: *Betula pubescens* (a), *Molinia caerulea* (b) and *Narthecium ossifragum* (c) growing in the vicinity of wooden survey posts on which birds have perched and defecated during more than 10 years. [Reprinted in colour on page 189]

In contrast to Dutch bogs, large-scale invasion of more nutrient-demanding species has not been observed on bogs in the Irish midlands, where total N deposition rates are relatively low (0.6 - 0.8 g m^{-2} yr^{-1}; Tomassen *et al.* 2004). Stands of *Betula* are, however, common in many parts of Ireland on cut-over and dried-out bogs, or are sometimes found to be associated with flushes or soaks (Cross 1987), which are areas of nutrient enrichment. On several Irish bogs, growth of atypical species, such as *M. caerulea* and *Betula* trees, can be observed in the immediate vicinity of wooden survey posts which were placed there about 10 years ago (Figure 7.1a,b). Birds regularly perch on these wooden posts (personal observations) and the increased influx of nutrients from their droppings may be responsible for the observed changes in vegetation composition in the immediate vicinity of the posts. Several studies have reported the effect of large densities of waterfowl on the availability of nutrients (N and P) in oligotrophic waters (Leentvaar 1967; Gould & Fletcher 1978; Paffen 1990). Information about the effects of bird droppings on terrestrial vegetation is limited, although there have been some studies in Antarctica (e.g. Cocks *et al.* 1998; 1999), and Bazely and Jefferies (1985) found increased production and shoot N concentrations of salt-marsh vegetation as a result of goose faeces.

We thought that the input of nutrients originating from bird droppings might locally affect the vegetation composition and development of ombrotrophic bogs. To test

this hypothesis, we measured influx of major nutrients by bird droppings on an Irish bog. The effects of this influx on the vegetation were determined by comparing the growth of and nutrient concentrations in vegetation exposed to bird droppings with those on nearby unsupplemented reference sites, on three Irish bogs. The effects of bird droppings on the vegetation of ombrotrophic bogs are discussed and put in a larger context.

Materials and methods

Field sites

We studied the effects of bird droppings at three ombrotrophic bogs in the midlands of Ireland: Clara bog, Raheenmore bog and Sharavogue bog. The Irish midlands have a mean annual precipitation of 800 - 1000 mm (Jordan 1997). The mean annual temperature (1951 - 1980) is 9.3 °C and the July and January means are 14.8 °C and 4.4 °C, respectively. Clara bog (County Offaly; 53°19′N; 7°37′W) is situated in a depression adjacent to a complex of esker ridges, and its overall depth is in most cases around 10 m (Warren *et al.* 2002). Clara bog (665 ha) is 4.2 km long and 1.2 km wide and is divided by a road into a western and an eastern part. Raheenmore bog (County Offaly, 53°20′N; 7°21′W) developed in a depression between a number of low hills, and its peat reaches depths of up to 15 m (Warren *et al.* 2002). The actual bog covers approximately 160 ha. In 1990 - 1991, wooden posts were placed on both Clara bog and Raheenmore bog for hydrological research (Van der Schaaf 1999). Nowadays, the vegetation near these posts is dominated by atypical species such as *Molinia caerulea*, *Betula pubescens*, *Pinus sylvestris*, *Juncus effusus* and *Agrostis canina* (Figure 1a,b). Sharavogue bog (County Offaly, 53°02′N; 7°54′W) is characterised by several small *Pinus sylvestris* stands on the bog (223 hectares). The understorey vegetation of these stands includes species such as *Molinia caerulea* and *Juncus effusus*, which indicate more eutrophic conditions.

Vegetation

At Clara bog, the vegetation on sites affected by bird droppings (in the vicinity of survey posts) and on reference sites (2 m from the survey posts) was described in terms of the percentage cover of vascular plants, bryophytes and lichens. In the case of species with an insignificant cover (< 5 %), the number of individuals was estimated (+ = several individuals; # = large number of individuals).

Input of nutrients by bird droppings

Input of nutrients by bird droppings was measured around three wooden survey posts on Clara bog East by collecting precipitation and bird droppings using a polyethylene container (10 l) connected to a circular funnel (22 cm diameter). Background input of nutrients ($n = 3$) was measured by collecting bulk precipitation using a black polyethylene bottle (2 l) connected to a funnel (9.75 cm diameter) according to Tomassen *et al.* (2004). The collectors were dug in the peat and contained 2 ml of a 200 mg l^{-1} $HgCl_2$ solution to inhibit microbial activity. Between September 2000 and September 2001, rainwater + bird droppings and rainwater were collected eight times (November 2000, February, March, April, May, June, July and September 2001) and the volume was measured in the field. Part of the bird dropping samples was filtered through a Whatman GF/C filter (pore size 1.2 μm). This method allowed us to measure only the soluble part of the nutrients from the bird droppings and will underestimate the total nutrient input by bird droppings. Samples were stored at -20 °C until further analysis.

Effect of bird droppings on the nutrient concentrations in the vegetation

On Clara bog, nutrient concentrations in the top layer of the peat (0 – 10 cm) were measured in the immediate vicinity of the survey posts ($n = 5$) and at reference sites (2 m from the posts; $n = 5$). The peat was stored in airtight plastic bags at 4 °C until chemical analysis. Uptake of bird-derived nutrients by *Sphagnum* mosses was determined by comparing nutrient concentrations in the capitula (0 - 2 cm) of *Sphagnum magellanicum* or *S. papillosum* at sites with bird droppings ($n = 11$) and at reference sites without bird droppings ($n = 6$). In addition to *Sphagnum* from Clara bog (posts), we also analysed *Sphagnum* plants from Raheenmore bog (around posts) and Sharavogue bog (underneath *P. sylvestris* trees). For *Narthecium ossifragum* L. the length of the longest leaf and foliar nutrient concentrations (15 plants per location) were measured in the immediate vicinity of wooden posts ($n = 4$) and at reference sites (2 m from the posts; $n = 2$) at Clara bog.

Sharavogue bog is characterised by several small groups of *Pinus sylvestris* trees with an understorey dominated by *Calluna vulgaris, Molinia caerulea, Betula pubescens, Vaccinium myrtillus* and *Juncus effusus*. The larger trees supported nests of the hooded crow (*Corvus corone cornix*) and bird droppings were obvious. All the small *Pinus* trees were established in 1989, which was an extremely dry year (Mills 2000). During this drought there was a fire which probably destroyed all *Pinus* seedlings. The young *Pinus* trees at a larger distance from the 'mother' tree were much smaller, though they all became established directly after the fire (1989). In order to determine the effects of bird droppings on the growth of these trees, tree height and lifespan of the needles (number of year classes present) were determined in three transects

going out from a 'mother' tree. On Clara bog East, the effects of bird droppings on *Pinus sylvestris* were examined experimentally by planting small *Pinus* trees near wooden posts ($n = 4$) as well as at a distance of 5 metres from these posts ($n = 4$). Trees were planted in September 2000. The *Pinus* trees (height: 52 ± 5 cm) were collected in a nearby cut-away. In July 2002, the growth of the trees was determined by measuring the increase in height, the length of the current year's shoot and needles, and the lifespan of the needles. Needle samples were taken for analysis of major nutrient concentrations.

Chemical analysis

To analyse nutrient concentrations in plant tissue and peat, dried samples (48 hours at 70 ºC) were ground up in liquid nitrogen. Nitrogen and carbon concentrations were measured in dried samples with a CNS analyser (type NA1500; Carlo Erba Instruments, Milan, Italy). Potassium and phosphorus concentrations were determined by digesting 200 mg of dried material in sealed Teflon vessels in a Milestone microwave oven (type: mls 1200 Mega, Sorisole, Italy) after addition of 4 ml HNO_3 (65%) and 1 ml H_2O_2 (30%). After dilution, the digests were kept at 4 ºC until analysis.

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

Potential CO_2 production rates of the peat were measured by incubating 50 g of fresh peat in air-filled 250 ml infusion flasks, sealed with airtight stoppers. Duplicate incubations were made. The flasks were kept in the dark at 20 °C, and over a period of four weeks CO_2 concentrations in the headspace were measured weekly. CO_2 concentrations were measured using an infrared carbon analyser (model PIR-2000, Horiba Instruments, Irvine, USA). CO_2 production rates were calculated by linear regression of the measurements and expressed on a dry mass basis.

Statistical analysis

Prior to statistical analysis, data were log-transformed to make the variance less dependent on the means and so that the data were approximately Gaussian. All statistical analyses were carried out using the SPSS for Windows software package (version 10.0.7; SPSS Inc., Chicago, USA). Differences between sites with bird

droppings and reference sites without bird droppings were tested with an independent samples *t*-test. Correlations between height and lifespan of the needles of the small *Pinus sylvestris* trees and the distance from the 'mother' tree were analysed with a Pearson correlation test, and described by a linear regression analysis. For clarity of presentation, the means and standard errors (SE) presented in the tables are for the untransformed data.

Table 7.1: Vegetation cover (%) at a reference site 2 m from the posts and in the immediate vicinity of wooden survey posts at Clara bog; *n* = 5. Key: + = several individuals; # = large number of individuals but no significant cover.

	Reference					*Post*				
	1	2	3	4	5	1	2	3	4	5
Cladonia portentosa (Dufour) Coem.	+		+		20					
Dicranum scoparium Hedw.			+		+					
Drosera rotundifolia L.	#		10	5	#					
Menyanthes trifoliata L.	+									
Rhynchospora alba (L.) Vahl	+	5	5		+					
Vaccinium oxycoccos L.		#								
Andromeda polifolia L.			+	+	+				5	+
Calluna vulgaris (L.) Hull	5	10	5	35	+		10		+	
Carex echinata Murray	+					#				
Carex panacea L.					+					10
Erica tetralix L.	5	20	5	5	5	+		5		
Eriophorum angustifolium Honck.	#	#	#	#		#		+		+
Eriophorum vaginatum L.	#	#	#	#	#			#	25	#
Narthecium ossifragum (L.) Huds.	10	30	#	#	5					30
Potentilla reptans L.	+					+	+			
Sphagnum cuspidatum Hoffm.	10	20				5				
Sphagnum magellanicum Brid.	25	15	30	10	+		#			
Sphagnum papillosum Lindb.	35	#	+		10	5				
Sphagnum rubellum Wils.	10		40	45	60					5
Agrostis canina L.						#				35
Cerastium fontanum Baumg. ssp. *vulgare* (Hartm.) Greuter & Burdet									10	+
Comarum palustre L.							+			
Epilobium hirsutum L.							+			
Holcus lanatus L.								85		
Hydrocotyle vulgaris L.							+			
Molinia caerulea (L.) Moench						90	80			
Poa annua L.									40	
Polygonum persicaria L.										20
Polytrichum commune Hedw.							10			
Prunus sp.						+				+
Pteridium aquilinum (L.) Kuhn							+			
Rubus fruticosus L.								10	5	
Sambucus nigra L.									+	
Trifolium repens L.									5	

Results

The vegetation around the wooden posts on Clara bog was frequently dominated by atypical species, including *Molinia caerulea, Agrostis canina, Epilobium hirsutum, Rubus fruticosus, Holcus lanatus, Poa annua* and *Trifolium repens* (Table 7.1), all of which were absent from the reference sites. Some typical bog species, such as *Sphagnum* mosses, had lower cover; others such as *Drosera rotundifolia* and *Rhynchospora alba* were absent altogether from the areas around the posts (Table 7.1).

Table 7.2 shows that the influx of N, P and K in bird droppings was tens or hundreds of times greater than that in precipitation. This difference was reflected in the peat (Table 7.3), though the concentration at the site with bird droppings was only a few-fold greater than at the reference site. At sites with bird droppings peat C:N and C:P ratios were significantly lower, and the potential CO_2 production rate was increased (Table 7.3). Increased input of N, P and K by bird droppings significantly affected nutrient concentrations in *Sphagnum* (Table 7.4). The N:P ratio in *Sphagnum* was significantly lower at the site with bird droppings than at the reference site. *Narthecium ossifragum* plants were significantly taller and P concentrations and N:K ratios were significantly greater at the site with bird droppings than at the reference sites (Table 7.5 & Figure 7.1c). Foliar N concentrations were unaffected, but K concentrations and N:P ratios were significantly lower at the site with bird droppings.

Table 7.2: Mean ($\pm$ SE, n = 3) influx from precipitation and precipitation + bird droppings (September 2000 – September 2001) of N (NH_4^+ and NO_3^-), P and K at Clara bog. *** $P \leq 0.001$ (*t*-test)

	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	P	K
	$g\ m^{-2}\ year^{-1}$	$g\ m^{-2}\ year^{-1}$	$g\ m^{-2}\ year^{-1}$	$g\ m^{-2}\ year^{-1}$
Influx from precipitation	0.3 ± 0.1	0.14 ± 0.00	0.02 ± 0.00	0.2 ± 0.1
Influx from bird droppings	18.4 ± 2.3	0.04 ± 0.00	3.52 ± 0.76	6.0 ± 1.6
	***	***	***	***

Table 7.3: N, P and K concentrations on a dry mass basis, C:N and C:P ratios and CO_2 production rate (mean $\pm$ 1 SE) of superficial peat (0 – 10 cm) at a reference site at 2 m from the posts and in the immediate vicinity of posts at Clara bog; n = 5. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ (*t*-test).

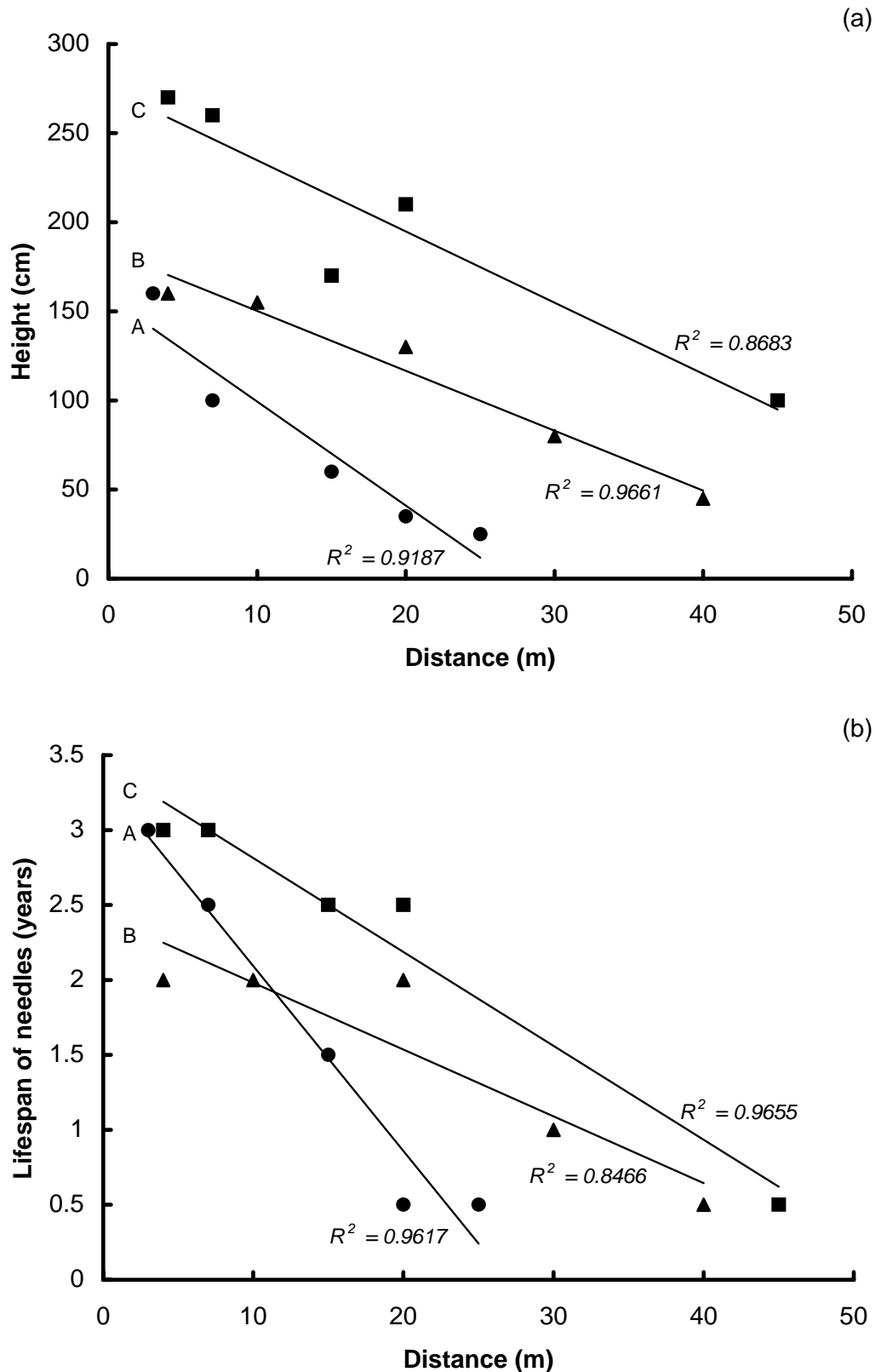|	N	P	K	C:N ratio	C:P ratio	CO_2 production rate
	$(\mu mol\ g^{-1})$	$(\mu mol\ g^{-1})$	$(\mu mol\ g^{-1})$	$(g\ g^{-1})$	$(g\ g^{-1})$	$\mu mol\ g^{-1}\ d^{-1}$
Reference	879 ± 78	13 ± 2	8.3 ± 0.8	40 ± 4	1246 ± 119	11.7 ± 5.9
Post	1832 ± 274	57 ± 16	28.6 ± 2.8	21 ± 3	350 ± 66	25.3 ± 12.7
	**	***	***	**	***	*

Table 7.4: Capitulum N, P and K concentrations on a dry mass basis, and N:P and N:K ratios (mean ± 1 SE) in *Sphagnum* from reference sites (*n* = 6) and sites affected by bird droppings (*n* = 11) at three Irish bogs (Clara bog, Sharavogue bog and Raheenmore bog). n.s. *P* > 0.05, *** *P* ≤ 0.001 (*t*-test).

	N	P	K	N:P ratio	N:K ratio
	($\mu mol\,g^{-1}$)	($\mu mol\,g^{-1}$)	($\mu mol\,g^{-1}$)	($g\,g^{-1}$)	($g\,g^{-1}$)
Reference	412 ± 28	9 ± 1	78 ± 4	22 ± 2	1.9 ± 0.1
Post	1086 ± 58	49 ± 4	128 ± 7	11 ± 1	3.2 ± 0.3
	***	***	***	***	n.s.

Table 7.5: Length of longest leaf, N, P and K concentrations on a dry mass basis, and N:P and N:K ratios (mean ± 1 SE) in *Narthecium ossifragum* from a reference site 2 m from the posts (*n* = 2) and the immediate vicinity of posts (*n* = 3) at Clara bog. n.s. *P* > 0.05, * *P* ≤ 0.05, ** *P* ≤ 0.01, *** *P* ≤ 0.001 (*t*-test).

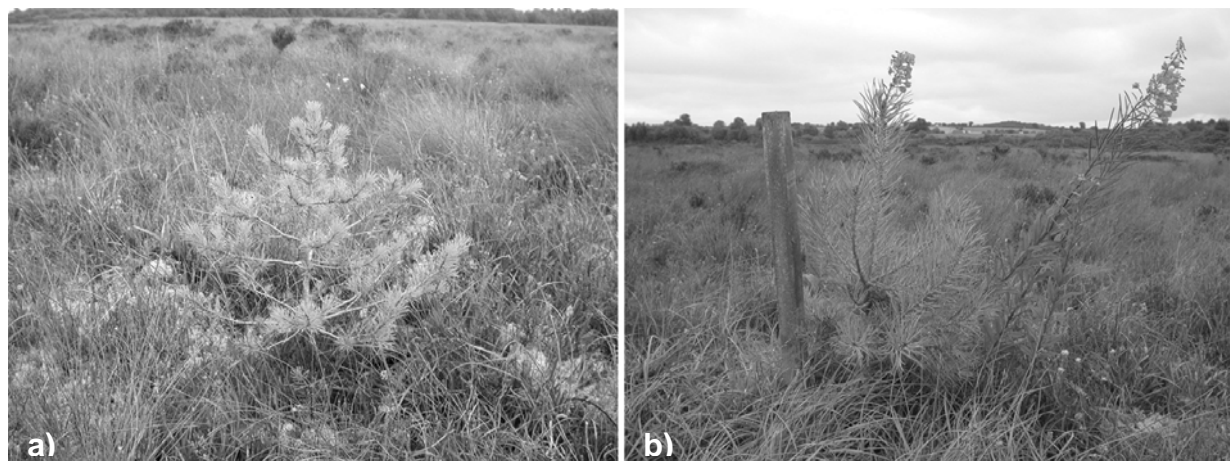|	Length longest leaf	N	P	K	N:P ratio	N:K ratio
	(mm)	($\mu mol\,g^{-1}$)	($\mu mol\,g^{-1}$)	($\mu mol\,g^{-1}$)	($g\,g^{-1}$)	($g\,g^{-1}$)
Reference	86 ± 5	1089 ± 43	12 ± 1	326 ± 25	41 ± 4	1.2 ± 0.0
Post	206 ± 7	1291 ± 57	24 ± 1	194 ± 18	24 ± 1	2.4 ± 0.3
	***	n.s.	**	*	**	*

At Sharavogue bog, the small *Pinus sylvestris* trees of similar age were significantly taller closer to the 'mother' tree than at greater distances (Figure 7.2a). The lifespan of the needles was significantly longer close to the 'mother' tree (Figure 7.2b). Overall, the trees were more vigorous closer to the 'mother' tree. On Clara bog, transplantation of small *P. sylvestris* trees to the immediate vicinity of the wooden posts significantly stimulated the growth of these trees compared to trees transplanted to reference sites (Table 7.6). The trees growing around the posts were more vigorous (Figure 7.3) and the length of the current year's shoots (of the 2002 growing season) was significantly greater, while the needles had a longer lifespan (Table 7.6). The total height increase of the trees between September 2000 and July 2002 was 13% at the reference sites, against 80% around the posts. The length of the current year's shoots in 2002 was 47% smaller than that for 2000 at the reference sites and 201% larger next to the wooden posts (Table 7.6). P concentrations measured in the current year's needles (2002) of the *Pinus* trees were significantly increased in the immediate vicinity of the posts, whereas N and K concentrations remained unaffected (Table 7.6). N:P ratios in the needles were significantly greater at the reference sites.

Figure 7.2: Height (a) and lifespan of the needles (b) of *Pinus sylvestris* trees measured in three transects (A – C) starting from the 'mother' tree (0 m) at Sharavogue bog, September 2000. All trees were 11 years old. Linear regression yields: height: P_A = 0.01, P_B = 0.003 and P_C = 0.02; lifespan of needles: P_A = 0.003, P_B = 0.03 and P_C = 0.003.

Table 7.6: Growth variables, nutrient concentrations on a dry mass basis, and ratios in current year's needles (mean ± 1 SE) of *Pinus sylvestris* in July 2002, 2 years after transplantation (September 2000) to reference sites 2 m from the posts and to the immediate vicinity of survey posts, at Clara bog; *n* = 4. n.s. *P* > 0.05, * *P* ≤ 0.05, ** *P* ≤ 0.01, *** *P* ≤ 0.001 (*t*-test).

	Reference	*Post*	
Length of current year's shoot (cm)	4.0 ± 0.5	28.5 ± 3.5	***
Total height (cm)	59 ± 10	86 ± 3	*
Length of needles (cm)	3.0 ± 0.5	5.9 ± 0.4	**
Lifespan of needles (yr)	1.6 ± 0.1	2.8 ± 0.1	***
Increase in height between 2000 and 2002 (%)	13 ± 3	80 ± 21	*
Change of length of current year's shoot between 2000 and 2002 (%)	-47 ± 15	201 ± 64	*
N (μmol g^{-1})	1104 ± 172	811 ± 59	n.s.
P (μmol g^{-1})	17 ± 3	37 ± 8	*
K (μmol g^{-1})	84 ± 25	65 ± 8	n.s.
N:P ratio (g g^{-1})	33 ± 8	10 ± 1	*
N:K ratio (g g^{-1})	5.8 ± 1.5	4.7 ± 0.8	n.s.

Figure 7.3: *Pinus sylvestris* trees growing (a) at a reference site 2 m from the post and (b) in the vicinity of a wooden survey post at Clara bog East, 2 years after transplantation. [Reprinted in colour on page 189]

Discussion

Bird droppings and nutrient limitation in ombrotrophic vegetation

As mentioned in the Introduction, ombrotrophic bogs are oligotrophic ecosystems and the production of pristine bogs is limited by N (Aerts *et al.* 1992). The total N deposition rates in the Irish midlands are below the critical N load for ombrotrophic bogs (0.5 – 1.0 g m^{-2} yr^{-1}; Bobbink & Roelofs 1995; Risager 1998; Tomassen *et al.* 2003),

and no invasion of atypical species such as *Molinia caerulea* and *Betula pubescens* has been observed. By contrast, more nutrient-demanding atypical species were able to grow in the immediate vicinity of wooden survey posts (Figure 7.1). Although wooden posts can leach nutrients, this is very likely to be a slow process, whereas the input of nutrients by bird droppings was extremely high (Table 7.2). Only the input of soluble inorganic N was measured, but even then bird droppings increased the total inorganic N input rate to 18.5 g m^{-2} yr^{-1}. The actual input of N will be much higher since bird droppings contain large amounts of organic N compounds (e.g. urea) and some of the N could have been volatilised.

The input rate of inorganic N is by far exceeding the critical N load for ombrotrophic bogs. Above this critical N load, changes in the vegetation composition can be expected, as has been found in the Netherlands, where total N deposition rates are extremely high (approx. 4 g N m^{-2} yr^{-1}; e.g. Tomassen *et al.* 2003). At high N deposition rates, the production of the vegetation of ombrotrophic bogs is often limited by P instead of N (Aerts *et al.* 1992). The sites with bird droppings were characterised by increased availability of all major nutrients, and the top peat layer had significantly higher N, P and K concentrations (Table 7.3). Cocks *et al.* (1999) also found that soils near snow petrel nests contained high levels of N and P (K was not measured) and that the effects of the bird droppings were no longer noticeable at a distance of 2 metres. We also found that the influence of the bird droppings was very local.

Given that nutrient availability usually limits the activity of decomposing organisms, it seems likely that decomposition processes are stimulated when C:N, C:P or C:K ratios are low and N, P or K concentrations are high (e.g. Swift *et al.* 1979; Updegraff *et al.* 1995; Smolders *et al.* 2002). In our study, the potential CO$_2$ production rate of the peat was greater around the posts, where nutrient concentrations were significantly increased (Table 7.3). The effects of changes in peat quality on decomposition and nutrient cycling result in stimulated mobilisation and availability of nutrients for the vegetation. The NPK fertilisation by bird droppings had a remarkable effect on the vegetation near the posts, within a relatively short period (Table 7.1). Maksimova & Yudina (1999) showed that even much lower NPK fertilisation rates (0.5, 1 and 0.75 g m^{-2} yr^{-1}, respectively), applied for six years in a Sedge-*Sphagnum* bog, led to rapid changes in the vegetation composition, even 15 years after their application ceased.
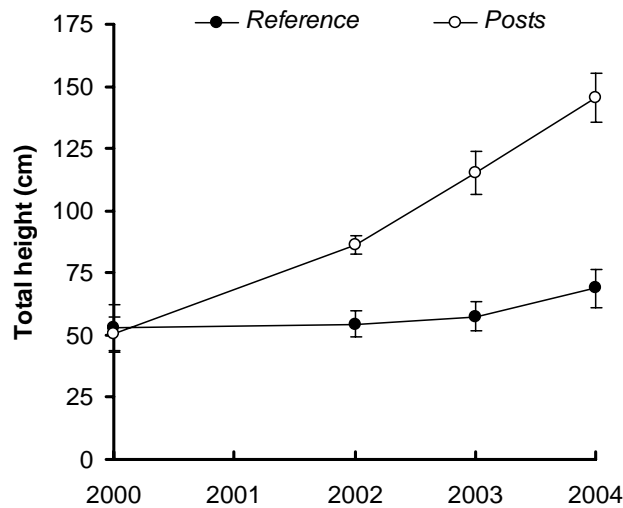
The increased input of nutrients around the posts was also reflected in much higher N, P and K concentrations in the capitula of *Sphagnum* mosses (Table 7.4). A stable isotope study in Antarctica showed that bird droppings were a major source of N for the vegetation (Cocks *et al.* 1998). The tissue N:P ratio exceeded 16, indicating that growth of *Sphagnum* at the reference sites was limited by P and growth at the sites with bird droppings by N (N:P ratio < 14; Koerselman & Meuleman 1996). Limpens *et al.* (2004) also found that growth of *Sphagnum* in Ireland (Clara bog) is not limited

by N but by P. Although N deposition rates in Ireland are below the critical N load for ombrotrophic bogs, *Sphagnum* productivity is nowadays limited by P. If, however, K is also taken into account, *Sphagnum* growth at the reference sites was co-limited by P and K, while growth at the sites with bird droppings was limited by K alone (a tissue N:K ratio above 1.4 indicates K limitation; Hoosbeek *et al.* 2002). According to Hoosbeek *et al.* (2002), *Sphagnum* growth in bogs of north-western Europe is often limited (or co-limited) by K.

At low nutrient availability, *Sphagnum* immobilises most of the nutrients, leading to relatively low nutrient availability in the rhizosphere of vascular plants (Lamers *et al.* 2000; Berendse *et al.* 2001). Increased nutrient input will ultimately lead to higher nutrient availability for (more nutrient-demanding) vascular plants as the *Sphagnum* filter becomes saturated (Lamers *et al.* 2000). Although the vegetation around the posts was frequently dominated by atypical species, *Narthecium ossifragum*, which is a characteristic plant for bogs, was also able to profit from the increased input of nutrients by increasing its length (Figure 7.1c & Table 7.5). The growth rate of *N. ossifragum* on mires in the UK has been found to be determined by the supply and uptake of phosphate and calcium (Summerfield & Rieley 1975). Based on the N:P ratio we measured, growth of *N. ossifragum* was limited by P at both the reference and bird dropping sites (Koerselman & Meuleman 1996). A fertilisation experiment in southern Sweden showed also that P is a growth-limiting nutrient for *N. ossifragum* (Malmer *et al.* 2003). At the sites with bird droppings, we measured significantly lower foliar K concentrations, and the N:K ratio above 1.4 indicates that K could be co-limiting growth (Table 7.5). We must consider, however, that strongly increased growth paired with decreasing nutrient concentrations can indicate a considerable increase in nutrient uptake, although at a lower rate than the rate of biomass accumulation. Tissue nutrient concentrations are therefore not the best measure of the effect of bird droppings because the bird droppings had a strong effect on the biomass (measured as length of the longest leaves) of *N. ossifragum.*

Intact Irish bogs are characteristically devoid of trees such as *Betula* species and *Pinus sylvestris*. Sharavogue bog, however, features small groups of *P. sylvestris* trees. The larger trees had several nests of the hooded crow and the understorey indicated more eutrophic growth conditions. Tissue nutrient concentrations in the *Sphagnum* growing underneath the *Pinus* trees were increased compared to those at the reference sites (Table 7.4). All the smaller trees appeared to have become established in the same year, although their height differed significantly (Figure 7.2). Abiotic conditions were obviously more suitable for tree growth closer to the 'mother' tree, probably due to increased nutrient availability from bird droppings. A 40-year study in south-central Sweden revealed that vascular plant species, including tree species, increased in frequency probably as a result of increased availability of N and drier conditions (Gunnarsson *et al.* 2002).
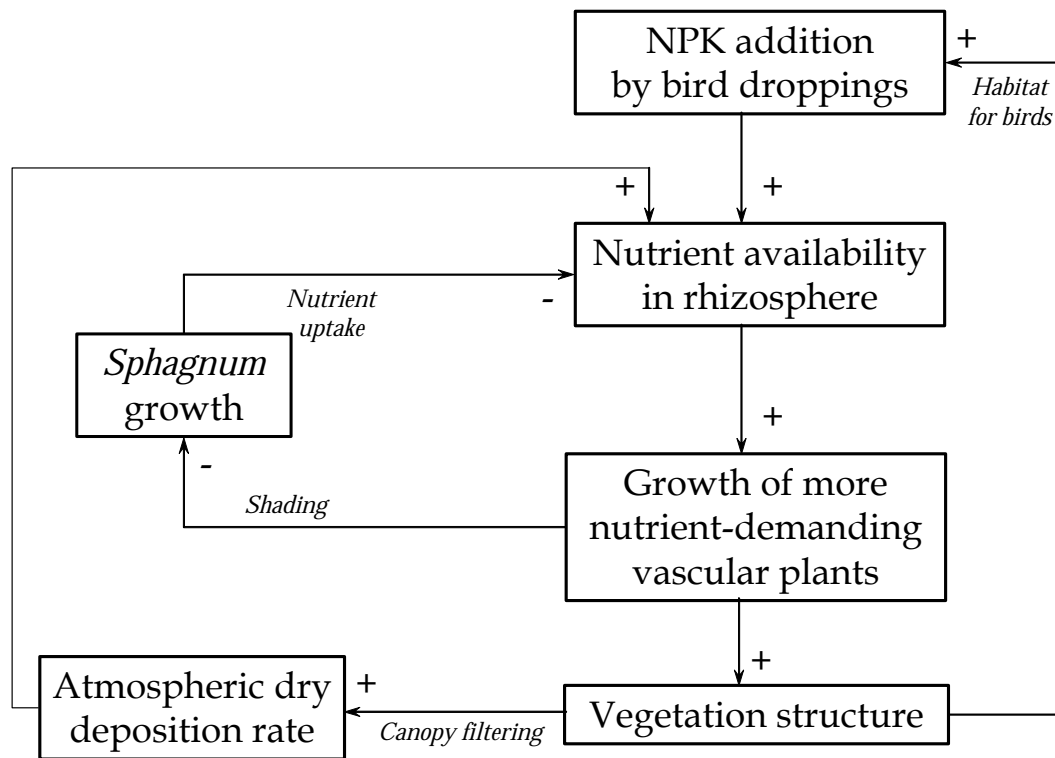
Figure 7.4: Change in total height (mean ± 1 SE; *n* = 4) of *Pinus sylvestris* trees between September 2000 and June 2004, 4 years after transplantation to reference sites 2 m from survey posts and to the immediate vicinity of the posts at Clara bog. Results for GLM for repeated measures are as follows: bird dropping effect *P* < 0.01, time effect *P* < 0.001 and interaction bird dropping * time *P* < 0.01.

On Clara bog, the trees which had been planted in the immediate vicinity of the survey posts were also able to grow very fast and appeared very vigorous (Figure 7.3b). In contrast, the small *Pinus* trees we planted at the reference sites showed very poor growth and had yellowish needles (Figure 7.3a & Table 7.6). Recent measurements of the height of the *Pinus* trees, 4 years after transplantation, show that the difference in growth rate of *Pinus* at reference sites and in the vicinity of survey posts has become even more pronounced (Figure 7.4). The average relative proportions of nutrients (N = 100) in the current year's needles of *Pinus* at the reference sites were 100 N, 3 P, 21 K, 21 Ca and 9 Mg (in g). These proportions indicate that P and K concentrations were too low for optimal growth, viz. 100 N, 14 P, 45 K, 6 Ca and 6 Mg (Ingestad 1979). Around the wooden posts (100 N, 10 P, 22 K, 44 Ca and 11 Mg) only K seemed supra-optimal for growth. These findings are supported by the threshold values of nutrient concentrations for nutrient deficiency in *Pinus sylvestris* as given by Houdijk & Roelofs (1993). Growth of *P. sylvestris* on Clara bog was limited by both P and K, whereas growth at the sites with bird droppings was only limited by K, due to the huge input of P from bird droppings. Finér (1992) also found that the growth of trees on bogs is often limited by P and K, as PK and NPK fertilisation increased the growth of *P. sylvestris* growing on an ombrotrophic pine bog. Once established, an increased tree cover can have radical effects on the bog ecosystem (Frankl & Smeidl 2000; Ohlson *et al.* 2001). *P. sylvestris,* for instance, is capable of creating and maintaining a fundamentally different environment in bogs, and brings inevitably an end to *Sphagnum* dominance by

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altering local hydrological conditions and increased accumulation of litter (Frankl & Smeidl 2000; Ohlson *et al.* 2001).

Figure 7.5: Simplified scheme of the effects of bird droppings on ombrotrophic bog vegetation.

Effect of bird droppings on ombrotrophic bogs

Based on our results, we conclude that non-characteristic herbs and trees are only able to grow on ombrotrophic bogs if nutrient availability, especially that of P and K, is sufficiently high. Sites on bogs characterised by increased nutrient availability include the so-called flushes or soak systems (Kelly 1993), but also sites eutrophicated by the presence of birds. Avian communities of typical open raised bogs are generally poor in species. Increased structural complexity of the vegetation, particularly in terms of the vertical component, increases the density and foraging and nesting opportunities for birds (Bölscher 1995). The plants that most notably affect the increase in structural complexity in bogs are *Molinia caerulea, Myrica gale, Juncus effusus, Rubus fruticosus, Betula pubescens* and *Pinus sylvestris,* but the wooden survey posts also proved to attract birds. The major increase in nutrient availability from bird droppings allowed these plant species in particular to grow on the bogs. This increase in vegetation structure provides birds with foraging and nesting opportunities, which leads to greater densities of birds and a further increase in nutrient availability. This increased nutrient availability in turn further increases the

vegetation structure and finally leads to even higher densities of birds (Figure 7.5). We assume that this self-enhancing process may result in small patches of bog forest in the long term.

In addition, the increased structure of the vegetation results in more efficient filtering of dry atmospheric deposition thus is increasing the total deposition rate (e.g. Ivens 1990). Increased canopy density can also intercept larger amounts of rainfall and reduces the amount of water reaching the peat surface (H.B.M. Tomassen unpublished data; chapter 8). Since most Irish bogs suffer from the effects of water table draw-down as a result of small-scale peat extraction and the presence of drainage ditches, reduced water input from precipitation may enhance the water shortage for ombrotrophic vegetation. In *Sphagnum* suffering from water stress, gas exchange is suppressed, resulting in reduced photosynthetic rates and growth (Hayward & Clymo 1983; Schipperges & Rydin 1998), reduced immobilisation of nutrients by the *Sphagnum* layer (Aldous 2002) and increased availability of nutrients for vascular plants. All these processes will ultimately stimulate the growth of atypical grasses and trees and are disastrous for the characteristic ombrotrophic species.

We conclude that once a tree has established on a bog, this tree can have significant effects on the vegetation composition of its direct vicinity due to bird droppings, since they serve as a 'growth nucleus' for bog woodlands. Bird droppings may have a large impact on the vegetation composition, organic matter decomposition and structure of ombrotrophic bogs, which may even result in the development of small patches of bog forest.

Acknowledgements

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Chapter **8**

General discussion

Clara bog (Ireland)

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General discussion

In the Netherlands, the entire area of raised bogs has been virtually lost during two millennia of human impact (Joosten 1994). The importance of nature conservation was first recognised in the Netherlands in the 20th century. Since the early 1960s, much effort has been invested in counteracting water shortage in bog relics by improving their hydrological conditions. Although blocking drainage ditches and constructing bunds appeared to be successful in retaining precipitation, the recovery of *Sphagnum*-dominated vegetation, unfortunately, often failed. It was observed that *Sphagnum*-dominated vegetation developed well at locations where rewetting resulted in floating peat. The key processes involved in the development of these floating rafts, and methods to stimulate the buoyancy of peat, form one of the two main research themes of this thesis. The other theme relates to nitrogen loads. In addition to the detrimental effects of drainage, Dutch bog relics have been exposed to high levels of atmospheric nitrogen (N) input since the middle of the 20th century. Increased N inputs have greatly altered the vegetation composition in various ecosystems (Bobbink *et al.* 1998, 2003). The rapid invasion of Dutch bogs by Birch trees (*Betula* sp.) and Purple moor grass (*Molinia caerulea*) is likely to be the result of high N availability. This General discussion chapter provides a synthesis of the key processes involved in the buoyancy of peat in bog restoration projects and of the role of N in the invasion of bogs by *Betula* and *Molinia caerulea*. In addition, it discusses the implications of the research findings for bog restoration and management. The chapter concludes with the answers to the questions posed in the General introduction.

Characteristics of floating peat

Floating peat provides suitable growing conditions for *Sphagnum*, since it allows water-saturated conditions close to the surface to be maintained throughout the year (Lamers *et al.* 1999; Smolders *et al.* 2003). Complete flooding of cut-over peatlands without buoyant peat provides a less appropriate starting condition because it creates the serious risk of periodical desiccation during the summer or insufficient irradiance as a result of the dystrophic water layer (Wheeler & Shaw 1995; Lamers *et al.* 1999; Smolders *et al.* 2003). The buoyancy of floating peat rafts is caused by methane (CH_4) bubbles accumulating in the peat (Scott *et al.* 1999; Lamers *et al.* 1999; chapters 2, 3 & 4 of the present thesis) and CH_4 production rates therefore play an important role in the buoyancy of residual peat after the rewetting of bog relics. Methane production rates were found to depend on both physical and chemical peat

characteristics (chapters 2, 3 & 4). Methane production rates are usually highest in poorly decomposed peat, leading to a relatively low bulk density (Van den Pol - Van Dasselaar & Oenema 1999; Bozkurt *et al.* 2001; chapters 2 & 4). Since the activity of decomposing micro-organisms is usually limited by the nutrient availability, CH_4 production rates are hampered when peat N, P or K concentrations are low and when peat C:N, C:P or C:K ratios are high (Swift *et al.* 1979; Updegraff *et al.* 1995; Beltman *et al.* 1996; chapter 2). The potential decay rates of *Sphagnum* peat are most strongly determined by P-related variables for litter chemistry (Aerts *et al.* 2001; chapter 2). Selective removal of the more easily metabolised carbon compounds by decomposer organisms during decomposition results in larger proportions of resistant organic compounds, such as lignin (Bozkurt *et al.* 2001). Lignin may retard the activity of decomposing organisms (Swift *et al.* 1979; Bozkurt *et al.* 2001), resulting in a lower methane production rate (Yavitt *et al.* 1997; chapters 2 & 3). In addition, a recent study showed that cation limitation due to the high cation binding capacity of anoxic deep catotelm peat may also prevent the decay of strongly humified peat (Thomas & Pierce 2004).

In large parts of the Netherlands, the groundwater has high levels of sulphate (Lamers *et al.* 1998). Where water tables are close to the peat base, groundwater quality may strongly influence processes in remaining peat layers, especially if these layers are thin. Sulphate can hamper methanogenesis because sulphate-reducing and methanogenic bacteria compete for substrates (Bhattacharya *et al.* 1996; chapter 3). In addition, sulphate increases the mobilisation of nutrients by internal eutrophication (Roelofs 1991; Smolders & Roelofs 1993; Lamers *et al.* 1999; Lucassen *et al.* 2004). Sulphate-containing groundwater may therefore have a negative effect on floating raft development and the re-establishment of ombrotrophic vegetation (Lamers *et al.* 1999; chapter 3).

Even if the substrate quality is adequate, CH_4 production rates may be low due to the highly acidic nature of the formerly desiccated remaining peat, as desiccation of peat can lead to a further acidification due to acid-producing oxidation processes (Lucassen *et al.* 2002). Increased pH is known to enhance CH_4 production by stimulating the activity of methanogenic bacteria (Williams & Crawford 1984; Dunfield *et al.* 1993; Segers 1998; chapter 3) and the availability of organic substrates for methanogenic bacteria (Lamers *et al.* 1999; chapter 3).

Overall, peat buoyancy was found to depend on high methane production rates, which are determined by both chemical and physical peat characteristics. In general, buoyant peat has a low bulk density ($\leq$ 75 g DW l^{-1} FW), a relatively high pH ($\geq$ 4.0) and low lignin-to-nutrient ratios (lignin:P ratio $\leq$ 1000; lignin:N ratio $\leq$ 20). Where these peat characteristics prevail, buoyancy of the residual peat after rewetting is very likely (chapter 2), providing favourable starting conditions for bog restoration.

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Restoration of cut-over bogs by development of floating rafts

In Dutch cut-over bogs, the quality of residual peat is often inadequate for floating raft formation, since it is mostly composed of strongly humified catotelm peat which is left after peat harvesting. This peat does not become buoyant after inundation. If it is impossible to achieve waterlogged conditions or shallow inundation, a good alternative is to introduce poorly humified peat, with the appropriate characteristics, from nearby sites (chapter 4). This measure is similar to returning the removed *bunkerde* after peat harvesting, a procedure which was carried out in the past. Although this poorly humified substrate may have the appropriate composition to warrant high CH_4 production rates, the low pH of the peat may retard methanogenesis (Segers 1998). Methanogenesis can be successfully stimulated by mixing in small amounts of lime (2 g kg^{-1} fresh peat) to raise the pH (up to approximately 5.0) and thereby stimulate CH_4 production rates and thus the buoyancy of the peat (chapter 4). The amount of lime that has to be added depends on the acidity of the substrate. Lime addition levels should not be higher than necessary, as there is some evidence that calcium toxicity may occur when calcium concentrations exceed 500 μmol l^{-1} (Money 1995; Sliva & Pfadenhauer 1999). Furthermore, the addition of very large amounts of lime might lead to eutrophication due to excessive decomposition rates of the peat (Lamers *et al.* 1999; chapter 3).
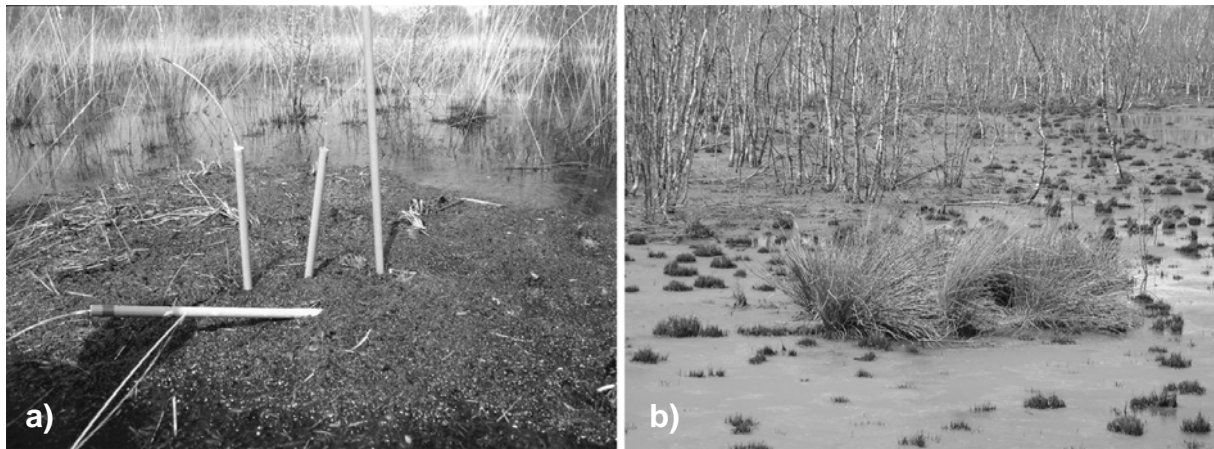
Higher pH values not only lead to increased CH_4 production rates but also to higher CO_2 production rates. Additionally, the dissolution of added calcium carbonate leads to CO_2 release. Increased benthic CO_2 concentrations may be beneficial, as they are known to stimulate the growth of *Sphagnum* establishing on the newly formed floating rafts (Lamers *et al.* 1999; Turetsky & Wieder 1999; Smolders *et al.* 2001).

It can be concluded that in inundated peat remnants where the amount of poorly humified peat left is insufficient, the introduction of (limed) substrates might be a valuable method to stimulate floating raft formation. Obviously, its feasibility will greatly depend on the availability and quality of donor substrate.
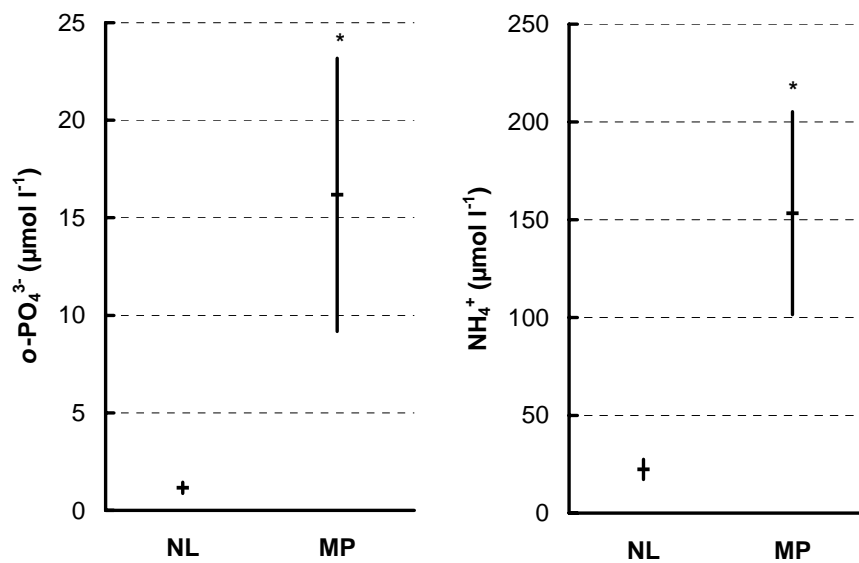
Long-term perspectives for floating rafts

Successful long-term restoration requires the colonisation of the newly formed rafts by *Sphagnum*, since high methane production rates depend on the continuous availability of poorly humified peat. New floating rafts are normally colonised first by *Sphagnum cuspidatum* (Figure 8.1) or *S. fallax*. Other *Sphagnum* species and vascular plants may establish later. Due to differences in decomposability between the *Sphagnum* species that are characteristic of hollows (*S. cuspidatum* and *S. fallax*) and species forming hummocks (e.g., *S. magellanicum* and *S. papillosum*), peat

accumulation proceeds much more slowly when only species of hollows are present (Johnson & Damman 1991; Limpens & Berendse 2003a).

Figure 8.1: Rewetting measures taken at the Mariapeel nature reserve stimulated the development of floating rafts colonised by *Sphagnum cuspidatum* (a). Within a few years, however, *Molinia caerulea* and *Juncus effusus* invaded the raft (b) due to high nutrient availability (both N and P). [Reprinted in colour on page 188]

Figure 8.2: Concentrations (means ± 1 SE) of phosphate and ammonium at depths of 0 - 10 cm at various Dutch bogs, not including the Mariapeel bog) NL; n = 12) and at the Mariapeel bog (MP; n = 8). * $P \leq 0.01$ (*t*-test) [Tomassen & Smolders, unpublished data].

In addition to the species-dependent decay of *Sphagnum*, nutrient concentrations of the *Sphagnum* litter influence decomposability (Coulson & Butterfield 1978; Aerts *et al.* 2001; Limpens & Berendse 2003a; chapter 2). High N deposition rates affect *Sphagnum* litter N concentrations (chapter 6) and thereby N release (Limpens &

Berendse 2003a). Stimulated mobilisation of N results in increased availability of N for nitrophilous vascular plants, including *Molinia caerulea*. High availability of both N and P in the Mariapeel bog, for instance, led to a rapid colonisation of newly developed rafts by *Molinia* and *Juncus effusus* (Figure 8.1 & Figure 8.2). This highly productive and dense vegetation may hamper *Sphagnum* growth by shading (Hayward & Clymo 1983; Heijmans *et al.* 2001; Limpens *et al.* 2004) and thereby constrain peat accumulation. In addition, this dense vegetation increases the weight of the floating raft and hampers the accumulation of CH_4 because large amounts of the CH_4 produced are vented into the atmosphere via the aerenchyma of these vascular plants (Lloyd *et al.* 1998). Ultimately, these unfavourable processes may lead to the sinking of the raft (personal observations). Eutrophication is therefore expected to affect the perspectives for floating bog formation in many locations.

Ombrotrophic vegetation and high N deposition rates

Molinia and *Betula* can only dominate bogs when nutrient availability in the rhizosphere is increased. The presence of a vital *Sphagnum* layer can immobilise large amounts of N (Aerts *et al.* 1992; Limpens *et al.* 2003a; chapter 6) and thus act as an N buffer or filter (Lamers *et al.* 2000; Berendse *et al.* 2001). N uptake by *Sphagnum* mosses is influenced by environmental factors including moisture availability, shading by vascular plants and the availability of other nutrients (especially P) (Hayward & Clymo 1983; Aldous 2002; Limpens *et al.* 2004) and C (Smolders *et al.*, 2001). As long as growing conditions are optimal for *Sphagnum* and N deposition rates are not too high, N concentrations in the rhizosphere will remain low or very low (Lamers *et al.* 2000; Limpens *et al.* 2003a; chapter 6) and nitrophilous vascular species will be unable to become dominant. In Dutch bogs, however, nitrogen deposition loads are too high and growing conditions are frequently suboptimal in terms of water supply and vascular plant density (Limpens *et al.* 2004; chapter 5). When such conditions are limiting the growth of *Sphagnum* mosses and/or N deposition rates are high, a surplus of N becomes available. At first, *Sphagnum* can immobilise the N overload by producing N-rich free amino acids (Nordin & Gunnarsson 2000; Limpens & Berendse 2003b; chapter 6). Accumulation of free amino acids, including arginine, asparagine and glutamine, by *Sphagnum* is the first sign of N saturation, although N concentrations in the rhizosphere will still be relatively low. If the assimilation of N into free amino acids cannot keep up with the N uptake, toxic NH_4^+ accumulates in the cells and leads to reduced *Sphagnum* growth (Limpens & Berendse 2003b). Amino acid accumulation results in higher capitulum N concentrations, increasing the sensitivity of *Sphagnum* to infections with its fungal parasite *Lyophyllum palustre* (Limpens *et al.* 2003b).

Finally, the imbalance between N availability and N uptake by *Sphagnum* mosses results in increased availability of N in the rhizosphere. Vascular plants such as *Betula* and *Molinia* are able to profit from the extra N and may invade ombrotrophic bogs. Whether invasion by *Molinia* and *Betula* actually takes place depends on the availability of not only N but also other nutrients, especially P. Our N addition experiments revealed that *Betula* was not able to profit from the surplus of N, probably due to P limitation (chapters 5 & 6). Peat water o-PO_4^{3-} concentrations in Dutch bogs proved to be much higher than in Irish bogs (chapter 5). The combined high availability of N and P, together with the often sub-optimal growing conditions for *Sphagnum* mosses, results in the invasion of Dutch bogs by *Molinia* and *Betula*. Increased growth of trees, particularly *Pinus sylvestris*, has also been observed in a south-central Swedish *Sphagnum* bog (Gunnarsson *et al.* 2002). This increased tree cover, probably triggering further changes in plant cover, was the result of a drier mire surface and increased availability of nitrogen (Gunnarsson *et al.* 2002).

Just like atmospheric N deposition, bird droppings were found to reduce nutrient (P, N and K) limitation for non-characteristic vascular plants. Bird droppings have been found to stimulate the growth on bogs of *Pinus sylvestris* trees, which are normally severely limited by P and K (Finér 1992; chapter 7). Invasions by tree species of the genera *Pinus* and *Betula* change hydrological conditions for other species within the ecosystem (Frankl & Schmeidl 2000) and are even able to destroy the *Sphagnum*-dominated environment (Ohlson *et al.* 2001).

Lichens are even more sensitive to atmospheric pollution than *Sphagnum*, and have almost completely disappeared from ombrotrophic bogs in the Netherlands. Various studies have reported the disappearance of lichens from heathlands at high N availability (e.g., Søchting & Johnsen 1987; Jonasson 1992; Press *et al.* 1998; Carroll *et al.* 1999; Gordon *et al.* 2001). A similar deleterious effect of N deposition on the lichen flora was found for lichens growing on ombrotrophic bogs (chapter 5). In our experiments, a total N deposition rate of 2.7 g m^{-2} $year^{-1}$ (the lowest N treatment level) affected N concentrations and the regrowth of *Cladonia portentosa* within 3 years (chapter 5). Tissue N concentrations of the lichen *Cladonia portentosa* correlate with N deposition rates (Søchting 1990; Hyvärinen & Crittenden 1998; chapter 5), and may therefore be used as bioindicators for the assessment of critical N loads.

Vegetation structure and the interception of precipitation and atmospheric deposition

Invasion of ombrotrophic bogs by vascular plants like *Molinia* and *Betula* at high N deposition rates makes the vegetation structure more complex and increases the density, and high densities of vascular plants can hamper *Sphagnum* growth by

shading (Hayward & Clymo, 1983; Heijmans *et al.* 2001; Malmer *et al.* 2003). Limpens *et al.* (2004) showed that under favourable growing conditions, interception of more than approximately 50% of the light depresses *Sphagnum* growth. In addition, high densities of vascular plants such as *Betula* and *Molinia* also affect the interception of rainwater and the input from dry deposition. A thinning experiment in a *Pinus sylvestris* stand at the 'Ravenvennen' nature reserve, for instance, clearly showed the effects of canopy density on the interception of precipitation. The tree canopy of the non-thinned *Pinus* stand reduced bulk precipitation by 41%, while the thinned *Pinus* stand still intercepted 23% of the precipitation (Figure 8.3). Bogs invaded by *Molinia* and *Betula* have an increased canopy density and the interception of precipitation results in a reduced input of precipitation onto the bog. In Dutch bogs already suffering from the effects of water table draw-down, increased interception stimulates further desiccation.

Figure 8.3: Amount of throughfall (means ± 1 SE; *n* = 10) in a non-thinned *Pinus sylvestris* stand (canopy density 59 ± 2 %), a thinned stand (canopy density 43 ± 4 %) and a clear-cutting (canopy density 0 %) at the 'Ravenvennen' nature reserve, between May 1997 and May 1998. Different letters indicate significant differences ($P < 0.05$) between treatments in May 1998 (one-way ANOVA). A positive correlation was found between canopy density and interception ($P < 0.05$; Spearman's rho = 0.54) [Tomassen unpublished data].

Increased canopy density and vegetation structure are also known to affect the input of dry deposition. Whereas wet deposition and sedimentation of heavy aerosols are barely influenced by the characteristics of the surfaces on which they are deposited (Bobbink & Heil 1993), the quantity of dry deposition resulting from the absorption of gases and particles directly from the atmosphere is strongly affected by the
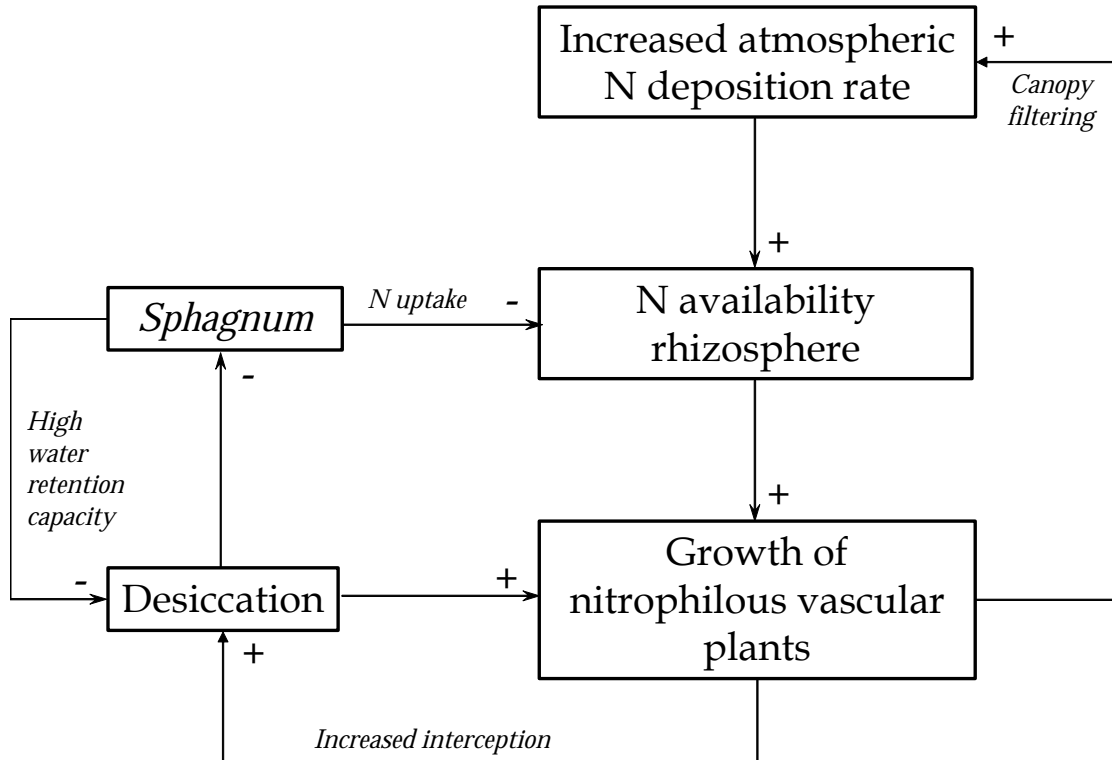
roughness of the deposition surface (e.g., Ivens 1990; Draaijers 1993). Not only forests, but also low shrub vegetations possess surface structures that greatly promote the dry deposition of N and sulphur (S) (e.g., Van Breemen *et al.* 1982; Heil *et al.* 1988; Ivens 1990). The clear-cutting experiment showed higher total N and S deposition rates for the non-thinned and thinned *Pinus* stand than for the clear-cut area (Table 8.1). It can be concluded that in areas with high N deposition rates, the changes in canopy density and structure of the bog vegetation caused by high densities of *Betula* and *Molinia* increase the interception of water and N by the vegetation.

Table 8.1: Ammonium, nitrate, total nitrogen and sulphur deposition rates (g m^{-2} year^{-1}; means ± 1 SE; *n* = 2) in a non-thinned and a thinned *Pinus sylvestris* stand and in a clear-cut area at the 'Ravenvennen' nature reserve between May 1997 and May 1998. Different letters in each column indicate significant differences ($P < 0.05$) between treatments (one-way ANOVA) [Tomassen unpublished data].

Treatment	N-NH$_4^+$	N-NO$_3^-$	Total N	Total S
	g m^{-2} year^{-1}	*g m^{-2} year^{-1}*	*g m^{-2} year^{-1}*	*g m^{-2} year^{-1}*
Non-thinned	3.13 ± 0.07[a]	1.51 ± 0.05[a]	4.64 ± 0.11[a]	2.43 ± 0.12[a]
Thinned	1.60 ± 0.08[b]	1.02 ± 0.01[b]	2.62 ± 0.09[b]	1.57 ± 0.15[b]
Clear-cutting	1.11 ± 0.08[c]	0.76 ± 0.01[c]	1.87 ± 0.06[c]	0.97 ± 0.06[c]

Reduced precipitation inputs and concomitant water shortage may increase aerobic mineralisation rates and reduce the growth of *Sphagnum* mosses, resulting in increased nutrient availability (Hayward & Clymo 1983). In desiccated *Sphagnum*, gas exchange is suppressed, resulting in reduced photosynthetic rates and growth (Schipperges & Rydin 1998), reduced immobilisation of nutrients by the *Sphagnum* layer (Aldous 2002) and increased availability of nutrients for vascular plants. Therefore, the productivity of *Sphagnum* mosses during periods of surface water stress may be much lower than that of vascular plants (Malmer *et al.* 1994). Saplings of *Betula*, for instance, are less rapidly overgrown by *Sphagnum* suffering from water stress. Drainage projects of peatlands for forestry have shown that the succession towards forest vegetation started soon after drainage, with increasing tree stand volumes and decreasing coverages of *Sphagnum* species (Laine *et al.* 1995). Desiccation alone, however, is probably not sufficient to trigger the dominance of non-characteristic grass and tree species, since severely desiccated bog areas in the Irish midlands were not invaded (chapter 5). The effects of desiccation on the invasion of bogs by *Molinia* and *Betula* are likely to be smaller than those of increased N availability (Figure 8.4).

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Figure 8.4: Schematic summary of the effects of increased nitrogen deposition rates on ombrotrophic bog vegetation. The increased availability of nitrogen in the rhizosphere produces a number of feedbacks, intensifying the unfavourable changes in ombrotrophic bog functioning.

Figure 8.5: Estimated cover (*n* = 3; means ± 1 SE) of the total vegetation, vascular plants and *Sphagnum* in September 2002, 8 years after various management measures were taken at the Pikmeeuwenwater bog (the Netherlands). In the sod-cut plots, the top layer (30 cm) of the peat was removed, including the vegetation. Peat removal means that the entire peat layer was removed (approx. 80 cm) resulting in new peat pits [Tomassen & Lamers unpublished results].

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Thus, increased canopy density and the concomitant desiccation and N eutrophication can be expected to stimulate the growth of *Betula* and *Molinia* even more, and hamper the growth of *Sphagnum* species (Figure 8.4). This is a self-enhancing process, which might be interrupted by measures like Birch tree removal or mowing the vegetation.

Experimental management measures to cope with *Betula pubescens* and *Molinia caerulea* were taken at the 'Pikmeeuwenwater' floating bog to prevent further decline of ombrotrophic species (started 1994; Lamers 1995). The reduction in vascular plant cover after mowing significantly increased the total *Sphagnum* cover (Figure 8.5), which consisted of *Sphagnum papillosum, S. magellanicum, S. rubellum* and *S. fallax.* The cover of *Molinia* and *Betula* was only temporarily reduced by mowing, but the total vascular plant cover (especially that of *Calluna vulgaris*) was drastically reduced, thereby reducing shading effects on *Sphagnum* mosses (Heijmans *et al.* 2001). *Sphagnum* cover was also increased by the other two management measures (Figure 8.5), but *Sphagnum cuspidatum* was the dominant species.

Critical N load for ombrotrophic bog vegetation

Based on observations of changes in the structure and functioning of ecosystems, empirical critical loads for N have been established for various natural and semi-natural ecosystems (Bobbink *et al.* 1992, 2003). The critical N load for ombrotrophic bogs has been estimated to be in the range of 0.5 – 1.0 g N m^{-2} year^{-1}. Our laboratory N addition experiment (chapter 6) was set up to further assess the reliability of this critical N load, which had been classified as 'quite reliable' (Bobbink & Roelofs 1995). Recent studies (Table 8.2) indicate that critical levels of N are in the range of 0.7 – 1.6 g N m^{-2} yr^{-1}, indicating that the suggested load of 0.5 – 1.0 g N m^{-2} yr^{-1} is still reliable. Based on these recent studies, an expert workshop under the Convention on Long-range Transboundary Air Pollution covering the region of the UNECE has recently changed the status of the critical load for bog ecosystems to 'reliable' (Bobbink *et al.* 2003). These studies used various *Sphagnum* variables as indicators of exceedance of the critical level, such as increased production, increased capitulum N concentrations and accumulation of N-rich free amino acids in the capitulum of *Sphagnum* (Table 8.2 & chapter 6). Indicators of N saturation seem to be particularly useful tools to estimate the critical N load (Krupa 2003). In addition, environmental conditions such as the availability of other nutrients (P, K and C) and water affect critical N loads. Bogs receiving high effective precipitation are probably less sensitive to N pollution than bogs in drier areas (Roelofs & Woodin 2003).

Table 8.2: Overview of proposed critical levels of N deposition for ombrotrophic bogs.
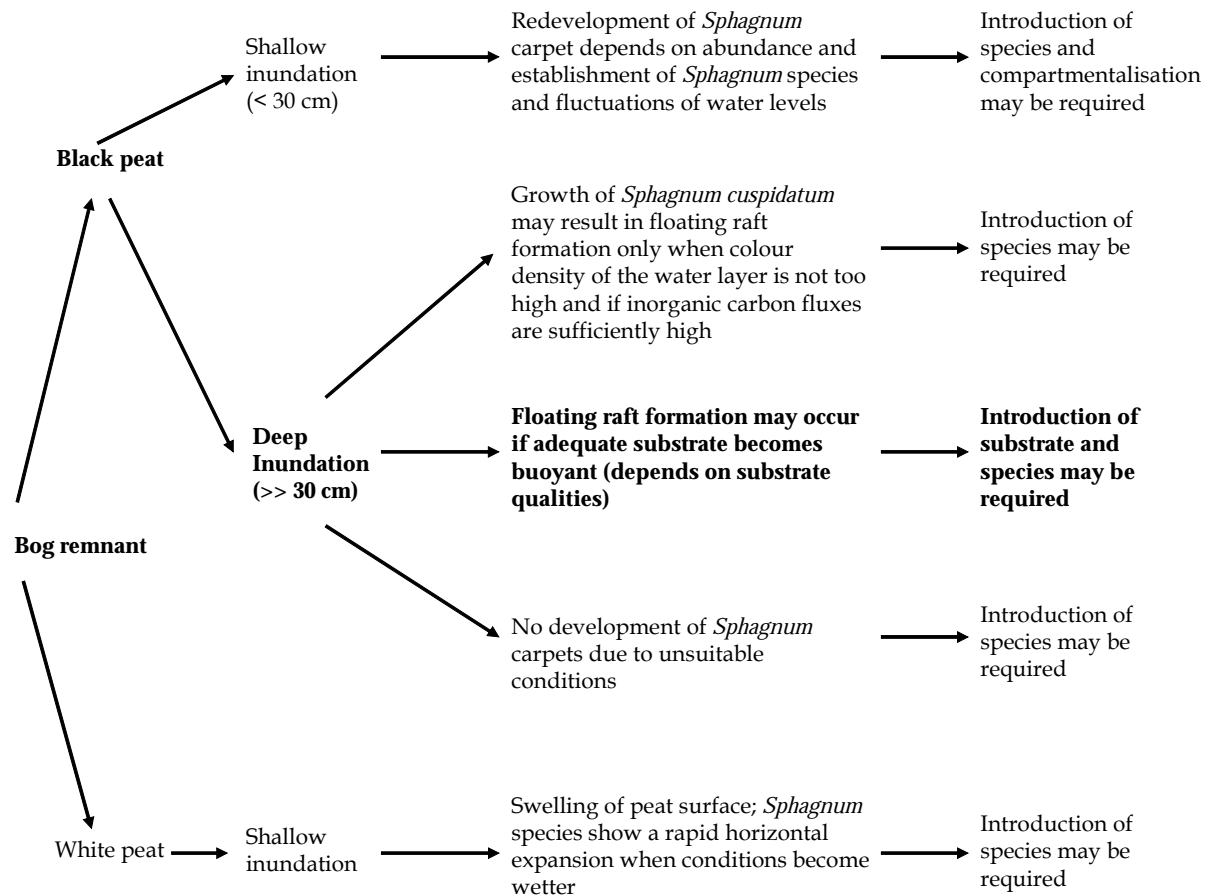
Indicator of N stress	Critical level of total N deposition	Reference
Sphagnum growth	0.7 g m^{-2} year^{-1}	Risager (1998)
Increased *Sphagnum* production	Below 1 g m^{-2} year^{-1}	Gunnarsson & Rydin (2000)
> 15 mg capitulum N g^{-1} DW	Level was not determined	Van der Heijden *et al.* (2000)
> 2.0 mg amino acid N g^{-1} DW	Below 1 g m^{-2} year^{-1}	Nordin & Gunnarsson (2000)
Increased capitulum N concentration	1.2 g m^{-2} year^{-1}	Lamers *et al.* (2000)
Increased net primary *Sphagnum* production	1.48 – 1.57 g m^{-2} year^{-1}	Vitt *et al.* (2003)
Leaching ^{15}N from *Sphagnum* layer	1.3 g m^{-2} year^{-1}	Nordbakken *et al.* (2003)
Accumulation of N-rich amino acids	0.5 – 1 g m^{-2} year^{-1}	chapter 6

Implications for raised bog restoration and conservation

Have all the efforts to restore and preserve Dutch bogs been useless? The answer to this question must be: certainly not! For one thing, nature management organisations have learnt that retaining as much water as possible is not always the best strategy. For instance, large water bodies on locations where only strongly decomposed peat (black peat) is left are not desirable. The results of the present research programme have improved our understanding of the most appropriate rewetting measures, based on the type of peat that is still present (Smolders *et al.* 2003; Figure 8.6). Based on this, expensive rewetting measures can be restricted to locations with good prospects for the development of *Sphagnum*-dominated vegetation. It is better to invest in relatively small areas with optimal growing conditions than in large areas with sub-optimal or poor growing conditions for *Sphagnum*. Parts of the bog relics may serve as water reservoirs or overflow compartments to create optimal growth conditions for *Sphagnum* in other parts. Successful restoration of bogs, however, requires long-term monitoring of restoration projects for a better understanding of the right way to carry out restoration measures (Schouten *et al.* 2002; Gorham & Rochefort 2003).

Even when optimal hydrological and hydrochemical conditions for *Sphagnum* growth are achieved, successful restoration is not guaranteed (e.g., Money & Wheeler 1999). An important prerequisite for successful restoration is the development of a hydrologically self-regulating acrotelm (Joosten 1995; Wheeler & Shaw 1995). Key species in this development are *Sphagnum magellanicum*, *S. papillosum* and *S. rubellum*. Since *Sphagnum* forms a persistent spore bank with a potential longevity of several decades (Sundberg & Rydin 2000), rewetting is usually sufficient to restore the growth of the key *Sphagnum* species if the original acrotelm is still present in a desiccated bog relict (Smolders *et al.* 2003). In the Netherlands, however, these typical hummock and lawn species are often absent or poorly represented because the viable diaspore bank was removed with the upper peat layers, and only the deeper catotelm peat still remains. The key species are usually very slow colonisers

compared to species of hollows, and introduction of these species in carpets dominated by species of hollows or on bare substrates appears to be very successful, indicating that dispersion and colonisation is the main constraint (Smolders *et al.* 2003).

Figure 8.6: Mechanisms involved in the restoration of bog remnants (adapted from Smolders *et al.* 2003). Conclusions based on results presented in this thesis are shown in bold.

The high atmospheric nitrogen deposition levels in the Netherlands are more difficult to cope with. Fortunately, N deposition levels are decreasing as a result of effective measures against the emission of N compounds taken in recent decades (Erisman *et al.* 2003). A reduction in deposition can be effective in the short term. In one case, a *Sphagnum* vegetation returned to its nutrient-poor status within two years after the source of enrichment was taken away (Limpens *et al.* 2003a). The best prospects for ombrotrophic bogs undoubtedly lie in further reduction of the emission of N compounds, as N deposition levels still greatly exceed the critical N level of 0.5 – 1 g m^{-2} year^{-1}. The production of *Sphagnum* and the role of this species as an ecosystem engineer (Van Breemen 1995; Malmer *et al.* 2003) form the key to the continued existence of bog vegetation despite the excessive N deposition levels

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(Limpens 2003). Under optimal growing conditions (water availability, available P and C, low vascular plant cover) the S*phagnum* layer should be able to cope with a total N deposition of approx. 2.5 g N m^{-2} year^{-1}, keeping the availability of N to vascular plants low (Limpens 2003). Additional management measures aimed at optimising overall growth conditions for *Sphagnum* can help to avoid an important part of the unfavourable effects of N.

Table 8.3 summarises the answers to the questions posed in the introduction to this thesis. The general question whether the revival of Dutch bogs is a reasonable perspective prompts the following comments. The limited space for nature in the Netherlands obviously makes it impossible to return to a situation with large areas of raised bog in the near future. However, there are good opportunities not only to preserve but even to expand the area covered by actively growing *Sphagnum*-dominated vegetation. A further reduction of N emissions, the application of the appropriate rewetting measures (based on the remaining peat type) and the re-introduction of key species (e.g., *S. magellanicum* and *S. papillosum*) could definitely turn this dream into reality!

Table 8.3: Main questions dealt with in this thesis and the corresponding answers.

Question	*Answer*
What are the key processes involved in the development of floating rafts after rewetting of cut-over bogs?	Buoyancy of the residual peat layers depends on sufficiently high methane production rates, requiring poorly humified peat characterised by low bulk density ($\leq$ 75 g DW l^{-1} FW), relatively high pH ($\geq$ 4.0), and low lignin-to-nutrient ratios (lignin:P ratio $\leq$ 1000, lignin:N ratio $\leq$ 20) (chapters 2, 3 & 4).
What is the influence of buffered groundwater on the development of floating peat?	The increased pH which results from the presence of buffered groundwater stimulates methane production rates and affects buoyancy of the peat, on condition that the peat has the appropriate physico-chemical characteristics (chapters 3 & 4).
Can the presence of poorly humified peat stimulate the development of floating rafts?	Since high methane production rates in poorly humified peat result in buoyancy of the peat, the presence or introduction of poorly humified peat stimulates the development of floating rafts (chapter 4).
Is the invasion of bogs by nitrophilous vascular plants, *Betula* and *Molinia* in particular, the result of high N deposition rates?	The imbalance between N availability and N uptake by *Sphagnum* mosses results in increased availability of N in the rhizosphere of vascular plants (chapter 6). Whether invasion by *Molinia* and *Betula* actually takes place depends not only on the availability of N but also on that of other nutrients, especially P. The N addition experiments revealed that *Betula* was not able to profit from the surplus of N, probably due to P limitation (chapters 5 & 6). Peat water o-PO$_4^{3-}$ concentrations in Dutch bogs proved to be much higher than in Irish bogs (chapter 5). The combined high availability of N and P, together with the often sub-optimal growing conditions for *Sphagnum* mosses, has resulted in the invasion of Dutch bogs by *Molinia* and *Betula*. (chapters 5 & 6).
What is the impact of nutrients derived from bird droppings on the vegetation composition and structure of bogs?	Growth of non-characteristic species such as *Betula* and *Pinus* is limited by P and K. Increased availability of P, K and N from bird dropping stimulates the growth of non-characteristic species such as *Molinia*, *Betula* and *Pinus* on ombrotrophic bogs (chapter 7).
Is the revival of Dutch *Sphagnum* bogs a reasonable perspective?	Suitable rewetting measures and further reduction of N deposition rates provide a reasonable perspective for Dutch *Sphagnum* bogs! (chapters 2 - 7)

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Chapter 8

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Samenvatting

De wederopstanding van Nederlandse *Sphagnum*-venen: een aannemelijk vooruitzicht?

Drijftilonderzoek in de Mariapeel met behulp van cilinders (2000)

*Deze Nederlandse samenvatting is speciaal bedoeld voor niet-ecologen en anderen wordt
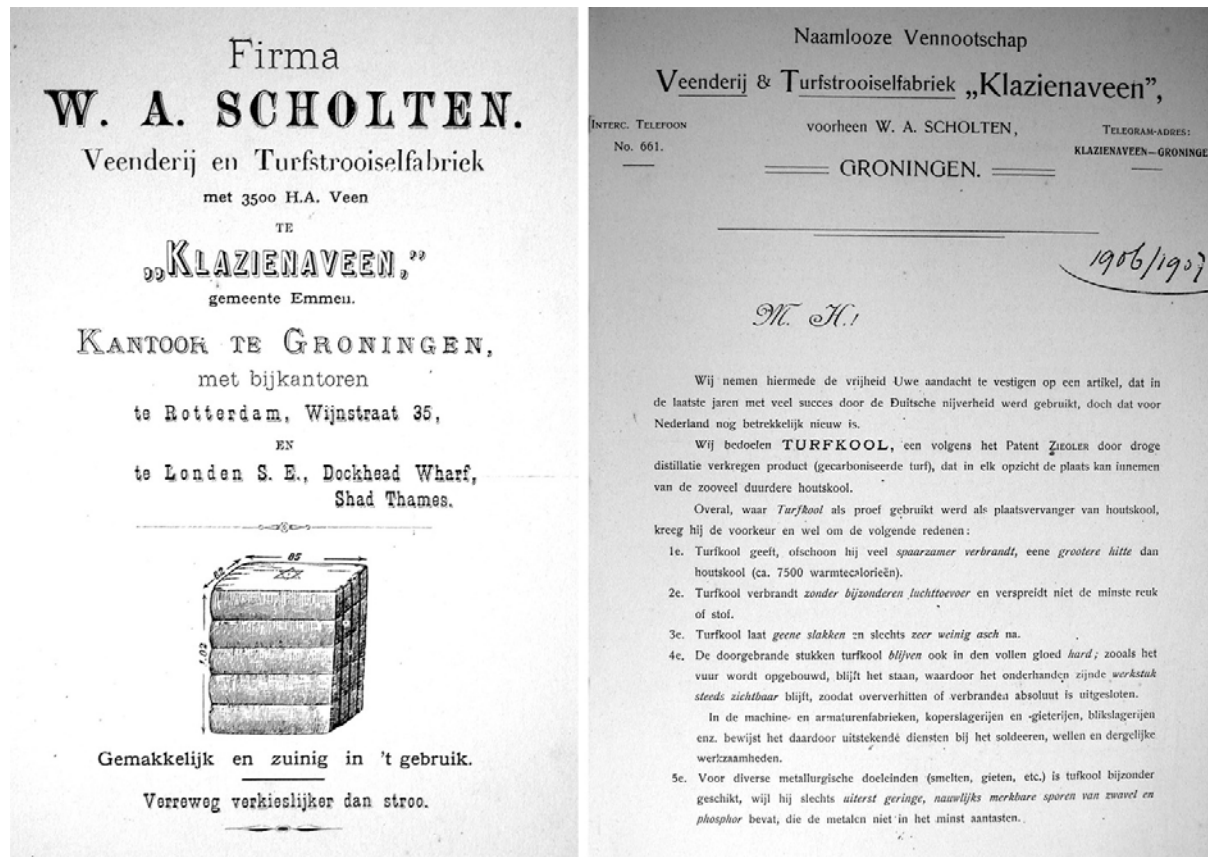daarom aangeraden de General discussion te lezen.*

Samenvatting

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Samenvatting

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Samenvatting

Hoogvenen in Nederland

Grote delen van hoog Nederland, maar ook van laag Nederland, waren ooit bedekt met uitgestrekte hoogvenen. Na de laatste ijstijd (ongeveer 12.000 jaar geleden) zijn deze hoogvenen gevormd in permanent natte laagtes in het landschap. De natte omstandigheden zorgden ervoor dat het gevormde en afgestorven plantenmateriaal goed geconserveerd werd, met andere woorden er trad veenvorming op. Wanneer dit veenvormende systeem in contact staat met het kalkhoudend (minerotroof) grondwater, ontwikkelt zich een laagveen (Engels: *fen*). Als het veen boven het kalkhoudend grondwater uitstijgt ontstaat een hoogveen (Engels: *bog*) dat voornamelijk afhankelijk is van regenwater (ombrotroof). De afname van de invloed van het kalkhoudende water stimuleert de veenvorming. Dit komt doordat de bij het veenvormingsproces gevormde organische en anorganische zuren niet meer geneutraliseerd worden, zodat het milieu verzuurt. De vegetatie van zulke hoogvenen wordt gedomineerd door veenmossen (*Sphagnum* soorten; zie Figuur 1.1 op pagina 188) die moeilijk afbreekbaar zijn en voor een snelle opbouw van het veen zorgen. Veenmossen verzuren het leefmilieu nog verder en vertragen hiermee de afbraak, doordat bij de opname van positief geladen voedingsstoffen (zoals ammonium en kalium) zuurionen worden afgegeven. Dit alles bij elkaar opgeteld maakt duidelijk dat hoogveen een zeer bijzonder ecosysteem is, gekarakteriseerd door aan een natte en zure omgeving aangepaste flora en fauna.

Al in de Middeleeuwen werd ontdekt dat het hoogveen-veen door de mens goed gebruikt kon worden als brandstof (turf) en bodemverbeteraar (Figuur 1). Het hoogveen dat zich in duizenden jaren had gevormd, werd in enkele eeuwen vrijwel geheel afgegraven. De overgebleven stukjes van deze voormalige hoogvenen liggen voornamelijk in het Noordoosten van het land en in Limburg en Brabant (Figuur 2). Deze hoogveenrestanten hebben echter hun karakteristieke eigenschappen grotendeels verloren en bestaan voornamelijk uit verdroogde heiden, vaak gedomineerd door Berken (*Betula*) en grassen als Pijpenstrootje (*Molinia caerulea*; zie Figuur 1.3 op pagina 188).

In de tweede helft van de vorige eeuw ontstond het besef dat deze zeer waardevolle en voor Nederland karakteristieke natuur bijna geheel verloren was gegaan. Langzaamaan werd de vervening stopgezet en werden de overgebleven stukken hoogveen aangekocht door natuurbeschermingsorganisaties. Deze hoogveen-restanten waren echter sterk verdroogd doordat er tijdens de vervening vele ontwateringskanalen waren aangelegd zodat het turf makkelijker gestoken kon worden. Om de verdroging tegen te gaan werden de kanalen en sloten gedempt en werden dammen aangelegd om het regenwater vast te houden in het gebied.

Ondanks het feit dat er weer voldoende water aanwezig was bleef de ontwikkeling van een door veenmossen gedomineerde vegetatie vaak afwezig.

Figuur 1: Reclameposters voor turfproducten uit het begin van de vorige eeuw (foto's genomen in het Veenmuseum in Barger-Compascuum).

Om inzicht te krijgen in de sturende processen voor een succesvol herstel van Nederlandse hoogveenrestanten werd in 1998, binnen het natuurbeschermingprogramma OBN (*Overlevingsplan Bos en Natuur*) van het ministerie van LNV (*Landbouw, Natuur en Voedselkwaliteit*), het onderzoeksproject OBN Hoogvenen gestart. Het OBN Hoogvenenproject omvat onderzoek naar de effecten van atmosferische stikstofdepositie op de vegetatie (deels in mijn proefschrift), de hydrologie, de kwalitatieve hydrologie & veenkwaliteit (deels in mijn proefschrift) en de aquatische ongewervelde dieren (macrofauna). Op basis van de resultaten van het onderzoek wordt getracht herstelmaatregelen te formuleren voor een succesvol herstel van hoogvenen in Nederland.

Figuur 2: Ligging van de belangrijkste natuurgebieden waar onderzoek is uitgevoerd in het kader van het onderzoeksproject OBN Hoogvenen.

Figuur 3: In het Haaksbergerveen zijn na vernatting mooie drijftillen ontstaan met karakteristieke soorten als Hoogveen-veenmos (*Sphagnum magellanicum*), Wrattig veenmos (*Sphagnum papillosum*), Kleine Veenbes (*Oxycoccus palustris*) en Lavendelhei (*Andomeda polifolia*).

Vernatting van hoogveenrestanten en drijftilvorming

In sommige hoogveenreservaten zoals het Haaksbergerveen en het Meerstalblok (deel van het Bargerveen) ontwikkelde zich na vernatting een zeer mooie vegetatie op locaties waar het nog aanwezige veen kwam opdrijven (drijftilvorming; zie Figuur 3). Drijvend veen levert optimale groeiomstandigheden voor veenmossen doordat het oppervlak permanent met water verzadigd blijft. Het is zeer lastig om permanent natte omstandigheden te creëren op het vaste veen. In de zomermaanden zakt het grondwaterpeil vaak diep weg in het veen en in de winter staat het waterpeil te hoog. Fluctuerende waterstanden remmen de groei van veenmossen en beperken dus het herstel van het hoogveen. Drijftilvorming lijkt daarom een ideale uitgangssituatie voor hoogveenherstel. De sturende processen bij de vorming van drijftillen vormen één van de twee onderzoeksthema's beschreven in mijn proefschrift.

Op de locaties waar na vernatting spontaan drijftillen gevormd zijn, blijkt na vervening vaak de bolster (de bovenlaag van het veen met de vegetatie) te zijn teruggestort. Deze bolster bestaat uit relatief weinig afgebroken plantenmateriaal waarin in principe gemakkelijk moerasgas (methaangas) gevormd kan worden. Dit gas, dat onder zuurstofloze omstandigheden gevormd wordt bij afbraak van het veen, fungeert letterlijk als 'drijfgas' voor het veen. Methaangas is zeer slecht oplosbaar in water en vormt kleine gasbelletjes die in het veen vastgehouden worden en het veen laten opdrijven. De productie van het gas blijkt sterk afhankelijk te zijn van de chemische en fysische samenstelling van het veen. De snelheid van methaanvorming wordt voornamelijk gestuurd door de pH[1], de soortelijke massa (volumegewicht), en de lignine- en fosforconcentratie[2] van het veen (hoofdstuk 2 & 3). Aan de hand van deze veeneigenschappen kan goed bepaald worden of het nog aanwezige veen zal komen opdrijven na vernatting.

Uit het onderzoek blijkt ook dat de pH van het veen in de verdroogde hoogvenen vaak verder verlaagd is door verzurende oxidatieprocessen (pH lager dan 4). Ondanks de aanwezigheid van voldoende afbreekbaar organisch materiaal is de methaanproductie laag doordat de activiteit van methaanproducerende bacteriën (methanogenen) geremd wordt bij een lage pH. De aanwezigheid van kalkrijk (grond)water in de veenbasis, zoals in het Haaksbergerveen, kan de methaanproductie stimuleren (hoofdstuk 3). Naast dit positieve effect van grondwater kan de aanwezigheid van grondwater negatieve effecten hebben door de aanwezigheid van sulfaat (een zwavelverbinding). Het grondwater kan hoge

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[1] pH is een maat voor de zuurgraad; hoe zuurder, hoe lager de pH.
[2] Lignine, houtstof, is een plantenbestanddeel dat moeilijk afbreekbaar is; fosfor is een belangrijke voedingsstof voor planten, schimmels en bacteriën.

concentraties sulfaat bevatten door de oxidatie van pyrietafzettingen[3] als gevolg van verdroging en nitraatuitspoeling en door de langdurige hoge zwavelneerslag ('zure regen') uit de atmosfeer. Sulfaat heeft nadelige gevolgen voor de ontwikkeling van drijftillen doordat sulfaatreducerende bacteriën en methanogene bacteriën vechten om dezelfde stoffen waardoor de methaanproductie geremd wordt. Sulfaat kan bovendien zorgen voor een overmatige afbraak van het veen en een zeer hoge beschikbaarheid van voedingsstoffen. Dit wordt 'interne eutrofiering' (vermesting) genoemd, omdat de voedingsstoffen al aanwezig waren in het veen. Verdroogde hoogvenen moeten om deze redenen nooit met sulfaatrijk water vernat worden.

De Nederlandse hoogveenrestanten zijn vaak verveend tot op het sterk afgebroken zwartveen. De eigenschappen van dit veen belemmeren een hoge productie van methaan waardoor drijftilvorming onmogelijk is. De bergingscoëfficiënt[4] van zwartveen is zeer laag en stabiele waterstanden zijn dan zeer moeilijk te realiseren. Uit praktische overwegingen worden zwartveenrestanten vaak geheel onder water gezet (geïnundeerd). Onderwatergroei van veenmossen verloopt echter vaak moeizaam door een gebrek aan licht en te lage concentraties koolstofdioxide (CO_2) in de thee-kleurige waterlaag. In deze situatie kan drijftilvorming gestimuleerd worden door het inbrengen van weinig afgebroken veen (vergelijkbaar met het terugstorten van de bolster). Indien de pH van het veen te laag is kan het veen vermengd worden met kleine hoeveelheden kalk om de methaanproductie te stimuleren (hoofdstuk 4). Kalk is alleen nodig om de methaanproductie op gang te brengen. Omdat het proces zelf zuur verbruikt, wordt het opnieuw verzuren tegengegaan.

Invasie van grassen en berken

De laatste tientallen jaren neemt de bedekking van grassen en berken in de Nederlandse hoogvenen sterk toe. Van heidesystemen is bekend dat de sterke vergrassing het resultaat is van de toegenomen neerslag (depositie) van stikstofverbindingen[5] uit de atmosfeer ('zure regen') gedurende de afgelopen eeuw. Aangezien hoogvenen voornamelijk worden gevoed via neerslag zal een toename van de beschikbaarheid van deze voedingsstof, die normaal beperkt aanwezig is, een grote invloed hebben op de hoogveenvegetatie. Hoogvenen in Nederland hebben daarnaast gedurende lange tijd ook veel te lijden gehad van verdroging en het is onduidelijk of de ongewenste invasie van grassen en berken het gevolg is van

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[3] Pyriet is een ijzersulfide verbinding (FeS_2) waarvan de kristalvorm erg veel op goud lijkt. Pyriet wordt daarom ook wel 'fools gold' of 'goud der dwazen' genoemd.

[4] De bergingscoëfficiënt is de verhouding tussen de verandering in de hoeveelheid opgeslagen water en de bijbehorende verandering in grondwaterstand. Als de toevoer van 1 mm water leidt tot een stijging van de grondwaterstand met 10 mm, is de bergingscoëfficiënt gelijk aan 0,1.

[5] Stikstof is een belangrijke voedingsstof voor planten, schimmels en bacteriën.

verdroging, hoge atmosferische stikstofdepositie of een combinatie van beide. Sterke verdroging van (niet afgegraven) hoogveen blijkt in Ierland, waar de stikstofdepositie veel lager is, niet te leiden tot vergrassing en 'verberking' van de vegetatie. Experimentele verhoging van de toevoer van stikstof op deze locatie leidt echter wel tot vergrassing (hoofdstuk 5). In tegenstelling tot de situatie in Ierland wordt de vegetatie van het Pikmeeuwenwater, een grote drijftil in nationaal park 'De Hamert', wel sterk gedomineerd door grassen en berken. Hydrologisch onderzoek toont aan dat de drijftil zeker niet blootgesteld is aan verdroging; de hele drijftil gaat immers als een dobber op en neer met de waterstand. Daarom is het waarschijnlijk dat de hoge depositie van stikstof dominantie van grassen en berken in Nederlandse hoogvenen mogelijk maakt. Dit proces wordt zeer waarschijnlijk versterkt door verdroging. Het andere onderzoeksthema beschreven in mijn proefschrift heeft als doel hierin duidelijkheid te verschaffen.

Veenmossen blijken een belangrijke rol te spelen bij het vastleggen van de overmaat aan beschikbaar stikstof. Bij een lage stikstofdepositie nemen veenmossen vrijwel al het stikstof op voor hun groei. Wanneer de depositie van stikstof hoger wordt en niet alles nodig is voor groei, dan gaan veenmossen het stikstofoverschot opslaan. Stikstof kan veilig ontgiftigd en opgeslagen worden in stikstofrijke vrije aminozuren[6] (zoals arginine, asparagine en glutamine; hoofdstuk 6). Bij nog hogere depositieniveaus, zoals in Nederland, is de veenmosdeken niet meer in staat om als een 'stikstoffilter' te dienen en gaat stikstof ophopen in de veenbodem onder de deken. In deze situatie kunnen snelle groeiers zoals grassen en berken gaan domineren, doordat ze nu wel voldoende stikstof krijgen. Verdroging remt de groei van veenmossen en beperkt hierdoor de capaciteit van veenmossen om stikstof vast te leggen. Een hogere bedekking van planten zoals Berk en Pijpenstrootje remt de groei van veenmossen tevens doordat er minder licht beschikbaar is (beschaduwing). Bovendien is er minder water doordat meer regenwater wordt weggevangen door de 'kroonlaag'.

De invasie van hoogvenen door deze snelle groeiers is wel afhankelijk van de beschikbaarheid van andere voedingstoffen die nu de groei limiteren. De beschikbaarheid van fosfor moet voldoende hoog zijn, vooral voor berken, om een invasie mogelijk te maken (hoofdstuk 5 & 6). De gevolgen van een hoge beschikbaarheid van alle belangrijke voedingsstoffen (stikstof, fosfor en kalium) op de vegetatie is waar te nemen in de directe omgeving van houten markeerpaaltjes op het Ierse hoogveen Clara bog (zie Figuur 7.1 op pagina 189). Nutriënten afkomstig van vogelpoep maken het voor soorten als Pijpenstrootje en Berk mogelijk om in korte tijd de karakteristieke vegetatie, inclusief de veenmossen, te verdrijven (hoofdstuk 7). Zo ontstaat er een piepklein stukje 'Nederlands' hoogveen in Ierland.

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[6] Aminozuren zijn organische verbindingen die de bouwstenen vormen van eiwitten.

De wederopstanding van Nederlandse *Sphagnum* venen: een aannemelijk vooruitzicht?

Van een echte, grootschalige wederopstanding van hoogvenen in Nederland kan natuurlijk geen sprake meer zijn. Eenderde van Nederland is ooit bedekt geweest met hoogveen en de ruimte voor natuur in Nederland is tegenwoordig zeer beperkt. Toch zijn er mogelijkheden om op kleine schaal mooie hoogveenvegetaties te herstellen en behouden. De afname van de stikstofdepositie sinds het afgelopen decennium blijkt nu al gunstige effecten te hebben op de vegetatie. Veenmossen kunnen, mits andere omstandigheden zoals de beschikbaarheid van water en licht optimaal zijn, op den duur de beschikbaarheid van voedingsstoffen voor snelle ongewenste groeiers laag houden doordat het 'filter' niet meer verzadigd is. Het OBN onderzoek aan hoogvenen heeft ook inzicht gegeven in de kansrijkdom van hoogveenrestanten en welke vernattingsmaatregelen het beste toegepast kunnen worden. Een hoogveenrestant zou eigenlijk zo ingericht moeten worden dat de omstandigheden voor veenmosgroei optimaal zijn op de meest kansrijke locaties. Andere delen van het gebied moeten eventueel hiervoor 'opgeofferd' worden, door bijvoorbeeld als watervoorraad te dienen.

Het antwoord op de vraag in de titel van mijn proefschrift, *De wederopstanding van Nederlandse Sphagnum venen: een aannemelijk vooruitzicht?*, zal daarmee moeten luiden: *Ja, op kleine schaal is herstel van hoogvenen een te realiseren en mooi vooruitzicht!*

Figure 1.1: Typical ombrotrophic bog vegetation dominated by bog mosses (*Sphagnum magellanicum, S. papillosum* and *S. rubellum*), *Vaccinium oxycoccus, Drosera rotundifolia* and *Eriophorum angustifolium*.

Figure 1.3: Invasion of the Bargerveen reserve by Purple moor grass (*Molinia caerulea*) and Birch trees (*Betula* sp.).

Figure 8.1: Rewetting measures taken at the Mariapeel nature reserve stimulated the development of floating rafts colonised by *Sphagnum cuspidatum* (a). Within a few years, however, *Molinia caerulea* and *Juncus effusus* invaded the raft (b) due to high nutrient availability (both N and P).

Figure 7.1: *Betula pubescens* (a), *Molinia caerulea* (b) and *Narthecium ossifragum* (c) growing in the vicinity of wooden posts on which birds have perched and defecated during more than 10 years.

Figure 7.3: *Pinus sylvestris* trees growing (a) at a reference site 2 m from the post and (b) in the vicinity of a wooden survey post at Clara bog East, 2 years after transplantation.

Colour section

. . . .

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Het Pikmeeuwenwater, een hoogveendrijftil gelegen in Nationaal Park De Maasduinen (Limburg)

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Curriculum vitae

Op 7 juni 1971 ben ik geboren in het Achterhoekse Wehl. Na de Martinusschool in Wehl ging ik naar de Gemeentelijke ScholenGemeenschap in Doetinchem (G.S.G.D.). In 1988 heb ik de HAVO met succes afgerond en ben ik begonnen met de Hogere Laboratorium Opleiding (H.L.O.) in Arnhem (1^e jaar) en Nijmegen (2^e en 3^e jaar). Na een jaar stage bij het Laboratorium Microbiologie Twente-Achterhoek in Enschede heb ik in 1992 de HLO-opleiding afgerond in de Medisch Microbiologische richting. In 1992 ben ik gestart met de opleiding Biologie aan de Katholieke Universiteit te Nijmegen. Als HBO-instromer werd ik direct bij aanvang geconfronteerd met de keuze voor een afstudeerrichting en ik heb voor een compleet andere richting gekozen: ECOLOGIE. Na een bijvak bij de afdeling Experimentele Plantenecologie (overstromingstolerantie van ruigtekruiden in relatie tot de ontwikkeling van ooibossen) en een hoofdvak bij de afdeling Aquatische Ecologie & Milieubiologie (interne eutrofiering door sulfaat in natte systemen), heb ik in 1995 mijn studie 'met genoegen' afgerond.

Van 1996 tot en met 2003 ben ik werkzaam geweest bij de afdeling Aquatische Ecologie & Milieubiologie. Eerst heb ik aan een aantal korte projecten gewerkt zoals de organisatie van een groot OBN symposium in opdracht van het ExpertiseCentrum van het Ministerie van Landbouw, Natuur en Voedselkwaliteit (EC-LNV) en een vooronderzoek in het natuurgebied 'de Ravenvennen' voor Stichting het Limburgs Landschap. Vanaf 1 januari 1998 heb ik gewerkt als 3^e geldstroom wetenschappelijk medewerker aan onderzoek naar de randvoorwaarden voor een succesvol herstel van hoogvenen in Nederland. Dit onderzoeksproject werd gefinancierd vanuit van het programma Overlevingsplan Bos en Natuur (OBN) van het Ministerie van LNV. Het onderzoek beschreven in dit proefschrift is het resultaat hiervan. Verder heb ik op diverse internationale congressen mijn onderzoek mogen presenteren, waaronder: Kopenhagen, Quebèc City, Sheffield, Budapest & Utrecht. Naast het verrichten van onderzoek heb ik ook meegewerkt in het onderwijs door het mede-organiseren van de cursus Toegepaste Ecologie en het begeleiden van een studentenexcursie naar het Ierse Connemara (2001). Momenteel werk ik als Ecoloog bij Onderzoekcentrum B-ware te Nijmegen aan vooronderzoek naar het herstel van het Wierdense Veld in opdracht van Landschap Overijssel en aan de 2^e fase van het OBN Hoogvenen onderzoek in opdracht van het EC-LNV.

Lough Roe (Clara bog, Ireland)