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Biogeochemistry and ecohydrology of peatlands in South America and New Zealand

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Contents

Introduction: *Sphagnum* in the New World

Christian Fritz

Why did Darwin 'skip' the exploration of *Sphagnum* in the New World?

Water permits peat forming plants like *Sphagnum* mosses to create peatlands, which are the most efficient terrestrial ecosystems for (long-term) carbon storage. Globally, living peatlands provide an important freshwater reservoir. Peatlands maintain biodiversity by supporting many specialised species adapted to harsh conditions and unique ecosystem types. Living peatlands gain importance in providing a refuge for species that are under pressure due to changing landuse and climate in non-peatland habitats (Parish *et al.*, 2008). During the last decades, peatlands have received growing attention by scientists and policy makers because of their important ecosystem services (e.g. carbon storage, water retention, biodiversity) and their high vulnerability to anthropogenic activities.

When Darwin set foot on Patagonia during his famous trip on the Beagle almost 200 years ago, peatlands, particularly *Sphagnum*-dominated bogs, had already received significant appreciation from scientists. Dau (1823) described their ombrotrophic character (e.g. peatland or vegetation exclusively fed by rain water) and recognised the ability of *Sphagnum* mosses to retain large quantities of water. Botanists had already explored the diversity of *Sphagnum* species, and it was questioned how *Sphagnum* mosses were able to flourish under conditions that are generally unfavourable for plant growth. However, Darwin's visit to the numerous bog ecosystems in South America apparently did not spark him to investigate *Sphagnum*. There were probably two reasons for this.

Firstly, many bogs in Patagonia were dominated by vascular plants (Fig. 1.1), mainly *Astelia pumila* and *Donatia fasciculares*, while *Sphagnum* mosses were scarce. Charles Darwin (1839) wrote:

> … for every patch of level ground is covered by two species of plants (Astelia pumila and Donatia magellanica), which by their joint decay compose a thick bed of elastic peat. In Tierra del Fuego, above the region of woodland, the former of these eminently sociable plants is the chief agent in the production of peat.

Secondly, Darwin got intrigued by the remarkable morphology of these cushion-forming species (Fig. 1.2):

> Fresh leaves are always succeeding one to the other round the central tap-root, the lower ones soon decay, and in tracing a root downwards in the peat, the leaves, yet holding their place.

In his diary Darwin linked the long roots of cushion-forming species (hereafter cushion plants) to their ability to form peat ('coal formation'). When writing about peat formation by cushion plants, which were unknown from wetlands in the Northern Hemisphere, Darwin concluded:

> Although every plant lends its aid, yet in most parts the Astelia is the most efficient. It is rather a singular circumstance, as being so very different from what occurs in Europe, that I nowhere saw moss forming by its decay any portion of the peat in South America.

In conclusion, Darwin seemed greatly amazed by cushion plants and their ability to form peatlands more efficiently than *Sphagnum* mosses were able to.

Darwin's reports raised attention among his colleague scientists. The Swiss botanist and geologist Leo Lesquereux questioned in his letter to Darwin:

> It is: that in the peat bogs of South America contrary to what happens in Europe, no species of mosses enters into the composition and formation of the peat? … I would like to know how you explain the slow combustion of the wood and its transformation into carbon under the influence of water, with those Southern plants. Do they absorb the humidity as the sphagnum does. Or is the atmospheric humidity so great that the oxigenation of the woody tissue is stopped by its action. (http://www.darwinproject.ac.uk/entry-4715#mark-4715.f1 accessed 5 December 2012)

Lesquereux also seemed to be puzzled by the reported absence of *Sphagnum* mosses as he mentioned:

> Moreover I have received many specimens of sphagnum of Terra del Fuego with the label: abundant.

Figure 1.1. Lawn of *Sphagnum magellanicum* (centre, left) invaded by *Astelia pumila* (Forster f.) Gaudich and *Donatia fasciculares* Forster and Forster in Patagonia (both foreground).

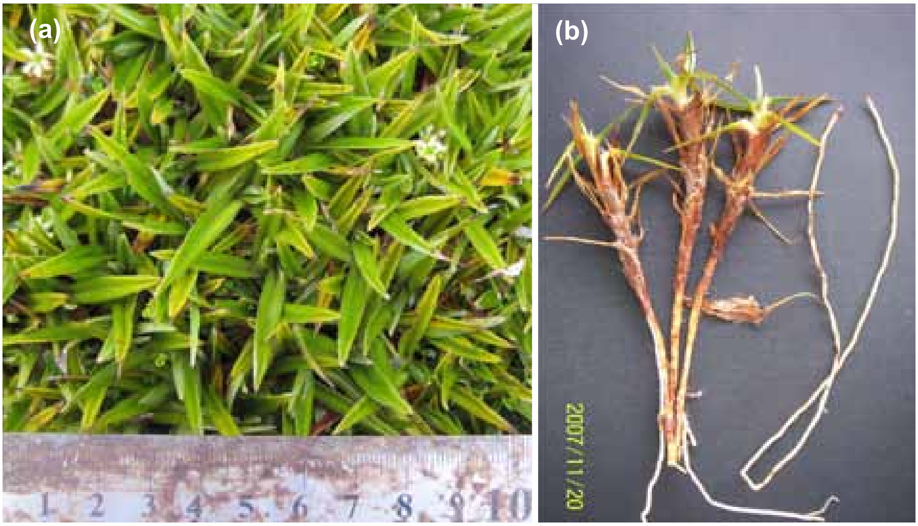

Figure 1.2. Left panel (a) shows a dense cushion formed by *Astelia pumila* – the dominating plant in Patagonian cushion bogs. Photo courtesy Susanne Abel. A close up of *Astelia pumila* is presented in the right panel (b). Large root biomass of cushion plants contrasts the few evergreen leaves that remain attach to the stem after senescence. Photo courtesy Annette Teltewskaya.

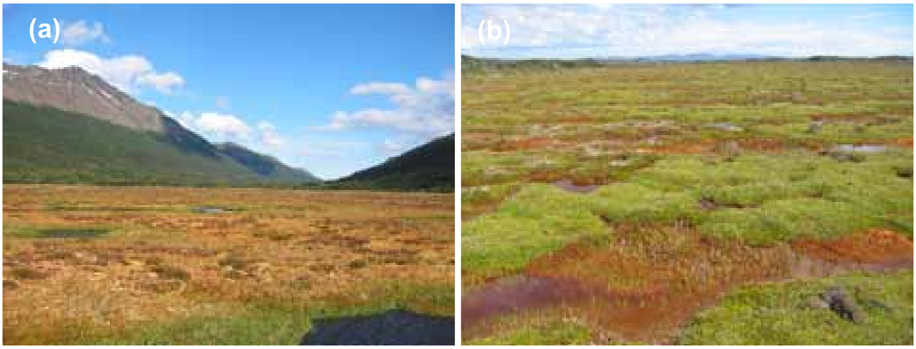

Figure 1.3. Left panel (a) shows the *Sphagnum* bog in the Andorra Valley (site 1 in Fig. 1.4). Vascular plants are limited by the growth of *Sphagnum* mosses and the accumulation of *Sphagnum* peat. In the right panel (b) a cushion bog of the Moat bogs (site 3 in Fig. 1.4) is presented. The vegetation is dominated by *Astelia pumila* (green foreground) and to a lesser extent by *Donatia fasciculares*. Patches of *Sphagnum* are limited to pool (margins).

What would have been Darwin's response to Lesquereux? It has never been found. Since then, little research has been dedicated to the role of cushion plants and *Sphagnum* mosses in the formation of bogs in Patagonia. Both poor accessibility, and the lack of infrastructure for (experimental) research are probably the main reasons for this.

Background on *Sphagnum* bogs

Sphagnum bogs are fascinating ecosystems that combine high accumulation rates of carbon and nutrients under conditions where the productivity of other plants is hampered by low nutrient availability (Fig. 1.3). In *Sphagnum* bogs nutrients are normally scarce because of rain being the main source of nutrients (ombrotrophy). The successful growth of peatlands is often linked to a combination of traits unique to *Sphagnum* mosses, which dominate bog and poor fen ecosystems in northern peatlands (Joosten, 1993; Rydin & Jeglum, 2006). *Sphagnum* has evolved a high nutrient use efficiency to cope with low input rates of nutrients (Van Bremen, 1995; Wiedermann *et al.*, 2009a). The atmospheric input of nutrients is effectively retained accordingly. At the same time, also atmospheric carbon is effectively retained because of the low decomposition rates in *Sphagnum* bogs. The main factors lowering decomposition are the high retention of rainwater, acidic conditions and slowly degradable organic matter (Clymo & Hayward, 1982; Aerts *et al.*, 1999). In addition, a substantial part of the carbon losses (including CH_4) are re-fixed in *Sphagnum* (Smolders *et al.*, 2001; Kip *et al.*, 2010). These traits enable *Sphagnum* to avoid the competition with vascular plants, as well as to form peat at high rates. The conservation of the carbon stored in peatlands depends on water tables being close to the surface (Couwenberg *et al.*, 2010). Under dry conditions (e.g. low water levels) *Sphagnum* mosses increase water storage, while under wet conditions water losses are facilitated by lateral run-off and pool formation (Couwenberg & Joosten, 1999; Couwenberg & Joosten, 2005). This concept of maintaining wet conditions is referred to as 'hydrological self-regulation'. Bog ecosystems are a classic example of this engineering trait because the peat forming *Sphagnum* mosses are central in the regulation of wet conditions (Ivanov, 1981; Joosten, 1993). Hydrological self-regulation has not yet been reported for vascular plants in open bogs.

In bogs suffering from (anthropogenic) drainage and nitrogen pollution, *Sphagnum* mosses are being outcompeted by vascular plants and peat formation is hampered (Laine *et al.*, 1995; Strack *et al.*, 2006; Wiedermann *et al.*, 2009b; Bobbink & Hettelingh, 2011). Under the dense canopy of vascular plants, mosses suffer from light limitation resulting in low biomass production and cover (Clymo, 1973; Hayward & Clymo, 1983; Minkkinen *et al.*, 1999). Vascular plants also impose indirect changes to the processes in *Sphagnum*-dominated ecosystems. The litter of vascular plants decomposes at higher rates than *Sphagnum* litter (Aerts *et al.*, 1999; Turretsky, 2003; Dorrepaal *et al.*, 2005). Next, water losses may also be enhanced by vascular plants due to increased evaporation (Lafleur *et al.*, 2005) and increased run-off (Ivanov, 1981; Chason & Siegel, 1986). In northern peatlands vascular plants are inferior to *Sphagnum* plants in promoting and regulating conditions that lead to extensive peat growth under ombrotrophic conditions. As a result, *Sphagnum* mosses may overgrow vascular plant stands slowly by peat formation (Malmer *et al.*, 1994). Under dry conditions or when mineral-rich water enters an acidic peatland *Sphagnum* species and higher plants, such as trees can co-exist for a long time (Minkkinen *et al.*, 1999; Murphy & Moore, 2010).

In contrast to bogs on the Northern Hemisphere, many bogs in high southern latitudes (Patagonia, New Zealand) are dominated by vascular plant species (Figs 1.1–1.3) despite nutrient poor and acidic conditions (Pisano 1983; Campbell, 1983; Clarkson *et al.*, 2004; Roig & Collado, 2004; Kleinebecker *et al.*, 2007). Vascular plants that dominate the bogs are cushion-forming genera like *Astelia* sp., *Donatia* sp., *Oreobolus* sp. (*Liliaceae, Stylidiaceae* and *Cyperaceae*, respectively), which are mainly found in the Southern Hemisphere, particularly to Patagonia, New Zealand and Tasmania (Gibson & Kirkpatrick, 1985; Kleinebecker *et al.*, 2007). A second group of important bog species are *Empodisma* sp. and *Sporodanthus* sp., which belong to the *Restionaceae* family (henceforth restiad plants). Restiad plants are restricted to the nutrient poor peatlands in New Zealand and Tasmania (Clarkson *et al.*, 2004; Hodges & Rapson, 2010). Peatlands dominated by cushion plants and restiad plants seem to be stable and peat accumulating (Heusser, 1995; Newnham *et al.*, 1995; Fesq-Martin *et al.*, 2004), but it remains uncertain if it is a particular plant species (e.g. *Astelia pumila*, *Empodisma minus*) that is responsible for the bulk of the peat formation as it is the case in *Sphagnum*-dominated bogs. Interestingly, *Sphagnum* species seem to have evolved mainly in the Northern Hemisphere as suggested by phylogenetic studies (Shaw *et al.*, 2010), while typical peatland species of cushion plants and restiads are restricted to the Southern Hemisphere.

Theoretically, the dominance of vascular plants in bogs in the Southern Hemisphere could arise from conditions that specifically favour vascular bog plants, or from conditions that specifically limit the growth of *Sphagnum* mosses, respectively. Limited *Sphagnum* growth would create a niche for vascular plants under nutrient deprived and wet conditions.

Factors favouring vascular plants in bogs

The water-logged conditions (anoxia) found in living peatlands (mires) and wetlands impede the growth of vascular plants mainly by damaging the roots and lowering their lifespan, which consequently restricts the uptake of nutrients and water (Emerson, 1928; Armstrong & Boatman, 1967; Armstrong *et al.*, 1991; Hodge *et al.*, 2009). Enhanced nutrient availability (particularly nitrogen) increases the cover and productivity of vascular plants in bogs and fens, probably by improving the efficiency of roots in water-logged soils (Tomassen *et al.*, 2004; Juutinen *et al.*, 2010; Koelbner *et al.*, 2010). Vascular plants also benefit from increased oxygen availability (lower water levels, higher water level fluctuations) in the root zone (Armstrong & Boatman, 1967; Strack *et al.*, 2006; Murphy & Moore, 2010). Furthermore, increased temperatures are beneficial for the growth of vascular plants (Hollister *et al.*, 2005), provided that they have a sufficient water supply. Factors that favour the growth of vascular plants (nutrients, oxygen, and temperature), also stimulate the decomposition of litter/peat and therefore the nutrient availability in the root zone. This auto-correlation complicates a detailed analysis of the importance of each factor. However, nutrients may play a key role in the dominance of vascular plants, which have intrinsically higher nutrient requirements than mosses (Van Bremen, 1995; Van der Waal *et al.*, 2005; Lindo & Gonzalez, 2010). A positive relationship between vascular plant cover and nutrient addition has been found in a number of field experiments (e.g. Shaver *et al.*, 1998; Limpens *et al.*, 2004; Tomassen *et al.*, 2004; Wiedermann *et al.*, 2009b; Juutinen *et al.*, 2010).

Factors limiting *Sphagnum*

The growth of *Sphagnum* mosses in bog habitats is primarily limited by water availability (Clymo, 1973; Robroek *et al.*, 2009) and shading (Hayward & Clymo, 1983; Minkkinen *et al.*, 1999; Bonnett *et al.*, 2010). In *Sphagnum* mosses that are desiccated, photosynthesis is rapidly reduced when the water content of the mosses drops below 7 gFW gDW^{-1} (e.g. Schipperges & Rydin, 1998). For its high water transport capacity, *Sphagnum* needs to form stands with densely packed branches (Clymo, 1973; Hayward & Clymo, 1982). In contrast, the competition for light with vascular plants requests fast growing and slender mosses (Malmer *et al.*, 1994), which may conflict with the anatomical requirements for an efficient water transport in mosses. Very wet conditions also limit *Sphagnum* growth (Belyea & Clymo, 2001). High water contents in moss tissue impose resistance to the diffusion of CO_2, thereby limiting photosynthesis (Clymo, 1970; Rice & Giles, 1996; Smolders *et al.*, 2001).

Excess amounts of nutrients (nitrogen, potassium, phosphorus) and their co-occurring salts, seem to be toxic to *Sphagnum* mosses that are adapted to mineral poor environments (Skene, 1915). Therefore, the addition of extra nutrients has a limited stimulating effect on *Sphagnum* growth (Aerts *et al.*, 2001; Limpens *et al.*, 2004; Gerdol *et al.*, 2007). For example, the role of nitrogen in *Sphagnum* growth seems to be ambiguous (Clymo, 1995): at low nitrogen availability the growth of *Sphagnum* will initially be nitrogen-limited (Aerts *et al.*, 1992; Twenhöven, 1992). In contrast, growth of *Sphagnum* is inhibited at increased availability of (reduced) nitrogen, such as ammonium (Press *et al.*, 1986; Rudolph & Voigt, 1986; Limpens & Berendse, 2003). The accumulation of nitrogen in moss cells seems to create adverse growth conditions by changing the nitrogen metabolism (e.g. formation of amino acids; Baxter *et al.*, 1992; Rudolph *et al.*, 1993; Nordin & Gunnarsson, 2000).

The enigmatic poor performance of *Sphagnum* in southern bogs

It seems puzzling that *Sphagnum* can become outcompeted in pristine bogs in Patagonia. Conditions seem to be extraordinarily favourable for *Sphagnum* bog growth: frequent rainfall leading to an atmospheric water surplus (Tuhkanen, 1992; Schneider *et al.*, 2003) and a very low atmospheric nitrogen deposition (Godoy *et al.*, 2003). The competitive strength of *Sphagnum* over vascular plants may be further strengthened by leaching of nutrients and cations as a result from high rainfall (Holdgate, 1961; Thomas *et al.*, 1999), by cold summers (Tuhkanen *et al.*, 1990) and by mechanic stress due to strong winds, respectively. Nevertheless, cushion plants dominate bog sites where factors like rainfall, wind and remoteness from nutrient sources (farm land, dust) are most pronounced (c.f. gradients of continentality in Kleinebecker *et al.*, 2007). This leads to the question if cushion plants are simply better adapted to these 'harsh' conditions than *Sphagnum* mosses.

Location of research sites

Mires and peatlands in Tierra del Fuego and southwest Patagonia are of global significance. They form the Southern Hemisphere's counterpart to the northern peatlands (boreal and subartic) that are found north of 50° N. As at these southern latitudes almost no other land is found, the large concentration of Patagonian peatlands is globally important (Iturraspe & Urciuolo, 2004; Blanco & de la Balze, 2004; Yu *et al.,* 2010; Iturraspe *et al.,* 2011). This importance is furthermore accentuated by the large diversity of landscape types in which the Patagonian peatlands are embedded. The large differences in altitude, bedrock, relief and soil conditions, and the extremely steep climatic gradients, have been the breeding ground for a rich variety of peatland types of which several are unique in the world (e.g. cushion bogs and *Sphagnum magellanicum* bogs).

This thesis concentrates on two peatland sites with contrasting vegetation (Figs 1.3, 1.4). The Andorra valley bog (Fig. 1.3a) is an outstanding example of bog vegetation dominated by *Sphagnum magellanicum* (Köpke, 2005; Grootjans *et al.,* 2010). The Moat mires, at the coast of the Beagle Channel, are dominated by cushion plants (Fig. 1.3b), which have invaded a former *Sphagnum* bog (Heusser, 1995; Roig & Collado, 2004). Differences in potential evaporation, wind and temperature dynamics between the two sites are significant (Table 1.1), but much smaller than the climatic gradient in other Patagonian

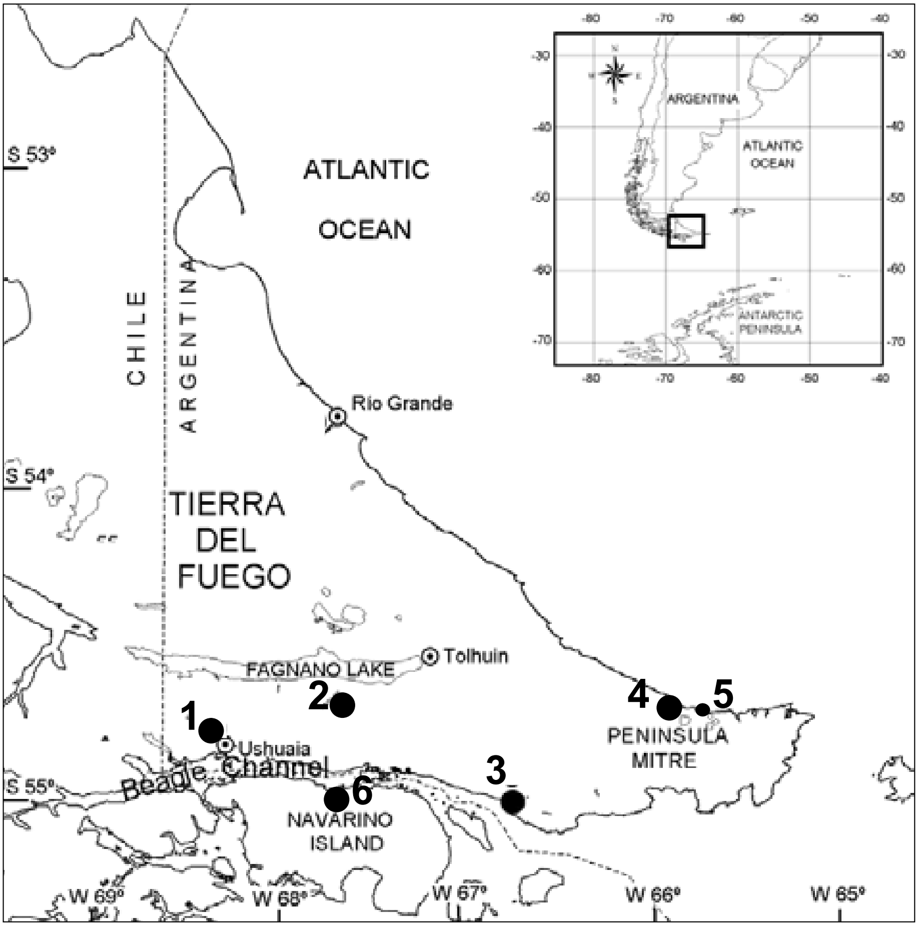

Figure 1.4. Peatland locations in Tierra del Fuego, Patagonia. At site 1 (Andorra valley bog) and site 3 (Moat bogs) field experiments and extensive measurements were conducted. Site 2 (Paso Garibaldi) was visited regularly to obtain measurements from an additional cushion bog. Peatlands 4–6 (Lago Rio Bueno, Policarpo and Paso de los Dientos Isla Navarino, respectively) were visited during a two-week expedition in March 2007.

bogs (cf. Kleinebecker *et al.*, 2007). Patagonian bogs are characterised by a high degree of oceanity, e.g. summer temperature below < 10–12 °C, strong desiccating winds and mild winters along the coast. This supports a long growing season and the occurrence of evergreen vegetation. To include bog vegetation dominated by restiad plants (*Empodisma minus*), chapter 4 reports on research conducted in the restiad bog Opuatia in New Zealand. Restiad peatlands in New Zealand are susceptible to dry spells, despite a frequent rainfall

Table 1.1. Climatic variables recorded at a cushion bog (Moat) and at a *Sphagnum* bog (Andorra) in Tierra del Fuego, Argentina.

	Moat	Andorra	units
period	April 2009 – March 2011	July 2009 – June 2011	–
air temperature	5.9	4.4	°C
humiditiy	71	74	%
wind speed	14.9	8.8	$m\ s^{-1}$
rainfall	503	529	$mm\ y^{-1}$
snow	>150	>150	$mm\ y^{-1}$
potential evaporation sum	1932	1414	mm
average evaporation	0.11	0.08	$mm\ h^{-1}$
average solar radiation	105	108	$W\ m^{-2}\ s^{-1}$

(600–2500 mm per year), similar to Patagonian bogs. The annual temperature of peatlands in New Zealand is 4–6 °C higher than in Patagonia.

Poor accessibility and limited financial support prevented us from including more than three field sites for the experimental research. Even so, I visited and sampled more than 40 bog complexes, including 10 cushion bogs, to place the insights from the experimental work in a broader context. For many observations on the ecology of dominant bog plants, my co-workers and I went by foot, horse and kayak. Poor accessibility is a valuable pre-requisite for pristine conditions.

Objectives and outline of this thesis

The main objective of the present thesis is to unravel how vascular plants are able to win the competition with *Sphagnum* mosses in pristine southern bogs. The focus is on conditions and traits that permit vascular bog plants to maintain a viable root biomass, facilitating the uptake and accumulation of nutrients and the ability to overgrow *Sphagnum* mosses (chapters 2, 4, 5 & 7). Furthermore, I assessed growth conditions of *Sphagnum* mosses, such as nutrient limitation, nutrient uptake, nitrogen stress, desiccation risk and CO_2-supply by methanotrophs in pools. My co-workers and I (henceforth we) studied the growth conditions by conducting long-term fertilisation experiments, field observations and laboratory trials (chapters 2, 3, 6 & 7).

Nutrient availability is reported to be very low in pristine southern bogs. Hence, we questioned whether *Sphagnum magellanicum* from Patagonia is sufficiently adapted to the low nitrogen concentration typical for the rain and porewater in pristine bogs (chapter 2). To answer this question, we measured the uptake rates of nitrogen in the laboratory at a wide range of concentrations and exposure times. Additionally, we compared the nitrogen uptake rates with the nitrogen demand for growth and with the uptake rates of Dutch *Sphagnum magellanicum* plants that have been exposed to high nitrogen deposition in the past.

In two fertilisation experiments, we studied the effects of phosphorus and nitrogen addition on the growth of *Sphagnum* mosses. Both experiments lasted 3 years. Nutrients were added to *Sphagnum* mosses and vascular plants in a *Sphagnum magellanicum* bog (i.e. Andorra; chapter 3) and in a cushion bog (i.e. Moat; chapter 7), respectively. Our hypothesis was that biomass production and growth of the bog vegetation was limited by nutrients. We expected nitrogen to increase the cover of vascular plants. We also investigated the negative effects of nitrogen on *Sphagnum* growth as well as physiological stress. Furthermore, we tested whether phosphorus could alleviate the negative effects induced by nitrogen (chapter 3).

Flooding and water-logged conditions hamper nutrient uptake via roots and thereby reduce the growth of vascular plants. We investigated water level fluctuations in relation to flooding of the root zone (e.g. oscillating/floating peat surfaces) in a restiad peatland in New Zealand, which was dominated by *Empodisma minus* and contained some *Sphagnum* mosses (chapter 4). We questioned if the plants could contribute to prevent flooding and benefit from water levels below the surface. By developing a new method we were able to obtain high-resolution chronosequence records of water level and surface level fluctuations. This new approach facilitated a better interpretation of the dominating mechanism that prevents flooding of the root layer, where the bulk nutrient uptake takes place (Agnew *et al.*, 1993, Clarkson *et al.*, 2009).

In chapter 5, we explore another important strategy of vascular plants to cope with water-logged conditions. In a 2 year-field study we investigated the extent of soil aeration by the roots of cushion plants. Methane emission measurements and laboratory incubations allowed us to study the effects of soil oxygenation on carbon dynamics in peat soils and on the availability of CH_4

for methanotrophic bacteria, often found in peat and associated to *Sphagnum* mosses, respectively.

Methanotrophic activity and diversity is investigated in chapter 6. An alternative source of CO_2 used by *Sphagnum* is provided by microbial CH_4 oxidation, which can be an important growth factor when *Sphagnum* is limited by CO_2 in case of flooding. We questioned if methanotrophic bacteria are limited by CH_4 in *Sphagnum* stands surrounded by cushion plants (chapter 6).

The final chapter synthesises data and conclusions from previous chapters with additional data such as fertilisation experiments, decomposition rates and nutrient uptake by cushion plants. We weighted the factors favouring vascular bog plants against conditions that reduce the growth of *Sphagnum* mosses (chapter 7). The final discussion focuses on mechanisms that enables vascular bog plants to outcompete *Sphagnum* mosses. This competition has a surprising outcome in the southernmost parts of the New World.

2

Adaptive nitrogen uptake in *Sphagnum magellanicum* mosses

A delicate trade-off between saturation and leaching

Christian Fritz, Muhammad Riaz, Leon van den Berg,
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Abstract. Under elevated nitrogen (N) deposition, carbon and nutrient sequestration is hampered in *Sphagnum*-dominated ecosystems as peat-forming *Sphagnum* mosses become outcompeted by vascular plants. N-uptake kinetics of mosses play a major role in regulating N-availability to vascular plants and consequently, plant competition. Factors reducing moss N-uptake are poorly understood. We, therefore, investigated the effects of exposure time (0.5, 2 and 72 h), N-form (^{15}N ammonium and nitrate) and concentration (1, 10, 100 and 500 µM) on the uptake kinetics in *Sphagnum magellanicum* from a pristine and a polluted area. Uptake rates for ammonium were higher than for nitrate and but adsorption of ammonium appeared negligible. During the first 0.5 h, N-uptake followed saturation kinetics revealing a high affinity (K_m 11 µM). Uptake rates decreased drastically with increasing exposure times, which implies that earlier short-term N-uptake experiments may have overestimated long-term uptake rates and ecosystem retention. Compared to the pristine site, *Sphagnum* from the polluted site showed lower uptake rates. *Sphagnum* seems highly efficient in using short N pulses (e.g. rainfall in pristine areas). This strategy has important ecological and evolutionary consequences: risk of N-toxicity seems to be reduced at the expenses of long-term filter capacity and, therefore, competitive advantage over vascular plants.

Introduction

Bogs are fascinating ecosystems that are capable of high storage rates for nutrients and carbon, while nutrient availability is very low due to ombrotrophic conditions limiting growth of vascular plants. A set of traits, unique to *Sphagnum* mosses, enable their dominance in bog ecosystems. *Sphagnum* has evolved a high nutrient use efficiency to cope with low input rates of nutrients (Rudolph *et al.*, 1993; Van Bremen, 1995). The atmospheric input of nutrients is efficiently retained and decomposition rates are low, due to the high retention of rainwater, acidic conditions and poorly degradable organic matter (Aerts *et al.*, 1999). In addition, a substantial part of the C losses (including CH_4) are refixed by *Sphagnum* (Turetsky & Wieder, 1999; Smolders *et al.*, 2001; Kip *et al.*, 2010). This combination of traits enables *Sphagnum* to avoid being outcompeted by vascular plants. However, increased (airborne) nitrogen availability favours vascular plants at the expense of *Sphagnum* mosses. Displacement of *Sphagnum* by vascular bog plants often leads to reduced storage of nutrients, carbon (peat) and water (Lafleur *et al.*, 2005; Gunnarsson *et al.*, 2008).

Bog vegetation reacts slowly to small increments of nitrogen availability as the living *Sphagnum* layer filters nitrogen by retaining and storing substantial amounts of nitrogen in biomass and peat (Lamers *et al.*, 2000; Moore *et al.*, 2005). This nitrogen filter prevents the build-up of airborne nitrogen and increased N availability in deeper soil layers. Cryptogams in general appear to be more efficient in retaining atmospheric nitrogen than other plant groups (Gundale *et al.*, 2011; Xing *et al.*, 2011). Moss biomass has even been found to be the major determinant of N retention capacity of ecosystems (Wanek & Portle, 2005; Curtis *et al.*, 2005). Therefore, mosses are thought to dampen effects of increased anthropogenic inputs of nitrogen (Lindo & Gonzales, 2010; Turetsky *et al.*, 2010). High nitrogen deposition rates were, however, found to have detrimental effects on biomass production of mosses (Arroniz-Crespo *et al.*, 2008; Limpens *et al.*, 2011). Negative effects of nitrogen can be direct (e.g. lower photosynthesis; increased metabolic costs) and indirect (e.g. shading and lower water availability due to increased cover of vascular plants). Direct physiological changes in (*Sphagnum*) mosses have been found upon increased availability of nitrogen (Rudolph *et al.*, 1993; Soares & Pearson, 1997; Pearce *et al.*, 2003). High uptake rates can cause a rapid saturation of nitrogen in *Sphagnum* mosses (Rudolph *et al.*, 1993; Lamers *et al.*, 2000), which is often followed by growth reduction (Baxter *et al.*, 1992; Gunnarsson & Rydin, 2000). It has been shown for *Sphagnum* mosses that photosynthetic rates decrease at nitrogen tissue contents above 930 μmol N g^{-1}DW (Granath *et al.*, 2009). A meta-analysis suggested a general decline in biomass production of *Sphagnum* mosses with increasing nitrogen tissue content (Limpens *et al.*, 2011). At present, it is poorly understood which mechanism causes negative effects of nitrogen (saturation) at the cellular level.

Indirect effects of nitrogen are mostly related to an increased cover of vascular plants and consequently shading of *Sphagnum*-dominated vegetation (Tomassen *et al.*, 2004; Wiedermann *et al.*, 2009b). Vascular plants can also lower water availability to *Sphagnum* mosses by increased ecosystem evaporation followed by lower water levels. Leaching of nitrogen through the moss layer plays a major role in the vegetation change by increasing the availability of N in the rhizosphere to vascular plants (Limpens *et al.*, 2003; Tomassen *et al.*, 2004). As a dense cover of vascular plants has the potential to decrease moss biomass, vascular plants may also indirectly increase nitrogen leaching and availability in the root zone, which has been suggested by field studies (Curtis *et al.*, 2005; Pilkington *et al.*, 2005). Leaching of nitrogen in bogs is also facilitated by the high porosity of the living *Sphagnum* layer and underlying layers of litter and recently formed peat. Low-intensity rainfall may have an average residence time of 10–30 minutes in the *Sphagnum* layer (Yazaki *et al.*, 2006; Fritz *et al.*, 2008; Holden, 2009). Excessive rainfall >5 mm may remain for only 0.5–5 minutes in the uppermost *Sphagnum* layer (Holden, 2009; Fritz pers. observation). Future scenarios for rainfall predict increases in excessive rain events in regions with a substantial cover of *Sphagnum* peatlands (Solomon *et al.*, 2007).

Leakage of nitrogen may also occur when mosses decrease their nitrogen uptake efficiency. Early studies found that nitrogen uptake can be induced by high pulses of nitrogen and seems to be variable in time (Woodin & Lee, 1987; Rudolph *et al.*, 1993). A slowing down of nitrogen uptake may result from nitrogen saturation at the cellular level as assumed by earlier studies (Melzer *et al.*, 1989; Lamers *et al.*, 2000). Alternatively, metabolic changes (e.g. amino acid formation, accumulation of free ammonium) may promptly slow down nitrogen uptake. It has been shown that prolonged exposure to elevated nitrogen deposition may result in a decreased uptake efficiency and nitrogen use efficiency for mosses (Aldous, 2002b; Granath *et al.*, 2009) and vascular plants (Vergeer *et al.*, 2008). In contrast, Swedish mosses from sites with elevated nitrogen deposition showed higher uptake rates of inorganic nitrogen than mosses from a low deposition site (Wiedermann *et al.*, 2009a). Considering these results, a better understanding of the nitrogen uptake kinetics of

Sphagnum mosses seems to be necessary to predict nitrogen retention time and leaching potential at elevated atmospheric nitrogen deposition and its interaction with hydrology (e.g. residence time of rain).

In this study we address the question whether increased exposure times to elevated nitrogen inputs (ammonium or nitrate) result in a reduction of nitrogen uptake efficiency and whether this can be related to nitrogen saturation. We therefore conducted a series of uptake experiments with the peat-forming species *Sphagnum magellanicum* using ^{15}N-ammonium and ^{15}N-nitrate, which mark the upper and lower range of uptake rates for inorganic and organic nitrogen forms, respectively. Long-term effects of nitrogen exposure were assessed by comparing uptake rates of *Sphagnum* mosses collected from a low-deposition site in Patagonia (N deposition 1–2 kg N ha^{-1} y^{-1}) and high-deposition site in the Netherlands (N deposition 30 kg N ha^{-1} y^{-1}). It was hypothesised that exposure time, as influenced by weather conditions and climate, interacts with concentration effects, and that acclimatisation to high N affects N-uptake kinetics.

Materials and Methods

Study site, climate and N-deposition

Experiments were conducted with *Sphagnum magellanicum* (Bridel) collected from lawns at two locations with contrasting histories of nitrogen deposition. Most uptake experiments were carried out using mosses from a pristine *Sphagnum* bog (54° 45′ S; 68° 20′ W) in southernmost Patagonia, Argentina (site description in Grootjans *et al.*, 2010; Fritz *et al.*, 2011), where atmospheric nitrogen deposition is estimated to be as low as 1–2 kg ha^{-1} y^{-1} (chapter 3). This site is hereafter termed the ‘low-deposition site’. The second location was a small bog in the State Forest of Dwingeloo, the Netherlands (52° 49′ N; 6° 25′ E; ‘high-deposition site’) with an estimated atmospheric N deposition ranging between 20 and 30 kg N ha^{-1} y^{-1}, but exceeding 40 kg N ha^{-1} y^{-1} in the period from 1970–2000 (Beijk *et al.*, 2007). Mosses from this site were used for one set of the nitrogen uptake experiments lasting 72 h. In April 2010, two months prior to our experiments, 10 cm thick sods of *Sphagnum magellanicum* were collected from carpets located at five randomly chosen parts in each peatland. Summer water levels ranged between 20 cm and 40 cm below the surface at all sampling locations. After removing vascular plants, we allowed mosses to acclimatise to lab conditions. During this period, the water table was kept 2–5 cm below capitula (photosynthetic active apex, 1 cm in size) for optimal water supply and light intensity was set at 200 µmol PAR m^{-2} s^{-1}.

Uptake experiments

Uptake rates were measured according to Wiedermann *et al.* (2009a). In brief, *Sphagnum* plants (n = 5) were submerged in the experimental solution containing ammonium or nitrate at different concentrations. We used entire *Sphagnum* mosses (capitulum + 20–25 mm living moss tissue below) since uptake takes also place in the tissue below the capitulum (Aldous, 2002a). In a subset of treatments, capitula and stem tissue were separated after the experiment and uptake rates were calculated separately and compared (see statistical analyses).

During the entire experiment, mosses where exposed to an artificial rain solution that resembled the ionic strength of rain and bog waters (150 µmol l^{-1} NaCl, 30 µmol l^{-1} $MgCl_2(6\times H_2O)$, 30 µmol l^{-1} KCl, 10 µmol l^{-1} $CaCl_2$, 10 µmol l^{-1} KH_2PO_4, pH 5.5–6.0), to which the different amounts of nitrogen were added. Plants were washed in 20 l of the artificial rain solution for 0.5 h before exposing them to a treatment solution, and placed in open vials filled with the test solutions containing nitrogen in excess. During the experiment, solutions were gently stirred (35 rpm) to prevent the formation of boundary layers. In the 72 h experiment, mosses were placed in 0.3 l open containers and fixed nitrogen concentrations were maintained by peristaltic pumps (Masterflex, 7015–20; Cole-Parmer, Vernon Hills, IL, U.S.A.). Flow rates varied according to the treatment: 1 µmol N l^{-1} at 1 l h^{-1}, 10 µmol N l^{-1} at 0.1 l h^{-1} and 100 µmol N l^{-1} at 0.01 l h^{-1}, respectively. Theses rates rendered an availability of 10 µmol N g^{-1}DW h^{-1}. The stock solutions were refreshed every 24 h. All treatments were replicated 5 times, and carried out at room temperature (18–21 °C) and 150 µmol PAR m^{-2} s^{-1}. Mosses in the 72 h experiment were exposed to 16 h of light and 8 h dark period per day.

After harvesting, mosses were blotted dry, washed for 5 minutes in 1 M KCl added to the artificial rain solution and blotted dry again before drying at 70 °C for 48 h. Oven dried mosses were ground in liquid nitrogen before being analysed for ^{15}N and total N using isotope ratio mass spectrometry (IRMS, see Kleinebecker *et al.*, 2009 for details). Results were corrected for measured background ^{15}N content (n = 10 per site). To obtain net nitrogen uptake we divided the uptake of ^{15}N by the enrichment of N-sources used: $^{15}NH_4Cl$ (10 atom% ^{15}N) and $Na^{15}NO_3$ (5 atom% ^{15}N). In addition, we estimated uptake rates in the 72 h experiment by means of depletion of nitrogen in experimental solution related to the dry weight (cf. Twenhöven, 1992a; Jauhiainen *et al.*, 1998). Samples of the experimental solution (24, 48 and 72 h after experiment started) were directly frozen (–20 °C) until colorimetrical analysis of ammonium (Traacs 800+ auto-analyzer). This allowed us to compare average uptake rates over 72 h with snap-shots of uptake (24, 48 and 72 h after experiment started).

To estimate the passive uptake, we measured nitrogen uptake in autoclaved (1 h at 120 °C) *Sphagnum* plants (passive uptake) and living *Sphagnum* plants (active+passive uptake). Following the procedure described above, plants were exposed for 0.5 h to ^{15}N-ammonium or ^{15}N-nitrate at 3 concentrations: 1 µM, 10 µM and 100 µM, respectively. After autoclaving, mosses maintained their morphology. Washing of the plants was different than described above. Samples were exposed to two different washing solutions: demineralised water (loosely attached N-fraction) and a 0.2 M $SrCl_2$ solution (complete exchange of cations by Sr^{2+} ions).

Uptake kinetics – effects of concentrations and increasing exposure time

We used Michaelis–Menten kinetics to describe the observed saturation of uptake rates with increasing strength of the nitrogen solution for mosses exposed for 0.5 h. Parameters of the Michaelis–Menten equation (Eq. 1) were fitted to the uptake data by performing a regression analysis (hyperbola, single rectangular, two parameters) in Sigma Plot 10.0 (Systat Software, Inc.).

$$v = (V_{max} \times [S]) / (K_m + [S]) \qquad \text{(Eq. 1)}$$

where v is the measured uptake rate at a given substrate concentration [S], V_{max} is the maximum uptake rate at substrate saturation and K_m is the Michaelis–Menten constant (substrate concentration at which uptake occurs at half of the maximal uptake – half saturation constant).

We also estimated the dependency of uptake rates on exposure time t for treatments receiving 10µM or 100µM ammonium. Parameters for the effects of exposure time (Eq. 2) were fitted to the uptake data by performing a regression analysis (hyperbola, hyperbolic decay, three parameters) in Sigma Plot 10.0 (Systat Software, Inc.).

$$V_t = [(V_{ini} \times b) / (t + b)] + C \qquad \text{(Eq. 2)}$$

where V_t is the uptake rate at the exposure time t and b is the time after which V_t decreased to half of the initial uptake rate (V_{ini}). Uptake rates at 24, 48 and 72 h were based on depletion of nitrogen in the experimental solution. The constant C represents the rate of background nitrogen uptake e.g. to supply nitrogen consumed by growth and natural nitrogen losses. Under field conditions the constant C may be related to 'critical N-uptake', that is the N-uptake rate needed to sustain the maximal growth rate. Aldous (2002b) estimated the annual nitrogen requirement of a growing *Sphagnum* carpet to range from 0.18–0.35 mol N m^{-2} y^{-1}. This is equivalent to an average hourly nitrogen consumption of 0.14–0.57 µmol N g^{-1}DW h^{-1} when assuming a capitulum biomass of 180–360 gDW m^{-2} and a 5-month growing season, which has been found by other studies (Twenhöven, 1992b; chapter 3).

Application of uptake kinetics – contextualising the relationship between nitrogen loads and exposure time

To estimate the exposure time of *Sphagnum* mosses to nitrogen for different nitrogen deposition loads, the following assumptions were made: a rainfall of 5 l m^{-2} and a living moss biomass of 500 gDW m^{-2}, which is equivalent to an average bulk density of 10 gDW l^{-1} in the upper 5 cm (cf. Aldous, 2002a; Bragazza *et al.*, 2004). As a conservative estimate we assumed that the entire moss biomass contributes to nitrogen uptake. The nitrogen pulse was diluted 4 times by the water content in *Sphagnum* mosses that often exceeds 10 times the dry weight of mosses (Schipperges & Rydin, 1998; Cagampan & Waddington, 2008). The resulting concentrations were used in equation (1) to calculate uptake rates on gDW^{-1} biomass basis. Different scenarios were used for maximum uptake rates (V_{max}), which were based on the uptake rates measured in the present study: ammonium_pulse 35 µmol N g^{-1}DW h^{-1}, ammonium_long 3.4 µmol N g^{-1}DW h^{-1}, nitrate_pulse 3.7 µmol N g^{-1}DW h^{-1} and nitrate_long 1.0 µmol N g^{-1}DW h^{-1}, respectively. For the scenarios, we assumed 11 µM as half saturation constant (K_m) except for the scenario 'nitrate-low affinity' where a K_m of 50 µM was assumed. Finally, we calculated the time it takes to deplete 90 % of a nitrogen pulse (volume rain times concentration) given the concentration specific uptake rates and biomass. Nitrogen concentrations in rain (µmol NH_4NO_3 l^{-1}) are related to yearly loads of nitrogen (kg ha^{-1}) by a factor of 0.105, assuming 750 mm rainfall.

Statistical analyses

Initial data analysis started by fitting multiple linear regressions, followed by a check for violation of statistical assumptions. In cases where heteroscedasticity was observed, we used linear regression with the generalized least squares (GLS) extension, which allowed us to benefit from retaining the original variance structure in the data (Pinheiro *et al.*, 2006; Zuur *et al.*, 2007). Model simplification to a minimal adequate model was based on AIC (Akaike Information Criteria; Sakamoto *et al.*, 1986), after backward selection using the likelihood ratio test. The importance of each explanatory factor in the minimum adequate model was assessed by comparison of this model with a reduced model (with all the terms involving the factor of interest removed), using the likelihood ratio test. All analyses were performed using the 'nlme' package (v. 3.1, Pinheiro *et al.*, 2006) in the 'R' (version 2.9.2) statistical and programming environment (R Development Core Team, 2009).

Results

Concentration effect and N-uptake in autoclaved plant material

In plants of *Sphagnum magellanicum* from the pristine Patagonian bog (low-deposition site), nitrogen uptake during the first 0.5 h increased with increasing nitrogen concentration. N-uptake could be described by Michaelis–Menten kinetics (Fig. 2.1) for both ammonium and nitrate. Mosses showed ten times higher uptake rates for ammonium (V_{max} of 35 µmol N g^{-1}DW h^{-1}) than for nitrate (V_{max} 3.7 µmol N g^{-1}DW h^{-1}). Ammonium and nitrate were taken up efficiently already at low concentrations, which can be inferred from K_m-values of 11 µM found for both forms of nitrogen. This means that at a concentration of 100 µmol N l^{-1} the uptake is already at 90 % of V_{max}. Uptake rates in living mosses were independent from the washing solution (demineralised water and $SrCl_2$, respectively). Thus, the fraction bound by adsorption to the cell wall is negligible compared to the fraction taken up into the cells.

When mosses were autoclaved, uptake rates decreased by 2 orders of magnitude compared to living mosses. Mosses maintained their morphology after being autoclaved. In general, autoclaved mosses revealed low nitrogen uptake that was a small fraction (1–5 %, $n = 50$) of the nitrogen uptake found in living mosses. Only the 100µM-nitrate treatment showed higher relative uptake rates (11–16 %, $n = 10$) compared to living mosses. Uptake in autoclaved mosses increased with nitrogen concentration of the experimental solution and was highest in the ammonium treatments. Comparing the two washing solutions suggested that 60–75 % of ammonium was removable with $SrCl_2$ in autoclaved plant material. The fraction washed-off by $SrCl_2$ may indicate the total of ammonium bound to adsorption sites (e.g. cation exchanger).

Effect of increasing exposure time

Nitrogen uptake rates decreased with exposure time. This decrease was most pronounced for ammonium. The concentration dependency found after 0.5 h (Fig. 2.1) was also observed when the duration of the experiment was increased to 2 h and 72 h, respectively (Fig. 2.2) Uptake rates were, however, much lower with increasing exposure time ($P < 0.001$, Table 2.1). Uptake rates after 2 h were 1.6 times lower for ammonium, but only 1.2 times lower for nitrate at both concentrations (10 µM and 100 µM). During 72 h uptake rates of ammonium were drastically decreased, and were 6–14 times lower than rates after 0.5 h.

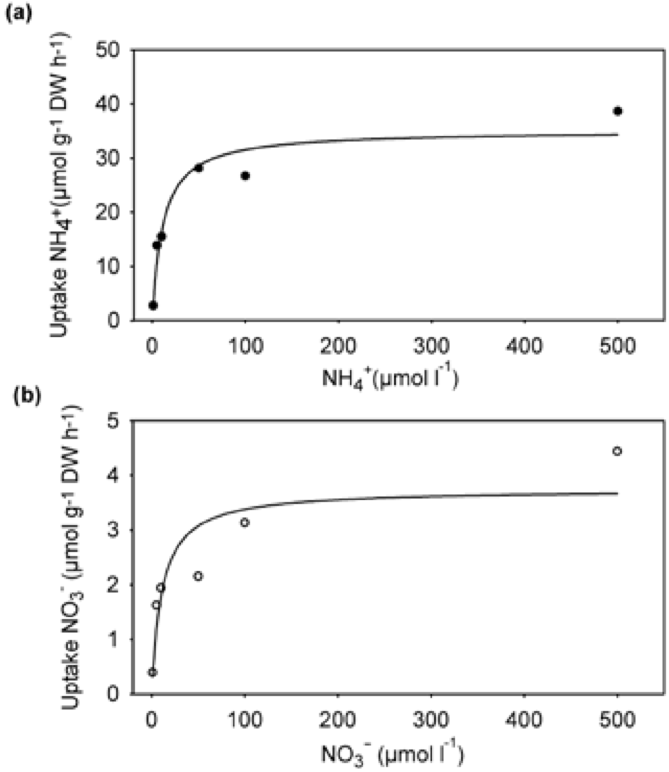

Figure 2.1. Dose response curves of (a) ammonium uptake rates (filled circles; µmol NH_4^+ g^{-1}DW h^{-1}) and (b) nitrate (open circles; µmol NO_3^- g^{-1}DW h^{-1}) of *Sphagnum magellanicum* ($n = 5$) from the low-deposition site. The rates are the average uptake after 0.5 h. Note that y-axes differ by one order of magnitude. Michaelis–Menten curves (Eq. 1) were fitted to rates: V_{max} of ammonium (35 µmol NH_4^+ g^{-1}DW h^{-1}) was higher than V_{max} of nitrate 3.7 µmol NO_3^- g^{-1}DW h^{-1}), whereas K_m-values were similar (11.3 µM for ammonium and 11.0 µM for nitrate, respectively).

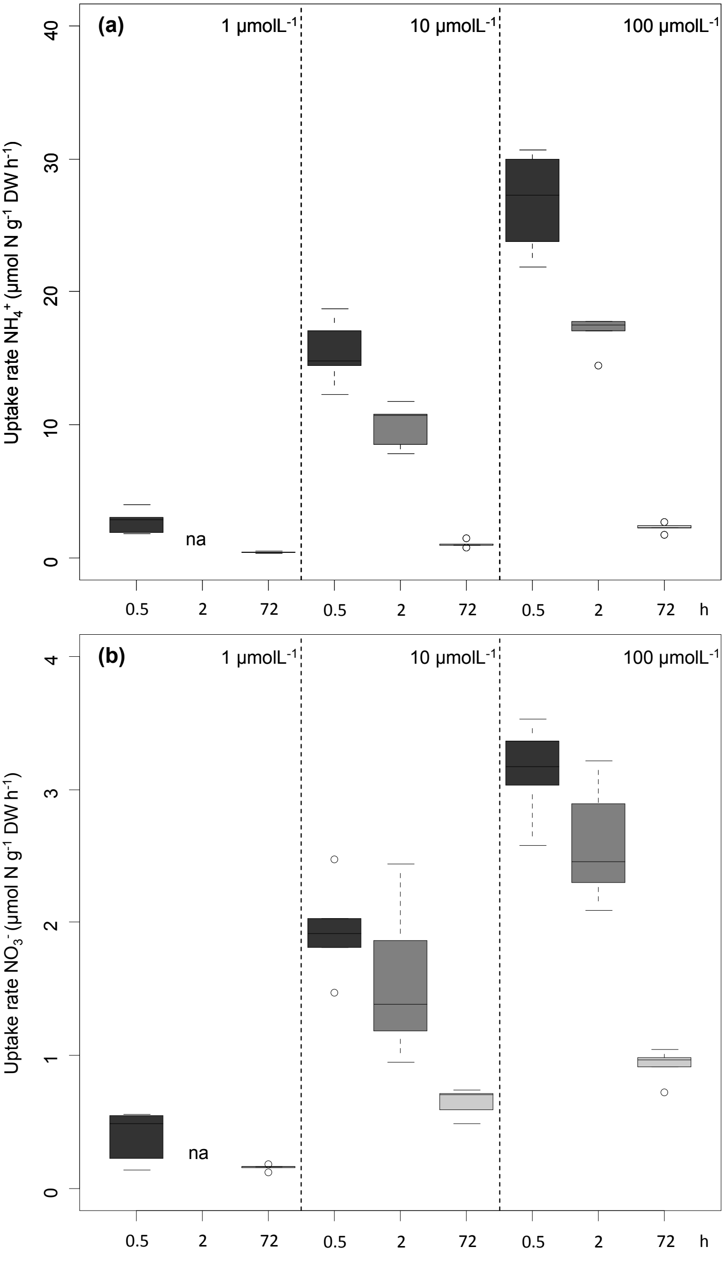

Figure 2.2. Average uptake rates (μmol N g^{-1}DW h^{-1}) after 0.5 h (dark grey), 2 h (grey) and 72 h (light grey) , respectively, in *Sphagnum magellanicum* (*n* = 5) from the low-deposition site. Upper panel (a) shows ammonium (NH_4^+) uptake. The lower panel (b) shows nitrate (NO_3^-) uptake.

Table 2.1. Minimal adequate models[1] for data presented in Fig. 2.2.

Uptake rate	Factor	Log L Ratio	*P*-value[2]
NH_4^+	concentration	58.34	<0.001
	exposure time	109.40	<0.001
	concentration × exposure time	22.69	<0.001
NO_3^-	concentration	42.06	<0.001
	exposure time	50.86	<0.001
	concentration × exposure time	13.43	<0.001

1. Nitrogen saturation of moss tissue was not a significant factor.
2. *P*-values represent the importance of the explanatory factor assessed by a comparison of the minimum adequate model with a reduced model using the likelihood ratio test.

In contrast, nitrate uptake efficiency was less affected by exposure time and lowered by a factor of 3. Consequently, the preference of ammonium uptake over nitrate uptake declined with increasing exposure time. Tissue nitrogen remained below 990 µmol N g^{-1}DW in treatments with lower uptake rates than the 100µM-ammonium treatment. Interestingly, uptake rates were independent from tissue nitrogen at the start of the experiment (indicator for N saturation), which ranged from 632–921 µmol N g^{-1}DW.

We investigated the uptake dynamics of ammonium into more detail during the 72 h experiment by means of ammonium depletion of the experimental solution. During the first 2 h, uptake rates slowed down to 60 % of the initial uptake rates (Fig. 2.3; b values were 2.1 h). The decline of ammonium uptake rates with increasing exposure time can be well described (r^2 = 0.99) by equation 2 (Fig. 2.3). Background nitrogen consumption (C in Eq. 2) ranged from 0.22–0.88 µmol N g^{-1}DW h^{-1}, which is similar to nitrate uptake rates over 72 h.

Long-term adaptation to increased nitrogen supply

Mosses from the pristine bog in Patagonia (low-deposition site) appeared to be more efficient in taking up ammonium and nitrate than mosses from the Netherlands (high-deposition site). 40–160 % higher uptake rates were observed in all treatments in the 72 h experiment with the exception of the 1µM-nitrate treatment (Fig. 2.4). Both sites showed the concentration effect as described above (Fig. 2.2). For the 1 µM-nitrate treatment, mosses from Patagonia had similarly low rates as mosses from the Netherlands. This resulted in an interaction effect between site and concentration (Table 2.2; df = 1, *F* = 4.35,

$P = 0.047$). Mosses from the low-deposition site were more efficient in taking up ammonium at concentrations below 100 µM, than mosses from the high-deposition site. This difference was most pronounced at very low ammonium concentrations (1 µM). At 1 µM ammonium mosses from the low-deposition site showed average uptake rates of 0.46 N g^{-1}DW h^{-1} that is still within 'criti-

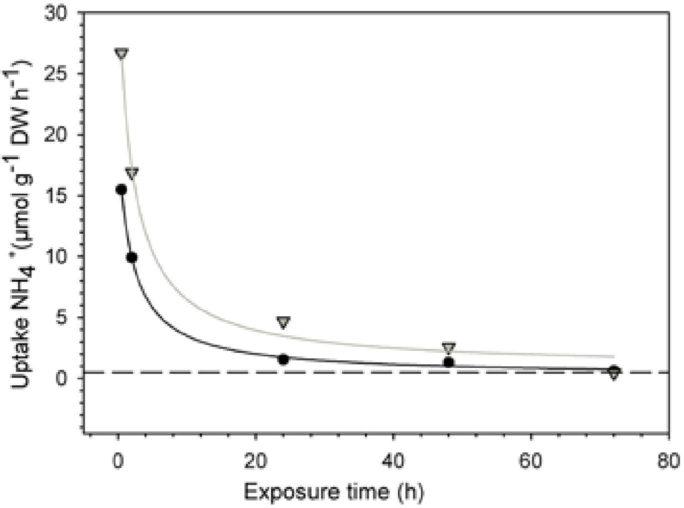

Figure 2.3. The effect of exposure time on ammonium uptake in *Sphagnum* from the low-deposition site. Eq.2 fitted well ($r^2 = 0.99$) the uptake data from both concentrations 10 µM (black circles) and 100 µM (grey triangles). The half-time value 'b' was 2.1 h for both concentrations. The constant (C) was lower in the 10µM-ammonium treatment (0.22 µmol N g^{-1} DW h^{-1}) compared to the 100µM-ammonium treatment (0.88 µmol N g^{-1} DW h^{-1}). Dashed line indicates critical N-uptake to maintain biomass production (0.8 µmol N g^{-1} DW h^{-1}). Note that rates at 24 h and later were based on the depletion of ammonium in the experimental solution, whereas other rates presented in this paper are average rates calculated from ^{15}N uptake.

Table 2.2. Accession effect: linear models of data presented in Fig. 2.4.

Uptake rate	Factor	*F*	*P*-value
NH_4^+	concentration	180.33	<0.001
	origin	24.39	<0.001
	concentration × origin	3.03	0.0936
NO_3^-	concentration	45.36	<0.001
	origin	20.91	<0.001
	concentration × origin	4.35	0.0469

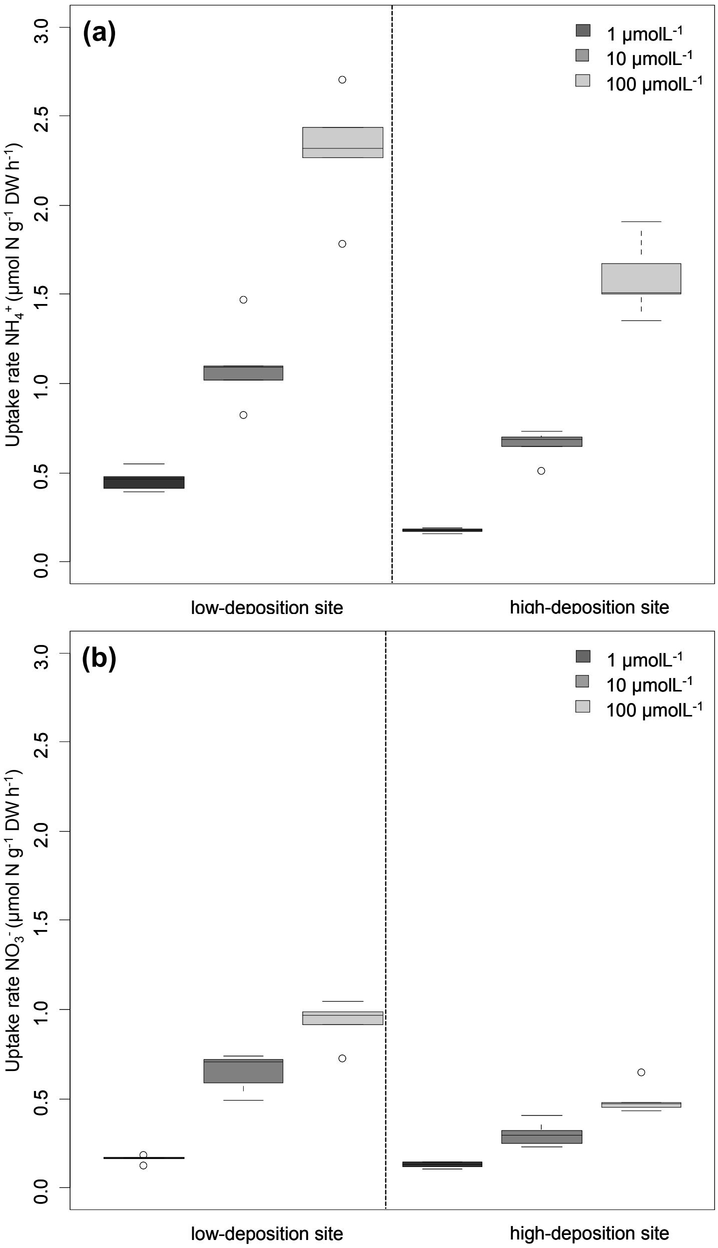

Figure 2.4. Accession effect. N-Uptake rates (μmol N gDW^{-1} h^{-1}) at 1 μM (dark grey), 10 μM (grey) and 100 μM (light grey), respectively, of the low-deposition site (Argentina, left) and the high-deposition site (The Netherlands, right). Upper panel (a) shows ammonium (NH_4^+) uptake. The lower panel (b) shows nitrate (NO_3^-) uptake. Experiments lasted 72 h.

cal N-uptake rates' (Figs 2.3, 2.4). Mosses from the high-deposition site were less efficient at concentrations of 1 μM ammonium or nitrate, resulting in a total uptake of < 13 μM N g^{-1}DW over 72 h. Such a low uptake is on average lower than the 'critical N-uptake' rate. In *Sphagnum* mosses from the high-deposition site, tissue nitrogen remained below 800 μmol N g^{-1}DW during the experiment showing, no signs of N-saturation.

Comparison of N-uptake in apical tissue and stem tissue

As rainwater usually infiltrates deeper than the upper 5–10 mm where the capitulum is situated, a larger part (30–35 mm) of the *Sphagnum* mosses were used in the uptake experiments. Lower stem tissue is likely to be in contact with soil moisture containing ammonium and nitrate for longer periods of time. We found 30 % higher uptake by stems than by capitula tissue for ammonium treatments with high concentrations (50 μM and 500 μM) (Fig. 2.5, Table 2.3). In the 1 μM-nitrate treatment the uptake rate of stems was higher than those of capitula by only 0.5 μmol N g^{-1}DW h^{-1} (against a range of rates 0–50 μmol N g^{-1}DW h^{-1}; Fig. 2.5, Table 2.3).

Conceptualised model of dose–exposure time relationship

In figure 2.6 the nitrogen uptake kinetics are translated to field conditions by assuming a *Sphagnum* moss biomass of 500 g m^{-2}. Initial uptake rates of an 'ammonium_pulse' (0.5 h uptake rates, Fig. 2.1) resulted in lowest time to remove 90 % of nitrogen from rain. Since the exposure time linearly decreases with V_{max}, the highest exposure times are found for the scenario with lowest V_{max} ('nitrate_long'). Simulations suggest that at natural nitrate concentra-

Table 2.3. Differences between uptake rates in capitula and stem tissue shown Fig. 2.5. The statistical parameter values are the result of *T*-tests. Significant differences are printed bold.

Capitula vs. Stem uptake	Concentration	Ratio Cap:Stem uptake	*T*	df	*P*-value
NH_4^+	1	ns	2.762	4	0.0507
	50	0.66	4.952	4	0.0158
	500	0.73	6.125	4	0.0036
NO_3^-	1	0.07	5.955	4	0.0040
	50	ns	−1.418	4	0.2291
	500	ns	−1.939	4	0.1245

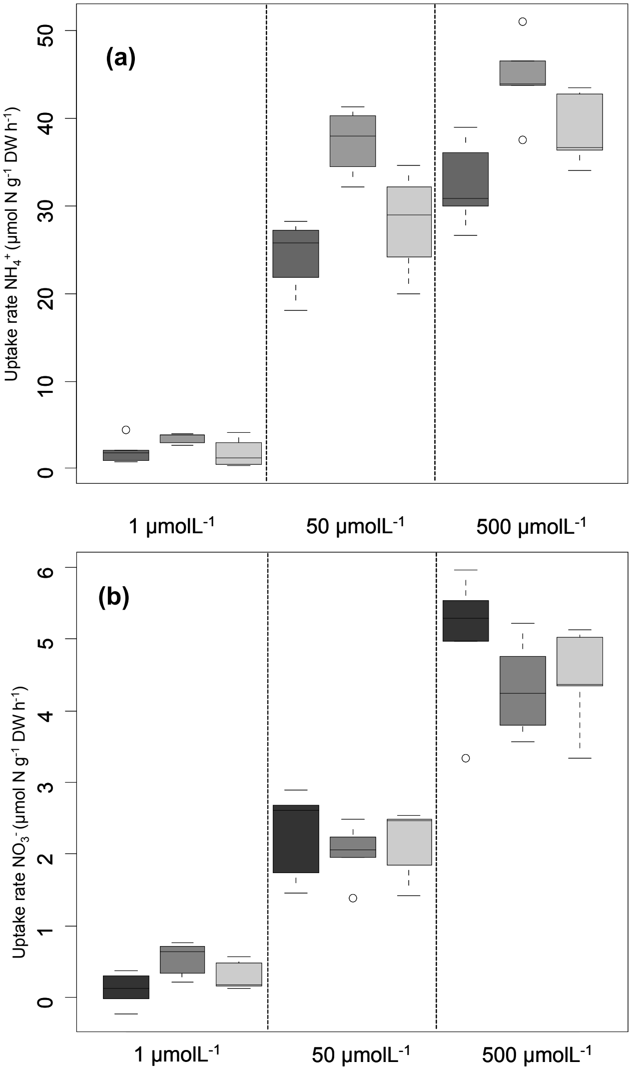

Figure 2.5. Differences between average uptake rates (µmol N g^{-1}DW h^{-1}) after 0.5 hours in different fractions of *Sphagnum magellanicum* from the low-deposition site. We analysed capitula and stem separately, while whole plant is the mean of both fractions weighted by dry biomass.

tions the exposure time will be above 30 minutes, which is close to the upper limit (grey dotted line) of average residence time of rain (Fig. 2.6).

Rain nitrogen concentrations below 10 µmol N l^{-1} (natural rain) had surprisingly little influence on exposure time. In contrast, exposure time increased linearly with N concentration in rain above 100 µmol N l^{-1} (rain from polluted areas). Both results underscore the effect of the half-saturation constant K_m that was 11 µM. Simulation applying higher K_m values proportionally increased the exposure time at nitrogen concentrations below 10 µmol N l^{-1} (uppermost curve Fig. 2.6). Uptake simulations at V_{max} lower than 4 µmol N g^{-1}DW h^{-1} resulted in exposure times exceeding those of rain for concentrations above 100 µmol N l^{-1}. Nitrogen doses used in many field application experiments (>2000 µmol N l^{-1}) require exposure times up to a hundred times higher than the residence time of rain, which permits the infiltration of nitrogen. The model provides additional information: exposure times increase lin-

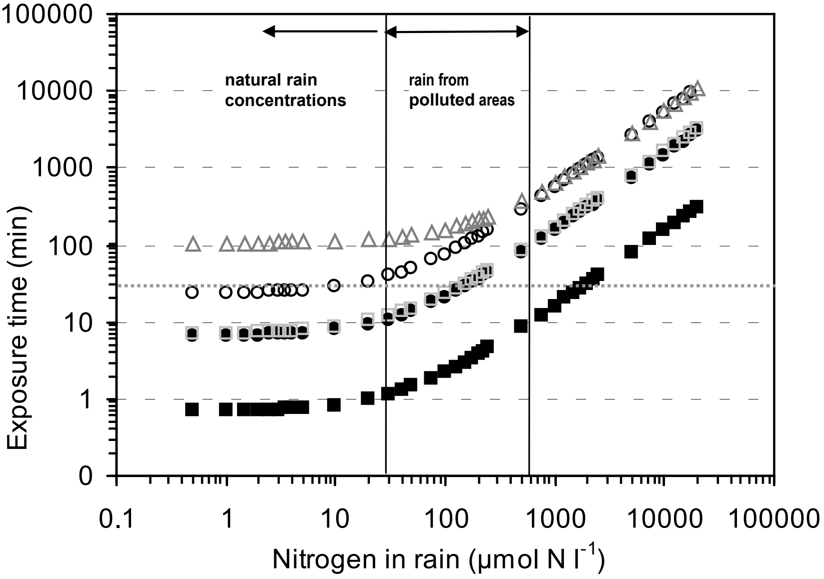

Figure 2.6. Conceptualised relationship (log–log) between nitrogen concentration in rain and exposure time. The y-axis displays the time a living Sphagnum layer would need to retain some 90 % of the nitrogen load. Maximal uptake rates V_{max} differ between scenarios: closed black squares 'ammonium_pulse' 35 µmol N g^{-1}DW h^{-1}, open squares 'ammonium_long' 3.4 µmol N g^{-1}DW h^{-1}, closed circles 'nitrate_pulse' 3.7 µmol N g^{-1}DW h^{-1}, open circles 'nitrate_long' 1.0 µmol N g^{-1}DW h^{-1}, and 1.0 µmol N g^{-1}DW h^{-1} open triangles 'nitrate_low affinity' respectively. The model was parameterised with a K_m of 11 µM for all simulations except 'nitrate_low affinity' where a K_m of 50 µM was assumed. An increasing potential of leaching is expected above the dotted grey line that indicates the average residence time of rain. Average nitrogen concentrations in rain (µmol N l^{-1}) relate to yearly wet deposition of nitrogen (kg N ha^{-1}) by a factor of 0.1.

early with the volume of rain at a fixed N concentration, but decrease linearly with biomass.

Discussion

To our knowledge, this is the first study that investigates the interactions between exposure time and substrate concentration for the uptake of ammonium and nitrate by peat-forming *Sphagnum magellanicum* mosses. High uptake rates found in the present study suggest immediate (within minutes) uptake of ammonium and nitrate from rain (Fig. 2.6), which has also been found during a tracer field study using labelled ammonium nitrate at the low-deposition site (Fritz & Caspers unpublished data). Long-term uptake rates (Figs 2.2, 2.3) seemed to be sufficient to maintain N-provision for growth (critical N-uptake rates). Higher uptake rates were found for stem tissue as compared to capitula tissue (Fig. 2.5). Stems showed a higher surface area per dry weight, which increased the relative surface of stems to the nitrogen solution. Nitrogen uptake by *Sphagnum magellanicum* from pristine Patagonia seemed to be more efficient at low concentrations (K_m = 11 μM) compared to arctic *Sphagna* (Kielland, 1997; K_m = 1001 μM or tropical non-*Sphagnum* mosses (Wanek & Portl, 2008; mean K_m = 59 μM over 10 species). Such a high uptake efficiency may reflect the nutrient deprived conditions in Patagonian bogs and serve as a benchmark for studies in polluted areas. Differences in N-uptake seems rather small between distinct peat-forming *Sphagnum* mosses (Wiedermann *et al.*, 2009a).

The highly efficient nitrogen uptake, however, provides a serious risk of over-saturation in case of a large nitrogen load and consequently high nitrogen availability in rain and surface moisture. Porewater concentration of dissolved inorganic nitrogen are often in the range of 5–50 μmol N l^{-1} at low to average nitrogen deposition sites (Twenhöven, 1992b; Limpens *et al.*, 2004; Blodau *et al.*, 2006), with ammonium being the dominating species due to the low pH. Our studies revealed substantial uptake rates for this range of porewater concentrations. Mosses would exceed their optimal nitrogen content (930 μmol N g^{-1}DW) within 36 h (extrapolating 0.5 h uptake rates at 10 μM ammonium (15.5 μmol N g^{-1}DW h^{-1}) and a nitrogen tissue content of 400 μmol N g^{-1}DW. Evidence for supra-optimal N-uptake was found in the 100μM-ammonium treatment over 72 h (Fig. 2.2). Detoxification of excess nitrogen, especially ammonium, is slow and demands photosynthesis products as well as energy (Rudolph *et al.*, 1993; Heeschen *et al.*, 1996; Kahl *et al.*, 1997). It is therefore very likely that a mechanism for rapid reduction of N-uptake has evolved in *Sphagnum* to prevent excessive uptake of nitrogen. We have indeed found evidence of such a decrease in uptake efficiency upon enhanced exposure time to nitrogen (Figs 2.2–2.4).

Exposure time effect

Exposure time significantly decreased nitrogen uptake rates at all N doses (Figs 2.2, 2.3). This exposure time effect increased with nitrogen dose (Table 2.1). We expected uptake rates to remain constant or to decline only modestly at low nitrogen doses (i.e. low uptake rates) as earlier studies assumed a fixed upper threshold of nitrogen uptake which is related to nitrogen saturation of moss tissue (Melzer *et al.*, 1989; Lamers *et al.*, 2000). In contrast to expectations, the present study suggests a decrease in uptake efficiency independent from tissue nitrogen concentration but present along the entire range of total nitrogen uptake (12–166 μmol N g^{-1}DW) over 72 h (Fig. 2.3). Nitrate uptake efficiency also decreased, but substantial differences were only found at the scale of hours to days (Fig. 2.2). The observed reduction in nitrate uptake occurred at the same time scale as reported declines of nitrate reductase activity, a key enzyme in the nitrate assimilation (Woodin *et al.*, 1985; Rudolph *et al.*, 1993). A lower activity of nitrate reductase may cause an accumulation of symplastic nitrate (Melzer *et al.*, 1989) and possibly a less steep import gradient.

Interestingly, uptake rates decreased also at low concentrations of nitrogen and consequently resulted in low total uptake. We hypothesise that the observed rapid reduction in ammonium uptake (40 % in the first 2 h; Fig. 2.3) is related to the saturation of temporal storage pools (vacuole, cell wall, amino acid production). Once these pools are saturated, ammonium may be taken up at rates similar to nitrogen consumption in biomass production and amino acid/protein synthesis. Conclusions are difficult as there is little known about kinetics of the amino acid metabolism in *Sphagnum* mosses. A slow accumulation of glutamine has been reported at rates of 35 μmol g^{-1}DW over 72 h (Rudolph *et al.*, 1993). Also Kahl *et al.* (1997) found the activity of glutamine synthetase was substantially lower than ammonium uptake rates in *Sphagnum* mosses. Biomass production, the most important long-term sink of nitrogen, sequesters on average 0.1 – 0.6 μmol N h^{-1} per gram growing apex (capitulum) when converting seasonal to hourly growth rates. These 'critical N-uptake' rates are in stark contrast to the actual nitrogen uptake rates observed during the first two hours, but seems to be in agreement with N-uptake rates found during the 72 h experiment (Figs 2.3, 2.4).

The cation exchange at the cell wall could represent an additional ammonium storage. The results with autoclaved mosses suggested that < 1 μmol NH_4 g^{-1}DW could be adsorbed to the cell wall when mosses where exposed to a medium containing 260 μeq l^{-1} competing cations (see methods). This low adsorption of ammonium is in agreement with other studies (Brehm, 1968; Breuer & Melzer, 1989). The capacity to adsorb ammonium is shown to be lowered by competing cations like K^+, Na^+ and Ca^{2+} (Breuer & Melzer, 1989).

Environmental changes increase the risk of leaching

Adaptation to high nitrogen loads may not only occur on the scale of hours but also after years or decades of increased nitrogen deposition (Vergeer *et al.*, 2008). Our results provide evidence that the N-uptake efficiency of *Sphagnum magellanicum* decreases by a factor 1.4–2.6 in mosses that experienced long-term exposure (decades) to high nitrogen deposition (Fig. 2.4). A decrease of nitrogen retention would be exacerbated when *Sphagnum* moss density is reduced by nitrogen (Aldous, 2002a; Bragazza *et al.*, 2004; chapter 3). Therefore, we expect a substantially lower nitrogen retention by stands of *Sphagnum* from polluted sites compared to pristine mosses, which may enhance further nitrogen leaching in N polluted sites even when lowering nitrogen deposition. Further studies are needed to unravel mechanisms causing the reduction in uptake efficiency.

We propose a conceptual model (Fig. 2.6) that relates uptake efficiency to both dose and exposure time. Our model suggests enhanced leaching (lower effective retention) with increasing loads, which explains well the leaching found in gradient studies (Bragazza & Limpens 2004; Curtis *et al.*, 2005), pulse loading studies (Woodin & Lee, 1987; Twenhöven, 1992b; Aldous, 2002a) and fertilisation studies (Tomassen *et al.*, 2004; Xing *et al.*, 2011). The decrease in uptake efficiency of mosses (Fig. 2.2) within 72 h results in substantially higher exposure times (Fig. 2.6), by which both ammonium and nitrate are at increased risk to leach even at natural nitrogen concentrations in rain. Substantial leaching of nitrate may occur with the short residence times associated with continuous rainfall, typical for the large oceanic bogs of North and South America (Gignac & Vitt, 1990; Kleinebecker *et al.*, 2007).

Nitrogen uptake kinetics also suggest that global change may interact with N-retention efficiency (Fig. 2.6). Excessive rainfall and extended dry spells may become more frequent, and in combination will result in rapid and deep infiltration of rainwater and solutes (Holden & Burt, 2002; Solomon *et al.*, 2007; Holden, 2009). Dry conditions may also lower uptake efficiency as a result of low metabolic activity and growth of mosses after desiccation (Marschall, 1998; Robroek *et al.*, 2009). N-loads typically applied in field addition experiments (> 1000 μmol N l^{-1}; 5–40 kg N ha^{-1} per application) would remain available for days to weeks in the living *Sphagnum* layer (Fig. 2.6), which has been found in field studies (Bayley *et al.*, 1987; chapter 3). At exposure times of days, availability of applied nitrogen depends largely on hydrological changes (dilution by rain, infiltration), which may partly account for the high variation found in nitrogen uptake by *Sphagnum* mosses (Limpens *et al.*, 2011).

Sphagnum mosses have developed a rapid uptake mechanism to deal with low availability of nitrogen, where other species in ombrotrophic environments have dealt with the same constraint by becoming insectivorous or particularly nutrient-conservative (Aerts, 1995). Nitrogen uptake of *Sphagnum* appears to be highly efficient and very well adapted to natural rain events (1–20 μmol N l^{-1}), a trait that has been selected for during the evolution of this 34–102 million year old genus (Shaw *et al.*, 2010). However, *Sphagnum* has to deal with a delicate trade-off between preventing potential adverse effects of high N-uptake (Fig. 2.2), and promoting the competitive strength of vascular plants by substantial leaching (Fig. 2.6) and accumulation of nitrogen in the rhizosphere (Lamers *et al.*, 2000; Malmer *et al.*, 2003). This trade-off extends across several scales, from cell to peatland. In case *Sphagnum* biomass decreases, leaching of nitrogen is strongly increased (Curtis *et al.*, 2005). Once the accumulation of nitrogen promotes the expansion of vascular plants (Limpens *et al.*, 2003; Tomassen *et al.*, 2004), *Sphagnum* growth is impeded by shading and desiccation on the ecosystem scale (Clymo, 1973; Berendse *et al.*, 2001; Lafleur *et al.*, 2005).

The present study indicates that long-term nitrogen retention maybe tightly linked to nitrogen assimilation via biomass production of *Sphagnum* mosses (Fig. 2.3). Productivity of *Sphagnum* mosses is often limited by water availability (Robroek *et al.*, 2007 and literature therein) and we therefore expect a strong interaction between moisture availability and nitrogen retention. The expected hydrological extremes (e.g. Bragazza, 2008), as a result of climate, change increase the negative effects of high nitrogen availability and can thereby stress *Sphagnum*-dominated vegetation under already low nitrogen loads. Therefore, both types of global change in concert (climate and anthropogenic N input) are expected to severely change the functioning of *Sphagnum*-dominated peatlands, and complicate the conservation and restoration of these self-regulating ecosystems (Joosten & Clarke, 2002).

Nutrient additions in pristine Patagonian *Sphagnum* bogs

Can phosphorus addition alleviate (the effects of) increased nitrogen loads?

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Abstract. *Sphagnum*-bog ecosystems have a limited capability to retain carbon and nutrients when subjected to increased nitrogen (N) deposition. Although it has been proposed that phosphorus (P) can dilute negative effects of nitrogen by increasing biomass production of *Sphagnum* mosses, it is still unclear whether P-addition can alleviate physiological N-stress in *Sphagnum* plants. A 3-year fertilisation experiment was conducted in lawns of a pristine *Sphagnum magellanicum* bog in Patagonia, where competing vascular plants were practically absent. Background wet deposition of nitrogen was low (~0.1–0.2 g N m^{-2} y^{-1}). Nitrogen (4 g N m^{-2} y^{-1}) and phosphorus (1 g P m^{-2} y^{-1}) were applied, separately and in combination, 6 times during the growing season. P-addition substantially increased biomass production of *Sphagnum*. Nitrogen and phosphorus changed the morphology of *Sphagnum* mosses by enhancing height increment, but lowering moss stem density. In contrast to expectations, phosphorus failed to alleviate physiological stress imposed by excess nitrogen (e.g. amino acid accumulation, N-saturation and decline in photosynthetic rates). We conclude that despite improving growth conditions by P-addition, *Sphagnum*-bog ecosystems remain highly susceptible to N-additions. Increased susceptibility to desiccation by additional nutrients may even worsen the negative effects of excess nitrogen especially in windy climates like in Patagonia.

Introduction

Peatlands store substantial amounts of carbon (Gorham, 1991; Yu *et al.*, 2010) and therefore their conservation is considered of global importance. Cycling of carbon in peatlands is mainly controlled by peatland hydrology (Belyea & Baird, 2006; Couwenberg *et al.*, 2010), which is largely driven by the vegetation and litter formed therein (Ivanov, 1981; Couwenberg & Joosten, 1999; Fritz *et al.*, 2008). *Sphagnum* mosses are important peat-forming plants because of their high capacity to retain water and nutrients, which results in water-logged conditions and recalcitrant litter (Van Breemen, 1995; Rydin & Jeglum, 2006). Under pristine conditions *Sphagnum* mosses can outcompete vascular plants through monopolising the nutrient pool which is primarily derived from atmospheric inputs (Clymo & Hayward, 1982; Curtis *et al.*, 2005; Phuyal *et al.*, 2008; Wiedermann *et al.*, 2009a). As peatlands dominated by *Sphagnum* mosses are adapted to extremely nutrient-poor conditions, they are highly threatened by increased atmospheric nitrogen (N) deposition (Lamers *et al.*, 2000; Rydin & Jeglum, 2006; Limpens *et al.*, 2011).

High nitrogen loads exceed the natural absorption (filter) capacity of *Sphagnum* mosses (Lamers *et al.*, 2000; Bobbink & Hettelingh, 2011). This results in leaching of nitrogen through the upper moss layer which increases the availability of nitrogen to vascular plants (Limpens *et al.*, 2003a; Tomassen *et al.*, 2004). As a consequence, *Sphagnum*-dominated ecosystems change into non-peat-forming phanerogam-dominated systems (Limpens *et al.*, 2008; Gunnarsson *et al.*, 2008; Juutinen *et al.*, 2010). This can already occur at nitrogen (N) loads higher than 1 g N m^{-2} y^{-1} (Bobbink & Hettelingh, 2011), but in several parts of the world atmospheric nitrogen deposition have exceeded 4 g N m^{-2} y^{-1} (Galloway *et al.*, 2008). The decline of *Sphagnum*-dominated vegetation is further stimulated by damage of *Sphagnum* mosses due to ammonium toxicity (Baxter *et al.*, 1992; Rudolph *et al.*, 1993). Increased susceptibility to pests has also been found after nitrogen enrichment (Limpens *et al.*, 2003b; Wiedermann *et al.*, 2007).

The most detrimental effects of increased nitrogen deposition are probably due to imbalances in nutrient stoichiometry (Bragazza *et al.*, 2004; Güsewell, 2004; Limpens *et al.*, 2011). Under high nitrogen deposition, a lack of phosphorus seems to limit *Sphagnum*-dominated vegetation (Aerts *et al.*, 1992; Lund *et al.*, 2009). *Sphagnum* mosses subsequently saturate with nitrogen as a result of limited biomass production (Lamers *et al.*, 2000; Limpens *et al.*, 2011). Lamers and co-workers (2000) expected that negative effects of nitrogen can be diluted by stimulated biomass production after adding limiting nutrients. It is still debated whether a better balanced nutrient stoichiometry (cf. Bragazza *et al*., 2004) can alleviate negative effects of excess nitrogen or not. Field experiments have indicated that the addition of phosphorus can increase biomass production of (*Sphagnum*) mosses, which may alleviate the detrimental effects of nitrogen (Pilkington *et al*., 2007; Limpens *et al*., 2011). Other field studies observed only modest and temporary effects of P-addition (Aerts *et al*., 1992; Aerts *et al*., 2001) or did not observe alleviating effects of phosphorus (Juutinen *et al*., 2010). The present paper emphasises the role of phosphorus (P) in improving the nutrient balance and alleviating stress imposed by excess nitrogen on *Sphagnum* mosses.

Increased availability of (airborne) nitrogen substantially changes the physiology of (*Sphagnum*) mosses (Rudolph *et al*., 1993; Soares & Pearson, 1997; Pearce *et al*., 2003). High uptake rates can cause a rapid saturation of the nitrogen metabolism in *Sphagnum* mosses (Rudolph *et al*., 1993; Wiedermann *et al*., 2009a), which is often followed by growth reduction (Baxter *et al*., 1992; Gunnarsson & Rydin, 2000; Limpens *et al*., 2011). Physiological parameters (e.g. nitrogen concentration, amino acid concentration and photosynthetic efficiency) have been suggested as early indicators for increasing metabolic stress preceding impeded growth and a decreased cover of (*Sphagnum*) mosses (Baxter *et al*., 1992; Arroniz-Crespo *et al*., 2008; Wiedermann *et al*., 2009b; Limpens *et al*., 2011). The formation of amino acids is thought to prevent an accumulation of toxic ammonium caused by a saturation of nitrogen in mosses (Rudolph *et al*., 1993; Wiedermann *et al*., 2009b). In contrast, growth of vascular plants is stimulated by enhanced nitrogen uptake (Tomassen *et al*., 2004).

Increased nitrogen deposition also stimulates the formation of chlorophyll in *Sphagnum* mosses (Tomassen *et al*., 2003; Granath *et al*., 2009). Upland mosses receiving high nitrogen loads increased chlorophyll concentrations, but surprisingly the biomass production declined (Arroniz-Crespo *et al*., 2008). Arroniz-Crespo and co-workers (2008) suggested that photo-inhibition caused damages to the chloroplast, which reduced the efficiency of photosystem II (PSII). Photo-inhibition can be related to increased chlorophyll levels: Photo-inhibition occurs when the photosynthetic system becomes ‘over-excited’ when the amount of photons absorbed exceeds photosynthesis, which can be limited by other stress factors (e.g. shortage of CO_2 under wet conditions or water shortage during desiccation). Under such conditions a high chlorophyll content makes plants more susceptible to damage caused by photo-inhibition, if the concentration of xanthophyll and other pigments preventing photostresss is insufficient. Raven (2011) summarised how photo-respiration and photo-inhibition may consequently prevent an increase of net C-sequestration (biomass production or growth) despite increased chlorophyll concentrations, particularly in areas where a cold climate (like in Alaska and Patagonia) limits photo-respiration (Murray *et al.*, 1993). In summary, nitrogen stress indicators (e.g. nitrogen, amino acids, photosynthesis) may be used to predict detrimental effects of elevated nitrogen deposition (Tomassen *et al.*, 2003; Arroniz-Crespo *et al.*, 2008; Wiedermann *et al.*, 2009b).

Our study focuses on the potential of phosphorus (P) to alleviate excess nitrogen stress by increased biomass production. We conducted a fertilisation experiment in the field to unravel the effects of P-addition on the physiology of *Sphagnum* plants that were stressed by high loads of nitrogen. It was tested whether biomass production lowered the concentration of nitrogen stress indicators. The experimental design of the present study follows Limpens *et al.* (2004) and Pilkington *et al.* (2007) to facilitate comparisons with these earlier experiments that found alleviating effects of phosphorus on biomass production. Experiments were conducted in a pristine bog in southernmost Patagonia with a very low atmospheric nitrogen deposition (Godoy *et al.*, 2003; Kleinebecker *et al.*, 2008; Schmidt *et al.*, 2010), and consequently with very few vascular plants (cover < 2 %; Fritz *et al.*, 2011), thus avoiding interactions with higher plants (Malmer *et al.*, 2003).

Materials and Methods

Study site, background N-deposition and experimental design

A 3-year fertilisation experiment was conducted in a *Sphagnum*-dominated bog in the Andorra valley mire, Patagonia, Argentina (54° 45′ S; 68° 20′ W; 200 m a.s.l.) within the deciduous forest zone (Grootjans *et al.*, 2010). Growing season lasts from October till April. Average daily air temperatures are 4–5 °C with cold summers (maximum average summer temperature 10 °C) characteristic for this type of boreal climate (Iturraspe *et al.*, 1989). Annual precipitation exceeds 600 mm, evenly distributed over the year, providing wet conditions and high air humidity levels (Iturraspe *et al.*, 1989). Groundwater level fluctuations during the growing season range around 25 cm, with lowest levels found in late summer. Strong desiccating winds are characteristic to Patagonian peatlands (Kleinebecker *et al.*, 2007).

In the present study bulk nitrogen deposition was collected in black polyethylene bottles equipped with funnels (0.01 m^2). Adding 1 mg $HgCl_2$ inhibited microbial alteration of samples. Total inorganic N in rain water averaged 8 µmol N l^{-1} (SD 4; $n = 10$) during two growing seasons (2007 and 2008) suggesting a nitrogen deposition as low as 0.1–0.2 g N m^{-2} y^{-1}. Such low concentrations are often found in Southern Patagonia with estimated bulk N-deposition below 0.1 g N m^{-2} y^{-1} (Godoy *et al.*, 2003). In general, Patagonian peatlands show very low nutrient concentrations and have preserved their pristine character with little human influence (Kleinebecker *et al.*, 2008).

The bog vegetation was dominated by *Sphagnum magellanicum* Brid. with < 2 % cover of vascular plants (e.g. *Rostkovia magellanica, Empetrum rubrum, Nothofagus antarctica, Carex magellanica, Marsippospermum grandiflorum* and *Tetroncium magellanicum*). Aboveground biomass of vascular plants was low (15 g DW m^{-2}; SD 7.7; $n = 10$) which equals approximately 1 % of living *Sphagnum* biomass. Interestingly, *S. magellanicum* occupies all hydrological niches from pool to hummock. Latter may rise up to 1 metre above the summer water table. Other *Sphagna* like *Sphagnum falcatulum (S. cuspidatum* coll.) and *Sphagnum cuspidatum* co-exist in pools (cf. Kleinebecker et al. 2007). In poor fens *Sphagnum fimbriatum* can be found next to *S. magellanicum* (Grootjans *et al.*, 2010 and literature therein).

The effects of applied nitrogen (4 g N m^{-2} y^{-1}, NH_4NO_3, N-treatment) were studied in comparison with a Control (bog water only, C-treatment) and addition of phosphorus (1 g P m^{-2} y^{-1}, NaH_2PO_4, P-treatment). Additionally we also applied both nitrogen and phosphorus (1 g P m^{-2} y^{-1}, NaH_2PO_4 and 4 g N m^{-2} y^{-1}, NH_4NO_3, NP-treatment) to unravel effects of phosphorus on N-addition (N×P interaction effect). Fertilisers were dissolved in 10 dm^3 sieved (1 mm mesh size) bog water which was sprayed over the 1 × 1 m plots ($n = 5$ per treatment, resulting in 20 plots randomly assigned to treatments). Fertilisers were applied 6 times (monthly) during each growing season (usually November–April). One hour after fertilising, *Sphagnum* heads were gently washed with 5 dm^3 sieved bog water to reduce possible toxic effects of concentrated salts (Wilcox, 1984; Tomassen *et al.*, 2004). The experiment lasted from December 2006 to April 2009.

Field measurements and chemical analysis

Height increase of *Sphagnum* mosses was measured by marking ($n = 50$ per treatment, $n = 10$ in two subplots 0.1 x 0.1 m per plot) the first branch below the capitulum with a small cable tie in March 2008. One year later the distance between the first branch below the capitulum and the cable tie was measured according to Krebs & Gaudig (2005). The bulk density of the two subplots was determined after harvesting. Annual biomass production of *S. magellanicum* in the third year of the experiment was estimated by multiplying height increment by bulk density of moss stems (first 40 mm below capitulum). Cover of vascular plants was estimated as percentage cover before the experiment started and after each year. In February 2010 we harvested vascular plants to determine dry weight of biomass (48 h at 70 °C) in the Control and the NP-treatment.

Net photosynthesis of the *S. magellanicum*-dominated plots was measured on 6 March 2009. We used a clear Plexiglas® climate-controlled chamber occupying a surface of 0.063 m^2 and a volume of 8.4 dm^3. With an infrared gas analyser (Vaisala CarboCap GMP343, Vanta, Finland) we recorded changes in CO_2 concentration in 15 seconds intervals in the static chamber (Workshop University of Groningen, The Netherlands) that was carefully placed 15 minutes prior to a measurement cycle. At every plot CO_2 exchange was measured for 5 minutes under light and under complete darkness in duplicates. Light levels were around 300 µmol PAR $m^{-2}\ s^{-1}$, close to light-saturated photosynthesis in *Sphagnum* sp. (Petersen, 1984; Harley *et al.*, 1989). The light level was continuously measured with a Li–Cor quantum photometer (Model LI–250; Lincoln, NE) and filter sheets were used to maintain light levels constant (300 µmol PAR $m^{-2}\ s^{-1}$ or complete darkness). The change of CO_2 concentrations over time suggested a linear relationship that was tight in all measurements ($r^2 > 0.8$). Chambers warmed by about 5 °C during light measurements. Water level was 15–20 cm below the surface.

All chemical analyses were performed on material harvested in March 2009, at the end of the 3rd growing season. In each plot mosses were harvested at 3 randomly assigned 10 by 10 cm subplots with a total volume of 1 dm^3. Before drying the *Sphagnum* mosses were divided in three functional fractions: capitula (average length 8 mm, photosynthetically active tissue), stem below capitula (40 mm, living moss tissue) and decaying tissue (first 50 mm below stem section, litter). Capitula were picked manually (300–500 per dm^2). The decaying tissue fraction mostly coincided with signs of disintegration and pale colour of the *Sphagnum* tissue. Vascular plants were harvested in 0.5 m^2 per plot. All plant samples where dried (48 hours at 70 °C) and ground with liquid nitrogen. For amino acid and chlorophyll analysis, fresh capitula material was used. We placed sods of intact bog vegetation (20 × 20 × 20 cm) in chilled opaque containers and transported them back to the laboratory.

Porewater of the living *Sphagnum* layer (uppermost 50 mm) was collected by connecting syringes (60 ml) to soil moisture samplers ($n = 2$ per plot, 10 cm

Rhizon SMS; Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands). The two subsamples were pooled and pH was measured. Part of the sample was stored after addition of $HgCl_2$ to a final concentration of 5 mg l^{-1} to prevent denitrification. All water samples were stored in polyethylene bottles (20 ml) at –18 °C until further analysis.

Nutrient concentrations in plant tissue were analysed in dried samples. Nitrogen and carbon concentrations were measured on a CNS analyser (Model NA1500; Carlo Elba Instruments, Milan Italy). Homogenised portions of 200 mg dried material were digested with 4 ml HNO_3 (65 %) and 1 ml H_2O_2 (30 %), using an Ethos D microwave labstation (Milestone srl, Sorisole, Italy). Digests were diluted and concentrations of P and (micro-)nutrients (Al, Ca, Fe, Mg, Mn, Na, S, Si, Zn) were determined by an ICP spectrometer (IRIS Intrepid II, Thermo Electron Corporation, Franklin, USA). Potassium in digests and porewater was measured on a FLM3 Flame Photometer (Radiometer, Copenhagen, Denmark). Concentrations of nitrate, ammonium and phosphate in rain water and porewater were measured colourimetrically on a Traacs 800+ auto-analyser as described by Tomassen *et al.* (2003).

Recovery of nutrients was determined per fraction (capitula, stem and decaying tissue). We multiplied density of a fraction with the difference in nutrient concentrations between a treatment plot and the means of the Control treatment rendering g N m^{-2} and g P m^{-2}. The sum of all three fractions (capitula, stem and decaying tissue) was divided by the total amount of nutrients applied over three years (12 g N m^{-2} and 3 g P m^{-2}).

Free amino acids were extracted from fresh capitula in duplicate using ethanol according to Van Dijk & Roelofs (1988). Twenty amino acids 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 (Tomassen *et al.*, 2003).

Leaf pigment concentrations were determined in frozen and homogenised fresh capitula tissue shaken for 24 h (4 °C) with 96 % ethanol. Leaf pigment concentrations in the supernatant fraction were measured spectrophotometrically according to Lichtenthaler (1987). For 'specific photosynthetic rates' we divided photosynthetic rates (μmol CO_2 m^{-2} s^{-1}) measured under field condition by chlorophyll a and chlorophyll b (mmol Chl m^{-2}) following Petersen (1984). Chlorophyll was determined 3 weeks after taking photosynthesis measurements.

The effects of N and P were tested using linear models in R 2.92 (R Development Core Team, 2009) which was followed by model justification procedures (Crawley, 2007). If fitted models were not justified, generalised linear models (GLMs) with different error structures and distributions were fitted, or data were log-transformed before analysis. Next, we conducted regression analysis to test the potential of biomass production to dilute the parameters of N metabolism. We included only plots receiving additional N (n = 10) for this analysis as they revealed a large range in productivity (243–657 g DW m^{-2} y^{-1}) and signs of stress induced by excess nitrogen.

Results

Ecosystem effects of nutrient addition

Sphagnum absorbed the majority of nutrients added. In contrast, vascular plants did not show substantial changes in cover or nutrient content after 3 years of nutrient addition. After application, nutrients depleted quickly in the porewater already after few days but showed a high variation. After one week only ~ 1 % of the added ammonium (leading to a slight elevation of the concentration to 23 µmol l^{-1}) could be demonstrated. In contrast, nitrate and phosphate remained longer available at increased levels. Even fourteen days after application we found elevated nitrate concentrations (31 µmol l^{-1}) in treatments receiving N and substantial concentrations of ortho-phosphate (26 µmol l^{-1}) in treatments where phosphorus (P) was added. After two months, porewater concentrations were below 5 µmol l^{-1} for ammonium and nitrate and below 1 µmol l^{-1} for total phosphorus.

A large fraction of the 12 g N m^{-2} applied during the entire experiment was found in living *Sphagnum* tissue (53–59 %) and the decaying fraction (15–18 %) which stored together 8.5–8.8 g N m^{-2} (SD 0.78) more than the Control. The total recovery of phosphorus was lower, averaging 47–53 % of total P applied.

Cover and composition of vascular plants remained unaffected by nutrient addition. *Rostkovia magellanica* (Lam.) Hook. f., a common rush in Patagonian peatlands, dominated the assemblage of vascular plants in all plots. Nutrient additions did not affect nitrogen concentrations in foliar tissue (mean 14.5 mg N g^{-1}DW) of *R. magellanica*. The addition of phosphorus slightly increased P tissue concentrations (P-effect; df = 1, $F = 6.7243$, $P = 0.018$) from 1.17 mg P g^{-1}DW (SD 0.49) in the Control to 1.56 mg P g^{-1}DW (SD 0.17) in the P-treatment and 1.54 mg P g^{-1}DW (SD 0.02) in the NP-treatment. In contrast, P tissue concentration in *S. magellanicum* increased by a factor 5–7 upon P-addition (Table 3.1). N:P ratios declined upon P-addition (P-effect; df = 1, $F = 4.7975$, $P = 0.042$). In the Control N:P ratios of *R. magellanica* were higher (13.1 g g^{-1}) than in the P-treatment (9.4 g g^{-1}) and NP-treatment (10.2 g g^{-1}).

Nutrient stoichiometry and growth response

The addition of nutrients changed both the total concentrations of nutrients in *S. magellanicum* plants and their distribution between capitula and stem tissue. Intermediate N:P ratios (10–14 g g^{-1}) in capitula tissue were associated with high biomass production in the NP-treatment (Fig. 3.1). In contrast, the Control showed higher N:P ratios (26.5 g g^{-1}), which were increased when fertilising with nitrogen (Table 3.1; df = 1, $F = 64.284$, $P < 0.001$). The addition of P increased the tissue P cocentrations and lowered the N:P ratio, which was lowest in the P-treatment (Table 3.1; df = 1, $F = 448.819$, $P < 0.001$). Also in the plots receiving the NP-treatment the N:P ratios were significantly lower than in the Control, but still higher than the N:P ratio (4:1) of the nutrient solution added. N:P ratios in stem tissue were similarly affected by nutrient additions (Table 3.1) but showed generally lower ratios than found in capitula tissue. Only the N-treatment revealed higher N:P ratios in stem tissue than in capitula tissue.

The addition of 1 g P m^{-2} y^{-1} stimulated height increment (growth) of *S. magellanicum* by 4 mm in the third year (Table 3.1). Addition of N had little effects on growth rates. In contrast, highest growth rates (mean 32 mm y^{-1}, SD

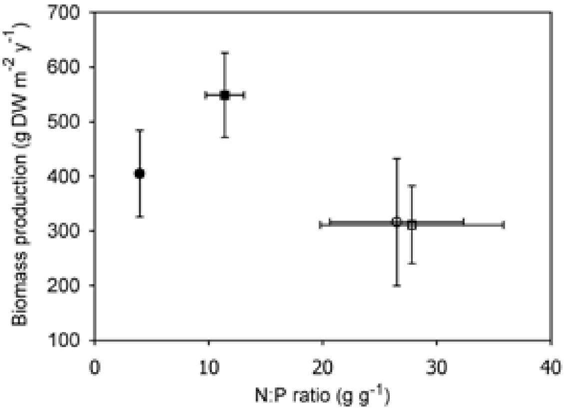

Figure 3.1. Biomass production of *Sphagnum* moss in relation to N:P ratio, means and SD are represented for each treatment: P (1 g P m^{-2} y^{-1}) filled circle; NP (1 g P m^{-2} y^{-1} and 4 g N m^{-2} y^{-1}) filled square; N (4 g N m^{-2} y^{-1}) open square; C (Control without nutrient addition) open circle. Five plots per treatment were installed at the field experiment in a bog, dominated by *Sphagnum magellanicum*, in Tierra del Fuego.

Table 3.1. Nutrient concentrations and growth of *Sphagnum magellanicum* after 3 years of treatment. For treatment details, see Fig. 3.1. Mean of 5 plots per treatment. Results from GLMs are indicated: ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

	Treatment				*P*-value		
	C	N	P	NP	N effect	P effect	N×P
P content capitula (mg P g^{-1}DW)	0.27	0.57	1.84	1.32	ns	***	***
N:P ratio capitula (g g^{-1})	26.5	27.8	4.0	11.4	***	***	***
N:P ratio stem (g g^{-1})	15.9	33.9	2.9	6.5	***	***	ns
Growth rate (mm y^{-1})	14	15	18	32	**	***	**
Biomass production (gDW m^{-2} y^{-1})	316	311	405	549	ns	**	ns
Capitula biomass (gDW m^{-2})	266	290	306	309	ns	**	ns
Photosynthesis (μmol CO_2 m^{-2} s^{-1})	2.40	2.42	2.62	2.87	ns	*	ns
Stem density (g l^{-1})	23.3	20.2	21.6	16.8	***	*	ns

3 mm) occurred in the NP-treatment which suggests a significant interaction effect between N and P (df = 1, F = 10.065, P = 0.006). Together with the positive effect on plant height, P also stimulated net biomass production (Table 3.1; df = 1, F = 13.659, P = 0.002). In both the NP-treatment (549 g DW m^{-2} y^{-1}; SD 77) and the P-treatment (405 g DW m^{-2} y^{-1}; SD 79) more biomass was produced than in the Control (316 g DW m^{-2} y^{-1}; SD 117) (Fig. 3.1, Table 3.1). The increase of capitula biomass (mean 40 g DW m^{-2} over 3 years) following P-addition (df = 1, F = 8.3817, P = 0.0096) was small compared to the increase in total biomass production. Net photosynthesis at nearly light saturation was increased by P-addition, resulting in a 14 % higher photosynthetic rate (Table 3.1; df = 1, F = 7.9836, P = 0.011). Photosynthesis on the basis of capitula biomass was similar in all treatments and averaged 89 nmol CO_2 g^{-1}DW s^{-1} (SD 12).

The addition of nutrients changed the morphology of *S. magellanicum*. The density of the *Sphagnum* layer affects both water transport and water conservation. A decrease in bulk density reduces the upward water transport by capillary flow (Clymo, 1973). Both nutrients caused stem density to decrease (Table 3.1). Growth in length of *Sphagnum* mosses correlated well with the observed decrease in stem density (r^2 = 0.50, df = 1,18; F = 19.97, P < 0.001). In contrast, mosses in the N-treatment revealed only a small decrease in stem density without additional growth compared to the Control (Table 3.1). The increased growth of *S. magellanicum* was associated with a large drop in bulk density when both N and P were added. In the NP-treatment mean density of the stem section was 16.8 g l^{-1} while in other treatments the density was always above 20 g l^{-1} (Table 3.1). We observed indicators for desiccation (white-colouration of capitula) in the NP-treatment after water tables had dropped to 35 cm below the surface in January 2009 (Fig. 3.2).

Indicator of excess N-stress in *Sphagnum* mosses

After 3 years of N-addition we observed substantial changes in physiological parameters that are related to stress because of excess N in mosses (Table 3.2). Addition of P had no significant effect on the concentration of excess N-stress indicators. N concentrations in capitula doubled by 7.2 mg g^{-1}DW upon N-addition (Table 3.2; df = 1, F = 413.96, P < 0.001). In stem tissue we observed a similar increase. Doubling N concentrations in capitula coincided with a sevenfold increase in concentration of free amino acids (df = 1, F = 1526.6, P < 0.001). Especially amino acids with a low C:N ratio (arginine, asparagine) accumulated substantially. The concentrations of glutamine, serine, aspartic

Table 3.2. Indicators for stress induced by excess nitrogen in tissues of *Sphagnum magellanicum*. For treatment details, see Fig. 3.1. Mean of 5 plots per treatment. Results from GLMs are indicated: ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

	Treatment				***P*-value**		
	C	N	P	NP	N effect	P effect	N×P
N content capitula (mg N g^{-1}DW)	7.0	14.5	7.2	14.4	***	ns	ns
N content stem (mg N g^{-1}DW)	3.6	10.0	3.8	10.7	***	ns	ns
Chl *a* (mmol g^{-1}DW)	0.71	1.65	0.72	1.52	***	ns	ns
Chl *b* (mmol g^{-1}DW)	0.67	1.25	0.66	1.25	***	ns	ns
Specific photosynthesis (µmol CO_2 mmol Chl a+b^{-1} s^{-1})	6.6	2.9	6.8	3.4	***	ns	ns
Amino acid content (µmol N g^{-1}DW)	18.6	122.3	18.0	129.4	***	ns	ns
Arginine (µmol N g^{-1}DW)	4.93	81.57	5.39	71.12	***	ns	ns
Asparagine (µmol N g^{-1}DW)	3.31	19.29	3.04	35.47	***	ns	*
Glutamine (µmol N g^{-1}DW)	3.70	7.33	3.49	8.31	***	ns	ns
Serine(µmol N g^{-1}DW)	0.88	1.66	0.75	2.07	***	ns	ns
Aspartic acid (µmol N g^{-1}DW)	0.65	1.26	0.65	0.97	***	ns	ns
Glutamic acid (µmol N g^{-1}DW)	1.22	2.40	1.20	2.13	***	ns	ns
Potassium content capitula (mg K g^{-1}DW)	2.9	2.9	3.3	3.9	ns	**	ns

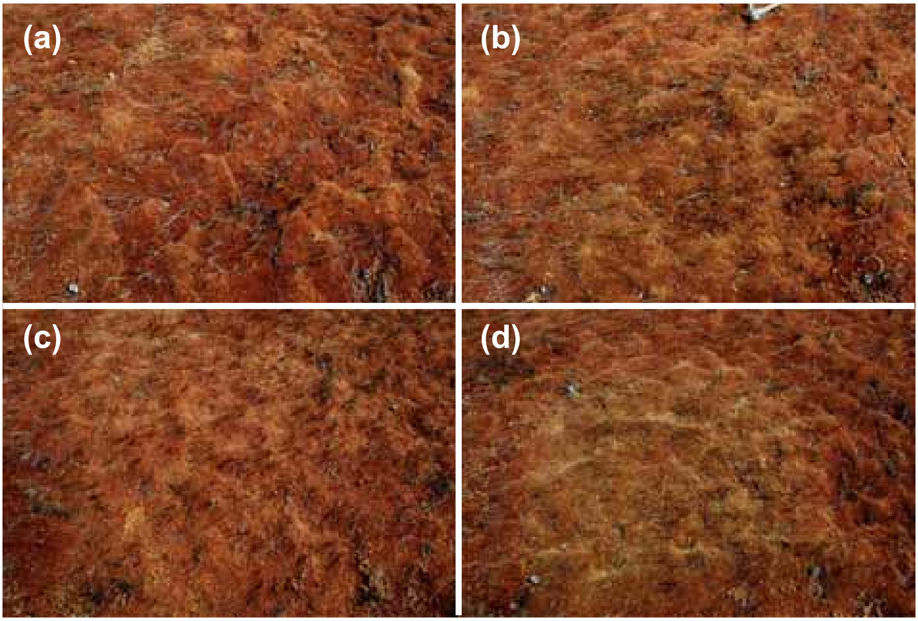

Figure 3.2. Visible effects of treatments [Control (a), N-treatment (b), P-treatment (c), NP-treatment (d)] on Sphagnum magellanicum–dominated plots. Oblique photographs were taken after a dry spell in January 2009, when water levels dropped 35 cm below the surface, 15 cm lower than the summer average water level. For treatment details, see Fig. 3.1.

acid and glutamic acid increased by 100 % (Table 3.2), similar to the observed increase in total N.

N-addition also doubled chlorophyll and carotenoids concentrations (Table 3.2), while the Chl*a* to Chl*b* ratio increased from 1.08–1.25. Although nitrogen stimulated the production of photoactive pigments, the photosynthetic rate per capitulum (gram dry weight) did not increase. As a result, specific photosynthesis (i.e. photosynthesis per unit Chl) was negatively affected by N-addition ($df = 1$, $F = 72.611$, $P < 0.001$) in both the N-treatment and the NP-treatment. Treatments receiving nitrogen showed increased potassium concentrations in the porewater (mean > 50 µmol l^{-1}) compared to the Control and P-treatment (mean below < 20 µmol l^{-1}). However, tissue concentrations of potassium remained unaffected by N-addition ($df = 1$, $F = 2.1738$, $P = 0.160$; Table 3.2).

Table 3.3. Concentrations of micro-nutrients in capitula tissue *Sphagnum magellanicum*. For treatment details, see Fig. 3.1. Mean of 5 plots per treatment. Results from GLMs are indicated: ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

	Treatment				*P*-value		
	C	N	P	NP	N effect	P effect	N×P
Al (μmol g^{-1}DW)	2.01	3.46	3.83	2.42	ns	ns	***
Ca (μmol g^{-1}DW)	3.57	3.98	6.44	2.58	***	ns	***
Fe (μmol g^{-1}DW)	2.66	3.91	4.61	2.96	ns	ns	***
Mg (μmol g^{-1}DW)	8.25	10.78	18.32	10.02	*	***	***
Mn (μmol g^{-1}DW)	0.45	0.45	0.88	0.38	**	ns	**
Na (μmol g^{-1}DW)	26.08	34.95	53.88	36.82	ns	***	***
S (μmol g^{-1}DW)	5.57	9.94	11.42	7.99	ns	**	***
Si (μmol g^{-1}DW)	1.36	2.78	3.16	2.08	ns	**	***
Zn (μmol g^{-1}DW)	0.09	0.18	0.22	0.14	ns	***	***

Alleviation of N-stress through biomass

Based on the assumption that increased biomass production can, by dilution, mitigate stress induced by excess nitrogen, we expected lower concentrations of N-stress indicators (amino acids, photosynthesis) in the NP-treatment compared to the N-treatment. However, the increased biomass production following P-addition failed to reduce concentrations of N-stress indicators. No significant correlation was found between N stored in amino acids (N_AA) and biomass production in plots of NP-treatment and N-treatment (df = 1,8; $F = 0.1917$, $P = 0.6731$). Other N-stress indicators (total N, chlorophyll) were also not correlated with biomass production (data not shown). Neither did we find an interaction effect between nitrogen and phosphorus for N-stress indicators (Table 3.2). In contrast to an expected dilution, we even found asparagine concentrations to increase with biomass production (df = 1,8; $F = 6.306$, $P = 0.036$). While the N-metabolism remained unaffected by additional P, increased biomass production resulted in a ‘dilution’ of moss stem density, which was associated with a moisture deficit in capitula after an episode of low water levels (Fig. 3.2).

Effects of nutrient addition on micro-nutrients

In capitula tissue the addition of P doubled concentrations of elements such as Mg, Na, Si, Zn and S (Table 3.3). The increase upon N-addition was less pronounced. The NP-treatment revealed element concentrations lower than the P-treatment and N-treatment but exceeding those of the Control. For all elements the interaction between N and P reduced element concentrations (Table 3.3). We furthermore found a biomass dilution effect (negative correlation between biomass and element concentration) for Al, Ca, Fe, Mg, S, Si and Zn. For example, S concentrations declined with increasing biomass (df = 1,8; $F = 10.1$, $P = 0.013$).

Discussion

This is the first study on the effects of N and its interaction with P in pristine bog vegetation in the Southern Hemisphere. Natural nutrient concentrations in *S. magellanicum* where comparable with other Patagonian bogs (Schmidt *et al.*, 2010). Low P availability in porewater < 10 µmol P g^{-1}DW and N:P ratios close to 30 in moss tissues highlight the scarcity of P. This is in accordance with the stimulated growth and biomass production following the addition of P (Fig. 3.1). P-limitation has also been suggested for bogs in New Zealand (Clarkson *et al.*, 2005)

Despite scarce nutrients, growth rates observed in the Control plots were similar to the global average of *S. magellanicum* (reviewed in Gunnarsson, 2005). Physiological parameters (e.g. nitrogen, amino acids, photosynthetic rates) were also similar to Northern Hemispheric *Sphagnum* mosses (Tomassen *et al.*, 2003; Rydin & Reglum, 2006; Rice *et al.*, 2008). From litter production in Control plots we estimate a loss of 0.36 g N m^{-2} y^{-1}, which suggests an additional nitrogen source next to a bulk deposition of 0.1 g N m^{-2} y^{-1} found in this experiment. Micro-organism, such as (methanotrophic bacteria and cyanobacteria), can fix nitrogen at substantial rates (0.2 g N m^{-2} y^{-1}) in North American *Sphagnum* bogs (Hemond, 1983; Markham, 2009) which may result in high N:P ratios even in pristine bogs. Bacteria having the potential for nitrogen fixation were indeed found at our experimental site (chapter 6).

Increased loads of N (4 g N m^{-2} y^{-1}) resulted in a doubling of tissue N concentrations to 14.5 mg N g^{-1}DW and increased N:P ratios. At this tissue N-concentration, saturation of nitrogen metabolism is known to occur resulting in impeded growth (Nordin & Gunnarsson, 2000; Limpens & Berendse, 2003; Tomassen *et al.*, 2003). Several physiological parameters (e.g. amino acids, specific photosynthesis) indicated that the *Sphagnum* mosses were stressed by N-addition. The accumulation of N resulted in an increased production of metabolically costly (cf. Rudolph *et al.*, 1993) amino acids, predominantly arginine and asparagine, and chlorophyll (Table 3.2). The accumulation of free amino acids is associated with a suppressed productivity and cover in *Sphagnum* mosses (Nordin & Gunnarsson, 2000; Wiedermann *et al.*, 2009b).

Assuming that increased biomass production potentially alleviates nitrogen stress, we expected lower concentrations of nitrogen and nitrogen-related stress indicators (Table 3.2) in mosses receiving additional P (NP-treatment). Although N:P ratios were low (10–14 g g^{-1}) and biomass production increased, we found, however, strong indications of excess nitrogen stress in NP-treated mosses, similar to N-treated mosses. In agreement with the expected dilution through biomass production, a lowering of element concentrations was observed for micro-nutrients (e.g. Ca, Fe and S; Table 3.3). In contrast to other elements, the nitrogen metabolism apparently remained unaffected by the addition of P, although biomass production was stimulated. Other studies also found that *Sphagnum* mosses became saturated by nitrogen irrespective of P-addition (Limpens & Heijmanns, 2008; Bu *et al.*, 2011). These earlier findings and the present study, therefore, suggest an accumulation of stress by excess nitrogen that may exceed a toxic threshold given a sufficiently long exposure time (Wiedermann *et al.*, 2009b).

At our Patagonian site N-addition doubled chlorophyll levels without effects on photosynthetic rates. Increased chlorophyll levels have also been found in experiments applying various N-levels (Tomassen *et al.*, 2003; Granath *et al.*, 2009). Increased chlorophyll levels do not automatically result in increased net photosynthesis as they may result in photo-inhibition at low CO_2-availabilities, which are typical for wet pristine bogs (Rice, 2000; Smolders *et al.*, 2001). Low temperatures can increase negative effects due to photo-inhibition as shown for *Sphagnum* vegetation in Alaska (Murray *et al.*, 1993). Also Arroniz-Crespo and co-workers (2008) linked detrimental effects of excess nitrogen to damage caused by photo-inhibition in mosses. Field evidence is needed to test the hypothesis if nitrogen depresses growth by exacerbating negative effects of photo-inhibition in cold climates of Patagonia. Micro-nutrients may also become limiting after N-addition as the seem to become diluted by increased biomass production in the NP-treatment (Table 3.3). The accumulation of ammonium in the porewater can facilitate the wash-out of cations like potassium, magnesium and iron, all important nutrients. Moisture availability and low temperatures during the growing season may furthermore lower growth of *Sphagnum* mosses.

We found that adding nutrients also changed the growth form of *Sphagnum* plants. Increased N availability lowered the bulk density of moss stems, which suggests an impeded branch formation or etiolation of the branches (Fig. 3.1, Table 3.1). This negative effect of extra nutrients on stem density was also found in N-addition experiments in *Sphagnum* bogs (Gunnarsson & Rydin 2000; Manninen *et al.*, 2011) and along a wide gradient of nitrogen deposition rates in European bogs (Bragazza *et al.*, 2004) and Northern American bogs (Aldous, 2002) .

As *Sphagnum* lacks internal water-conducting tissues, water transport takes place via capillary action, which strongly depends on stem density (Clymo, 1973). Lower stem density reduces the vitality of *Sphagnum* vegetation by lowering both the water-holding capacity (Hayward & Clymo, 1982; Titus & Wagner, 1984; Thompson & Wadington, 2008) and water transport (Clymo, 1973; Price & Whittington, 2008) resulting in reduced photosynthesis of mosses (Schipperges & Rydin, 1998; Robroek *et al.*, 2009). In the longterm, mosses growing at low densities are more susceptible to damage during droughts (Clymo, 1973; Murray *et al.*, 1989; Robroek *et al.*, 2007). In conclusion, water shortage resulting from changes in the architecture of the moss carpets will impose constraints on *Sphagnum* mosses vitality in addition to the physiological stress of excess nitrogen. We found evidence of severe desiccation in the NP-treatment at the end of a 3-week dry spell two years after the experiment ended. Mosses in the NP-treatment showed low capitula water contents (3.1 g FW g^{-1}DW) at which photosynthesis in *S. magellanicum* is impaired due to desiccation (Schipperges & Rydin, 1998). In contrast, the denser mosses in the Control showed higher capitula water contents (6.8 g FW g^{-1}DW; df = 8, $T = 4.2327$, $P = 0.0029$), suggesting near-optimal photosynthetic rates (cf. Schipperges & Rydin, 1998). Stem density and water levels in 2011 were similar to values found in 2009 (Fig. 3.2.) .

Increased stem biomass production is insufficient to mitigate the negative effects of excess N on the physiology of *Sphagnum magellanicum*. The absence of an effect of dilution of N by the increase in biomass of the stem only, could be due to the lack of a substantial increase in capitulum biomass. Our data suggest that the metabolic burden of nitrogen cannot be alleviated by increased biomass production stimulated by P-addition alone. This is in agreement with longterm studies showing a sudden decline in *Sphagnum* cover when experiments lasted more than 5 years (Wiedermann *et al.*, 2007; Juutinen *et al.*, 2010). We therefore expect mosses to become poisoned by nitrogen when exposed to additional stressors, which limit photosynthesis and the N-metabolism (e.g. droughts). Strong growth reduction has been observed in several experiments after increased water losses due to droughts or increased temperatures (Aerts *et al.*, 2001; Gerdol *et al.*, 2008; Breeuwer *et al.*, 2009). This raises the question if the alleviating effects of phosphorus, found in earlier studies (Limpens *et al.*, 2004; Pilkington *et al.*, 2007), were only temporary. We hypothesise that *Sphagnum magellanicum* is not able to increase its net photosynthetic rate sufficiently under natural CO_2-availability and therefore is not able to profit from an increased availability of nutrients. The pristine Patagonian bogs experience an additional stressor in the form of desiccating strong winds.

Acknowledgements

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Oscillating peat surface levels in a restiad peatland, New Zealand

Magnitude and spatiotemporal variability

Christian Fritz, Dave Campbell & Louis Schipper

4

Hydrological Processes (2008): 22(17) 3264–3274

Abstract. Hydrology, particularly the water table position below the surface (relative water level, RWL), is an important control on biogeochemical and ecological processes in peatlands. The surface elevation (SE) in a peatland oscillates in response to changes in effective stress on the peat matrix mainly caused by water level fluctuations. This phenomenon is called peatland surface oscillation (PSO). To investigate the spatiotemporal variability of PSO, surface elevation and the water level above sea level (AWL) were measured monthly (23 sites) over one year in a warm-temperate restiad peatland, New Zealand. At one site peat surface elevation was measured indirectly by monitoring AWL and RWL continuously with pressure transducers. Annual PSO (the difference between maximum and minimum surface elevation) ranged from 3.2 to 28 cm (mean 14.9 cm). Surface elevation changes were caused by AWL fluctuations. Spatially homogenous AWL fluctuations (mean 40 cm among sites) translated into RWL fluctuations reduced 27–56 % by PSO except for three sites with shallow and dense peat at the peatland margin (7–17 %). The SE–AWL relationship was linear for 15 sites. However, eight sites showed significantly higher rates of surface elevation changes during the wet season and thus a non-linear behaviour. We suggest flotation of upper peat layers during the wet season causing this non-linear behaviour. Surprisingly, PSO was subjected to hysteresis: the positive SE–AWL relationship reversed after rainfall when the surface slowly rose despite rapidly receding water levels (AWLs). Hysteresis was more prominent during the dry season than during the wet season. Total peat thickness and bulk density together could only explain 50 % of the spatial variability of PSO based on manual measurements. However, we found three broad types of SE–AWL relationships differing in shape and slope of SE–AWL curves. These oscillation types reflected patterns in vegetation and flooding.

Introduction

The surface level in many peatlands oscillates reversibly by several centimetres per year (Ingram, 1983) and changes of the surface level affect the position of the water table relative to the surface. Hydrology, particularly the water table position, is an important control on biogeochemical and ecological processes in peatlands. Rates of processes in peatlands such as lateral seepage (Ivanov, 1981), evaporation (Lafleur *et al.*, 2005), methane emission (Moore *et al.*, 1998; Blodau, 2002) and peat accumulation (Belyea, 1996; Blodau, 2002) tend to decrease when water tables are well below (> 30 cm) the surface. A review on carbon cycling in peatlands suggests that CO_2 emission rates increase with increasing water table fluctuation (Blodau, 2002). Plant species composition also depends on the water table position in peatlands (Clymo & Hayward, 1982; Wierda *et al.*, 1997; Kotowski *et al.,* 1998).

The position of the water table is dependent on peat water storage changes, total storativity and surface elevation changes. Storage changes translate into water table fluctuations magnified by the total storativity, which is defined as the volume of water released from an aquifer per unit surface area per unit decline in water table with respect to a fixed datum. The water table position can be defined in two ways (Fig. 4.1, Table 4.1): the water table position above an absolute elevation datum e.g. mean sea level (absolute water level, AWL) and the water table depth below the surface (relative water level, RWL). RWL indicates the thickness of the unsaturated zone (Heikurainen *et al.*, 1964; Schlotzhauer & Price, 1999; Barber *et al.*, 2004), the moisture content of which controls soil aeration and chemistry (de Mars &Wassen, 1999; Barber *et al.*, 2004). RWL has been used as a surrogate for measurements of redox and moisture status in peatlands and is therefore the focus of greenhouse gas and ecological studies (e.g. studies reviewed by Wierda *et al.* 1997; Regina *et. al* 1999; and Blodau, 2002).

The difference between AWL and RWL fluctuation is assumed to be negligible in mineral aquifers. However, peat is very compressible on account of its fragile architecture and porosity (MacFarlane, 1965; Price *et al.*, 2005). Peat compresses when the water table recedes, as the peat matrix is no longer supported by porewater pressure, which increases the effective stress in the dewatered and submerged peat layers (Ivanov, 1981; Kennedy & Price 2005). As a result AWL fluctuations cause changes in the peat surface elevation (SE), which in turn reduce water table fluctuations with respect to the surface, because the surface 'sticks' to the water table. Changes in surface elevation have been shown to reduce the range of RWL fluctuations, relative to AWL fluctuations, by 3–100 % (Nuttle *et al.*, 1990; Roulet, 1991; FechnerLevy & Hemond, 1996; Van der Schaaf, 1999; Van Seters & Price, 2001; Whittington & Price, 2006). For example floating peatlands display the smallest RWL fluctuations because as the surface elevation follows the AWL closely.

Surface elevation changes in peatlands include changes of the peat surface level above a fixed elevation datum (e.g. sea level) due to reversible volume changes of the peat deposit as a result of compression, shrinkage and flotation.

Of special interest are surface elevation changes resulting from gas volume dynamics in the peat matrix. Free gas in the peat matrix affects the surface elevation by flotation, buoyancy, porewater pressure and ebullition. Some 5 % vol. gas in most peats result in a wet bulk density smaller than 1 g cm^{-3} allowing flotation (cf. Hogg & Wein, 1988a; Strack *et al.*, 2005). The equilibrium of forces on the peat matrix is sensitive to changes in buoyancy and porewater pressure resulting from a fluctuating gas volume: a higher gas volume causes the peat matrix to expand and hence the surface to rise (Ivanov, 1981; Hogg & Wein, 1988a; Kellner *et al.*, 2005; Rosenberry *et al.*, 2003; Strack *et al.*, 2005). The release of free-phase gas in the form of bubbles (ebullition) inflates the peat surface before the surface elevation rapidly decreases (Glaser *et al.*, 2004; Strack *et al.*, 2006). Ebullition occurs on timescales of minutes to hours. The effect of free gas on surface elevation is generally in the range of millimeters (or small compared to the effect of water table fluctuations) (Hogg & Wein, 1988a; FechnerLevy & Hemond, 1996; Strack *et al.*, 2005; Strack *et al.*, 2006; Comas *et al.*, 2007). In contrast, Glaser *et al.* (2004) detected surface deformation of 10–30 cm in 4 h in a North American peatland by using a GPS antenna mounted to a tree, and interpreted this as a large ebullition event.

Reversible surface elevation changes have been termed peatland surface oscillation (PSO) among other terms. PSO is the focus of this study and refers also to the total range of reversible surface elevation changes over a certain period. In contrast, irreversible surface elevation changes can result from peat accumulation (Clymo, 1984), irreversible subsidence due to compaction and oxidation subsequent to drainage (Prus–Chacinski, 1962; Eggelsmann, 1978; Schipper & McLeod, 2002), peat cutting, volume changes of underlying aquifers (Whelan *et al.*, 2005) and geological crust movement.

A linear relationship between peat surface (SE) and water table elevation (AWL) is suggested by a number of studies in peatlands (Nuttle *et al.*, 1990; Baumann, 2006) especially for floating peatlands (Van Wirdum, 1991; Roulet *et al.*, 1992; FechnerLevy & Hemond, 1996; Price & Schlotzhauer, 1999). Linear-

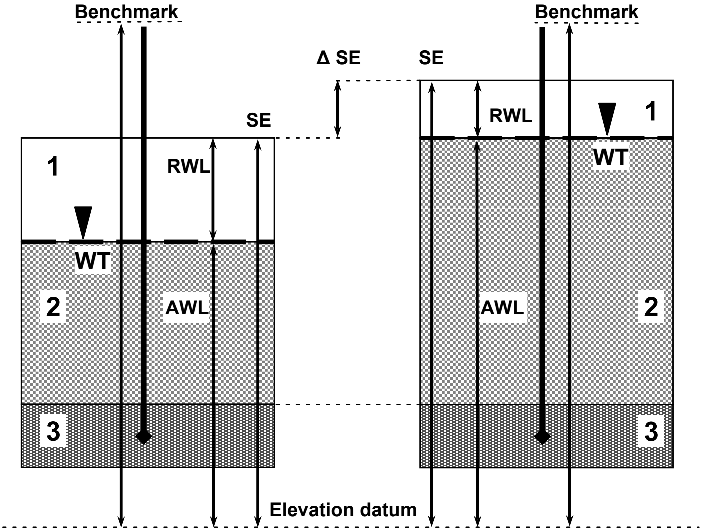

Figure 4.1. Definition diagram showing how differences in water table (WT) positions in a peatland depend on changes of the surface elevation (SE) where a benchmark (e.g. steel rod) anchored in firm substratum (3) provides a stable datum. In this illustration peat thickness increases by the surface elevation change (Δ SE) from time 1 on left to time 2 on right. Δ SE coincides with a rise of the absolute water level (AWL). The saturated zone thickness (2) increases at the expense of the relative water level (RWL–water table position with respect to the moving surface). The WT would reach the surface at time 2 (right) without ΔSE. In contrast, the increasing peat volume prevents a sharp decrease of the unsaturated zone thickness (1). Thus the difference in RWL from time 1 to time 2 is reduced. The total fluctuation in surface elevation is called peatland surface oscillation (PSO) if surface elevation changes are more-or-less reversible.

ity implies that surface elevation changes are directly proportional to changes in AWL. Hence, the slope of the SE–AWL relationship remains independent of the actual AWL position. However, there is doubt that the SE–AWL relationship is linear in all cases. The magnitude of surface elevation changes decreases substantially when the water level exceeds the peat surface (Koerselman, 1989; Swarzenski *et al.*, 1991) and at low AWLs (Green & Pearson, 1968; Schwintzer, 1978; Swarzenski *et al.*, 1991). Seasonal differences in surface elevation changes for an equivalent fluctuation in AWL, e.g. larger surface elevation changes in spring than in late summer, have been reported by Kennedy & Price (2005) who speculated that winter frosts increased the compressibility of peat, which then decreased during the growing season. Peat pore gas dynamics and atmospheric pressure changes may partly account for residuals of a linear rela-

Table 4.1. Definition of terms and abbreviations

Abbreviation	Term	Explanation
PSO	Peatland surface oscillation	Reversible peatland surface elevation changes and in particular the distance between maximum and minimum surface elevation for a site
AWL	Absolute water level	Vertical distance between water table elevation and stable elevation datum e.g. mean sea level
RWL	Relative water level	Vertical distance between water table and peat surface
SE	Surface elevation	Peat surface elevation above stable elevation datum, also SE = AWL – RWL
OSC	Oscillation coefficient	Ratio between the total SE range and total range of AWL fluctuations

tionship between surface level and water level observed in a poor fen (Strack *et al.*, 2006).

Surface elevation changes for an equivalent fluctuation in AWL are spatially variable within a peatland (e.g. Roulet, 1991; Gilman, 1994; Price & Schlotzhauer, 1999; Tanneberger & Hahne, 2003; Whittington & Price, 2006). However, only a handful of studies have addressed major controls on that spatial variability: Almendinger *et al.* (1986) concluded that surface elevation changes were related to peat thickness in a North American peatland complex and studies in temperate bogs found higher surface elevation changes in the centre than at the margin (shallow, dense peat) (Van der Schaaf, 1999; Baumann, 2006). In contrast, other studies have not observed a relationship between peat thickness and surface elevation changes (Buell & Buell, 1941; Schwintzer, 1978; Gilman, 1994; Price, 1994). Whittington and Price (2006) observed differences of several centimetres in surface elevation changes on the scale of hummocks, lawns and pools with presumably no significant differences in peat thickness: their lawn site showed higher surface elevation changes (6.5 cm) than their hummock/pool site (1 cm) being only several metres apart and subjected to the same AWL fluctuations (7.5 cm). Bulk density may also control surface elevation changes: increasing bulk density coincides with decreasing magnitude of surface elevation changes (Holm *et al.*, 2000; Whittington & Price, 2006). Strack *et al.* (2006) speculated that differences in peat architecture were responsible for observed spatial differences in surface elevation fluctuations within a poor fen.

Wetlands dominated by peat forming plants of the family Restionaceae ('restiad') are predominantly confined to New Zealand (Campbell, 1983). Peat surface oscillation in these systems has not been systematically studied despite the regulative role it may play during the dry season. Indeed, water table fluctuations below the surface in restiad peatlands are surprisingly small despite pronounced droughts (Campbell & Jackson, 2004). The rush-like Restionaceae produce a dense living root zone that is some 30 cm thick and rich in gas filled tissue (aerenchyma) (Campbell, 1964; Campbell, 1975). Deeper peat, comparable to sedge peats found in the Northern Hemisphere, consists of fibrous remains and rhizomes and is highly compressible when little decomposed (Campbell, 1964; Davoren *et al.*, 1978).

Our objective was to determine the spatiotemporal variability of PSO in a restiad fen. We investigated if the relationship between water table and surface elevation was linear or seasonally variable. We also examined the relationship between PSO spatial variability and peatland characteristics including AWL fluctuation, peat thickness, bulk density and vegetation cover.

Materials and methods

Study area

The study was conducted in a warm-temperate peatland (Opuatia wetland) 80 km south of Auckland, North Island New Zealand (37° 26′ S, 175° 04′ E). This 950 ha peatland fills a narrow valley basin next to the Opuatia River, a minor lowland tributary of the Waikato River (mean annual flow 375 $m^3 s^{-1}$ at the closest river gauge, Environment Waikato 2006). Opuatia wetland is occasionally inundated with a recurrence interval of 15–30 years because of back-flooding of the Waikato River as observed in 2004 (Browne, 2005). Average peat thickness is 7 m with well-preserved root peat in the upper 3.5 m underlain by highly decomposed silicate rich peats and flood deposits over impermeable clays commonly found in the region (Davoren *et al.*, 1978; Edbrooke, 2001). The peat is not drained but the surrounding hill country is used for intensive dairy farming. The 30-year average annual temperature of the closest climate station was 13.7 °C with average January and July temperatures of 18.9 °C and 8.9 °C, respectively (NIWA, 2006). Mean (30 years) annual total precipitation was 115 cm, typically with a late summer drought lasting 2–3 months.

The vegetation of large parts in the centre of the peatland is open consisting of poor fen species: restiad rushes, i.e. *Empodisma minus* (Restionaceae) on high relief elements and sedges, mainly *Baumea* sp. (Cyperaceae), in habitats with water tables exceeding the surface. Shrubs, mostly *Leptospermum scoparium* and *Epacris pauciflora*, are scattered. The nutrient rich margins and the flood plain are dominated by trees such as introduced *Salix* sp. and native *L. scoparium*. Moss is neither abundant in the present vegetation nor preserved in the peat. Hummocks of *Sphagnum cristatum* can be found below the dense canopy of *Empodisma minus*.

Methods

The spatial variability of surface elevation changes was assessed at 23 sites 50 m apart on two perpendicular transects: The transect EW (east–west), 450 m long, bridged from dryland to dryland and transect NS (north–south) reached 650 m from margin to centre of the peatland (Fig. 4.2). Peat thickness, determined using a D-Section corer (ID 4.5 cm), increased from shallow peat at the peatland's margins (< 3 m) to the centre with deep peat (10–12 m) (Fig. 4.2). GPS coordinates (NZ grid) of the transects' ends were: NS0 (N638379.6, E327104.7) to NS650 (N637788.2, E326946.3) and EW0 (N637974.5, E327330.8) to EW450 (N638065.7, E326896.8).

Every site was equipped with a benchmark consisting of a metal rod set firmly into the substratum (clay). We determined the elevation of every benchmark above mean sea level using a RTK GPS system (TRIMBLE RL 4000, ± 3 cm vertical accuracy). The constancy of benchmarks (± 1.5 cm) was confirmed by two elevation surveys deploying a water filled level gauge constructed from clear plastic hose (accuracy ± 1 cm) as used by Van Wirdum (1991). The peat surface was marked with a wooden plate fixed to the first 5 cm of the peat with galvanised wire and water tables were measured in slotted PVC pipes driven 1 m deep in the peat. Surface elevation and absolute water level (AWL) data were collected monthly (August 2005–August 2006) by measuring the distance between benchmark and peat surface or water table, respectively. Consequently, RWL was calculated from the difference between surface elevation and AWL (Fig. 4.1).

To measure the short-term to seasonal oscillation of surface elevation and AWL, high resolution vibrating wire pressure transducers (Geokon, Lebanon, NH, model 4580–2v–2.5: 0.2 mm precision and 0.4 mm accuracy) were deployed at site NS400 (Fig. 4.2) following the design of manual measurements. The transducer measuring AWL was fixed to the metal rod and the second transducer was free to move with the peat being attached to the peat surface with a wooden board (25 × 18 cm) that was wired onto the surface of the fibrous peat matrix. Surface elevation changes were then calculated by subtracting RWL from AWL (Fig. 4.1) so that the water table served as the relevant benchmark for every measurement. Pressure transducers were connected to a data logger (Campbell Scientific, Logan, UT, model CR10X) to monitor water levels every 15 min. The pressure transducers were calibrated and paired in the laboratory showing no systematic differences in response either before or after deployment. Comparing manual measurements of surface elevation ($n = 12$) with data from water level transducers the standard error amounted to ± 2 mm with no indication of a seasonal trend. Differences were probably

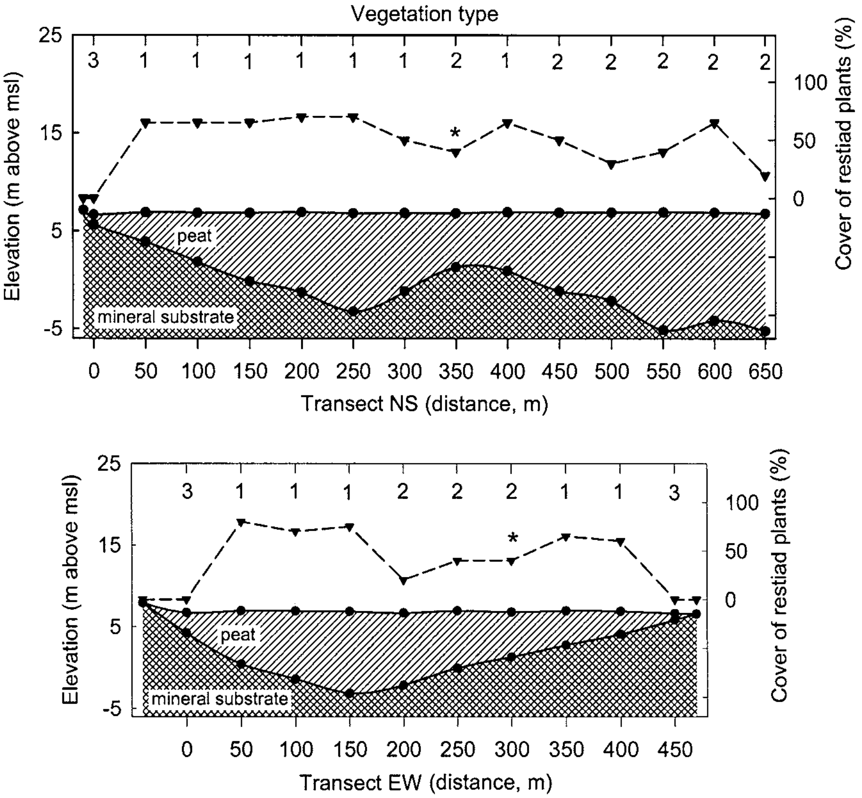

Figure 4.2. Surface elevation (upper circles), elevation of the peat base (lower circles) and cover (%) of Empodisma minus (triangles) for all sites along transect NS (top) and EW (bottom). Transects intersected at asterisks. The numbers 1–3 at top indicate the vegetation type for each site defined in the text.

caused by the combined inaccuracy of the measuring tape (± 2 mm) and the water level transducers (± 0.3 mm).

Peat cores were collected from surface peat (0–5 cm) and standard methods were used to calculate bulk density. We estimated the canopy cover of trees, shrubs, sedges, restiad rushes and other vascular plants, in vegetation plots (4 × 4 m) in units of 10 % cover.

Meteorological data (precipitation, air temperature, solar radiation, humidity) were measured at 10 s intervals with an automatic weather station and then recorded as half-hourly averages using a Campbell Scientific CR10X data logger. Of particular interest in this study, rainfall was measured with a tipping bucket rain gauge (Hydrological Services, Liverpool Australia, 0.2 mm tip^{-1}).

Data analysis

To describe peatland surface oscillation at longer time scales we calculated the ratio between the total surface elevation range and total range of AWL fluctuations. This ratio is termed the oscillation coefficient (OSC) and is used to analyse the spatial variability of manually measured surface elevation fluctuations. If the SE–AWL relationship is more-or-less linear, OSC is equivalent to the slope of that relationship.

To determine whether vegetation cover could explain the spatial variability of OSC we distinguished vegetation types using agglomerative hierarchical clustering of standardised (zero mean and unit variance) cover percentages of vegetation formations using the Euclidian distance as measure for similarity and Ward's method as clustering algorithm. Statistical analysis was performed with SPSS 10.0.

Results

Seasonal variability of water storage and surface elevation

Rainfall between 20 August 2005 and 20 August 2006 totalled 144.2 cm with an extended summer drought between January and April 2006 (Fig. 4.3). Total rainfall during the summer drought was 11.8 cm. The average air temperature was 13.5 °C.

During wet seasons (June–November) surface elevation was strongly linked to AWL at site NS400 (Fig. 4.3). In contrast, surface elevation showed little fluctuation during and immediately after the summer drought (February–April). The rate of surface elevation change slowed during summer while

AWL dropped sharply. The unsaturated zone (cf. RWL, shaded region in Fig. 4.3) reached a maximum thickness in late summer 2006 (30.7 cm), when evaporation and run-off exceeded rainfall substantially forcing AWL to draw down. A large rise in AWL (24 cm) in response to rainfall decreased the unsaturated zone sharply in April and May because the surface level rose only slightly (5 cm). Subsequently, the surface responded rapidly to rising AWL (10 cm) increasing some 7 cm in one week from 10 May 2006 onwards. In summary, for the annual period, the range in AWL was 47 cm, coinciding with 22 cm total range in surface elevation, which reduced RWL fluctuations to 25 cm (equivalent to 53 % of AWL fluctuations). The unsaturated zone thickness at site NS400 was never less than 4 cm, so that the water table never exceeded the surface during the study period.

The same seasonal trends can be inferred when surface elevation is plotted against AWL (Fig. 4.4): low surface elevation and AWL prevailed during the dry season (lower left segment) and high surface elevation and AWL during the wet season (upper right segment). Three general types of relationships were observed between surface elevation and AWL for different times (Fig. 4.4): firstly there was a more-or-less 1:1 relationship between surface elevation and AWL for AWLs above 690 cm above mean sea level (msl) common for the wet

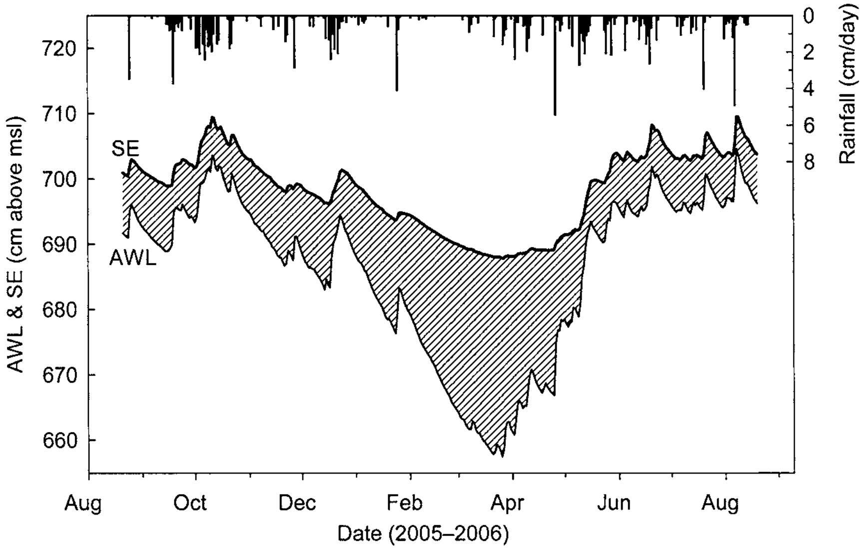

Figure 4.3. Time series of daily mean surface elevation (SE), absolute water level (AWL) and daily rainfall sum (bars) for a one year period starting 20 August 2005. The shaded region represents the unsaturated zone and its thickness equals the relative water level (RWL).

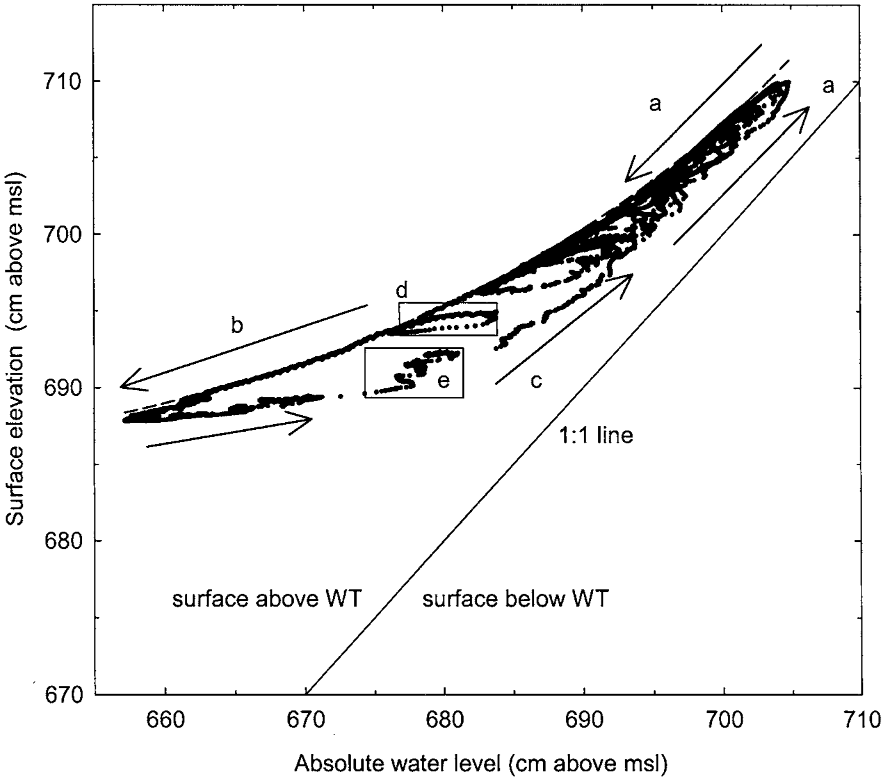

Figure 4.4. Peat surface elevation (SE) plotted against absolute water level (AWL) at site NS400 for a one year period starting 20 August 2005 comprising raw data (measuring interval was 15 min). The vertical distance between plotted data and the 1:1 line indicates the thickness of the unsaturated zone. Note that ranges of axes differ. Letters are referred to in the text. The upper boundary (dashed curve, visible at ends) matches with the 'drying curve' and was fitted to arbitrarily selected data points using a non-linear approach. Arrows indicate direction of time series sequence.

season (part 'a'). Part 'b' comprises the summer drought, resulting in a continuous AWL draw down and surface elevation subsidence. The slope of the SE–AWL curve decreased continually during this period approaching zero. There is very little scatter along part 'b' suggesting a distinct 'drying curve'. Rewetting of the upper peat started in April 2006 (part 'c') with a large delay of the surface elevation to rising AWL. The delayed rise of surface elevation prevailed until the 'rewetting curve' joined part 'a' in mid-May 2006 as a result of rapid surface elevation changes. To highlight the continuous character of the 'drying curve' an upper boundary was fitted to the SE–AWL curve using

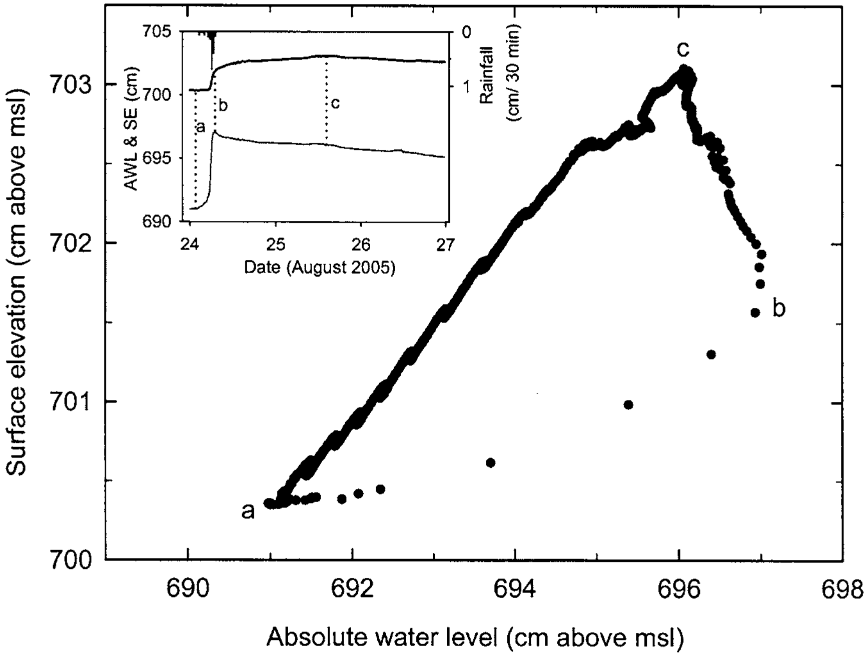

Figure 4.5. Hysteretic surface elevation (SE)–absolute water level (AWL) relationship for a single rain event (3.5 cm in 6 h) at site NS400 in August 2005 based on 15-min data (single point). Labels referred to in the text. Inset shows time series of rainfall (bars), AWL (lower line) and surface elevation (upper line) for the first part of the dataset shown in the main graph. Dotted vertical lines and labels coincide with labelled points on main graph.

a non-linear approach: 43 % of all data are within a 0.5 cm range of that upper boundary. Remaining data points were recorded during or after rain indicating hysteresis when the peat was rewetting.

Hysteresis of surface elevation changes

Increasing AWL following rain events shifted the SE–AWL relationship away from the drying curve because the response of the surface elevation to an AWL increase was delayed (Fig. 4.4). Hysteresis occurred on different time scales because AWL increased during the day, during rain events (several days) and at the beginning of the wet season. Examination of one rain event in winter 2005 demonstrates hysteresis on the scale of days (Fig. 4.5). Initial rainfall (3.5 cm) caused an immediate rise in AWL (6 cm) and surface elevation (1.8 cm) (Fig. 4.5, segment a–b). However, the surface continued rising for a period of 38 h after rainfall despite slightly receding AWL (b–c). The surface abruptly ceased rising (at c), then both AWL and surface elevation declined (c–a), approaching the 'drying curve' of Figure 4.4. This delayed, hysteretic response of the surface consequently results in many 'loops' in the SE–AWL curve (e.g. part 'd' in Fig. 4.4).

Along the 'rewetting' curve shown in Figure 4.4 wavy sections occurred when the water table drew down subsequent to rain events, whereas the surface level was still rising or receding very slowly (e.g. part 'e'). Hysteresis was more pronounced during dry than wet months: drying and rewetting curves were furthest apart (13.3 cm) in the dry season (lower left segment Fig. 4.4) and least (3.2 cm) for high surface elevations during the wet season (upper right segment in Fig. 4.4).

Spatial variation of surface and water levels

Water and surface level fluctuations varied along the transects with little surface elevation fluctuation next to the peatland's margin and the largest ranges of surface elevation at sites with high cover of *Empodisma minus* plants (Fig. 4.6). Maximum and minimum AWL and surface elevation were recorded during visits in October 2005 and in March 2006, respectively. However, three

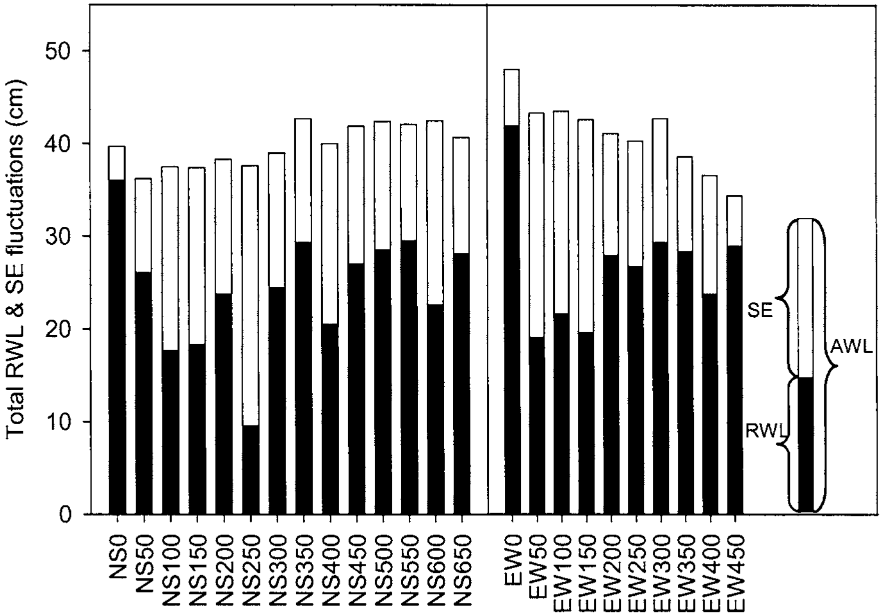

Figure 4.6. Spatial variability of total fluctuations of relative water level (RWL), surface elevation (SE) and absolute water level (AWL – sum of both bars) based on manual measurements.

sites showed slightly lower surface elevation in April 2006 presumably due to hysteresis.

Total annual surface elevation and AWL fluctuations derived from manual measurements may have been underestimated because dates of measurements

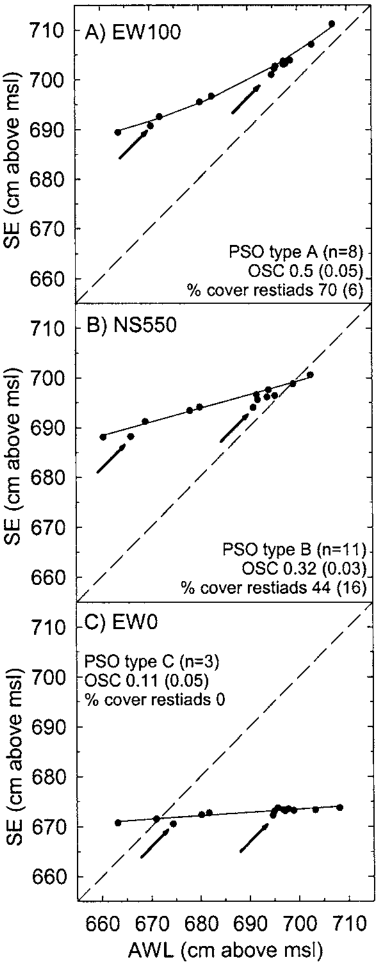

Figure 4.7. Spatial variability of the surface elevation (SE)–absolute water level (AWL) relationships among sites. Each graph is an example of an oscillation type (A–C). OSC is the ratio between total range of surface elevation changes and total range of AWL changes and is presented here as the mean (SD) of sites per type. Also for every type the mean (SD) percentage cover of restiads, i.e. *Empodisma minus*, is provided. Solid lines represent regression models based on measurements on days (n = 8), coinciding with the drying curve on Figure 4.4. Dashed lines are 1:1. Arrows point to measurements taken in April (left) and May 2006 (right) indicating hysteretic behaviour of PSO.

and dates of extreme levels differed: At site NS400 ranges derived from the continuous record of surface elevation and AWL exceeded ranges calculated from manual measurements by 3 cm and 8.5 cm, respectively. AWL fluctuations averaged 40 cm (SD 2.8 cm) amongst the sites and most sites (> 75 %) ranged within 37–43 cm (Fig. 4.6). In contrast to relatively homogeneous AWL fluctuations, surface elevation changes varied greatly among sites with no spatial trend (Fig. 4.6). Surface elevation changes averaged 15 cm with a higher standard deviation (± 6 cm) than AWLs.

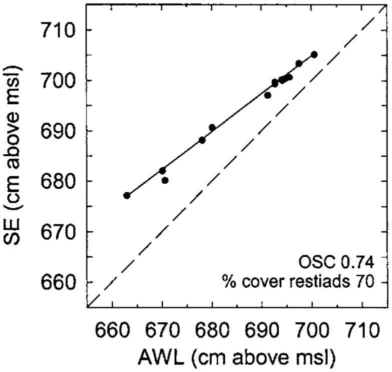

Figure 4.8. Surface elevation (SE)–absolute water level (AWL) relationship close to 1:1 as recorded by manual measurements at site NS250, which was omitted in the classification of sites (cf. Fig. 4.7). The solid line represents a regression model based on measurements on days (*n* = 8), which coincided with the drying curve on Figure 4.4. Dashed line is 1:1.

Examining the SE–AWL relationship for all sites using manually collected data we delineated three broad types (Fig. 4.7). Type criteria were shape and slope of the SE–AWL relationship. A non-linear relationship was found for type A (cf. continuous record at site NS400, Fig. 4.4) with an upper slope more-or-less parallel to the 1:1 line and a lower slope approaching zero. Conversely, types B and C suggested linear relationships. Sites of type A kept the surface above the water table (*n* = 8, OSC = 0.5 ± 0.05, mean ± standard deviation). The water table exceeded the surface during high AWL at sites of type B as a result of low OSC (*n* = 11, OSC = 0.32 ± 0.03) and a mean RWL closer to the surface than other types. Type C comprises sites next to the dryland, where RWL was above the surface much of the year. Type C sites showed little change in surface elevation (< 6 cm) and thus, very small OSC (*n* = 3, OSC = 0.11 ± 0.05). However, this classification failed to fit site NS250 that showed highest surface elevation changes (Fig. 4.6) but a linear SE–AWL relationship (Fig. 4.8). Site NS250 also showed the highest OSC (0.74) of all sites.

Total RWL fluctuations were spatially variable despite relatively homogenous AWL fluctuations (Fig. 4.6) as a result of spatially variable PSO. RWL fluctuations at type A sites (mean 20 cm) were always less than 24 cm, but the RWL fluctuated more than 24 cm at type B (mean 27 cm) and type C sites (mean 36 cm). Water tables were at least 2 cm below the surface for all sites in summer. Wet season water tables were close to or above the surface for most sites. However, some sites (NS250, NS400, EW100, and EW150) sustained an unsaturated zone exceeding 4 cm throughout the year.

Vegetation at the 23 sites can be grouped into three vegetation types showing some spatial trend. Restiads, i.e. *Empodisma minus*, were abundant in type 1 (> 60 % cover), which dominated the northern half of transect NS and was also abundant on transect EW (Fig. 4.2). Vegetation type 2 was characterised by a higher sedge cover (> 20 % cover) and an abundance of shrubs. Most of the vegetation type 2 sites concentrated on the southern half of transect NS. Vegetation type 3 was dominated by high growing trees as well as shrubs and was limited to sites at the nutrient rich margins. There was a close match between vegetation types and oscillation types. For example all sites of oscillation type A (cf. a non-linear SE–AWL relationship) belonged to vegetation type 1, i.e. high cover of restiads. Vegetation type 2 sites belonged exclusively to oscillation type B (except for NS300) with a mean water table close to or above the surface. Oscillation type C was restricted to margins that were dominated by type 3 vegetation.

Bulk density averaged (range) 0.09 (0.05–0.18) g cm^{-3} and showed no spatial trend except for sites next to the dryland exceeding 0.1 g cm^{-3} (data not shown).

Discussion

Non-linearity of SE–AWL relationship and hysteresis

A linear relationship between absolute water level (AWL) and surface elevation (SE) in peatlands has been suggested (e.g. Nuttle & Hemond, 1988; Price & Schlotzhauer, 1999) particularly for floating peatlands (Koerselman, 1989; Roulet, 1991; Roulet *et al.*, 1992; FechnerLevy & Hemond, 1996). However, the SE–AWL relationship was non-linear for 35 % of the sites in this study (Figs 4.4, 4.7). In the case of non-linearity, PSO was large during the wet season for the equivalent fluctuation in AWL resulting in a reduction of RWL fluctuations of up to 80 % compared to the dry season.

The magnitude of PSO will depend on the mechanism driving surface elevation changes. For example flotation results in substantially higher PSO than compression or shrinkage of peat for the same fluctuations in AWL (e.g. Roulet, 1991). Therefore, seasonal shifts between mechanisms may result in nonlinear SE–AWL curves. The slope of the SE–AWL curve at site NS400 ('drying curve' in Fig. 4.4) was significantly flatter at low AWLs (0.2) compared to high AWLs (0.8). We suggest that at times of highly fluctuating surface elevation, flotation of upper peat layers was the main cause of PSO. Conversely, low surface elevation fluctuations were recorded when mainly reversible compression and shrinkage may have caused PSO. Sites belonging to oscillation types B and C (Fig. 4.7) had less PSO presumably due to a lack of flotation. However, site NS250 could not be encompassed by any oscillation type because of the steep slope (0.74) of the linear SE–AWL curve (Fig. 4.8). The large PSO at this site may have resulted from a floating-like behaviour that was not restricted to the wet season.

To find a distinct layer of water (cf. water cushion), which allows flotation, the peat profile at site NS400 was cored during the wet season in 2005. While no explicit layer of free water was found, the peat between 50–150 cm depths was very soft and compressible indicating high moisture content. Temporary flotation implies that upper peat layers are subjected to grounding and lifting, which has only been described for floating peatlands comprising large water bodies in the peat profile (Green & Pearson, 1968; Swan & Gill, 1970; Schwintzer, 1978; Swarzenski *et al.*, 1991; Van Wirdum, 1991). In contrast, Strack *et al.* (2006) found a more-a-less 1:1 relationship between surface elevation and AWL, typical for floating peatlands, but no indication for a distinct layer of water, in a poor fen in Canada. Temporary flotation has been proposed on a conceptual level for non-floating peatlands in Eastern Europe (Kulczynski, 1949).

We propose that the slope of the SE–AWL relationship is diagnostic of mechanisms causing PSO. Slopes close to unity and displaying a linear relationship suggest flotation. Much flatter slopes will be observed when reversible compression and shrinkage are operating. Surface elevation changes due to gas volume dynamics can be diagnosed (detected) as scatter along the 'drying curve' during periods of fluctuating atmospheric pressure and temperature (FechnerLevy & Hemond, 1996). Non-linearity may occur as the compressibility of peat is limited (MacFarlane, 1965; Hobbs, 1986; Kennedy & Price, 2005) and as switches between dominant mechanisms occur e.g. temporary flotation. Therefore, the concept of a floating peatland is only applicable for a defined range of space and time. In other words, floating peatlands can cease to float, whereas the surface peat in 'fens' and 'bogs' may temporarily float. In order to estimate the relative importance of flotation and peat volume changes (compression/shrinkage) causing surface elevation changes, it would be necessary to measure elevation changes of peat layers at various depths (e.g. Eggelsmann, 1981; Gilman, 1994; Price, 2003).

The non-linearity of the SE–AWL relationship impacts the accuracy of models that predict the hydrological response of peatlands to lower AWL, which may be caused by human impact on peatlands or changing weather patterns: Roulet *et al.* (1992) predicted a ~22–28 cm AWL draw down for a northern boreal peatland under a CO_2 climate scenario. They assumed that an increase of the unsaturated zone would be continuously mitigated by 50 % due to surface elevation changes (OSC = 0.5). Manual measurements reported here indicate that the majority of sites may exhibit a linear SE–AWL relationship but with varying slopes. However, our continuous dataset at site NS400 strongly suggests that a linear model e.g. a linear regression through the 'drying curve' (Fig. 4.4), slope = 0.46, substantially deviates from observed SE/RWL. This linear model overestimates surface elevation changes and the reduction of RWL fluctuations by up to 135 % for low AWL. Conversely, surface elevation changes are underestimated for high AWL. Therefore, extrapolation and generalisation of sparse manual measurements (limited frequency or observation period) are prone to errors. Non-linear behaviour should always be considered a possibility. Future models of the hydrological response to climate change need to incorporate surface elevation changes that cause the temporal variability of hydraulic parameters such as porosity and permeability (Chow *et al.*, 1992; Kennedy & Price, 2005; Camporese *et al.*, 2006; Whittington & Price, 2006). These hydraulic parameters govern material fluxes (e.g. total carbon and nutrients in porewater) along a hydraulic gradient that depends on AWL.

Hysteresis of peatland surface oscillation

A hysteretic response of the surface elevation to AWL changes was found on all time scales (seasonally, episodically and daily) during continuous monitoring (Figs 4.4, 4.5). Manual measurements also indicate that PSO was seasonally hysteretic for most sites (Fig. 4.7). However, hysteresis was less obvious because of the paucity of manual measurements during the rapid rewetting phase. Generally, the main drivers of surface elevation changes—moisture movement and peat porosity—show hysteretic behaviour (Heikurainen *et al.*, 1964; Price & Schlotzhauer, 1999; Tsuboya *et al.*, 2001; Schwärzel *et al.*, 2002;

Schindler *et al.*, 2003; Naasz *et al.*, 2005). We hypothesise that cause (i.e. AWL changes altering the effective stress, as defined by Terzaghi, 1943) and effect, (i.e. structural changes of the peat matrix induced by effective stress e.g. Hobbs, 1986; Kennedy & Price, 2005), operate on different time scales. Water level fluctuations occur in the range of minutes to hours but it may take hours to days until forces imposed on the peat matrix equilibrate. For example, Eggelsmann (1981) reported hysteresis of surface elevation changes (total 20 cm) in a drained peatland that lasted for several months subsequent to rapid lowering (200 cm) and recovery of AWL.

The spatial variability of PSO rates may cause horizontal drag on the peat matrix that requires time to be evened out. The extent of hysteresis may depend on the main mechanism forcing the surface to oscillate as inferred from different slopes of the SE–AWL relationship. For example, hysteresis was at a maximum for low AWLs, when surface elevation changes occurred presumably only due to compression/shrinkage (Fig. 4.4). In contrast, hysteresis was minor during the wet season, when the peat appeared to be floating. No hysteretic behaviour of the surface elevation was reported from a free floating peatland in the USA (FechnerLevy & Hemond, 1996). We hypothesise that ideal flotation (no lateral or horizontal drag) results in non-hysteretic surface elevation changes. The SE–AWL relationship would then be parallel to the 1:1 line assuming no changes in buoyancy of the peat.

Clearly, water and surface level monitoring need to adjust to this hysteretic behaviour e.g. higher measuring frequency subsequent to large rain events and 'outliers' need to be treated with care (Fig. 4.7).

Spatial variability of peatland surface oscillation and controls

Annual surface elevation changes of all sites were reversible, which is a defining criterion for peatland surface oscillation (PSO). We measured PSO larger (10–28 cm) than values reported for other fens (0.4–10 cm) (Almendinger *et al.*, 1986; Nuttle *et al.*, 1990; Kellner *et al.*, 2005; Price, 1994; Glaser *et al.*, 2004; Whittington & Price, 2006) except for three marginal sites with little PSO. This may have resulted from high AWL fluctuations (mean 40 cm). Also, high OSC values (≥ 0.5 for 8 of 23 sites in the present study) have also been reported for floating peatlands (Touber, 1973; Roulet, 1991; Swarzenski *et al.*, 1991), which furthermore supports our hypothesis that surface elevation changes of these sites are partly due to flotation.

Peatland surface oscillation was found to be spatially variable (Fig. 4.6). Results of this study support a positive relationship between peat thickness and

PSO as hypothesised by Almendinger *et al.* (1986), but this relationship was weak ($r^2 = 0.27$, $P < 0.05$). Peat thickness and bulk density explained together less than 50 % of spatial variation in PSO. In contrast, the cover of the restiad plant *Empodisma minus* explained a substantial part of the spatial variability ($r^2 = 0.64$, $P < 0.001$). Additionally, the cover of *Empodisma minus*, peat thickness and bulk density together explained 73 % of the spatial variability. However, all three variables were likely auto-correlated and true drivers of spatial variability of PSO are difficult to determine in this study.

We observed OSC to be higher in central parts of the peatland than at the margin (Fig. 4.6) (c.f. Touber, 1973; Price, 1994; Van der Schaaf, 1999; Holm *et al.*, 2000; Tanneberger & Hahne, 2003; Baumann, 2006). Measurements of elevation changes in individual peat layers in northern hemisphere peatlands suggest that surface elevation changes are confined to the upper 1 to 1.5 m of peat (Gilman, 1994; Price, 2003). Thus, we suggest that only a limited part of the peat profile contributes substantially to surface elevation changes by compression/shrinkage. As thickness of this crucial part of the peat body may not have varied significantly along the transects the control of peat thickness on PSO became overwritten by other peatland variables such as vegetation type. Similarly, the bulk density of the upper 5 cm of peat has little explanatory power in respect to the entire peat body but it was found to be a good mean of the bulk density of the 30 cm living root layer at two sites (data not shown) and therefore included in the spatial analysis. Sampling below the root layer is hampered by the weak architecture of the peat.

Implications for the water-plant relationship in peatlands

The mean position and fluctuation of the water table below the surface controls the composition of dominant plants (e.g. Ivanov, 1981; Clymo & Hayward, 1982; Wheeler and Shaw, 1995; Wierda *et al.*, 1997; Kotowski *et al.*, 1998). Despite relatively homogenous AWL fluctuations (Fig. 4.6) there was a significant site-to-site variation in mean RWL position and fluctuations as well as vegetation. Spatially variable OSC causes site-to-site variations of RWL fluctuations resulting in a range of water level regimes, which provide a range of habitats that would support different plant communities. Consequently, sites of oscillation type A, i.e. high OSC, have a very small probability of inundation because RWL fluctuations are reduced by flotation, whereas type B and C sites have a higher probability of inundation due to larger RWL fluctuations.

The frequency of water tables above the surface may control the vegetation at Opuatia wetland. *Empodisma minus*, the dominant peat former (Campbell,

1983) and the only restiad species in the study area, grows on high (dry) relief elements avoiding full saturation of the root zone (Johnson & Brooke, 1998). *Empodisma* plants form a dense matrix of highly specialised cluster roots in the first 7–10 cm of the peat that consists of living, gas filled tissue (aerenchyma) (Campbell, 1964; Agnew *et al.*, 1993; Neumann & Martinoia, 2002). The high percentage cover of *Empodisma* plants at sites with large PSO suggested that *Empodisma* benefited from the reduction of inundation and RWL fluctuations (Fig. 4.7). More specifically, flotation prevented the complete saturation of the root zone (cf. Figs 4.3, 4.4 & 4.8). Hogg and Wein (1988b) showed that the root system of *Typha* sp. can contributed up to 20 % of the buoyancy within the root zone, raising the surface level of floating mats in North American wetlands. Further investigation is required to determine whether *Empodisma minus* can engineer its environment via flotation given the high volume of gas filled plant tissue in the near surface peat.

Conclusion

We reported the magnitude of peatland surface oscillation (PSO) measured for one year in a temperate restiad peatland that is little affected by human activities. The surface elevation (SE) oscillated by 10–28 cm for 20 of 23 sites, which is in the upper range of reported values for peatlands. PSO was controlled by the absolute elevation of the water table (AWL) and PSO reduced fluctuations of the water table below the surface (RWL) by 30–50 % for 19 of 23 sites. We discovered that the relationship between surface elevation and AWL was not uniform due to hysteresis, which occurred at a range of time scales following a rise of AWL. Delayed adjustment of the peat matrix to changes in effective stress may cause this hysteresis. Also, for 35 % of 23 sites the SE–AWL relationship was non-linear: PSO was up to four times higher during the wet season than during the dry season for equivalent fluctuations in AWL. Therefore, the increase of the unsaturated zone during the dry season was proportionally larger compared to the wet season. We propose that a switch in PSO mechanism (from compression-driven to flotation) causes this non-linear behaviour as well as seasonal changes in hysteresis. The slope of the SE–AWL relationship can be a useful diagnostic tool to detect the mechanisms, which govern magnitude and hysteresis of surface elevation changes.

Hydrological monitoring and modelling need to allow for an oscillating surface because differences between fluctuations in AWL and RWL can be substantial. Otherwise, calculated water balances or RWL dynamics may be incorrectly interpreted depending on the differences between AWL and RWL dynamics. Although a linear approach facilitates a simple approximation of the SE–AWL relationship, hysteresis and non-linearity need to be considered, especially when interpreting surface elevation changes with respect to gas dynamics.

Peatland surface oscillation is likely also important for ecological processes. The site-to-site variability of RWL fluctuations and thus peatland plant species composition is controlled by PSO if AWL fluctuations are homogenous. Where restoration of peatlands is hampered due to flooding (Money, 1995; Schipper *et al.*, 2002), promoting flotation of upper peat layers may be beneficial. Further investigation is needed to determine to what extent plant species can control PSO through litter inputs and root morphology. The control of PSO on drainable porosity and hydraulic conductivity also needs to be quantified, when the temporal variability of these hydraulic parameters would be incorporated in hydrological models.

Acknowledgements

Ken Whitney is thanked for generously allowing access to the privately owned Opuatia wetland. Environment Waikato, in particular Edmund Brown, provided financial and logistical support for some of the work reported here. Craig Burgess was a staunch field assistant and technical wizard, sadly we have lost a valued friend and colleague as a result of his passing away in February 2007. Chris McKinnon and Anna Jesussek provided invaluable laboratory and field technical support.

Zero methane emission bogs

Extreme rhizosphere oxygenation by cushion plants in Patagonia

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Abstract. Vascular wetland plants may substantially increase methane emissions by producing root exudates and easily degradable litter, and by providing a low-resistance diffusion pathway via their aerenchyma. However, model studies have indicated that vascular plants can reduce methane emissions when soil oxygen demand is exceeded by oxygen released from roots. We tested whether these conditions occur in bogs dominated by cushion plants. Root–methane interactions were studied by comparing methane emissions, stock and oxygen availability in depth profiles below lawns of either cushion plants or *Sphagnum* mosses in Patagonia. Cushion plants, *Astelia pumila* and *Donatia fascicularis*, formed extensive root systems up to 120 cm depth. The cold soil (< 10 °C) and highly decomposed peat resulted in low microbial activity and oxygen consumption. In cushion plant lawns, high soil oxygen coincided with high root densities, while methane emissions were absent. In *Sphagnum* lawns methane emissions were substantial. High methane concentrations were only found in soils without cushion plant roots. This first methane study in Patagonian bog vegetation reveals lower emissions than expected. We conclude that cushion plants are capable of reducing methane emissions on an ecosystem scale by thorough soil and methane oxidation.

Introduction

Wetlands are favourable habitats for methanogenic archaea that form methane during the decomposition of organic material. These methanogens require environments with no oxygen and abundant organic matter, both of which are present in wetland conditions (Segers, 1998). Peatlands dominated by *Sphagnum* mosses (bogs) are known for the slow decomposition of dead organic matter. The refractory nature of *Sphagnum* litter is mainly responsible for this slow decomposition, as other plants, including typical bog species, decompose much faster than mosses (Aerts *et al.*, 1999; Woodin *et al.*, 2009). Therefore, if nutrient availability permits the dominance of vascular plants the potential production of methane is strongly increased by a high production of vascular plant biomass, which results in an increased input of more easily decomposable litter (Whiting & Chanton, 1993; Joabsson & Christensen, 2001). Belowground vascular plant tissue can also transport labile carbon compounds into anoxic soil layers (Joabsson & Christensen, 2001; Ström *et al.*, 2003; Chanton *et al.*, 2008). Such increased substrate stocks for methanogenic archaea may be crucial because methane production is frequently substrate limited (reviewed in Whalen, 2005). Next, aerenchymatous roots can strongly stimulate the export of methane by creating shortcuts to the atmosphere (Van der Nat & Middelburg, 1998; Kutzbach *et al.*, 2004; Whalen, 2005).

The presence of roots, however, may also decrease the release of methane. Oxygen diffuses through the aerenchyma of vascular plants from the atmosphere into the roots and subsequently leaks into the rhizosphere (Armstrong *et al.*, 1991; Armstrong *et al.*, 2006). Under such oxic rhizosphere conditions, methane production can be reduced by two orders of magnitude (reviewed in Segers, 1998). Additionally, when oxygen is present methane stocks can be lowered by oxidation via methanotrophic bacteria (King, 1994; Sorrell *et al.*, 2002; Raghoebarsing *et al.*, 2005). The passage through a thick aerobic soil–atmosphere interface (i.e., 3–20 cm of aerobic soil) can thus oxidise most of the methane (Roulet *et al.*, 1993; Daulat & Clymo, 1998; Hornibrook *et al.*, 2009). Our study investigated methane release from bog lands that have such high root densities, and provides evidence that certain wetland ecosystems do not produce nearly as much methane as do most temperate and tropical wetlands.

The extent to which the rhizosphere can become aerated depends on various conditions: root density, rate of oxygen loss from the roots, soil oxygen consumption, and the diffusion coefficient of oxygen in the soil. Under most conditions the combination of high oxygen consumption (high temperature, suitable substrate and high microbial activity) and limited oxygen release (limited oxygen conduction capacity and low root density) will result in a very thin oxic rhizosphere. Therefore, in wetland soils a large fraction of the substrate surrounding a root remains anoxic despite root oxygen loss (Armstrong *et al.*, 1991; Armstrong *et al.*, 1992). Such incomplete oxidation of organic soils promotes the coexistence of roots and methane (Grosse *et al.*, 1996), ultimately resulting in increased emission of methane (Watson *et al.*, 1997; Ding *et al.*, 2005).

Depending on the type of vegetation, the potential to lower methane emission by creating oxic soil conditions varies from 16 to 95 % (Laanbroek, 2010). Extensive rhizospheric oxidation requires a dense root biomass (Grosse *et al.*, 1996; Smolders *et al.*, 2002), which in turn provides extra carbon for methane production. However, many studies ignore such additional methane production fuelled by plant litter and root exudates when estimating oxidation potential of the rhizosphere. A root–methane interaction model of Watson and co-workers (1997) required high root biomass to find considerable methane oxidation. Only thorough rhizospheric oxidation created a sufficiently large spatial separation of roots from methane to prevent aerenchyma mediated diffusion (Grosse *et al.*, 1996). Methane emissions can become temporarily decoupled from vascular plant cover when the water levels are low (Bubier, 1995; Couwenberg *et al.*, 2010). At these dry sites the water table dropped below the bulk root mass so that methane was oxidised before being released via plants.

In essence, the large majority of studies showed that vascular plants increase methane release from wetlands (Whiting & Chanton, 1993; Waddington *et al.*, 1996; Kutzbach *et al.*, 2004; Bortoluzzi *et al.*, 2006). Estimations reveal that approximately one third of global methane emission derives from wetlands, where minerotrophic wet peatlands and marshes dominated by vascular plant vegetation are the most important sources (Whiting & Chanton, 1993; Saarnio *et al.*, 2009; Koelbener *et al.*, 2010). Bypassing the aerobic soil–atmosphere interface will be the main cause for high, vascular plant-mediated emission rates. Via their aerenchyma, higher plants can conduct 50–95 % of the total methane emission (Ding *et al.*, 2005; Whalen, 2005).

Global change leading, for instance to nutrient availability and changes in soil wetness is believed to increase vascular plant cover in peatlands (Johansson *et al.*, 2006; Breeuwer *et al.*, 2010), which substantially feeds back on methane cycling in wetlands (see first two paragraphs). However, the importance of particular plant species on methane cycling remains highly variable (Joabsson *et al.*, 1999; Laanbroek, 2010). Part of that variation can be explained by varying dominance of plant functional types as shown in recent studies (Bouchard *et al.*, 2007; Kao-Kniffin *et al.*, 2010; Koelbener *et al.*, 2010). Plant functional types (reviewed in Ustin & Gamon, 2010) may efficiently combine differences of traits like litter production, root density and oxygenation potential (Sorrell *et al.*, 2001; Allen *et al.*, 2002; Van Bodegom *et al.*, 2005; Bouchard *et al.*, 2007). The functional type 'cushion plant' (cf. Gibson & Kirkpatrick, 1985) has not been studied in respect to methane despite their importance in forming peatlands in the Southern Hemisphere.

We hypothesised that in vascular plant dominated wetlands methane release may be decreased or even absent if the soil is thoroughly oxidised by extensive rhizosphere oxygen loss. In search of such wetlands, we targeted for methane producing wetlands (e.g. deep bogs) where oxygen consumption is low. The rainy cold parts of Patagonia harbour pristine bogs with very few nutrients (Kleinebecker *et al.*, 2008; Schmidt *et al.*, 2010). Already Darwin (1839) described bogs in Patagonia with deep rooting plants growing in dense cushion-like vegetation that forms extensive blanket bogs. Cushion plants like *Astelia* sp. and *Donatia* sp. form dense root systems consisting of shallow tap roots and aerenchymous roots exceeding 100 cm in length (Darwin, 1839; Grootjans *et al.*, 2010). Our objective was to elucidate if these roots negatively affected methane emission, by comparing densely rooted sites with sites covered only by moss species (*Sphagnum* sp.). Interactions of roots with soil methane cycling were studied by correlating the vertical distribution of methane stock, oxygen availability and methane oxidation potential, with root biomass density of cushion plants.

Materials and Methods

Sampling design & description of experimental sites

The effects of roots on methane (CH_4) were studied by comparing methane dynamics in different bog vegetation in southernmost Patagonia: 1. Cushion plant lawns in a cushion bog (high root biomass). 2. *Sphagnum magellanicum* lawns adjacent to the cushion plant lawns mentioned above (non-rooted sites in a system dominated by roots). 3. *Sphagnum magellanicum* lawns in a control bog with cover of vascular plants less than 1 % (non-rooted sites in a system with only few roots). Each type was represented by three replicates. In addition, we included two pools surrounded by cushion plants, but without roots in the soil, to estimate methane emissions independent from atmospheric oxygen (oxidation) and roots (gas transport).

Field measurements and experiments were performed in a cushion bog peatland in Tierra del Fuego (Moat, 54° 58′ S; 66° 44′ W; 40 m a.s.l.) where average daily air temperatures are 5–6 °C with cold summers (maximum average temperature 9 °C; Iturraspe & Fritz unpublished). July is usually the coldest month at 2 °C. The absence of a thermal summer is typical for oceanic bogs in Patagonia (Kleinebecker *et al.*, 2007 and literature therein). The control bog (Andorra, 54° 45′ S; 68° 20′ W; 200 m a.s.l.) shows slightly higher daily and seasonal temperature differences during summer due to its location at a valley bottom (Iturraspe *et al.*, 1989). Soil temperature was low and stable at both bogs, through out the growing season decreasing from 8–12 °C at 5 cm below surface to 4–8 °C at 100 cm depth. Soil temperature profiles were recorded during expeditions in spring 2006 and summer 2007. Annual precipitation assessed in the 1980s and from 2008 onwards exceeded 600 mm evenly distributed over the year in both peatlands providing wet conditions (Iturraspe *et al.*, 1989, Iturraspe & Fritz unpublished). Water levels fluctuated between 5 cm above and 20 cm below the surface at all lawn sites from spring to fall.

The cushion bog was dominated by lawns of evergreen cushion plants intermingled with patches (few square meters) of dominating *Sphagnum magellanicum* (Bridel) and scarcely vegetated pools (Roig & Collado, 2004; Gebser, 2008). Dominating cushion plants were *Astelia pumila* (Forster f.) Gaudich and *Donatia fasciculares* Forster and Forster covering more than 70 %. The soil below cushion plants was densely packed with tap roots (1–2 mm diameter) and fine roots exceeding depths of 120 cm (Grootjans *et al.*, 2010). In contrast, roots and vascular plants were almost absent at *Sphagnum* sites. Peat depth was comparable between sites ranging from 700 to 1000 cm, thus providing large stocks of carbon rich substrate. The densely rooted cushion plant peat was highly decomposed (H8–H10 on the Von-Post scale) contrasting with the well-preserved *Sphagnum* peat (Kleinebecker *et al.*, 2007; Gebser, 2008). Peat formed by cushion plants was three to five times denser than *Sphagnum* peat. At all sites *Sphagnum* peat was found at depths deeper than 300 cm. Peatlands studied remained unaffected by anthropogenic alteration such as drainage, agricultural use or elevated atmospheric nutrient deposition. Reviewing scarce deposition data from Patagonia Godoy and co-workers (2003) suggested bulk N deposition below 0.1 g N m^{-2} a^{-1} in coastal regions. The substrate was very low in nutrients with total P concentrations typically below 0.023 % in *Sphagnum* peat and 0.034 % in cushion plant peat. Porewater reflected acid conditions in both bogs (pH 3.8–4.2) with little variation in the upper 300 cm.

Methane stock and emission measurements

Methane and ethane headspace samples were measured on a Hewlett–Packard® 5890 gas chromatograph (Avondale, California) equipped with a flame-ionization detector and a Porapak Q column (80/100 mesh) operated at 120 °C with nitrogen as carrier gas, in the laboratory of the Radboud University, Nijmegen (accuracy 0.2 ppm). Injection volume was 0.1 ml for incubations and porewater samples and 0.5 ml for emission samples to improve the detection of low concentrations.

Methane stock and release were estimated by means of porewater concentration and emission into static chambers, respectively. Sampling took place over the growing season: December 2008 (spring), February 2009 (summer) and late March 2009 (autumn). For logistic reasons sampling was one week delayed in the control bog. Insights into inter-annual and seasonal variations in methane stock were addressed by sampling porewater 8 times from 2006 to 2009 at one site per vegetation type.

Porewater samples were drawn from seven depths (5 cm, 30 cm, 60 cm, 120 cm, 150 cm, 180 cm, 300 cm and 600 cm in the cushion bog and from five depths (5 cm, 50 cm, 150 cm, 300 cm and 500 cm) in the control *Sphagnum* bog, respectively. Anaerobic peat water samples were taken using 5 cm ceramic cups (Eijkelkamp Agrisearch Equipment®, Giesbeek, the Netherlands), connected to vacuum infusion flasks (40 ml) after sampling 150 ml to exclude internal stagnant sampler water. The 40 ml glass infusion flasks had a sample-headspace ratio of usually 1:2. As internal standard 1 ml of ultra pure ethane gas (Airliquide®, Eindhoven, the Netherlands) was added after sampling and flasks were stored at 4 °C during less than 2 weeks until analysis. Microbial modification of samples was hampered by adding 0.1 mg $HgCl_2$ (0.1 ml of 0.1 g l^{-1}). Methane and ethane concentrations were measured in the head space after vigorous shaking, releasing > 96 % of methane to the headspace.

Methane emissions were assessed using dark static PVC chambers (3700 cm^3, 15 cm high) with bleeds of 4 mm PVC hose. PVC frames were installed 2 months before measuring and removable chamber tops were sealed to the frame by the water filled rim. Gas samples were taken in the morning and in the afternoon at the same day at all sites per peatland. After placing chambers temperature differed less than 3 K between t = 0 and end of sampling. Gas samples were taken with a double sided needle during 60 minutes in 20 minute intervals in pre-vacuumed 12 ml glass vial with butyl stopper (Exetainer®, High Wycombe, UK). At sites with very low emissions, an additional sample was taken after 360 min. Samples were stored cool and analysed within one week. Emission data are presented for 53 of 62 measurements where the linear slope fitted $r^2 > 0.75$ or when methane head space concentration stayed constant (zero emissions). Rejected time series, mostly pool sites, were probably subject to ebullition as observed by unexpectedly high methane concentration at t = 20 min followed by depletion afterwards. After two months of additional storage more than 95 % of original methane was retrieved in porewater bottles. Emission samples maintained equal concentrations. Methane release by large-scale ebullition was estimated by surface elevation fluctuations measured with water level recorders attached to the surface and a stable benchmark as described by Fritz *et al.* (2008). Automatic recorders (Odyssey capacitance probes®, Dataflow Christchurch, New Zealand) were set-up to measure levels in one hour intervals during two years and confirmed with hand measurements during field visits.

Redox potential and oxygen measurements

Redox potential measurements were taken at five depths (30 cm, 60 cm, 120 cm, 150 cm, and 200 cm) 2–3 days after gas sampling. Per depth four platinum electrodes were gently pushed into a pre-made hole and allowed to equilibrate. Stable readings were generally obtained after 30–60 minutes. In most cases the drift was smaller than 1 mV per minute within 10 minutes. The redox potential (E_7) corrects the field measurements (E_{field}) for pH (pH_{soil}), absolute temperature in K (T) and the potential of the 3M AgCl/Cl reference electrode (E_{ref} 217 mV at 10 °C) using the following relationship,

$$E_7 = E_{field} + E_{ref} + 0.2\ T\ (pH_{soil} - 7). \qquad \text{(Eq. 3)}$$

Literature on redox processes (e.g. Laanbroek, 1990) suggested E_7 values above 330–350 mV as indication for free oxygen in soils. In figures showing redox data we highlight 350 mV as a threshold for occurrence of free oxygen also used by similar works (Visser *et al.*, 2000). However, other studies found some nanomoles of oxygen when for E_7 just above 300 (Lloyd *et al.*, 1998). Oxygen content in the soil was measured polarographically at 30 and 70 cm below the water table in the cushion bog in February 2009. For oxygen measurement we deployed platinum needle electrodes with a sensing tip of < 0.1 mm embedded in stainless steel (Microscale Measurements, The Hague, The Netherlands). The platinum tips remained protected by cellulose-nitrate membranes. Oxygen electrodes were connected to a custom-made nA-meter (Electronic Workshop, University of Groningen) and an AgCl/Cl reference electrode. To calibrate we used oxygen saturated bog water in the field. Zero point calibration was done in the lab in demineralised water flushed with nitrogen for at least 24 h. Long lasting rainfall prevented frequent measurements of oxygen and also establishing polarograms at various depth. We measured currents at some 450 mV, presettings of the equipment obtained from polarograms in Dutch bogs.

Root characteristics

Root density at cushion plant sites was determined by sampling in a Piston corer (ID 10 cm) to a depth of 80 cm and from 50 cm to 250 cm using a D-Section corer (ID 4.7 cm; Eijkelkamp Agrisearch Equipment®, Giesbeek, the Netherlands). Roots were dried at 70 °C for 2 days. Root density is expressed in g per litre soil. The presence of living fine roots of cushion plants (1–2 mm in diameter) usually coincided with a sharp change from black coloured peat to yellow brown peat below the (oxygenated) rooting zone. In this paper rooting zone comprises the entire volume of soil down to the maximum root depth. The proportion of rhizosphere to rooting zone depends on root density and the space that is affected by activity of individual roots. Integrating root density over the entire rooting zone rendered total dry root biomass expressed as g m^{-2}. Porosity of root material was determined in 1 cm increments using the microbalance method (Visser & Bögemann, 2003). To visualise oxygen loss in the rooting zone we exposed cushion plants to an anaerobic methylene blue solution (25 mg l^{-1} methylene blue, 0. 5 g l^{-1} Agar, 5 mM KCl, 0,05 mM $CaSO_4$) filled in glass cuvettes in the lab. Sodium dithionite ($Na_2S_2o_4$) was used to decolourize the dye. The leaves projected into the air while the surface of the solution was protected from the air by plastic and gently flushed with nitrogen (adapted after Armstrong *et al.*, 1992).

Clipping experiment

To highlight the functional role of cushion plants with respect to the redox state of the soil, the oxygen transport below the water table was hampered by removing green parts of cushion plants in January 2008. We chose to remove four large areas of 2 × 2 m to reduce the effects of surrounding cushion plants. Differences in methane dynamics were documented by methane porewater samples taken at three depths (60 cm, 150 cm and 300 cm) after one month, 13 months and 26 months as described above. The redox potential (E_7) was measured before and 13 months after removing green parts. Methane emissions were measured at three clipped sites two years after clipping. Regrowth was minimal within two years, which highlights the harsh growing conditions.

Methane production & consumption

To estimate differences in potential methane production and consumption we took peat cores in the cushion bog (06 December 2008). Samples (100 ml) were put in airtight plastic bags in the field and stored at 4 °C before being processed in the lab. Differences in potential production were measured in one pure *Sphagnum magellanicum* and one cushion plant site dominated by *Astelia pumila* at 20, 70 and 120 cm depth, respectively. Activity of methanotrophs was estimated by incubating peat along a profile at cushion plant site 1. Samples were collected at depths of 70, 120, 140 and 150 cm, respectively. Maximum rooting depth was 140 cm at that site.

The interior of the bulk peat was sub sampled by taking 20 g fresh weight of soil (some 1.2 g dry weight), which was incubated in 100 ml grey rubber-stoppered glass flasks in threefold at 22 °C. For potential methane production, flasks were flushed with nitrogen and vacuumed 8 times to remove methane and oxygen. For aerobic production we incubated with ambient air. Methane consumption incubations contained a headspace of ambient air and methane was added to a final concentration of 1.2 to 1.5 %. Methane head space concentration was frequently determined over seven weeks in both production and consumption incubation. Rates of methane production/consumption were derived from the linear part of the slope related to the weight of the sample after drying at 70 °C for 48 h. For the cushion plant site we present methane production rates for the beginning and end of the incubation period because rates differed by one magnitude. Oxygen depletion was regularly controlled by means of CO_2 head space concentrations determined on an Infrared Gas Analyzer (IRGA, ABB Advance Optima, Zürich, Switzerland). Bulk density samples were taken at the same locations using a D-Section corer (ID 4.7 cm) mentioned above and dried at 70 °C for 48 h. Rates of methane consumption and production are related to volume and to dry weight, respectively. Rates can be related to either the surface (volume) or substrate quality (dry weight).

Results

Methane emission and physical factors (temperature, water table)

Methane (CH_4) emissions were low but significant, reflecting the low temperature and nutrient status of the bog sites investigated (Fig. 5.1). In cushion plant lawns, however, emissions approached zero. One cushion plant site exhibited emission rates below 1 mg CH_4 m^{-2} d^{-1} on two occasions. In contrast to these virtually zero-emissions, in *Sphagnum* lawns of the cushion bog the methane emission rate was 1–14 mg CH_4 m^{-2} d^{-1} (95 % confidence interval), and similar to the control bog (1–11 mg CH_4 m^{-2} d^{-1}). Emissions of pools were in the same range as those of *Sphagnum* lawns (Fig. 5.1). The highest emissions were found where cushion plants had been clipped. The average water level during measurements were comparable between the different sites (ca. 5 cm and cushion plant lawns being slightly drier), but fluctuated seasonally by some 10 cm from the mean water level (9 cm below surface). Therefore, differences in water level did not correlate with methane emission rates ($r^2 = 0.05$, $n = 53$). The temperature was 8–12 °C in air and 10 °C in the first 10 cm of the soil varying less than 2 °C between measurements at the same day. Monitoring of surface elevation in cushion plant and *Sphagnum* lawns gave no indication for lifting of the peat surface by several centimetres within hours, which is associated by large-scale ebullition. Weak ebullition events could only be triggered in pools and *Sphagnum* growing in pools by jumping of the observer in the direct vicinity. Low

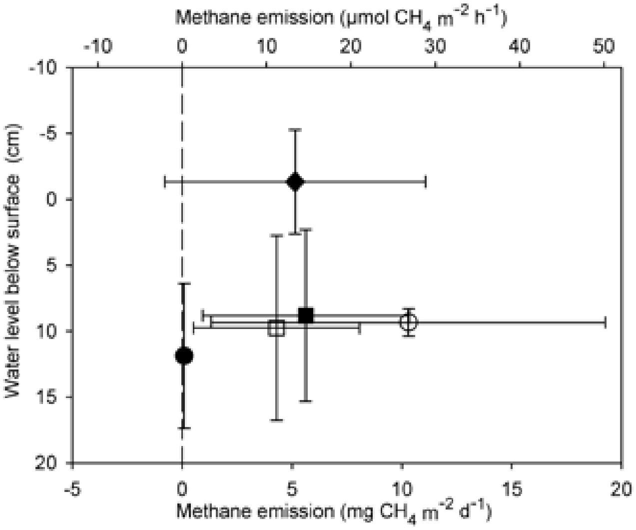

Figure 5.1. Dependency of methane (CH_4) emission from various Patagonian bog vegetation types on water level in the soil. Emissions were not related to the water level, but varied with vegetation type and clipping treatment after 26 months. Error bars indicate SD (n = 6–16). Cushion plant vegetation (filled circle) revealed zero emission when intact (dashed line), but highest emissions when clipped (open circle) ceasing oxygen transfer to the soil. *Sphagnum* lawns (filled square) had similar emission rates in the cushion bog as the control *Sphagnum* bog (open square) and pools (filled diamond) . Emission measurements were taken in the morning and early afternoon using dark chambers in spring, summer and autumn, respectively (i.e. December 2008, February 2009 and March 2009).

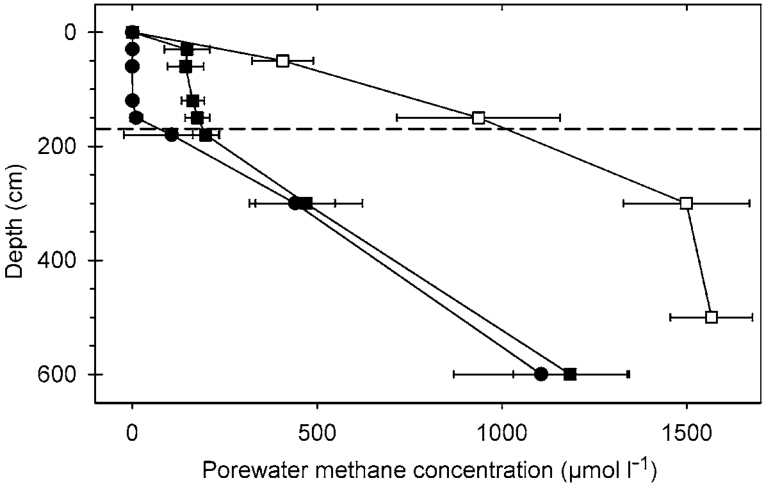

Figure 5.2. Depth profile of methane (CH_4) stock concentrations in various Patagonian bog vegetation types. Rooting depth of cushion plants in the cushion bog is indicated by the dashed line. Methane was thoroughly depleted in the rooting zone below cushion plants (filled circles) and significantly lower in *Sphagnum* lawns in the cushion bog (filled squares) compared to the control *Sphagnum* bog (open squares). Error bars indicate SD, ($n = 9$). Same sites and sampling interval as in Fig. 5.1. Seasonal variations of methane stock were low.

frequency of ebullition generally indicates low concentration of methane in upper peat layers.

Methane stock (porewater profiles)

Similar to emission rates, the methane stock varied strongly between different vegetation types, reflecting the presence of cushion plants and their deep roots (Fig. 5.2). Importantly, no methane (< 1 µmol l^{-1}) was found in the rooting zone of cushion plants while *Sphagnum* lawns stocked less methane in the cushion bog compared to the control bog. The linear increase in methane concentration with depth was similar between the cushion bog and control bog, however, there was a 170 cm offset between the two bog types, which coincided with the maximum depth of the rooting zone in the cushion bog (Fig. 5.2). Unexpectedly, offset in methane stock at this depth was also found in *Sphagnum* lawns less than 3 m adjacent to cushion plants. In the upper 170 cm, the mean methane porewater concentration of 166 µmol l^{-1} (SD 46, $n = 45$) remained stable in depth. This plateau of intermediated methane concentrations differed from generally increasing methane concentrations with depth (Fig. 5.2). It needs to be stressed that *Sphagnum* patches formed small islands closely surrounded (< 3 m) by cushion plants and their rooting zone. Lateral gradients of methane porewater fell in the same order of magnitude (50–150 µmol CH_4 l^{-1} m^{-1}) as gradients in depth (220–320 µmol CH_4 l^{-1} m^{-1}). A levelled surface of 1 % and vertical hydraulic head differences < 0.2 % (Gebser, 2008) suggested a substantial horizontal water movement. The horizontal methane gradients and water flow underline the connectivity of *Sphagnum* patches with their surrounding rooting zone of cushion plants. Additionally, intrusion of methane depleted, oxic rain water is most likely to occur in the sponge-like upper peat of *Sphagnum* lawns.

Absence of methane from the rooting zone of cushion plants becomes more visible when zooming at its lower boundary. At all three cushion plant sites the presence of methane was tightly linked to the lower boundary of the rooting zone of cushion plants. Remarkably, methane was always found merely 5–10 cm below the maximum root depth, which differed between sites (Fig. 5.3a,b). Below 300 cm many porewater samples indicated supersaturation of methane (mean 1499 µmol l^{-1}) in the control bog. Methane stock measurements taken between 2006 and 2009 revealed the same patterns. In general, seasonal and inter-annual variations were minor compared to the striking differences between the rooting zone of cushion plants and samples from non-rooted layers.

Presence of oxygen and roots

All three cushion plant sites were characterised by a dense root biomass (Fig. 5.3a,b). The average root biomass density was 2.15 g DW l^{-1} (SD 0.33, $n = 3$) in the upper 170 cm. Integrating root density along the rooting zone reveals that cushion plants maintained a total root biomass of 3590 g DW m^{-2} (SD 550, $n = 3$). Porosity of roots of the dominating cushion plant *Astelia pumila* was 60–70 % providing sufficient aerenchyma for rapid diffusion of oxygen. Lower porosity was only found within 5 cm from the root tip (apex). Staining experiments with methylene blue suggested modest oxygen release rates along the length of the root, being highest around the root tips. Root tips could be found scattered over the entire depth profile. However, the largest densities of root tips were confined to the upper 70 cm resulting in the highest potential to release oxygen in the upper half of the rooting zone (Fig. 5.3c). Less than 1 % of the fine root biomass was located close (< 15 cm) to accumulated methane in the soil (Fig. 5.3b). At *Sphagnum* sites the very few roots growing down to 30 cm reflected the very sparse cover of vascular plants.

The decrease of redox potential mirrored the increase in methane, being highly sensitive to the presence of roots of cushion plants (Fig. 5.4). Free oxygen in the rooting zone to a depth of 120 cm was indicated by redox potentials higher than E_7 330–350 mV (c.f. Laanbroek, 1990). The presence/activity of roots resulted in an increase in the redox potential of around 170 mV compared to the *Sphagnum* site. Beyond the maximum rooting depth the redox potential decreased rapidly to values comparable to *Sphagnum* sites. Root densities decreased pronouncedly with depth, whereas redox potentials varied little in the upper 120 cm. In contrast, a tight relationship between root density and redox potential was found at the bottom of the rooting zone. At 150 cm depth redox potentials varied substantially around 364 mV (SD 61, $n = 9$) suggesting that a smaller proportion of the substrate remained aerated. Here the coexistence of oxic and anoxic patches correlated with low numbers of root tips and low root densities (Fig. 5.3b). In the lower rooting zone only 2–5 root tips were found

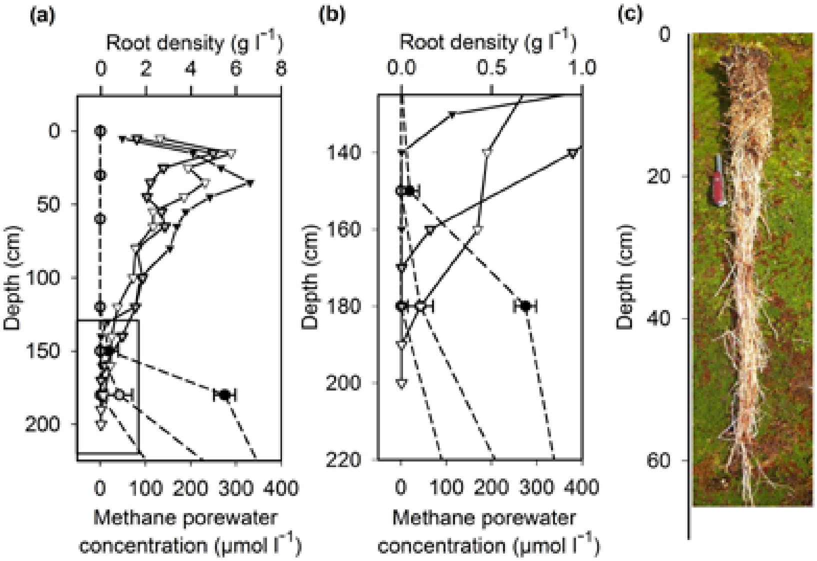

Figure 5.3. Methane (CH_4) stocks (dashed lines) were inversely related to root density profiles (solid lines) in three individual cushion plant sites: CP1 (black symbols, 140 cm deep roots), CP2 (grey symbols, 170 cm deep roots) and CP3 (open symbols, 190cm deep roots). Methane was always found merely 5–10 cm below the maximum root depth of individual sites. The box in panel A is by approximation the area of the graph expanded in panel B. Same methane data are presented in Fig. 5.2. Panel C shows root biomass of cushion plants retrieved from the upper 70 cm using a piston corer (ID 10 cm).

per litre peat substrate. Hence, surplus of oxygen (leading to presence of free oxygen) becomes more variable at these depths leading to a high spatial variation of redox potential. Seasonal variations of the redox potential were small compared to differences related to the density/presence of roots. In February 2009 oxygen sensitive mini-electrodes gave further evidence of oxygenated conditions. Oxygen concentrations up to 5 µmol l^{-1} were found at 30 cm and 70 cm below the water level in cushion plant lawns. Below *Sphagnum* vegetation oxygen was absent when measured by mini-electrodes.

Clipping of cushion plants caused a significant change in soil processes. Within one month after clipping, 3 µmol l^{-1} (SE 1.4, $n = 4$) methane accumulated in the uppermost rooting zone (Fig. 5.5a). After 13 months, porewater concentration increased from zero to around 78 µmol CH_4 l^{-1} (SE 49) at 60 cm and 102 µmol CH_4 l^{-1} (SE 39) at 150 cm. This increase in methane concentration was accompanied by a drastic drop of redox potential to < 300 mV suggesting a depletion of oxygen within a year (Fig. 5.5b). After 26 months mean methane emissions (10 mg CH_4 m^{-2} d^{-1}, $n = 6$) exceeded those of *Sphagnum* lawns (Fig. 5.1) and methane accumulated in the porewater to a concentration of 706 µmol CH_4 l^{-1} at 60 cm (SE 136, $n = 4$).

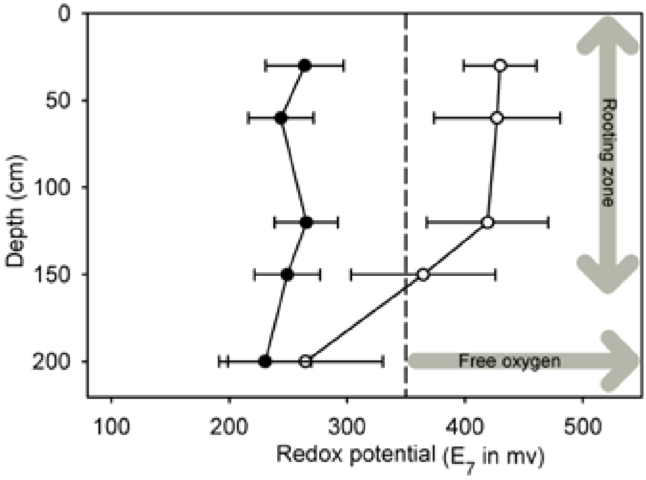

Figure 5.4. Surplus oxygen in the rooting zone of cushion plants (open circles, $n = 9$) as indicated by a redox potential > 350 mV (vertical dashed line). In *Sphagnum* lawns in the cushion bog (black circles) the redox potential was around 170 mV lower, suggesting anoxia. At each depth the potential was measured by four electrodes. Same sites and sampling interval as in Fig. 5.1. The vertical dashed line (350 mV) depicts the lower limit of redox potential for oxygen containing substrates (c.f. Laanbroek, 1990).

Methane oxidation and production

Activity of methanotrophs was found in the entire rooting zone of cushion plants. Mean activities ranged from 10 to 86 μmol l^{-1} d^{-1} and 0.2 to 1.2 μmol g^{-1}DW d^{-1}, respectively (Fig. 5.6). Highest oxidation rates were found at the lower part of the rooting zone where oxygen was in the vicinity of methane containing substrates (Figs 5.3, 5.4). Activity of methanotrophs (10–15 μmol l^{-1} d^{-1}) was also found above the methane–oxygen interface. In the rooting zone methanotrophs are methane limited (Fig. 5.3a), whereas below roots methane consumption became oxygen limited (Fig. 5.4). Oxidation rates in the field may be 62 % lower, assuming an average soil temperature of 8 °C and a Q10 of 2, found for methanotrophs in the control bog by Kip and co-workers (2010).

Mean potential methane production in the upper 120 cm ranged from 1 to 20 μmol l^{-1} d^{-1} and 0.04 to 0.36 μmol g^{-1}DW d^{-1}, respectively (Fig. 5.6). Based on volume, the highest production was found in the most recently accumulated parts, which was dense peat that was little decomposed and had the highest nutrient content. Because *Sphagnum* peat has a three to five times lower density (some 20–30 g l^{-1}), *Sphagnum* sites had a lower methane production

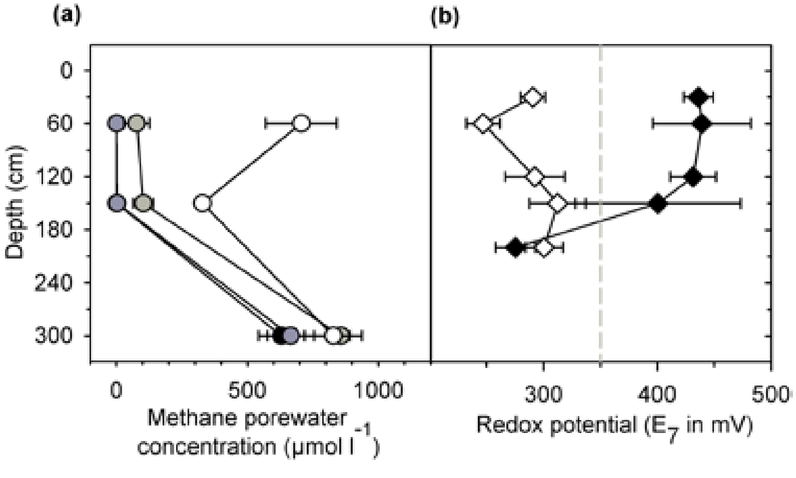

Figure 5.5. Methane (CH_4) concentrations (panel A) increased in the soil after cushion plant plots (2 × 2 m) were clipped resulting in soil anoxia (panel B). Methane accumulated over time (t = 0 black filled circles; t = 1 dark grey circles, t = 13 grey circles and t = 26 months open circles). The increase in methane coincided with decreasing redox potentials (panel B, t = 0 months black filled). After 13 months redox potentials (open diamonds) declined below $E_7 < 330–350$ mV indicating anoxic conditions (c.f. Laanbroek, 1990) comparable to *Sphagnum* lawns. Error bars indicate SE ($n = 4$). Compare also with methane stocks in Fig. 5.2 and redox potential in Fig. 5.4.

potential per surface area or volume compared to cushion plants (density of 50–120 g l^{-1}). In aerobic incubations methane production was below the detection limit. Thus, the actual methane production is assumed to be negligible in the rooting zone of cushion plants because of aerobic conditions (Fig. 5.4).

Samples taken from the rooting zone of cushion plants showed a time-lagged increase in production exceeding volume based rates of *Sphagnum* sites (Fig. 5.6). The time-lagged increase indicated that the community of methanogenic bacteria had adjusted to the anoxic conditions of the incubations. As the substrate from the rooting zone of cushion plants had been subjected to oxygen release, a low presence and activity of methanogens can be anticipated. Edwards and co-workers (1998) found that substrates from aerobic environments or exposed to oxygen after sampling exhibited hampered activity of methanogens.

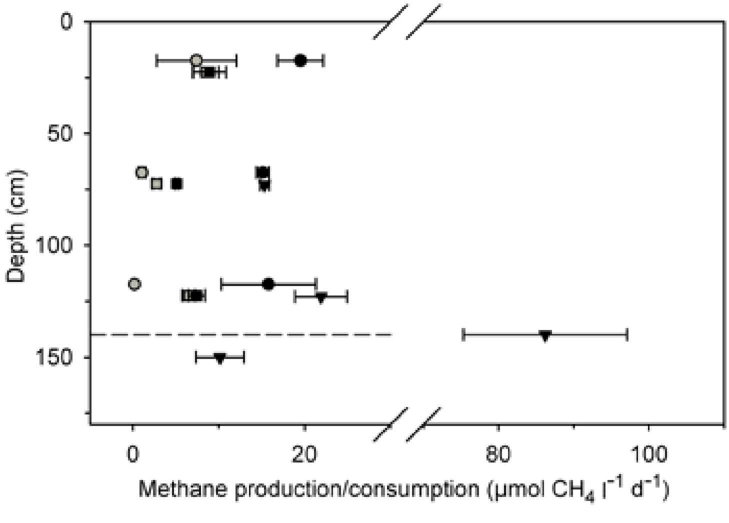

Figure 5.6. Depth profile of methane (CH_4) oxidation rates that exceeded rates of potential methane production at 22 °C. After two weeks methane production of cushion plant peat (grey circles) was lower than that of *Sphagnum* peat (grey squares). In contrast, after seven weeks cushion plants (black circles) revealed much higher potential methane production than *Sphagnum* peat (black squares). Methane oxidation rates (triangles) at 22 °C were substantially higher at the lower boundary of the rooting zone (dashed line) of cushion plant site 1 (CP1). Error bars indicate SD ($n = 4$).

Discussion

In this study, methane dynamics revealed a tight but inverse link to the presence of vascular plant roots. We found evidence that the specific conditions in cushion bogs lead to high oxygenation of the wetland soil well beyond the rhizosphere (> 150 cm), thus limiting methane production and methane release via plants. Crucial for extensive oxygenation are nutrient poor conditions of these sites, limiting soil oxygen demand, combined with high densities of very long and aerenchymatous roots. These data are the first on methane emissions and stocks in temperate bogs in the Southern Hemisphere and Patagonia.

Cushion plants are a significant part of wetland and mountainous vegetation in the Southern hemisphere (Gibson & Kirkpatrick, 1985; Blanco & de la Balze, 2004; Squeo *et al.*, 2006). Part of these bogs consist of *Sphagnum* vegetation, and methane emissions from these sites and from a pure *Sphagnum* bog were low (Fig. 5.1). Wet lawns of *Sphagnum* sp. emitted 1–14 mg CH_4 m^{-2} d^{-1}, which is in the lower range reported for Sphagnum-dominated vegetations (reviewed in Saarnio *et al.*, 2009). The observed slow carbon and methane turnover can be explained by summer temperatures below 10 °C (Daulat & Clymo, 1998; Segers, 1998), very low nutrient availability (Juottonen *et al.*, 2005; Schmidt *et al.*, 2010) and low pH (Segers, 1998).

The larger part of the cushion bogs consists of cushion plants, which are characterised by high densities of long aerenchymatous roots. These roots may function as a conduit for methane release. However, in the rooting zone of cushion plants no methane was present, and only around this zone methane levels steeply increased with (both horizontal and vertical) gradients of 200–300 μmol l^{-1} CH_4 m^{-1} (Figs 5.2, 5.3). So, although a large methane stock was present at the ecosystem scale, methane emissions at cushion plant sites were low approximating zero (Fig. 5.1). The main reason was oxygenation of the rooting zone by oxygen loss from the roots (Fig. 5.4). Root-derived oxygen suppressed methane production and increased methane oxidation, thus diminishing methane stocks in the rooting zone (Fig. 5.6). In contrast, studies on root–methane interactions revealed that roots remained in contact with methane. This resulted in methane emission rates exceeding those common for *Sphagnum*-vegetation (Popp *et al.*, 2000; Ding *et al.*, 2004; Strack *et al.*, 2006). Our study suggests that densely growing cushion plants have a higher potential to oxidise soil and methane than common wetland species e.g. *Phragmites* sp. (Van der Nat & Middelburg, 1998), *Oryza* sp. (Frenzel, 2000), *Carex* sp. (Popp *et al.*, 2000; Ding *et al.*, 2004) and *Sphagnum* sp. (Larmola *et al.*, 2010). As a consequence of incomplete oxygenation, the methane production in anoxic parts of the soil becomes fuelled by easily decomposing root exudates and litter (Joabsson & Christensen, 2001; Juottonen *et al.*, 2005). This 'fuelling-effect' of vascular plants was tested in this study by long-term clipping of cushion plants. After cutting off the oxygen supply to the roots, the redox potential dropped well below < 330 mV indicating anoxic conditions in the rooting zone (Fig. 5.5). Consequently, a substantial methane stock built up within a few months in the upper 150 cm fuelled by decomposing roots. After 2 years without oxygen supply, methane stocks exceeded those of *Sphagnum* vegetation (Figs 5.1, 5.6). Part of the built up methane stock may have resulted from decaying roots, especially in the beginning of the experiment.

In contrast, living cushion plants can thoroughly oxygenise the organic peat soil through oxygen leakage from hundreds of root tips per litre soil. Highly decomposed cushion bog peats are likely to consume little oxygen because of low soil temperatures (4–10 °C) (Haraguchi, 1995; Chapman & Thurlow, 1998; Allen *et al.*, 2002) and carbon densities (50 g C l^{-1}). The recalcitrant nature of highly decomposed peats (Chapman & Thurlow, 1998) and low nutrient availability like total P < 0.02 % (Reddy *et al.*, 1999) further reduce oxygen consumption. The aerobic state of the soil prevailed in the upper 120 cm despite a decrease in root density with depth (Figs 5.3, 5.4). At root densities as low as 2–5 tips l^{-1} found in the bottom 10 cm of the rooting zone an oxic state is unlikely to be maintained far beyond the root surface. However, this zone of low root density separates the bulk root surface from methane. We suggest that in the upper profile oxygen release rates are exceeding consumption. The surplus oxygen is transported by infiltrating rain water down the profile where deeper root layers thus receive additional oxygen, next to the *in situ* oxygen leakage. Lateral groundwater flow can convey fairly oxidising conditions beyond the rooting zone. This is indicated by lower methane stocks on the ecosystem level as suggested by low methane concentration in the upper 200 cm below *Sphagnum* patches (Fig. 5.2) and pools (data not shown) in the cushion bog. This is further indicated by methanotrophic activity, which is maximal in the bottom 10 cm of the rooting zone (Figs 5.3b, 5.6). Methanotrophic activity is highest where upward diffusion of methane meets available oxygen (Watson *et al.,* 1997, Edwards *et al.,* 1998). Despite the low root density, enough oxygen is present in the bottom 10 cm of the rooting zone to maintain methane oxidation (Figs 5.4, 5.6), which results in a spatial separation between roots and methane. When soil is aerated by deep drainage, a similar separation of roots and methane results in low or zero emissions (Roulet *et al.*, 1993; Bubier, 1995; Couwenberg *et* *al.*, 2010). A thorough oxygenation of the rooting zone is essential for sufficient separation between roots and methane stock and consequently for complete cessation of methane emission. This has also been suggested by physical models of root–methane interactions (Watson *et al.*, 1997; Segers *et al.*, 2001). Such oxygenation of wetland soils by an extensive and deep root biomass requires sufficient nutrients (Van Bodegom *et al.*, 2005; Koelbener *et al.*, 2010) that pristine bogs usually lack (Van Bremen, 1995; Kleinebecker *et al.*, 2008). Higher nutrients levels also increase oxygen consumption because litter/peat formed under nutrient rich conditions breaks down faster than recalcitrant *Sphagnum* litter from pristine sites (Aerts *et al.*, 1999; Chapin *et al.*, 2003). Incomplete oxygenation because of high soil oxygen consumption then permits the co-existence of roots and methane resulting in methane emissions.

However, cushion plants like *Astelia* sp. and *Donatia* sp. have specific traits allowing them to develop a dense root system while still outcompeting *Sphagnum* at low nutrient levels. These traits are a dense apical growth, high root to shoot ratio, very porous roots, low intrinsic growth rate, being evergreen and having efficient nutrient recycling (Gibson, 1990; Schmidt *et al.*, 2010; Fritz unpublished). Due to the high nutrient use efficiency and a low biomass turnover, a dense root system can be maintained even in the very nutrient poor Patagonian bogs (Kleinebecker *et al.*, 2008; Schmidt *et al.*, 2010). We show that cushion plants can form 2–4 times more biomass of fine roots (3590 g DW m^{-2}) than other bog vegetation (Moore *et al.*, 2002). A similar growth strategy is known from isoetid species growing at the bottom of nutrient-poor softwater lakes. Isoetid species (such as *Littorella uniflora* and *Lobelia dortmanna)* exhibit a dense root system while growing slowly and also oxidise entire mineral soil layers (Smolders *et al.*, 2002). The mutual interaction between plant (traits) and soil conditions warrants further investigation.

Conclusion

From our study, we conclude that under specific circumstances vascular plants are capable to oxidise the bulk of soil methane that might otherwise be released via the root aerenchyma. We highlight an outstanding example of an inverse relation between root density and methane release. The clear spatial separation of methane from cushion plant roots resulted from low oxygen consumption that was exceeded by oxygen loss from roots. The influence of vascular plants on methane cycling depends on traits such as formation of dense root biomass in spite of nutrient deficient conditions. Rising temperatures and habitat losses of cushion plants are expected to stimulate future methane emissions from Patagonian bogs.

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Methanotrophs in Patagonia

Activity and diversity in different *Sphagnum magellanicum* dominated habitats

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* Kip and Fritz contributed equally to the present manuscript.

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Abstract. *Sphagnum* peatlands are important ecosystems in the methane cycle. Methanotrophs living in and on the *Sphagnum* mosses are able to act as a methane filter and thereby reduce methane emissions. In contrast to the Northern Hemisphere, peat ecosystems in temperate South American peat bogs are dominated by one moss species: *Sphagnum magellanicum*. This permitted a species-independent comparison of *in situ* methane concentrations and the corresponding activity and diversity of methanotrophs in different bog microhabitats. Potential methane oxidising activity was found in all *Sphagnum* mosses and a positive correlation was found between activity and *in situ* methane concentrations. Substantial methane oxidation activity (23 µmol CH_4 g^{-1}DW d^{-1}) was associated with *in situ* methane concentrations > 35 µmol CH_4 l^{-1} porewater. Low methane oxidation activity (< 0.5 µmol CH_4 g^{-1}DW d^{-1}) was observed in living *Sphagnum* mosses from lawns and hummocks. Methane oxidation activity was relatively high (> 4 µmol CH_4 g^{-1}DW d^{-1}) in *Sphagnum* litter situated around the water table and rich in methane. The methanotrophic communities were studied using a *pmo*A microarray and a complementary *pmo*A clone library. The methanotrophic diversity was similar in the different habitats of this study and comparable to the methanotrophic diversity found in the Northern Hemisphere peat mosses. The *pmo*A microarray data indicated that both alpha- and gammaproteobacterial methanotrophs were present in all *Sphagnum* mosses, even in those mosses with a low initial methane oxidation activity. The high abundance of methanotrophic *Methylocystis* species in the most active mosses suggests that *Methylocystis* species might be responsible for the bulk of methane oxidation.

Introduction

Micro-organisms play an important role in the biogeochemical cycles of peatlands and the knowledge about the microbial diversity offers tools to improve our understanding of the carbon and nutrient turnover. Productivity of (semi)-aquatic ecosystems including peatlands can be limited by the supply of CO_2 (Smolders *et al.*, 2001; Spierenburg *et al.*, 2010; Patberg, 2011). Methane, produced under water-logged conditions, can become an important carbon source for photosynthesis in peatlands when oxidised to CO_2 by methanotrophic bacteria (Raghoebarsing *et al.*, 2005; Kip *et al.*, 2010; *et al.*, Liebner 2011). The methanotrophs were shown to be present on and inside *Sphagnum* mosses and can act as a filter for methane, thereby recycling carbon and reducing methane emissions. Highest methane oxidation rates have been found in submerged *Sphagnum* mosses from pools, where the mosses are most limited by CO_2 (Raghoebarsing *et al.*, 2005; Kip *et al.*, 2010).

Peat bogs are a harsh environment for microbes to live in because of the low pH (around 4.5) and the low nutrient content. Bacterial and methanotrophic communities have been studied in some *Sphagnum* dominated peat bogs (Dedysh, 2009; Opelt & Berger, 2004). Microbial communities can be investigated with molecular tools based on the bacterial 16S rRNA genes (Stackebrandt & Goebel, 1994), while methanotrophic communities can be characterised using functional genes like the methane monooxygenase genes, *pmo*A and *mmo*X (Murrel & Jetten, 2009). These genes encode sub units of the methane monooxygenase enzyme, which catalyses the first step in the methane oxidation pathway and can only be found in methanotrophs. A fast screening tool to study diversity is a *pmo*A based microarray that can be used to analyse the methanotrophic community of an ecosystem (Bodrossy *et al.*, 2003).

Methanotrophs occur within the *Proteobacteria,* NC10 phylum and the *Verrucomicrobia* (Conrad, 2009; Op den Camp *et al.*, 2009; Ettwig *et al.*, 2010). The recently described, extremely acidophilic methanotrophic members of the *Verrucomicrobia* phylum (Op den Camp *et al.*, 2009) may be well adapted to acid conditions natural to oligotrophic peatlands. Methane oxidising *Gammaproteobacteria* belong to the type I methanotrophs, which use the ribulose monophosphate pathway for formaldehyde fixation. *Alphaproteobacteria* belong to the type II methanotrophs, which use the serine pathway for formaldehyde fixation. This genus includes the *Methylocystis–Methylosinus* genera and the acidophilic methanotrophs of the genera *Methylocella* and *Methylocapsa*. *Methylocella* species were the first isolated facultative methanotrophs (Dedysh *et al.*, 2000) and recently facultative *Methylocystis* and *Methylocapsa* species were isolated as well (Im *et al.*, 2010; Dunfield *et al.*, 2010; Belova *et al.*, 2011). These facultative methanotrophs have been shown to be able to survive periods without methane in the presence of acetate, an important carbon source in peat ecosystems (Belova *et al.*, 2011). Moreover, nitrogen has been suggested to be an important limiting factor for methanotrophic activity and growth in certain ecosystems (Bodelier & Laanbroek, 2004). Several methanotrophic bacteria are capable of fixing nitrogen, allowing them to adapt to the shortage in nitrogen (Dedysh *et al.*, 2004a; Buckley *et al.*, 2008; Khadem *et al.*, 2010) which would be beneficial in peatlands that are naturally deprived in nitrogen (e.g. rain fed bogs; Lamers *et al.*, 2000).

Most studies on methanotrophy in peatlands have been performed in the Northern Hemisphere (Dedysh, 2009), while peatlands in the Southern Hemisphere have so far received less attention. Most South American peatlands can be found in remote areas with limited human influence and differ ecologically from their northern counterparts (Grootjans *et al.*, 2010; Blanco & de la Balze, 2004). In the Northern Hemisphere many different *Sphagnum* species occur that cover the different habitats, such as pool, lawn and hummock (Rydin & Jeglum, 2006). In contrast, many oligotrophic peatlands in Patagonia, southernmost South America, are dominated by a single peat moss species, *Sphagnum magellanicum*, covering the entire range of habitats (mean water levels; Kleinebecker *et al.*, 2007). The presence of only one moss species enables species-independent analyses of different peat habitats. Patagonian bogs also harbour cushion forming vegetation that oxidises methane before reaching porewater and pools at the peat surface (Fritz *et al.*, 2011). Effects of low methane availability on the association between *Sphagnum* mosses and methanotrophs remain yet to be investigated. There are numerous studies that link the presence of cushion forming vegetation (e.g. *Astelia* sp. and *Donatia* sp.) to a low cover or decline of *Sphagnum* mosses (Heusser, 1995; Roig & Collado, 2004; Kleinebecker *et al.*, 2007).

The present study describes methane oxidation activity in *S. magellanicum* of various habitats along a gradient of methane availability in Patagonian bog vegetation at two peatland sites. The methanotrophic communities of *S. magellanicum* mosses at one peatland site were investigated using a *pmo*A microarray and a *pmo*A clone library.

Materials and methods

Study sites description

Field measurements and sampling of *Sphagnum* mosses were performed in two bog complexes in Patagonia, Argentina: a pure *Sphagnum* bog, called 'high CH_4' bog (54° 45′ S; 68° 20′ W; 200 m a.s.l.) and a mixed-cushion bog, called 'low CH_4' bog (54° 58′ S; 66° 44′ W; 40 m a.s.l.). Annual average daily air temperature is 5–6 °C with cold summers around 9 °C. July is usually the coldest month with a mean temperature of 2 °C. Soil temperature was low and stable at both bogs, throughout the growing season decreasing from 8–12 °C at 5 cm below surface to 4–8 °C at 100 cm depth. The *Sphagnum* bog was dominated by *Sphagnum magellanicum* (Brid) with < 1 % cover of vascular plants like *Empetrum* sp., *Nothofagus* sp., *Rostkovia* sp., *Carex* sp., *Marsippospermum* sp. and *Tetroncium* sp. *Sphagnum magellanicum* occupies all hydrological niches from pools to hummocks rising up to 1 m above the summer water table. Other *Sphagna*, like *Sphagnum falcatulum* (*S. cuspidatum* coll.) and *Sphagnum cuspidatum*, only co-exist in pools (Kleinebecker *et al.*, 2007).

Table 6.1 (continued on facing page). Overview of ecological parameters (mean ± 1 SD) obtained at the 'high CH4' bog and 'low CH4' bog. Sampling depths relative to water levels are positive when a sample was taken above the water level (living *Spagnum* moss) and negative when the sample was taken from below the water level (litter and *Sphagnum* moss from pools). Sample depths are indicated as measured at the day of sampling and relative to the mean summer water level. NA indicates no data available. Water content is expressed in % of the wet weight.

Peatland	Microhabitat	Sample depth [cm]	Water level (mean) [cm]	Methane porewater [µmol $CH_4 l^{-1}$]	Water content [%]
'High CH_4' bog	pool	−1	−1	35.4 ± 10.9	96.8 ± 0.2
'High CH_4' bog	lawn	15	25	1.2± 1.0	94.0 ± 0.4
'High CH_4' bog	hummock	50	70	NA	90.7 ± 0.2
'Low CH_4' bog	pool	−1	1	1.4 ±1.5	97.7 ± 0.2
'Low CH_4' bog	lawn	10	8	1.0 ± 0.9	94.8 ± 0.3
'Low CH_4' bog	hummock	30	25	NA	91.5 ± 0.5
'High CH_4' bog	30 cm lawn	−15	−5	111± 56	92.4
'High CH_4' bog	80 cm lawn	−65	−55	407 ± 83	94.6
'High CH_4' bog	80 cm hummock	0	−10	23.1 ± 6.3	92.9

In poor fens, *Sphagnum fimbriatum* can be found next to *S. magellanicum* (Grootjans *et al.*, 2010).

In contrast, the mixed-cushion bog consists of little *Sphagnum magellanicum*. The *Sphagnum* mosses occupy margins of pools and form also small lawns (few square meters) embedded in a matrix of evergreen cushion plants (Roig & Collado, 2004). Dominating cushion plants were *Astelia pumila* (Forster f.) Gaudich and *Donatia fasciculares* Forster and Forster covering > 70 %. The soil below cushion plants is densely packed with tap roots (1–2 mm diameter) and fine roots exceeding depths of 120 cm (Fritz *et al.*, 2011). Cushion plant roots release oxygen at rates that are sufficient to oxidise the bulk of methane produced in deeper layers. Vascular plants were abundant in lawns of *Sphagnum magellanicum* mosses in the mixed-cushion abundantly compared to the *Sphagnum* bog. Porewater was acid in both bogs (pH 3.5–4.5). Studied peatlands remained unaffected by anthropogenic alteration such as drainage, agricultural use or elevated atmospheric nutrient deposition.

Methane availability and emissions

Porewater was sampled at depths of 5–10 cm from pools and lawns prior to collecting moss and litter samples for incubation (more details in Fritz *et al.*, 2011). Anaerobic peat water samples were taken using 5 cm ceramic cups (Eijkelkamp Agrisearch Equipment®, Giesbeek, the Netherlands), connected

Table 6.1 (continued).

Bulk density [g l^{-1}]	Methane emission [μmol C m^{-2} d^{-1}]	pH	Methane oxidation at 10 °C [μmol CH_4 g^{-1} DW d^{-1}]	Methane oxidation at 20 °C [μmol CH_4 g^{-1} DW d^{-1}]	Methane production at 10 °C [μmol CH_4 g^{-1} DW d^{-1}]
13.7 ± 1.6	474 ± 219	4.0–4.3	23.5 ± 20	46.7 ± 39.3	
24.5 ± 1.6	105 ± 100	3.5–4.2	0.22 ± 0.25	0.15 ± 0.10	
26.5 ± 3.3	NA	3.5–4.2	0.01 ± 0.005	0.04 ± 0.03	
19.4 ± 3.0	321 ± 369	3.6–4.0	0.47 ± 0.53	0.43 ± 0.43	
28.3 ± 6.8	351 ± 294	3.4–4.0	0.25 ± 0.38	0.44 ± 0.24	
45.5 ± 2.3	NA	3.4–3.6	0.04 ± 0.001	0.14 ± 0.10	
22.3	NA	4.0–4.5	10.5± 2.8	13.1 ± 1.9	0.02 ± 0.01
24.4	NA	4.3–4.6	5.2± 1.9	10.5 ± 2.6	0.54 ± 0.20
28.4	NA	NA	3.8± 0.4	7.0 ± 0.4	0.01 ± 0.00

to vacuum infusion flasks (40 ml) after sampling 150 ml to exclude internal stagnant sampler-water. For deeper samples, we estimated methane concentrations by adding/subtracting the linear increase (some 11 μmol CH_4 l^{-1} cm^{-1} depth; Fritz *et al.*, 2011) to methane concentrations determined 30 cm below the water level. Additional water samples were drawn with 60 ml syringes. Values of pH were determined one day after collection using a handheld (Consort® C933, Turnhout, Belgium) and a standard pH electrode (SP10T, Consort®, Turnhout, Belgium).

Details on protocols to estimate methane emissions are given in Fritz *et al.* 2011. Briefly, diffusive methane fluxes were measured by using dark static PVC chambers (3700 cm^3, 15 cm high) with bleeds of 4 mm PVC hose. At lawns in the *Sphagnum* bog, two collars were installed (additional sites than reported earlier in Fritz *et al.* 2011) and one collar at pool sites. Gas samples were taken in the morning and in the afternoon on the same day at all sites per peatland. Sampling took place in December 2008 (spring), February 2009 (summer) and late March 2009 (autumn) allowing for some variability in the water level. Gas samples where taken with a double sided needle during 60 minutes in 20 minute intervals in pre-vacuumed 12 ml glass vial with butyl stopper (Exetainer®, High Wycombe, UK). Water content and bulk density at cushion plants sites were determined by sampling carefully the upper 5 cm of mosses in a 10 cm × 10 cm box. Remnants of vascular plants were excluded. Deeper samples were taken using a D-Section corer (ID 4.7 cm; Eijkelkamp Agrisearch Equipment®, Giesbeek, the Netherlands). The samples were dried at 70 °C for 48 h.

Samples of mosses were taken in December 2007, March 2008 and April 2008. Litter samples (dead plant material forming peat) were taken in April and December 2008. For all the samples, the depth below the water table and below the surface on the day of sampling was measured. The depth below surface served as the reference for the depth of the sample below mean summer water table. Negative depths stand for submerged samples (Table 6.1).

Methane oxidation and production tests

Whole *Sphagnum* mosses were thoroughly washed and incubated in 120 ml bottles with 1 ml of methane. Methane was measured on a Hewlett–Packard model 5890 gas chromatograph equipped with a flame-ionisation detector and a Porapak Q column (80/100 mesh). Methane production tests were performed in 60 ml bottles containing only dinitrogen gas. Methane oxidation tests in the dark were performed at 10, 15 and 20 °C.

Molecular analysis

Sphagnum mosses were washed with sterile demineralised water after sampling and kept frozen at –20°C. Genomic DNA isolation, *pmo*A microarray and mmoX analysis was performed as described before (Kip *et al.*, 2010). For the clone library, the PCR amplification of the *pmoA* gene was performed with two general *pmoA* gene primers: A682R and A189F (Holmes 1995). All PCRs were performed in a gradient from 50 to 60 degrees and PCR products were combined. All PCR products were purified using the QIAquick PCR Purifications Kit (Qiagen). DNA sequencing was performed with the primers used in the PCR. The *pmoA* gene primers used were specific only for known methanotrophic A*lpha*- and *Gammaproteobacteria* and were not expected to hybridise with the *pmoA* gene of methane-oxidising *Verrucomicrobia*. Therefore we also designed a new primer set based on the *pmoA* 1 and *pmoA*2 of all three verrucomicrobial methanotrophs (Op den Camp *et al.*, 2009):

VpmoA216: 5'-GGAAAGAymGrATGTGGTGGCC-3' (forward) and VpmoA622: 5'-GTTTCnACCATnCGnATrTAyTCAGG-3' (reverse). Initial validation using a pure culture of *Methyloacidiphilum fumarolicum* SolV resulted in a product of the expected size.

The pGEM-T Easy Vector System Kit (Promega) was used for ligation of the *pmoA* gene amplifications. Ligation was performed as prescribed by the manufacturer. *pmoA* gene ligation mix was transformed by heat shock exposure to XL-1 Blue competent *E. coli* cells, constructed as described by Inoue *et al.* (1990). pDNA with ligated *pmoA* gene was isolated with the E.Z.N.A.™ Plasmid Miniprep Kit (EZNA™). The *pmoA* gene sequences were sequenced with M13 forward and reverse primers (Invitrogen™), targeting vector sequences adjacent to the multiple cloning site. pDNA sequencing was performed by the sequencing facility of the UMC Sint Radboud, Nijmegen. Clone library sequences and their closest relatives were analyzed using MEGA version 4 (Tamura *et al.*, 2007). All sequences were aligned automatically using the alignment tool of MEGA4 (ClustalW). Phylogenetic trees were calculated using the Neighbour-joining method. The sequences of the pmoA gene clones were deposited in the Genbank database under accession numbers JF907375- JF907390.

Statistical analysis

Regression analyses (linear model) were performed in 'R' software packages 2.92 (R Development Core Team, 2009) followed by model justification procedures. We log-transformed potential methane oxidation rates before using the regression analysis. Multiple regressions were carried out starting with all environmental factors (Table 6.1) and subsequently simplified until an adequate model was reached (stepwise backward deletion). We included only methane oxidation rates found in living *Sphagnum* stands ($n = 6$). Models exhibited well-spread residuals despite the low number of measurements.

Results and Discussion

Methane oxidising activity

Initial methane oxidising activities of *Sphagnum* mosses varied between the two peatlands and microhabitats (i.e. water level; Table 6.1). The highest activity (23.5 μmol CH_4 g^{-1}DW d^{-1}) was found in mosses collected from methane rich pools (35 μmol CH_4 l^{-1}). Low methane oxidising activity was found in *Sphagnum* mosses from aerated habitats like hummock and lawns that were typically depleted in methane (e.g. < 2 μmol CH_4 l^{-1}; Fig. 6.1). Methane oxidation rates at 20 °C were twice as high as rates at 10 °C, resulting in a Q10 of around 2. Methane oxidising activity was low (0.5 μmol CH_4 g^{-1}DW d^{-1}) in methane-depleted pools from the 'low CH_4' bog. Such low rates were similar to rates found at drier habitats like lawns and hummocks (Table 6.1). Our

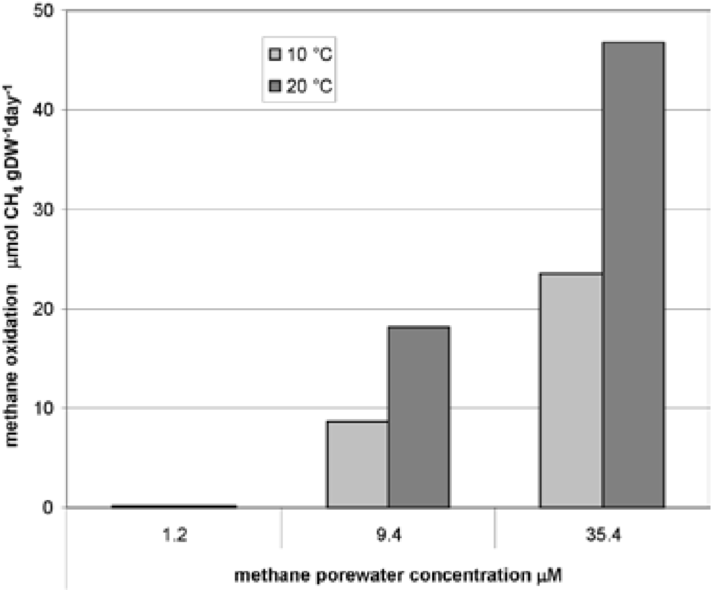

Figure 6.1. Methane oxidation rates were correlated to methane porewater concentrations of the pool samples 'high CH_4' bog. Initial methane oxidation was measured at two different temperatures: 10 °C (light grey bars) and 20 °C (dark grey bars). For SD see Table 6.1.

results indicate a significant correlation between the methane concentration in the porewater and the initial methane oxidation rates in the tested mosses. Methane porewater concentration could explain 68 % (df = 3, $P < 0.01$, $F = 60.5$) of the variation found in the potential methane oxidation rates along the different micro habitats of *Sphagnum magellanicum* (Fig. 6.1). Adding water level as a factor to the model increased the explained variation by 26 %. Also submerged litter samples showed substantial activities. Some studies have suggested a unimodal relationship between methanotrophic activity and water level with highest rates in the vicinity of the water-atmosphere interface (Watson *et al.*, 1997; Edwards *et al.*, 1998). However, in the present study, methane availability was the most important driver of the methanotrophic activity, which was best demonstrated by a lack of methanotrophy in the pools of the 'low CH_4' bog. Kip and co-workers (2010) showed a high variation of methanotrophic activity in *Sphagnum* mosses sampled at the height of the water table. The observed variability may have been caused by fluctuations in methane availability.

Since submerged *Sphagnum* mosses showed the highest methane oxidising activity, we measured oxidation and production of methane in *Sphagnum* litter (dead plant material forming peat) below the water level (Table 6.1). In these subsurface samples, both potential methane oxidation and methane production were found at high porewater methane concentrations. The potential methane oxidation rates of *Sphagnum* litter were substantial (3.8 to 10.5 μmol CH_4 g^{-1}DW d^{-1}). Even at greater depths (55 cm below the water table), potential methanotrophic activity was found. Measured methane oxidation rates are not representative *in situ* rates, due to the high oxygen concentration during incubation. Potential methane oxidation has also been found in litter samples (peat) that were anoxic under field conditions in a bog in the UK (Edwards *et al.*, 1998). Seasonal water level fluctuations influence methanotrophic and methanogenic activities (Lai, 2009). Methanogenic archaea, for example, recover slowly after exposure to oxic conditions following lower water levels (Edwards *et al.*, 1998). Similarly, we found that potential methane production rates well below the water table (55 cm) were 1–2 orders of magnitude higher than rates from samples collected in direct vicinity of the water table (Table 6.1). At a depth of 55 cm below the water level, *Sphagnum* litter has the potential to produce and consume methane depending on the availability of oxygen. The production and consumption activities at the same depth indicate the co-existence of contrasting micro-sites: It seems that co-existing oxic and anoxic conditions form contrasting microbial communities while there is not a real fixed aerobic-anaerobic interface. Microbial activity within a peat ecosystem is therefore not restricted to a fixed surface (e.g. opposing methane-oxygen gradients in a rice field; Lüke *et al.*, 2010). Evidence for co-existing microsites was also provided by in situ redox measurements. Depth profiles from Patagonian *Sphagnum* peats suggested high redox potential values close to oxic conditions (E_7 200–300 mV) whereas methane was abundant in porewater.

Methanotrophic activity in anoxic peat seems to be contradicting. In water saturated peatlands, oxygen usually penetrates less than 10 cm below the water table (Frenzel & Karofeld, 2000) coinciding with a steep drop in redox (Lloyd *et al.*, 1998). Input of oxygen into the system can come from severe water level drops, heavy rainfall events or temperature driven buoyancy flow (Rappoldt *et al.*, 2003). Wet *Sphagnum* peat decomposes slowly resulting in wide pores for infiltrating rain in Patagonia (Kleinebecker *et al.*, 2008; Price & Whittington, 2010). The slow decomposition causes a low oxygen demand (below 200 µmol O_2 l^{-1} soil d^{-1}, unpublished results), which can result in high redox potentials in *Sphagnum* peat under waterlogged conditions. Therefore, oxygen may episodically be present at depths below the water table enabling methanotrophs to remain viable and become active as soon as oxygen happens to enter at these depths (Roslev & King, 1995).

Induced methane oxidation in lawn and hummock mosses

Methanotrophic activity increased upon prolonged incubation in *Sphagnum* mosses from lawn and hummock with low initial activity. Although activity was still low compared to methane rich habitats, lawn mosses improved rates of methane oxidation substantially within 10–15 days while hummock mosses showed a small increase in activity after 22 days. Figure 6.2 shows an exponential increase in methane consumption, indicating a growth of methanotrophic bacteria. These data show that the flexibility of these methanotrophic species is large with a relatively fast response to more favourable conditions. Methanotrophs present in the lawn mosses reacted faster to the methane provided than those in hummocks. Under field conditions, water levels can reach lawn mosses increasing methane availability substantially in contrast to year-round dry hummocks. Activity of methanotrophic bacteria at low methane background concentrations was not studied. Latter may provide insights in the abundance of high affinity methanotrophs, which would be more common in methane depleted habitats like hummocks.

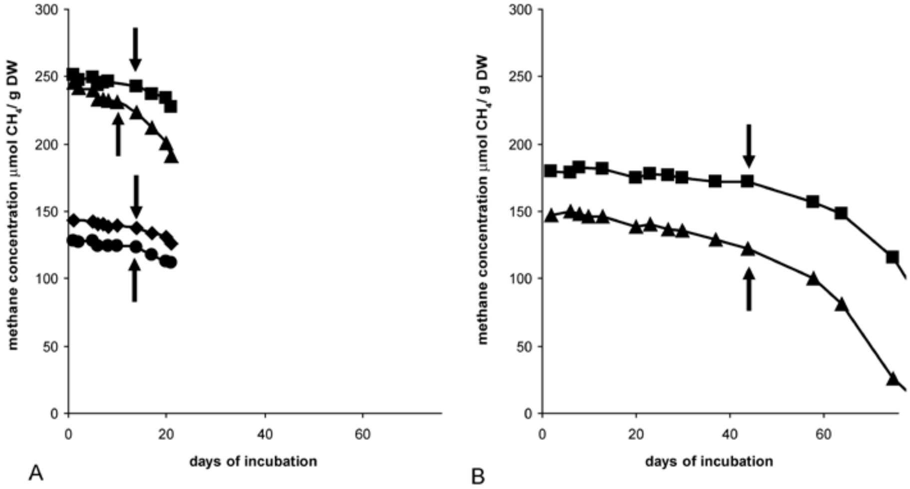

Figure 6.2. Methane consumption by Sphagnum mosses from different micro-habitats. Left panel (A) shows *Sphagnum* mosses from ‘high CH_4’ bog. Closed square and closed triangle: two different moss samples from the lawn micro habitat (average rates: 1.0 ± 1 [2–8 days] and 3.1 ± 1.7 [14–17 days] CH_4 $g^{-1}DW$ d^{-1}) closed diamant and closed circle: two different moss samples from the hummock micro habitat (average rates: 0.41 ± 0.0 [0–14 days] and 1.59 ± 0.0 [17–21 days] CH_4 $g^{-1}DW$ d^{-1}). Right panel (B) shows *Sphagnum* mosses from ‘low CH4’ bog. Filled square and filled triangle: two different moss samples from the lawn micro habitat (average rates 0.6 ± 0.2 [0–22 days] 1.1 ± 0.4 [23–64 days] 2.2 ± 0.8 [44–90days] CH_4 $g^{-1}DW$ d^{-1}).

Methane emission

Methane emission rates of both peatlands were generally low, reflecting high methanotrophic activity and a slow carbon turnover. Emission rates were found to be the highest in pools (> 300 µmol CH_4 m^{-2} d^{-1}) in the *Sphagnum* bog (‘high CH_4’ bog) coinciding with highest methane porewater concentrations (Table 6.1). Emissions were substantially lower when surface peat was depleted in methane. At low emission sites water levels were well below the surface facilitating the oxygenation of the surface peat resulting in nearly complete methane oxidation.

Lowest emissions were found in the lawn of the *Sphagnum* bog (‘high CH_4’ bog), which revealed a net consumption of 5.1 µmol CH_4 m^{-2} d^{-1} and 252 µmol CH_4 m^{-2} d^{-1} at two sampling occasions. At that lawn site, average water table was 5.5 cm deeper and vascular plant cover was extremely low (50–120 shoots m^{-2}) compared to the lawn in the ‘low CH_4’ bog (500–800 shoots m^{-2}). In the pool of the ‘high CH_4’ bog, the surface methane concentration was approximately 30 times higher than in the lawn, but emissions were only 4.5 times higher. This difference can be explained by methanotrophic consumption, which significantly reduced methane emissions to the atmosphere. The layer of potential methane oxidation is much thicker at lawn sites, starting from the highly active methanotrophic *Sphagnum* litter to a low activity by methanotrophs in the *Sphagnum* mosses in the aerated part. Therefore, lawns of *Sphagnum* mosses succeed to oxidise the bulk of methane produced in deeper layers like shown for Northern bogs (Frenzel & Karofeld, 2000; Daulat & Clymo, 1998). Methane emission is dependent on production, consumption and transport types, like diffusion, ebullition or vascular plant transport (Frenzel & Karofeld, 2000; Kutzbach *et al.*, 2004). The effectiveness of methanotrophs seemed to increase upon the low density of vascular plants that can create a shortcut between methane containing peat and the atmosphere. We conclude that methane oxidation associated to *Sphagnum* mosses is important at the ecosystem scale. The fraction washed off from the mosses before incubating revealed quasi zero rates of methane oxidation (Kip unpublished data). It is therefore likely that oxidation rates of washed mosses are similar to *in situ* unwashed mosses and peat samples, respectively (Freitag *et al.*, 2010; Fritz *et al.*, 2011).

***pmo*A microarray**

The *pmo*A microarray used in the present study represents *pmo*A gene sequences of all cultured and non-cultured methanotrophs known (Bodrossy *et al.*, 2003). *pmo*A PCR products were obtained from the pool and hummock samples and the litter samples of lawn (–55 cm below the water level) and hummock (–10 cm below the water level) from the 'high CH_4' bog. All the samples showed a rather similar methanotrophic community, as revealed by a similar hybridisation pattern on the microarray of the different samples (Fig. 6.3). The total *pmo*A diversity of the *Sphagnum* mosses was very high compared to other studies using the same microarray on peat soils (Chen *et al.*, 2008a), peat based upland soils (Cébron *et al.*, 2007), and rice fields (Vishwakarma *et al.*, 2009), but quite similar to the methanotrophic communities in *Sphagnum* mosses from other peat bogs around the world (Kip *et al.*, 2010). The microarray results showed abundance of both type I (*Gammaproteobacteria*) and type II (*Alphaproteobacteria*) methanotrophs and the presence of many different species.

The pool *Sphagnum* mosses showed a higher hybridisation with the type II probes over type I. The pool mosses and litter samples showed a similar hybridisation pattern indicating a similar methanotrophic community at the surface compared to the one below the water table. The type II methanotrophs seem to be more abundant in the litter samples from lawn and hummock than in pool mosses. The type II probes showed that both *Methylocystis* ssp. and *Methylosinus* ssp. were present in all the *Sphagnum* mosses, although the hummock *Sphagnum* mosses showed a much lower abundance and also a lower methane oxidation activity compared to all other samples. This indicates that these species may be largely responsible for the observed methane oxidation. A similar result was found for *Sphagnum* mosses in a Canadian bog (Kip *et al.*, 2010). A recent study on *Sphagnum* associated methanotrophs in peat ecosystems in Finland could also relate methane oxidising activity to the presence of a certain *Methylocystis* species (Larmola *et al.*, 2010).

The microarray also showed a strong signal with the gamma-proteobacterial probes targeting the genera *Methylomonas*, *Methylobacter* and *Methylomicrobium*. A broad diversity of *Methylomonas* and *Methylobacter* was detected. The specific probes, which target the type Ib (type X) methanotrophs (including the thermotolerant and thermophilic gammaproteobacterial genera *Methylococcus*, *Methylothermus* and *Methylocaldum* as well as environmental clades) showed a weaker signal, but still indicated a diverse community across the analyzed samples. So far, no acidophilic gammaproteobacterial methanotrophs have been isolated from any acidic ecosystem, which indicates that these are most probably new methanotrophic species.

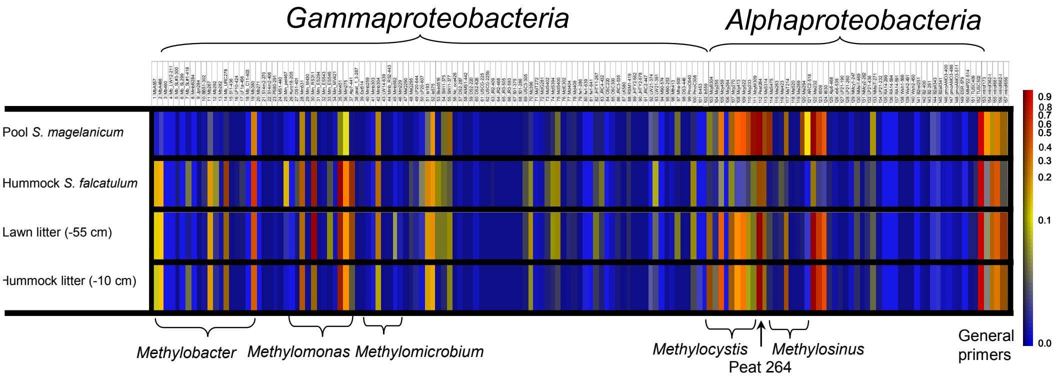

Figure 6.3. Representation of the results for the *pmo*A based microbial methanotrophic community analysis using a *pmo*A microarray. All samples originate from the 'high CH_4' bog. Colour coding bar on the right side represents obtainable signal for an individual probe (1 indicates maximum signal obtained, 0.1 indicates 10 % signal, i.e. only 10 % hybridisation to that probe and 0 indicates no signal).

As demonstrated above, the viability of the methanotrophs inside hummock and lawn mosses was indeed shown after a longer incubation time (Fig. 6.2). The microarray confirms the presence of methanotrophs in the hummock which showed very low initial methane oxidation. Unfortunately, there was not enough PCR product from the lawn mosses to perform the microarray. The methanotrophs detected in the hummock mosses could be present in a dormant state, waiting for the right growth conditions to come along.

Methylocella ssp., which are commonly found in Northern peatlands (Dedysh *et al.*, 2000; Dedysh *et al.*, 2004a) and the recently discovered *Methyloferula stellata* (Vorobev *et al.*, 2010) are the only known methanotrophs that do not have a *pmo*A gene and are therefore not detected in this microarray. These genera do have a soluble methane monooxygenase enzyme and part of the encoding gene (mmoX) can be used for phylogenetic analysis. Detection of *Methylocella* ssp. by screening with *mmo*X based PCR on the two pool samples did not result in any PCR product (Kip *et al.*, 2010), indicating *mmo*X-possessing methanotrophs are not abundantly present or that the PCR primers are not suitable. We designed new primers based on currently available verrucomicrobial sequences (Op den Camp *et al.*, 2009) but were not able to obtain any PCR product in the present study.

Community analysis of methanotrophs

A clone library was made from the *Sphagnum* pool mosses of 'high CH_4' bog: 14 clones were belonging to the *Alphaproteobacteria* and two clones belonged to the *Gammaproteobacteria* (Fig. 6.4). 13 clones showed the highest homology to *Methylocystis* ssp. (> 92 %, DNA homology), one clone showed the highest homology to *Methylosinus sporium* and two clones showed 80 % homology to isolated *Methylomonas* ssp. based on DNA sequences. These clones showed 87 and 88 % homology to an mRNA based environmental clone (EF644619) from *Sphagnum* peat soil from the United Kingdom (Chen *et al.*, 2008).

The *Methylocystis* related clones cluster phylogenetically together with the facultative *Methylocystis echinoides, Methylocystis heyeri* H2t, *Methylocystis* ssp. H2s and *Methylocystis* ssp. SB2 (Im *et al.*, 2010; Belova *et al.*, 2011). These species are not completely dependent on methane as a carbon source, which can be a great advantage under methane-deprived conditions in ecosystems. The abundance of *Methylocystis* ssp. in this *pmo*A clone library (13 out of 16 clones), is in line with other studies from peatlands (Dedysh, 2002; Chen *et al.*, 2008b). The two gammaproteobacterial clones might indicate the presence of a new species in this ecosystem. None of the gammaproteobacterial methanotrophs that were isolated in pure culture are able to grow below pH 5.5 (Bowman, 2006), while they have been found in other peat ecosystems using molecular tools (Kip *et al.*, 2010; Chen *et al.*, 2008a). Bacterial and methanotrophic communities in peatlands from the Southern hemisphere were comparable to those on the Northern hemisphere (16S rRNA clone library analysis

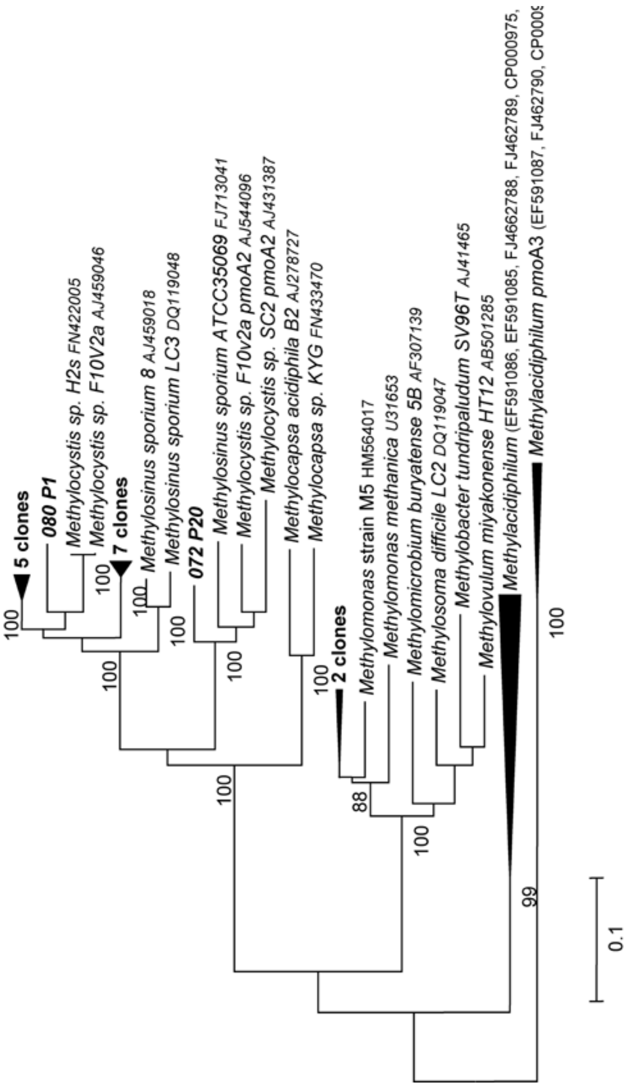

Figure 6.4. *pmo*A gene phylogenetic tree based on DNA showing the relationship of the clones to selected methanotrophs. The tree was based on Neighbour-Joining analysis using a bootstrap test of 500 replicates. Bootstrap values of > 60 are indicated at the node of the branch.

in Kip, 2011). The same families and genera were found, but it does not exclude the presence of new strains or species. In general, the low number of clones obtained in the *pmo*A clone library of the present study limits interpretation.

Adaptation of *Methylocystis* species to harsh conditions in bogs

In general, methanotrophs are believed to be obligate methanotrophs. However, several recent isolates have been shown to be able to metabolise additional carbon sources in the absence of methane (Belova *et al.*, 2011; Dedysh *et al.*, 2005). Using two different molecular tools, the presence of *Methylocystis* species has been indicated in *Sphagnum* mosses and litter in the present study. Several *Methylocystis* species are facultative methanotrophs and have been recently shown to be able to grow or survive on acetate (Im *et al.*, 2010; Belova *et al.*, 2011), which would be a great advantage in a peat ecosystems since methane and acetate are generally both present, although with fluctuating concentrations which often follow water level fluctuations. These *Methylocystis* species may be able to live on acetate at times of insufficient methane supply, while other obligate methanotrophs have to survive using different strategies like forming spores or capsules (Whittenbury *et al.*, 1970; Bouman *et al.*, 1993; Rothfuss *et al.*, 1997). Another advantage of several *Methylocystis* species is the presence of two different *pmo*A enzyme complexes which have different affinities for methane (Baani & Liesack, 2008). Most cultivated methanotrophs harbour only a one single type of methane-oxidising enzyme complex, enabling growth under high or low methane concentrations and this seemed to be species specific, until the *pmo*A2 gene was found in *Methylocystis* and *Methylosinus* species. The presence of *Methylocystis* species can result in flexible responses of the ecosystem to fluctuating methane levels.

Our study suggests that methanotrophic bacteria are viable and effectively reducing methane emissions under high, low and zero methane conditions and also during transitions between these conditions. Another important survival strategy of great importance in these pristine peat ecosystems with low nitrogen deposition is the ability to fix nitrogen (Hemond, 1983). Many methanotrophs are able to fix nitrogen (Dedysh *et al.*, 2004b; Bowman, 2006) and almost all *Methylocystis* species have been shown to contain the *nif*H gene, which codes for the dinitrogenase reductase. In general, this enzyme is oxygen sensitive, but some methanotrophs, e.g. *Methylocapsa* were shown to be able to fix nitrogen under complete aerobic conditions (Dedysh, 2004b). *Methylocystis* related species were shown to actively fix nitrogen *in situ* in soils (Buckley *et al.*, 2008). Nitrogen availability in Patagonian bogs (Schmidt *et al.*, 2010;

chapter 3) may be too low to sustain methanotrophic growth and therefore nitrogen fixation might be a necessity.

Conclusion

This study revealed a high activity and diversity of methanotrophic bacteria associated to the dominating moss, *Sphagnum magellanicum*, in Patagonian bogs with low methane emissions. In methane rich pools, highest methane oxidations rates were found in *Sphagnum* mosses, which consequently can benefit from extra carbon dioxide produced by methanotrophs while mosses provide extra oxygen via photosynthesis. In contrast, methane depleted pools and lawns revealed very low activity of methanotrophs probably lowering the carbon supply under high water contents typically found in moss tissue under submergence.

The high abundance of *Methylocystis* ssp. in the most active mosses and their low abundance in hummock mosses indicate that this group is responsible for a large part of the observed methane oxidation. *Methylocystis* ssp. maybe be highly successful in peatlands because of functional traits like using acetate as a carbon source, fixing nitrogen and being able to consume methane at both high and low concentrations (Buckley *et al.*, 2008; Belova *et al.*, 2011). Future work should incorporate isolation to test if *Methylocystis* ssp. present in Patagonian bogs are indeed capable of the above mentioned traits. Nitrogen fixation by methanotrophs may be an important nitrogen source in Patagonian bogs that are generally nutrient-deprived (chapter 3).

The microarray showed the presence of methanotrophs in the hummock mosses and the diversity of methanotrophs was surprisingly comparable between almost all the samples considering the origin of all the different micro habitats where methane and oxygen concentrations are very different. Activity of methanotrophs in *Sphagnum* was previously shown to differ between *Sphagnum* species (Larmola *et al.*, 2010). This variation may be related to anatomical differences of mosses or their release of organic compounds such as phenols, amino acids, carbohydrates, hydrocarbons and fatty acids (Fenner *et al.*, 2004) which could stabilise (facultative) methanotrophic communities during low methane conditions. The cooperation between methanotrophs and *Sphagnum* species make the ecosystem flexible since methanotrophs are able to adjust to changing conditions, like shifts in methane or oxygen concentrations. More research is needed to understand this plant-microbe interaction and their role in the regulatory ability of peatlands.

How *Astelia* bogs down *Sphagnum*

A synthesis

Christian Fritz

Introduction

Peat forming *Sphagnum* mosses have evolved a unique set of plant traits that facilitate high retention of water and nutrients (Clymo & Hayward, 1982; chapter 2). Therefore, *Sphagnum* is highly efficient in outcompeting vascular plants as long as nutrient availability is low and rainfall is sufficient (Clymo & Hayward, 1982; Van Bremen, 1995; Rydin & Jeglum, 2006). It is widely accepted that in bog ecosystems, growth of vascular plants is limited by the low input of (airborne) nutrients (Verhoeven, 1996; Lamers *et al.*, 2000) and by the low efficiency of roots due to anoxia resulting from water-logged conditions (Laanbroek, 1990; Armstrong *et al.*, 1991). Vascular plants become more abundant when nutrient availability increases (Malmer *et al.*, 2003; Limpens *et al.*, 2004; Tomassen *et al.*, 2004; Wiedermann *et al.*, 2009b), for instance, due to high atmospheric nitrogen deposition. Similarly, the growth and efficiency of roots improve when the stress from anoxia is reduced by lower water levels, especially since lower water levels also enhance mineralization and thus the nutrient availability in the root zone. Lower availability of water also favours vascular plants by reducing the competitive strength of *Sphagnum* mosses that prefer wet conditions (Rydin & Jeglum, 2006; Rice *et al.*, 2008). In Patagonia, however, my co-workers and I found that vascular plants remained unaffected by the addition of nitrogen and phosphorus, even after three years of fertilisation (chapter 3). Apparently, the conditions in southern hemispheric bogs (hereafter southern bogs) are so hostile that most vascular plants cannot easily win the competition with *Sphagnum magellanicum* (chapter 3).

Yet, southern bogs are often dominated by a special type of vascular plants: cushion-forming plants and restiad plants (Restionaceae), even under pristine conditions (Gibson & Kirkpatrick, 1985; Squeo *et al.*, 2006; Kleinebecker *et al.*, 2007; Hodges & Rapson, 2010). These plants are the main peat-formers in (temperate) Southern Hemisphere bogs, in contrast to Northern Hemisphere bogs where *Sphagnum* species take that role (Heusser, 1995; Kuder *et al.*, 1998; Turetsky, 2003; Rydin & Jeglum, 2006; Hodges & Rapson, 2010; Teltewskaya 2010). Cushion bogs cover a substantial part of the wetlands in the Southern Hemisphere (Yu *et al.*, 2010). In Patagonia, cushion plant dominated peatlands (i.e. cushion bogs, also called 'Magellanic moorland') are the most prominent peatland type. Here, *Sphagnum* is outcompeted by the vascular plants (Godley, 1960; Kleinebecker *et al.*, 2007). For instance, many bogs that used to be dominated by *Sphagnum* sp. are now invaded by *Astelia pumila*, a prominent peat-forming cushion plant (Fig. 7.1; Ch. Fritz personal observations in five Patagonian bogs; Heusser, 1995; Teltewskaya, 2010).

The present thesis concerns factors that favour cushion plants in the competition with *Sphagnum* mosses. In general, these factors may either work 'for' cushion plants or work 'against' *Sphagnum*. Nutrient availability or lower water levels supposedly promote extra growth of cushion plants. Alternatively, *Sphagnum* growth may be impeded by adverse growth conditions such as desiccation, lack of nutrients, low CO_2 availability, or toxicants like ammonium.

The insights on the functioning of cushion bogs presented here, are largely based on observations in cushion plant stands dominated by *Astelia pumila,* with a proportion < 30 % of other cushion plants such as *Donatia fasciculares*. Findings for *Astelia pumila* may or may not be applicable for *Donatia fasciculares*, but at least with respect to nutrient conditions, *Donatia fasciculares* and *Astelia pumila* are quite similar (Schmidt *et al.*, 2010). Additionally, all cushion plants are morphologically very much alike (e.g. dense growth, short leaves, long roots). For instance, the appearances of *Astelia* sp. and *Donatia* sp. in New Zealand and Tasmania resemble those of *Astelia pumila* and *Donatia fasciculares* in Patagonia (Gibson & Kirkpatrick, 1985; Ch. Fritz personal observation).

Roots – the main feature of cushion bogs

The success of cushion plants seems to be founded in their ability to form sufficiently long roots with which they can oxidise the water-saturated peat soil entirely. Such a high extent of soil oxygenation was not reported before and remains un-rivalled by other vascular bog plants. Chapter 5 provides multiple pieces of evidence for the large oxygenation capacity of cushion plants, such as thorough methane oxidation, redox potentials above 350 mV and free oxygen below the water table. The large root biomass establishes oxic conditions that enable the roots to survive longer resulting in a lower turnover of root biomass. However, the formation of long roots is a viable strategy restricted to weakly re-

Figure 7.1. Cut through dense cushion plant vegetation dominated by *Astelia pumila*, which invaded former *Sphagnum* vegetation (reddish peat).

Figure 7.2. Severe desiccation of *Sphagnum magellanicum* vegetation by the strong winds in Patagonia. Mosses surrounded by cushion plants showed white coloration despite water levels close (< 10 cm) to the surface. Potential evaporation rates were on average higher in the Moat cushion bog compared to the *Sphagnum* bog in Andorra (Table 1.1.).

ductive soil substrates with a low oxygen consumption (Armstrong & Boatman 1967; Armstrong *et al.*, 1991). The amount of oxygen lost to the surrounding soil should at least equal the amount that is consumed by the aerobic metabolism of soil (micro)biota. I hypothesise that low nutrient concentrations slow down decomposition and consequently oxygen consumption rates, which reduces the risk of root mortality due to anaerobiosis.

Aerobic incubations of peat from *Sphagnum* and cushion plants revealed low decomposition rates (Fig. 7.3), which were an order of magnitude lower than those found in studies in northern peatlands (Turetsky, 2004; Geurts *et al.*, 2009). Such low mineralization rates may be caused by particularly low nutrient concentrations (e.g. < 0.4 mg P g^{-1}DW) at which microbial activity is often hampered (chapter 5). Aerobic decomposition rates significantly decreased at lower contents of phosphorus and nitrogen in bog peat samples from Patagonia ($n = 31$, $P < 0.01$; $r^2 = 0.52$; linear regression). Phosphorus limitation on the community level, as found in chapter 3, may thus also limit microbial processes. The limiting role of phosphorus has been found in *Sphagnum* peats from Ireland (Tomassen *et al.*, 2004). Thus, only under very oligotrophic conditions, cushion plants may be able to maintain their characteristic long roots.

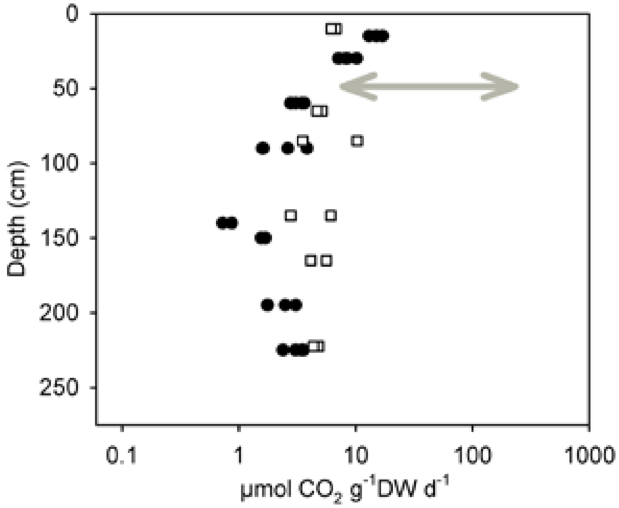

Figure 7.3. Aerobic decomposition rates (log scale) along peat profiles dominated by cushion plant peat (filled circles) and by *Sphagnum magellanicum* peat (open squares), respectively. The grey arrow indicates decomposition rates found in peatlands in Canada and Europe. Incubation temperature was 22 °C.

Their large oxygenation potential enables the roots of cushion plants to penetrate at least the upper first metre of the peat, which provides them access to nutrient stocks that are much larger than atmospheric supplies. As a consequence, cushion plants are able to profit from a much larger nutrient pool in the peat matrix than *Sphagnum* species. Important to note is that the atmospheric deposition of nutrients was generally low and comparable amongst the bogs studied (chapters 1 & 3).

Harsh hydro-climatic conditions lower the growth of *Sphagnum*

Sphagnum grew on average 15 mm y^{-1} ± 0.28 SE in the sheltered control bog (Andorra) (chapter 3). In the coastal cushion bog (Moat), *Sphagnum* growth appeared to be lower, namely 5–15 mm y^{-1}. Compared to *Sphagnum* species, cushion plants seem to have a low aboveground growth rate (Gibson, 1990; Gerdol, 1995; Mark & Wilson, 2005), however no data are available for *Astelia* sp. and *Donatia* fasciculares. My estimates for height increment of *Astelia pumila* are around 10 mm per year. In contrast to *Sphagnum*, which shows a vertical growth strategy under optimal conditions, cushion plants grow clonally and show a strong horizontal expansion (Mark & Wilson, 2005). For this reason, cushion plants can only establish and outcompete *Sphagnum* by shading when the vertical growth of *Sphagnum* is depressed.

Especially in coastal areas, *Sphagnum* tends to lose the competition with cushion plants. Exposure to harsh winds is a dominant feature of open coastal peatlands. Growth of *Sphagnum* mosses is certainly reduced by low water supply (desiccation) irrespective of nutrition and carbon supply (Clymo, 1973; Schipperges & Rydin, 1998; Robroek *et al.*, 2009; discussion in chapter 3). Desiccation of the capitula can be induced by the strong winds. Patagonia's westerly winds and a low humidity result in high evaporation rates (Paruelo, 1998; chapter 1). When the evaporation exceeded the rates of upward water transport (Clymo, 1973; Cagampan & Waddington, 2008), I observed desiccation of the *Sphagnum* mosses as indicated by the white coloration of the *Sphagnum* mosses (Fig. 7.2). Also other studies used white coloration of mosses as a diagnostic tool for growth inhibition related to water stress (Bragazza, 2008 and literature therein). White coloration (i.e. water contents < 5 gFW g^{-1}DW) usually occurs when the water table drops below 20–30 cm of the optimum of a particular *Sphagnum* species (Titus & Wagner, 1984; Rydin & Jeglum, 2006; Robroek *et al.*, 2007). In contrast, I frequently observed desiccation of mosses in coastal areas, even at water levels less than 10 cm below the surface (Fig. 7.2). In inland bogs, *Sphagnum* revealed a higher water holding capacity. I observed higher water contents (12 gFW g^{-1}DW, SD 0.3; $n = 5$) at even lower water levels (20 cm below surface). The large desiccation potential of coastal winds may be the main cause for these small-scale water losses at the moss surface.

Hummocks (e.g. *Sphagnum* stands > 20 cm above the water level) provide further evidence for the importance of desiccation in coastal bogs. In windy cushion bogs, *Sphagnum* hummocks were absent in the open and wind-exposed parts of the peatland whereas they were found in sheltered conditions, for example close to tree stands. This relationship was found in six cushion bogs and partly reported by Grootjans *et al.* (2010). Also paleoecological studies suggest desiccation as a mechanism limiting the growth of *Sphagnum* bogs: Dry surface conditions probably preceded an invasion of cushion plants. Dry surface conditions were indicated by a *Empetrum* dominated vegetation (Heusser, 1995; Teltewskaya, 2010).

Coastal peatlands in Patagonia experience cold summers with daily average temperatures < 10 °C (Gustafson 1997, Schneider *et al.*, 2003, Ch. Fritz & R. Iturraspe unpublished data). Low summer temperature reduces the rate of photosynthesis in *Sphagnum* mosses (Harley *et al.*, 1989) and also increases the risk of damage by photo-inhibition at a high solar radiation (Murray *et al.*, 1993; chapter 3). Cold snaps occur frequently during the growing season (Fig. 7.4) when peatlands are exposed to southerly winds (Schneider *et al.*, 2003). The growing season can exceed 300 days as winters are relatively mild, with the average temperature of the coldest month still exceeding 3 °C (Tuhkanen *et al.*, 1990). A long growing season is beneficial for evergreen plants like *Astelia pumila* and *Nothofagus betuloides*. During winter, seaborne airmasses are relatively warm. These mild winters at the sea prevent severe frost caused by temperature inversions, which frequently occur in valley bogs dominated by *Sphagnum* (R. Iturraspe, unplublished data; Roig & Collado, 2004; Kleinebecker *et al.*, 2007). Annual rainfall is evenly spread over the year (> 320 days with rain) and varies greatly within the distribution area of cushion bogs, from 600 mm to 5000 mm (Godley 1960; Tuhkanen 1992; Schneider *et al.*, 2003). However, severe dry spells can also occur and may last for 2–4 weeks (Schneider *et al.*, 2003; Ch. Fritz & R. Iturraspe, unpublished data).

The climatic conditions in coastal areas provoke adverse growth conditions for *Sphagnum* by desiccation and high rates of nutrient leaching. Additionally, seaborne air masses contain little phosphorus, which may intensify the phosphorus limitation observed in Patagonian bogs (chapter 3). A recent study

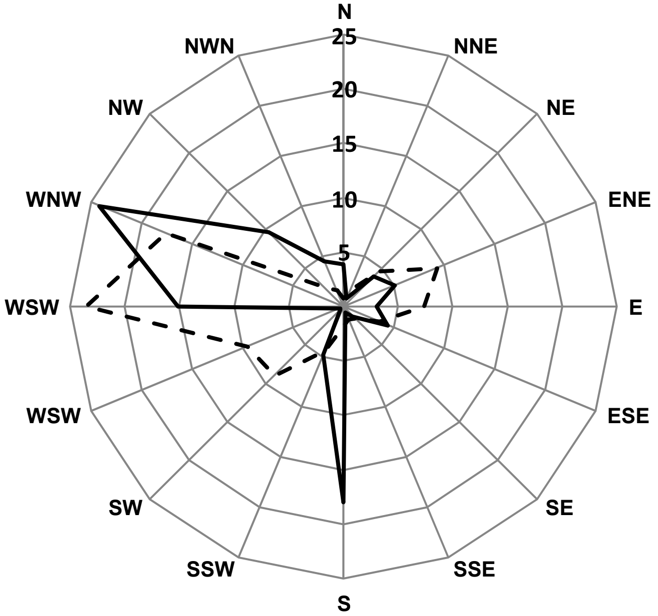

Figure 7.4. Wind speed (km h^{-1}) and prevailing wind direction measured from 2009 to 2011 in a *Sphagnum* bog (dashed line, Andorra) and in a cushion bog (black line, Moat). The cushion bog frequently experienced severe southerly winds that were cold and dry. Figure layout Till Kleinebecker.

of Patagonian bogs also found lower foliar concentration of phosphorus with increasing oceanity (rainfall) to coincide with the dominance of cushion plants over *Sphagnum* mosses (Schmidt *et al.*, 2010). Frequent rainfall leads to a higher dilution and a shorter residence time (washout by seepage) of nutrients in the moss carpet. In chapter 2, my co-workers and I found that the nitrate uptake rates at low concentrations (1 µM nitrate) may be lower than the nitrogen assimilation rates required to allow sufficient growth of mosses. The uptake of phosphorus may also be hampered due to the low P concentrations in rain and bog waters. Frequent rain fall, and as a consequence, high washout, can therefore reduce nutrient availability.

A relevant question is whether growth conditions can also become too wet for *Sphagnum* mosses. Field measurements from northern bogs showed that *Sphagnum* productivity steeply decreases when moss tissue gets saturated with water (Murray *et al.*, 1989; Belyea & Clymo, 2001). Frequent rainfall increases the water content of *Sphagnum* mosses (Cagampan & Waddington, 2008). Water contents > 10 g FW g^{-1}DW slows down photosynthesis by hampering the carbon supply rate (Clymo, 1970; Schipperges & Rydin, 1998; Smolders *et al.*, 2001; Rice *et al.*, 2008; Patberg, 2011). Carbon mineralization rates, and consequently the supply of carbon for photosynthesis under wet conditions, are low in Patagonian bog peats (Fig. 7.3; chapter 5). Low methane porewater concentrations may further limit the CO_2 supply deriving from methane oxidation (Raghoebarsing *et al.*, 2005; Kip *et al.*, 2010): evidence for this mechanism is provided in chapter 6, where the decrease in CO_2 supply was concluded from the low activity of methanotrophic bacteria. The activity of methane oxidising bacteria, and consequently CO_2 supply, was limited at low methane availability.

Next to sheltered positions, the location of persisting *Sphagnum* stands in cushion bogs was related to the influx of low-alkaline minerogenic water. Depth profiles showed that accumulated peats were richer in cations (e.g. calcium, iron, manganese) and nutrients (e.g. nitrogen, phosphorus). The most likely source of the minerals is a small input of groundwater as the degree of decomposition in the *Sphagnum* peat was similar or lower than that of the cushion plant peats. It has been shown that local cells of minerogenic water can be important sources of additional moisture and CO_2 supply (Lamers *et al.*, 1999) which may therefore reduce the growth constraints discussed above. I argue that growth of *Sphagnum* mosses was stimulated by the nutrients derived from minerogenic water. However, the positive effects of minerogenic water will be limited to small inputs of alkalinity (bicarbonate) and nutrients, as these factors may create adverse growth conditions for *Sphagnum* by stimulating the

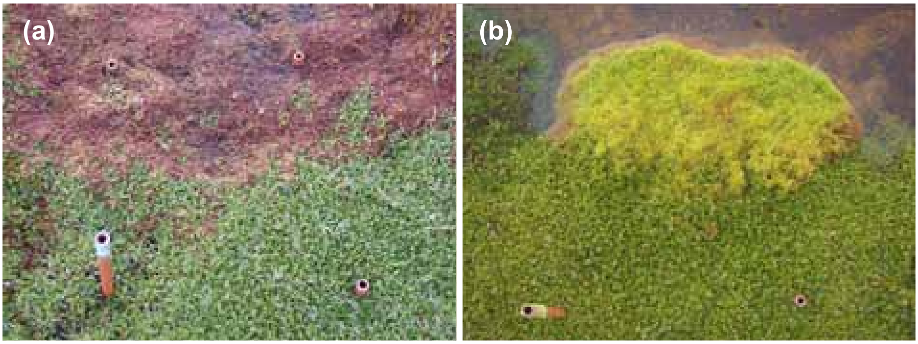

Figure 7.5. Effects of nutrient additions on the competition between cushion plants (lower part) and *Sphagnum* mosses (upper part) in the Moat cushion bog. Left panel (a) shows the control-treatment, where the surface of *Sphagnum magellanicum* is at the same level or lower than *Astelia pumila*. Right panel (b) shows a plot receiving nitrogen and phosphorus. Height increment of *Sphagnum magellanicum* was stimulated and the cover of *Astelia pumila* started decreasing. Method details in chapter 3.

growth of vascular plants such as sedges and trees (Lamers *et al.*, 1999; Granath *et al.*, 2010; Lanting, 2010).

Nutrient limitation of *Sphagnum* in a cushion bog

In a fertilisation experiment, lasting three years, my co-workers and I could show that the growth of *Sphagnum magellanicum* was nutrient limited in a pure *Sphagnum* bog (chapter 3). In contrast, vascular plants remained unaffected by the addition of nitrogen and phosphorus. In Northern Hemisphere bogs, vascular plant cover increases with extra nutrients, especially nitrogen (Limpens *et al.*, 2004; Tomassen *et al.*, 2004; Wiedermann *et al.*, 2009b). In Patagonia, the height increment of *Sphagnum* was substantially stimulated by phosphorus and the highest growth was found when both phosphorus and nitrogen were applied (chapter 3). I conducted a similar experiment in a cushion bog (Moat valley) with small patches of *Sphagnum*. Plots (0.5 × 0.5 m) were chosen so that half of the plot was covered with *Sphagnum* bordering a cushion plant vegetation (Fig. 7.5a). In the cushion bog, I found that *Sphagnum* growth was also limited by nutrients, similar to the *Sphagnum* bog. The P-treatment showed a slightly higher growth (27 mm in 2 years, SE 2.6) than the Control (21 mm in 2 years, SD 5) and the N-treatment (22 mm in 2 years, SE 1.1). Significant stimulation of height increment was found in the NP-treatment (50 mm in 2 years, SE 4.1), which was two times more than Control or single nutrient treatments. Field observations suggested that *Sphagnum* was able to overgrow cushion plants after three years in the NP-treatment (Fig. 7.5b). Surprisingly, cushion plants failed to increase their foliar nutrient contents upon fertilisation (Table 7.1). Also in the field, growth of cushion plants seemed to be little affected by the nutrient addition. Changes in growth and biomass production could not be sufficiently studied, however, for the cushion plants, which limits the conclusiveness of the foliar nutrient data.

I conclude that nutrient inputs by rain was similar at the Moat and Andorra site (i.e. rain chemistry and total rainfall were similar chapter 1). Porewater samples showed generally low concentrations of inorganic nitrogen and total phosphorus. The lowest phosphorus concentrations (< 0.5 µmol P l^{-1}) were found at cushion bog sites. This can be related to the large uptake potential by the roots of cushion plants and additionally, to the oxic conditions which immobilise phosphorus.

Table 7.1. Mean (SD) element concentration in leaves of *Astelia pumila* ($n = 5$). The fertilisation experiment in the cushion bog (Moat) lasted 3 years. Methods are described in chapter 3.

	Treatment			
	C	N	P	NP
N (µmol gDW^{-1})	1014 (82)	1021 (104)	942 (152)	1028 (71)
P (µmol gDW^{-1})	26 (3)	27 (3)	26 (2)	27 (2)
K (µmol gDW^{-1})	326 (36)	312 (45)	330 (65)	313 (49)
S (µmol gDW^{-1})	118 (28)	123 (37)	126 (15)	129 (13)
Mg (µmol gDW^{-1})	55 (11)	54 (15)	61 (12)	73 (16)
Mn (µmol gDW^{-1})	15 (5)	14 (3)	14 (6)	10 (2)
$\delta^{15}N$ (‰)	−6.87 (1.01)	−7.23 (0.81)	−7.02 (1.08)	−6.75 (0.61)

Nutrient uptake and recycling by cushion plants

Even if *Sphagnum* growth is nutrient limited, how can plants, which have an intrinsically higher nutrient demand, be more competitive? Cushion plants developed traits that enable efficient conservation and recycling of nutrients. The formation of dense cushions keep the plants and their roots close to their litter. The evergreen leaves of *Astelia pumila* have a lifespan of 4–10 years: on average, the slow growing cushion plants add one new leaf per growing season to a total of 3–6 green leaves. Leaves of cushion plants are avoided by grazers, which further reduces the leaf turnover.

Senescing leaves remain attached to the shoots and thereby form a large part of the cushion (Fig. 1.1 in chapter 1). The apical growth protects the litter from becoming removed through erosion agents like wind, water and snow. The detention of aging and dead leaves enables cushion plants to re-use nutrients at various (time) scales. We found that the nitrogen content in *Astelia pumila* leaves drops with the first signs of ageing (yellow colour), suggesting a resorption of 50 % (Ch. Fritz & B. Caspers, unpublished data). Resorption of nutrients is known to be of great importance in nutrient-deprived ecosystems (Small, 1972; Freschet *et al.*, 2010). The highest density of roots (4.9 g l^{-1} soil SD 0.8) was found just below the recently buried litter (chapter 5). These roots can filter the nutrients that are washed out from the cushion plants and the intermingled litter, respectively. Deeper roots (> 20 cm) have access to the

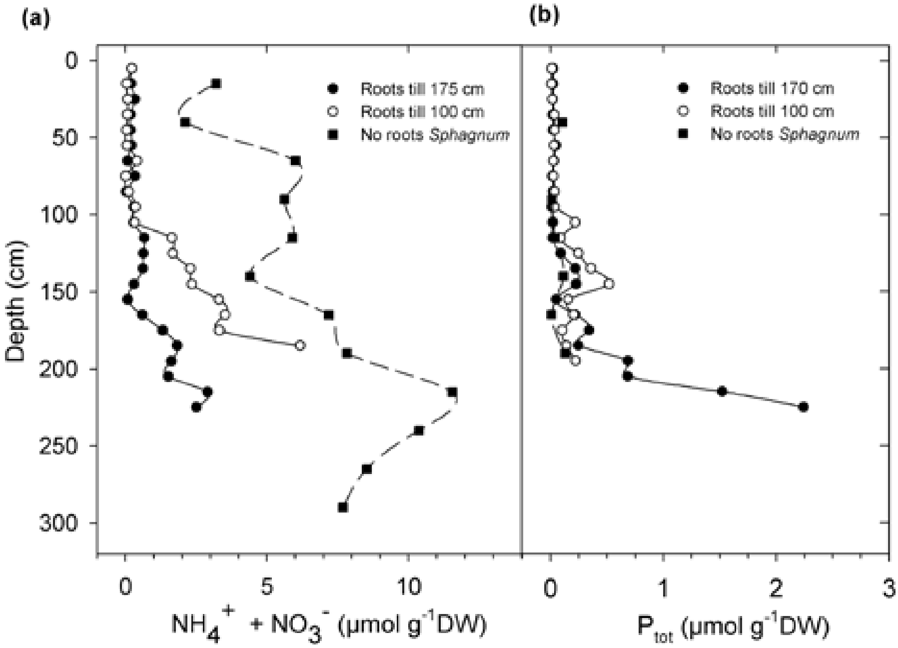

Figure 7.6. Relationship between rooting depth and plant available nutrients (salt extractable) in three profiles from the Moat cushion bogs. Sites differed in aboveground vegetation and belowground root biomass: dominance of *Astelia* with 175 cm deep roots (filled circles), a mix of *Astelia* and *Donatia* with 100 cm deep roots (open circles) and dominant *Sphagnum* vegetation (filled squares) without significant root biomass below 20 cm.

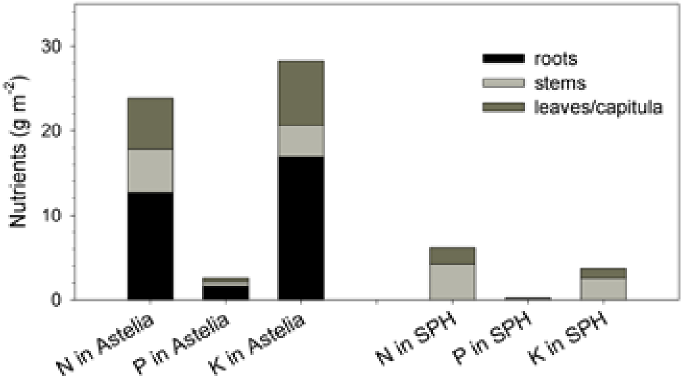

Figure 7.7. Comparison of mean nutrient stocks in the living tissue of *Astelia pumila* ($n = 5$) and SPH (i.e. *Sphagnum magellanicum*; $n = 3$) from Moat cushion bog. For nutrient stocks in a *Sphagnum* bog see Tables 3.1 and 3.2.

nutrients released from decaying peat, which is formed by accumulating litter over centuries.

I found multiple pieces of evidence for a substantial nutrient uptake by the roots of cushion plants. ^{15}N injected into the rooting zone could be traced back in the cushion plants: plants with their rooting zone (40–60 cm depth) exposed to $^{15}NH_4{}^{15}NO_3$ increased their ^{15}N content of the leaves to higher concentrations (delta 410 ‰, SD 600 ‰) than the untreated controls (delta −6.9 ‰, SD 1) and the plants (delta −7.0 ‰, SD 1.4) exposed to $^{15}NH_4{}^{15}NO_3$ below the rooting zone (200–220 cm), respectively.

Furthermore, the soil below cushion plants was depleted in nutrients. Analysis of salt extractable nutrients (0.2 M NaCl extract) in the upper 2 metres showed a thorough depletion of NH_4+NO_3 and PO_4 in the rooting zone (see chapter 5 and Fig. 7.6 a,b). In contrast, I observed nutrients to accumulate below *Sphagnum* stands and in peat below the rooting zone, which is commonly found in northern peatlands (Koerselmann *et al.*, 1993; Tomassen *et al.*, 2004; Crushell *et al.*, 2009). I conclude that cushion plants invest large amounts of resources in their roots that enable access to nutrients additional to atmospheric inputs, which are very low in Patagonia. Especially by the oxidation of the peat soil, cushion species can strongly stimulate the release of nutrients from the organic soil matrix (chapter 5).

The clonal organisation of cushion plants further reduces lateral nutrient losses. Cushion plants accumulate nutrients in litter and peat to concentrations that exceed the nutrient levels of *Sphagnum* peat (Fig. 7.7). As the litter of cushion plants becomes enriched in nutrients, the decomposition rate of the litter is likely to increase. I found a positive correlation between nutrient concentrations and peat decomposition in aerobic incubations of cushion plant peats ($n = 15$; $P < 0.05$; $r^2 = 0.53$; linear regression).

High productivity of cushion plants

As a result of efficient nutrient uptake and the conservation of the roots , cushion plants (e.g. *Astelia pumila*) can stock 6–10 times more nutrients than *Sphagnum* (Fig. 7.7; chapter 3). *Astelia* leaves contain up to six times more nitrogen than *Sphagnum* capitula (area based). The naturally high nitrogen content of *Astelia* (> 12 mg N g^{-1}) probably enables a large photosynthetic machinery and a high primary production (Xia & Wan, 2008). Cross sections of *Astelia* leaves show a high density of chloroplasts. This provides additional evidence for a high photosynthetic capacity.

Photosynthetic rates at field sites dominated by *Astelia pumila* were higher (mean 5.9 µmol CO_2 m^{-2} s^{-1} SD 1.4; *n* = 23) than adjacent plots (mean 3.1 µmol CO_2 m^{-2} s^{-1}, SD 0.6; *n* = 15) that were dominated by *Sphagnum* together with sedges (mostly *Tetroncium magellanicum*). Light saturation occurred between 400–600 µmol PAR m^{-2} s^{-1}. My results on photosynthetic rates are in agreement with a study by Gibson (1990) who found that the aboveground biomass production of Australian cushion plants (e.g. *Donatia* sp., *Astelia* sp.) amounted to 700 g m^{-2} y^{-1}, which is two times the biomass production of a *Sphagnum*-dominated vegetation (316 g m^{-2} y^{-1}; see chapter 3). In conclusion, efficient photosynthesis seems to be a unique feature of cushion plants as other vascular bog plants photosynthesise at equal or lower rates than *Sphagnum* mosses (Thorman & Bayley, 1997; Moore *et al.*, 2002).

Cushion plants can increase the uptake and conservation of nutrients, which in turn stimulates photosynthesis and productivity. As argued above, roots play a key role in building up nutrient stocks. However, the large belowground biomass also requires a high input of resources such photosynthetic products and nutrients. As an example, the roots of *Astelia pumila* alone, contain more nutrients than the entire living biomass of *Sphagnum* (area based). Moreover, roots and shoots account for 70–90 % of the total biomass imposing high respiration costs on cushion plants.

Persistence of cushion bog vegetation

Palaeoecological studies suggest that once cushion plants have established, *Sphagnum* is not coming back (Heusser, 1995; Teltewskaya, 2010). To my knowledge there are no (paleoecological) reports that describe present *Sphagnum* vegetation underlain by cushion plant peat. But there is much evidence for the ongoing invasion by *Astelia pumila*. In distinct peatlands (e.g. Paso Garibaldi and Lago Rio Bueno, Patagonia) I found small patches (10–15 m^2) of *Astelia pumila* invading a *Sphagnum* vegetation that was underlain by *Sphagnum*-peat and fen-peat (Figs 7.1, 7.8). The small number and size of the patches point toward a rather recent invasion compared to reports of an invasion thousands of years old (Heusser, 1995; Kilian *et al.*, 2003; Fesq–Martin *et al.*, 2004; Teltewskaya, 2010).

The growth strategy of *Astelia pumila* and other cushion plants (evergreen, large belowground biomass, slow growth) make them weak competitors for light (Grace, 1991; Grime, 2002; Funk & Vitousek, 2007). It can be argued that the adverse growth conditions in cushion bogs not only limit

Sphagnum mosses, but also other bog plants (ericaceous species, sedges, trees). The limited above ground competition may create a particular niche where large below-ground biomass and nutrient conservation pay off (Chapin *et al.*, 1993; Aerts 1995; Eckstein *et al.*, 1999). The growth strategy of cushion plants provides significant advantages over *Sphagnum* mosses and other competing vascular plants (e.g. sedges, trees). The closely packed cushion inhibits the establishment of seedlings, whereas the roots monopolise the nutrient stock in the soil. The dense cushion may also be a prerequisite to exclude other vascular plants from using the nutrients accumulated in the litter of cushion plants. This strategy, which allows cushion plants to monopolise nutrient pools, may relax the competition for light.

In a dense carpet of cushion plants, the re-establishment of *Sphagnum* can also be impeded by low water availability. The upper 10 cm of a cushion is significantly drier (6 gFW g^{-1}DW, SD 1) than of a *Sphagnum* stand (12 gFW g^{-1}DW, SD 1) at a similar water level (15–20 cm below surface). Higher evaporation, lower porosity and water retention capacity by cushion plants cause drier conditions in the cushions. The lowest water contents are found at the surface of the cushions, which were below 7 gFW g^{-1}DW. Under these moisture deficient conditions, *Sphagnum* mosses are inhibited by low water transport and desiccation (Clymo, 1973; Schipperges & Rydin, 1998). This is especially the case when evaporation stress is high due to strong winds (chapters 1 & 3). In order to rise above the water level, *Sphagnum* needs a continuous capillary transport of water, which becomes impaired once a dense vegetation of cushion plants establishes (Fig. 7.2). Experiments in which *Sphagnum* sods were placed on the surface of cushion plants revealed that *Sphagnum* dried out after several weeks. Only the sods that were in direct contact with the water acquired moisture for sufficient photosynthesis.

When the surface of cushion plants becomes dry, *Sphagnum* may still grown in pools where the conditions are still wet. These micro-habitats seem less suitable for cushion plants due to frequent flooding of the leaves and consequently, the lower oxygen transport (Gebser, 2008). In pools, however, *Sphagnum* can only grow and compete when there is a sufficient supply of carbon for photosynthesis (Smolders *et al.*, 2001; Kip *et al.*, 2010). Methane is crucial in the formation of a floating *Sphagnum* vegetation because it consequently improves the conditions for photosynthesis by increasing the availability of CO_2 and light (Smolders *et al.*, 2003). It was shown that cushion plants reduce the methane availability even in adjacent *Sphagnum* pools and lawns and thus on the ecosystem scale (chapters 5 & 6). The shortage of carbon di-

Figure 7.8. Small patch of *Astelia pumila* invading a pristine *Sphagnum* bog on Peninsula mitre, Argentina. Hans Joosten seems fascinated by cushion plants just like Charles Darwin 180 years before.

oxide for photosynthesis may explain the low growth of *Sphagnum* in pools, which was also found in northern peatlands (Belyea & Clymo, 2001; Smolders *et al.*, 2001). The pool environment forces *Sphagnum* mosses to grow with a lower bulk density than lawn mosses (chapters 3 & 6). A low bulk density implies a low water storage and high risk of desiccation when water tables drop below the surface level (Fig. 7.2; chapter 3). Desiccation is even more amplified, as *Sphagnum* accumulates little waterholding peat due to a marginal productivity under wet conditions (Belyea & Clymo, 2001). The lack of peat accretion implies that *Sphagnum* mosses fail to overgrow cushion plants under wet and low-productive circumstances.

The future of cushion plant bogs in Patagonia

Slow clonal growth and a large standing biomass are specific features of *Astelia pumila* and other cushion plant species. These strategies can be successful when adverse growth condition for other bog plants (e.g. *Sphagnum*) limit the competition for light. I have presented ample evidence that the factors reducing the growth of *Sphagnum* (e.g. superficial desiccation, nutrient shortage, low temperatures) are more pronounced in areas where cushion bogs dominate. The harsh growth conditions seem to offer *Astelia pumila* a 'window of opportunity', that permits the invasion of *Sphagnum* bogs (Heusser, 1995; Fesq–Martin *et al.*, 2004; Teltewskaya, 2010).

The distribution of today's cushion plant-dominated peatlands seems to be climate-related (Kleinebecker *et al.*, 2007). The main characteristics of the Magellanic Moorland are strong westerly winds and low mean summer temperature (< 10 °C) (Godley 1961; Schneider *et al.*, 2003). Pure cushion bogs and cushion bogs intermixed with (remnants of) *Sphagnum* cover large areas along the western and southern coasts of Patagonia and Tierra del Fuego (Godley, 1960; Roig & Collado, 2004). On the Peninsula Mitre, cushion plants are already invading the northern coast (Fig. 7.8, Fig. 1.4; Ch. Fritz personal observation in Lago Rio Bueno mires 54° 39′ 44 S, 65° 47′ 51 W and Policarpo mires 54° 39′ 44 S, 65° 35′ 48 W). During the Mitre expedition my fellow-travellers and I observed various indicators of erosion, probably caused by (postglacial) land-uplift in Peninsula Mitre (Bujalesky, 2007). The relative lowering of the sea level (local drainage base) with respect to the rising land causes increased water losses due to the increased discharge via small rivulets and macropores (soil piping c.f. Holden & Burt, 2002) cutting through the peatlands. High water losses increase the impact of the climatically induced growth inhibitions discussed above.

What prevents the worldwide domination of cushion bogs?

The niche of *Astelia pumila* and other cushion plants is probably very sensitive to disturbances. I found several indicators for this: flooding that lasted several months caused irreversible damage to the cushion plants (Fig. 7.9a). I observed a lack of re-colonisation two years after the uppermost 5 cm of *Astelia pumila* (Fig. 7.9b) had been removed. Furthermore, *Astelia* plants showed a lower survival rate after the root biomass was reduced by 50 % (Fig. 7.9c). These negative effects were more pronounced when *Astelia* plants were transplanted to wet conditions (Fig. 7.9d) or in areas with extended frost periods (Fig. 7.9e). Both wetness and frost increase the risk of plant damage, as these factors complicate gas and water transport in vascular plants. Severe winters in the inner parts of Patagonia (Tuhkanen *et al.*, 1992; Kleinebecker *et al.*, 2007) may impose too much stress (e.g. minimum temperature, frost duration, shading by snow) for the maintenance of cushion plants (Neuner & Pramsohler, 2006).

Slow growth and compact cushions are disadvantageous when plants have to compete for light. I observed an increase in the above ground biomass of cushion plants (leave length) in shading experiments. However, the resulting

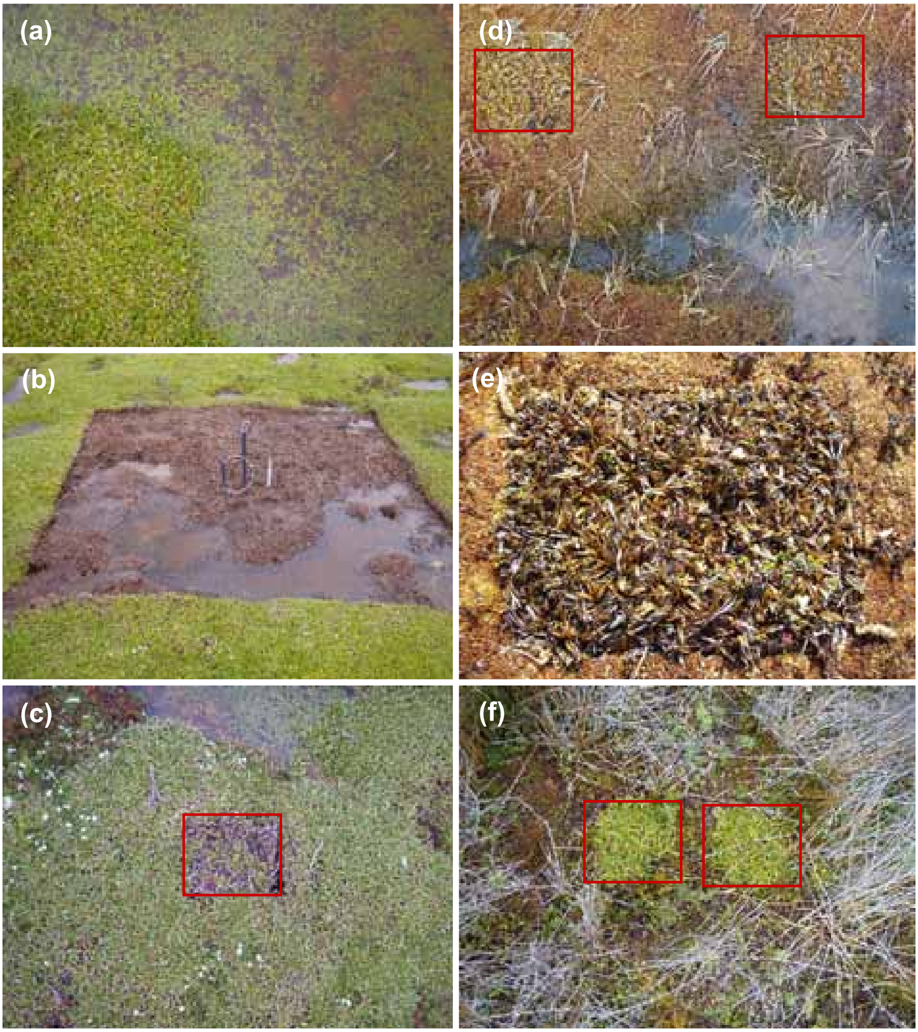

Figure 7.9. Conditions limiting growth of *Astelia pumila*. Upper-left panel (a) shows impeded growth following extensive flooding of low-lying cushion plant vegetation. Brown patches indicate irreversible damage. Mid-left panel (b) highlights the slow vegetation recovery. Cushion plants were removed two years before. Lower-left panel (c) shows a significant losses of cushion plants after their root biomass got removed a year before. Upper-right panel (d) shows losses (almost complete) of *Astelia pumila* transplants in a wet hollow of *Sphagnum*. Under dry conditions transplants showed much higher survival rates. Mid-right panel (e) shows transplants of *Astelia pumila* did not survive a severe winter in the *Sphagnum* bog (Andorra). Similar transplants performed well in a coastal bog with mild winters (Moat). Lower-right panel (f) gives an example that the growth of cushion plants is depressed in Patagonian fens, where nutrient influxes from groundwater favour sedges and shrubs as they are better competitors for light under these circumstances. See also figure 7.10.

surplus in height increment was < 2 cm, which is little compared to the growth rates of shrubs and trees in nutrient-rich peatlands. Furthermore, it was observed that the cover of *Astelia pumila* was low in fens, which provide better growth condition for other vascular plants by being richer in nutrients than cushion bogs (Ch. Fritz unpublished data; Fig. 7.9f). Addition of both nitrogen and phosphorus increased the cover of vascular plants like *Caltha dionifolia* and *Gaultheria* sp. that started to overgrow the cushion plants (Fig. 7.10). However, vascular bog plants, including cushion species, responded very slowly to nutrient addition in Patagonian bogs (Table 7.1). The slow response was probably caused by phosphorus limitation, high nitrogen retention capacity on ecosystem scale and low summer temperatures (chapters 2 & 3).

Little is known about the carbon accumulation rates of cushion bog vegetations (Teltewskaya, 2010). The oxygenation of the peat remobilises nutrients and thereby enables the growth of cushion plants. Under aerobic conditions ,decomposition rates increase by a factor of 3–10 (Ch. Fritz & E. Hoftijser unplublished data). As a result, peat accumulates at a lower rate, which increases the risk and duration of flooding when the surrounding accumulates peat at higher rates. Earlier on in this discussion, I showed that cushion plants suffer from permanent flooding, which would depress the peat growth even further. Therefore, I expect cushion plants to dominate in areas where nutrient and/or water availability restricts *Sphagnum* mosses to accumulate peat. At the peatland scale, a low peat accretion would reduce the risk of flooding for cushion plants as the water level rise following peat accumulation slows downs. Dense cushion plant roots may be able to stabilise the peat matrix and thereby prevent a compaction of the rapidly decomposing peat. In future studies, special attention should be directed to the factors influencing the growth (nutrient availability, flooding, shading, and increased soil temperatures) and the competitive advantages *Astelia pumila* holds over other bog plants.

Alternative strategies to outcompete *Sphagnum* mosses

Not only cushion plants, but also vascular bog plants of the Restionaceae family (restiads) can overgrow *Sphagnum* bogs. Restiad plants dominate the bogs in Tasmania and New Zealand where they have outcompeted *Sphagnum* (Campbell, 1983; Clarkson *et al.*, 2004; Hodges & Rapson, 2010). *Empodisma minus*, the most prominent restiad bog species, forms sponge-like upward-growing roots, also called 'cluster roots' (Agnew *et al.*, 1993; Hodges & Rapson, 2010). In chapter 4, my co-workers and I show the efficiency of peatland surface oscillation as a key mechanism to prevent the flooding of cluster roots. Keeping its roots dry is crucial as *Empodisma minus* lacks the capacity to oxidise the soil, in contrast to cushion plants (Sorrell *et al.*, 2000). The highest cover of *Empodisma minus* was associated with stable water levels close to, but still below, the surface. Combining upward-growing roots with peatland surface oscillation seems to be another viable strategy to protect roots from stress in wetland soils. Such adaptations to wet conditions provide additional advantages over vascular plants that struggle in wetlands. *Empodisma minus* is able to maintain the wet conditions in climates that also know long periods of water shortage (chapter 4; Coulter, 1973) by reducing water level fluctuations (chapter 4) and ecosystem evaporation (Campbell & Williamson, 1997; Thompson *et al.*, 1999). *Sphagnum* growth is inhibited by hydro-climatic conditions typical for restiad peatlands (e.g. warm and summer droughts). For example, small patches of *Sphagnum* are limited to stands below the protective canopy of restiad plants (Clarkson, 1984; Clarkson *et al.*, 2004).

The nutrient uptake strategy of *Empodisma minus* is similar to that of *Sphagnum* (chapter 2; Clarkson *et al.*, 2009). The cluster roots of *Empodisma minus* are very efficient in the uptake of nutrients that derive from atmospheric deposition, surface run-off and wash-out of the dense canopy (Agnew *et al.*, 1993; Clarkson *et al.*, 2009). Interestingly, restiad-dominated bogs seem to be phosphorus limited (Clarkson *et al.*, 2005), similar to Patagonian bogs (chapter 3). It remains unknown, whether deep roots of restiads play an important role in nutrient recycling (Hodges & Rapson, 2010). Similar to cushion plants, *Empodisma minus* also developed strategies to keep its characteristic roots under aerobic conditions, which enables an efficient uptake of nutrients.

Further research needs

The present thesis shows that experimentally increased nitrogen deposition does not result in the dominance of vascular bog plants over *Sphagnum* within 3 years. The opposite, extremely low nutrient availability, seems to be a prerequisite for the downfall of *Sphagnum* in southern bogs (Fig. 7.10). However, nitrogen may affect *Sphagnum* by increasing the risk of desiccation (chapter 3) and nitrogen can reach toxic levels in *Sphagnum* when it is rapidly accumulated (chapter 2). In conclusion, low nutrient availability and episodic water shortage (including possible drainage due to land-uplift) may be important factors in reducing *Sphagnum* growth in Patagonian peatlands. In New Zealand and Tasmania, water shortage may be more important in limiting

Sphagnum growth, as it provides restiad species with sufficient competitive advantage. More detailed studies are required to unravel the relationship between wind-induced evaporation, limits of water transport, architecture and productivity of *Sphagnum* stands.

Roots of cushion plants can easily penetrate the upper first metre of the peat, which provides them access to nutrients and water even under dry and nutrient-limited conditions. The functionality and metabolic costs of roots (cf. Hodge *et al.*, 2009; Mommer *et al.*, 2011) deserves further attention. Above

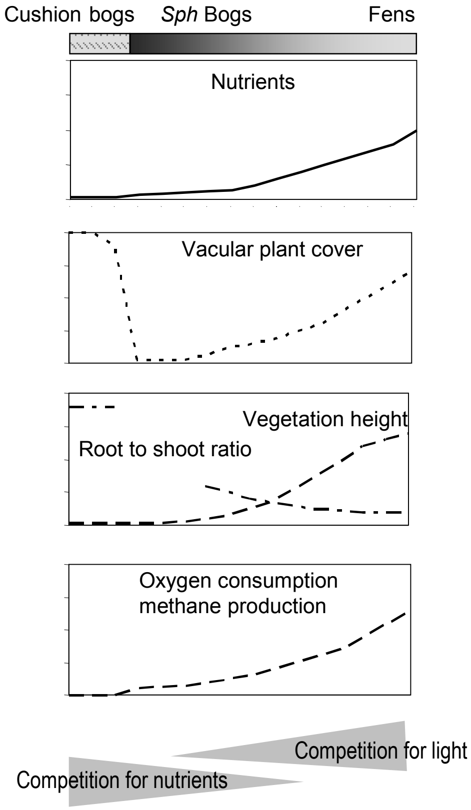

Figure 7.10. Conceptualized model of vegetation distribution in relation to nutrient availability and plant strategy/growth form. We propose that cushion plants occupy peatland ecosystems that are extremely low in nutrients. A low nutrient availability in combination with episodic desiccation limits the growth of *Sphagnum* bogs. In contrast, cushion plants can take advantage of the slow *Sphagnum* growth by accessing sufficient nutrients with their large root biomass. Low competition for light supports the characteristic high root to shoot ratio of cushion plants.

I have shown that with increasing cover, cushion plants extend their competitive advantage over *Sphagnum* mosses that are consequently forced to swerve to low-productive habitats. The same may be true for bogs dominated by restiad species.

Further investigations are encouraged to estimate the proportion of resources (nutrients and energy) invested in roots of cushion plants. It seems plausible that only plants with intrinsically low growth rates can invest in a large root biomass (Grime, 2002; Smolders *et al.*, 2002). These large investments are difficult to be repeated in a short period. In contrast, fast growing plants, which are often successful light-competitors, invest a large part of their resources in above-ground biomass production (Grime, 2002). The large below-ground biomass may actually restrict the dominance of cushion plants to temperate climates with mild winters as found in Patagonia, Tasmania, and New Zealand. Larger temperature differences between summer and winter may result in a frequent frost damage imposing large costs of recovery in cushion plants (Bannister, 2007).

Conservation needs

Mires and peatlands in Tierra del Fuego and south-west Patagonia are of global significance. They form the southern hemispheric counterpart to the northern mires and peatlands that are found north of 50° N in the boreal and subarctic climates. As in similar southern latitudes of the world, almost no land is found, the large concentration of the Patagonian mires and peatlands is globally important. Some mire types like cushion bogs and pure *Sphagnum magellanicum* bogs are unique to southern mires.

Mires in Patagonia are threatened by peat mining, drainage and land use changes (forest clearings, roads and fire), that alter their hydrology. The introduction of exotic species like the Canadian beaver has resulted in severe damage by causing erosion, drainage and flooding with nutrient-rich and alkaline water, respectively. Nitrogen deposition may also increase as life stock farming is intensifying in Chile and Argentina (Martínez–Lagos *et al.*, 2010). New Zealand has already lost almost all of its pristine peatland areas due to drainage, peat mining and invasive species. It is timely to explore the uniqueness of Patagonian peatlands before their precious secrets become irretrievably lost.

Summaries

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Summary

Peatlands are valuable to mankind. By accumulating peat they store carbon and water. Peat is formed when water hampers the decomposition of plant material. A classic example for a peatland is a *Sphagnum* bog (i.e. a peatland only fed by rainwater resulting in acid and nutrient poor conditions). In bogs of the Northern Hemisphere species of *Sphagnum* mosses usually win the competition over higher plants. The mosses retain scarce nutrients and water more efficiently than higher plants. The wet conditions in bogs are especially harmful to roots, which quickly suffer from oxygen deficiency below the water. Limited root growth and a low effective nutrient availability, therefore, restrain the productivity of higher plants in bogs. In contrast, low water levels and more nutrients increase higher plant biomass. A shift towards the dominance of higher plants has been observed in many bogs in Europe, North America and China. These bogs were often affected by man (e.g. drainage and increased atmospheric nutrient inputs).

Also in the Southern Hemisphere higher plants, e.g. cushion plants, dominate over *Sphagnum* mosses in many bogs. However, this dominance of higher plants is thousands of years old and not linked to man-made disturbances. In boreal and temperate climates in the Southern Hemisphere, *Sphagnum* bogs are replaced by an alternative peatland type: cushion bogs (i.e. bogs dominated by cushion plants). Cushion bogs cover large parts of the Patagonian coast and also some mountain peatlands in New Zealand. In Patagonia, dominant cushion plants are *Astelia pumila* and *Donatia fasciculares* (Liliaceae and Stylidiaceae, respectively). In New Zealand, 'restiad bogs' are formed by the higher plant *Empodisma minus* (Restionaceae family), which can replace *Sphagnum* bogs. *The present thesis focuses on Patagonian cushion bogs as model ecosystems to investigate what factors determine the competition between Sphagnum mosses and higher plants in southern bogs. Restiad bogs in New Zealand are also included to provide additional evidence for the dominance of higher plants in bogs.*

To investigate factors ruling the competition between *Sphagnum* mosses and higher plants me and my co-workers evaluated growth conditions of *Sphagnum* mosses with emphasis on nutrient availability, hydro-climate and gas availability for photosynthesis. We also investigated adaptations of higher plants, such as deep roots, to overcome the nutrient-poor and oxygen-poor conditions in bogs.

Patagonian *Sphagnum magellanicum* mosses are well adapted to nutrient-poor environments (chapter 2). Nitrogen is one such nutrient. Laboratory experiments revealed that nitrogen uptake efficiency of *Sphagnum magellanicum* is very high. Uptake of nitrogen decreased at high concentrations (Michaelis-Menten kinetics) and longer exposure times. Extrapolating these laboratory uptake rates suggested that nitrogen from rain is taken up within minutes by *Sphagnum magellanicum*. Such a thorough nitrogen immobilisation permits *Sphagnum* mosses to monopolise atmospheric nutrient inputs (chapters 2 & 3).

The monopolising effect was also found in a bog dominated by *Sphagnum magellanicum* in Patagonia. The growth of *Sphagnum magellanicum* increased upon nutrient addition (nitrogen and phosphorus) lasting for 3 years in Patagonia (chapter 3). In contrast, nutrient addition did neither affect higher plant's biomass nor foliar nutrient concentrations. This may have been a result of the rapid nutrient uptake shown in chapter 2 and wash-out by rain, respectively. However, we also observed that excessive nitrogen caused physiological stress in *Sphagnum* mosses (e.g. accumulation of amino acids and chlorophyll). Nitrogen stress remained unaffected by the co-addition of phosphorus. Interestingly, nutrient addition stimulated desiccation of *Sphagnum* by lowering the density of mosses (i.e. water holding capacity). We conclude that nutrients in excess can impair growth, especially in Patagonia where *Sphagnum* is exposed to desiccating winds (chapters 3 & 7).

Desiccation also limits growth of *Sphagnum* mosses in the warm-temperate climates of New Zealand and Tasmania. Surface droughts are frequently caused by substantial evaporation losses: water levels can drop by 40 cm during summer (chapter 4). Low water levels in bogs improve growth conditions for higher plants such as *Empodisma minus*. Roots of *Empodisma* suffer from flooding as high water levels reduce oxygen availability. We identified two mechanisms that protected roots of *Empodisma* from flooding stress (chapter 4). Firstly, the peat surface of *Empodisma* stands moves up when the water level rises. Secondly, cluster roots of *Empodisma* have the outstanding ability to grow upwards, thereby reducing the risk to get flooded. We hypothesise that

Empodisma plants 'themselves' can improve (i.e. engineer) the up-and-down movement of the surface by e.g. adding gas-filled roots to the peat matrix. First evidence may be found in the close relationship between the density of *Empodisma* plants and the efficiency of flood mitigation (chapter 4).

Gas transport and methane production are the focus of chapter 5. It was found that a large quantity of air-filled roots from cushion plants dominate wet soils in Patagonian peatlands. Cushion plant roots may become as long as 200 cm. Roots of cushion plants transport and release oxygen so effectively that soils became oxic, even below the water level. It was concluded that cushion plants 'themselves' provide (i.e. engineer) oxic soil conditions, upon which nutrient availability and root growth improves. As a result, cushion plants invest a large fraction of their resources in root biomass. Aboveground they are still winning the competition with *Sphagnum* mosses (chapters 5–7). The oxic soil conditions caused by roots provoked substantial changes in soil biogeochemistry by stimulating oxidation processes. Complete soil oxygenation suppressed methane production and methane emission (chapter 5). Impairing oxygen transport by cutting shoots reversed this pattern. Interestingly, cushion plants act as a long-term storage for carbon dioxide (i.e. peat) without fuelling methane emissions.

In Patagonian bogs, *Sphagnum* mosses live in association with methanotrophic bacteria (e.g. *Methylocystis* species; chapter 6). Methanotrophic bacteria oxidise methane to carbon dioxide which is used in the photosynthesis of mosses. Chapters 5 and 6 show a low activity of methanotrophic bacteria in bogs that were depleted in methane (e.g. *Sphagnum* mosses surrounded by cushion plants). It is hypothesised that cushion plants may improve their competitive advantage over mosses by reducing methane availability, which in turn reduces carbon dioxide supplied by methanotrophs, an element necessary for the photosynthesis of mosses.

Chapters 3–7 provide evidence for conditions which are sub-optimal for *Sphagnum* growth in southern bogs. Nevertheless, bogs studied in the present thesis remained unaffected by ecohydrological changes such as drainage. We suggest *Sphagnum* growth is depressed by natural forces like desiccating winds. The low atmospheric nutrient inputs also limit *Sphagnum* growth in Patagonia. In contrast, cushion plants have evolved several adaptations to harsh growth conditions (chapter 7). Thick leathery leaves grow close to the surface protecting cushion plants from cold and dry winds. Field and laboratory experiments proved that roots mobilise nutrients, which are taken up along the entire rooting zone (chapters 5 & 7). By growing in dense stands, cushion plants are able to conserve and recycle nutrients. Also, improved nutrition upon long roots was found to facilitate higher photosynthesis and biomass production necessary to maintain dense cushions. On the other hand, the large investment in root biomass makes cushion plants vulnerable to shading (e.g. by fast growing plants) and disturbances (e.g. flooding) (chapter 7).

The success of higher plants in Southern Hemispheric bogs is determined by two major factors: Firstly, *Sphagnum* mosses lack competitive strength due to adverse growth conditions such as drying winds and low nutrient input. Under these conditions *Sphagnum* is outcompeted by higher plants that have sufficient access to nutrients. As nutrients are scarce in bog ecosystems, this requires a particularly dense root biomass. Secondly, a large root biomass is only beneficial to higher plants as long as roots are protected from oxygen deficiency under wet conditions. Cushion plants (e.g. *Astelia pumila*) keep up with the water by creating widespread oxic conditions, which is facilitated by low oxygen consumption at low soil temperatures. Similarly, *Empodisma minus* escapes the water in restiad bogs via upward growth of roots and moving up following rising water levels. But what prevents these southern bog specialists to take over in peatlands situated in the Northern Hemisphere? Darwin might have known …

Resumen

Los turbales son ecosistemas muy valiosos para la humanidad ya funcionán como depósito de carbono y de agua. La turba es material vegetal parcialmente descompuesto que se va acumulando debido la baja descomposición por las condiciones de anoxia generadas por el agua. Un ejemplo clásico de turbales son las turberas ombrotróficas del musgo *Sphagnum* (es decir, turberas que son abastecidas solamente por el agua de lluvia, con condiciones de acidez y pobres en nutrientes). En las turberas de *Sphagnum* del Hemisferio Norte, en general, el musgo gana la competencia frente a las plantas vasculares. Los musgos retienen los pocos nutrientes y el agua en forma más eficiente que aquellas. Estas condiciones de humedad de las turberas son especialmente perjudiciales para las raíces, las cuales sufren la ausencia de oxígeno debajo del agua. De esta forma, el crecimiento radicular es limitado y la baja disponibilidad de nutrientes limitan la productividad de las plantas vasculares. Por el contrario, niveles bajos de agua y mayor cantidad de nutrientes incrementan la biomasa de las plantas vasculares. En Europa, América del Norte y China se ha observado un aumento en la dominancia de plantas vasculares en muchas turberas, a menudo debido a actividades humanas (p. ej., por drenaje y/o aumento en los aportes de nutrientes atmosféricos).

En el Hemisferio Sur las plantas vasculares también dominan sobre el *Sphagnum* en muchas turberas (p. ej., plantas que crecen en cojín o en forma compacta). Sin embargo, esta dominancia se remonta a miles de años de antigüedad y no está vinculada a disturbios provocados por actividades humanas. Las turberas compactas, es decir turberas dominadas por plantas en cojín, son probablemente la alternativa más frecuente a las turberas de *Sphagnum*, en climas templados y boreales/australes. Las turberas compactas cubren gran parte de la costa sur de la Patagonia y también se encuentran en zonas de montaña en Nueva Zelanda (restiad bogs). En la Patagonia, las especies dominantes son *Astelia pumila* y *Donatia fasciculares* (familia Liliaceae y Stylidiaceae, respectivamente). En Nueva Zelanda, son formadas por la especie *Empodisma minus* (familia Restionaceae) que forma turberas 'restiad'. *La presente tesis se centra en el estudio de las turberas compactas de la Patagonia como ecosistema modelo para investigar cuáles son los factores que determinan la competencia entre el musgo Sphagnum y las plantas vasculares en las turberas más australes. También se incluyen a las turberas de Nueva Zelanda dominadas por Empodisma sp, como una evidencia adicional de la dominancia de plantas vasculares en turberas.*

Para investigar los factores que rigen la competencia entre *Sphagnum* y las plantas vasculares, evaluamos las condiciones de crecimiento del *Sphagnum* poniendo énfasis en la disponibilidad de nutrientes, la hidrología, el clima y el ciclo del metano. También investigamos las adaptaciones de las plantas vasculares, tales como raíces profundas, para sobrellevar las condiciones de deficiencia en nutrientes y oxígeno de las turberas.

En Patagonia, el musgo *Sphagnum magellanicum* está bien adaptado a ambientes pobres en nutrientes (capítulo 2). El nitrógeno es uno de estos nutrientes. Experimentos en el laboratorio revelaron que la eficiencia de absorción de nitrógeno por *Sphagnum magellanicum* es muy alta. La absorción disminuye a altas concentraciones (cinética de Michaelis-Menten) y a mayor tiempo de exposición. Esta tasa de absorción obtenida en el laboratorio sugeriría que el nitrógeno atmosférico, aportado por la lluvia, es absorbido en pocos minutos por el *Sphagnum*. Esta alta inmovilización del nitrógeno permite al *Sphagnum* monopolizar la entrada de nitrógeno atmosférica (capítulos 2 y 3).

Este efecto monopolizador fue encontrado también en las turberas dominadas por *Sphagnum magellanicum* en Patagonia. El crecimiento del *Sphagnum* aumentó al agregar nutrientes (nitrógeno y fósforo) por 3 años en Patagonia (capítulo 3). En cambio, la adición de nutrientes no afectó la biomasa, ni la concentración de nutrientes foliar de las plantas vasculares. Este resultado podría deberse a la rápida incorporación de nutrientes por parte del musgo *Sphagnum*, mostrada en el capítulo 2, y al lavado por la lluvia. Sin embargo, también encontramos que el exceso de nitrógeno causó estrés fisiológico en el *Sphagnum* (p. ej., acumulación de aminoácidos y clorofila); este estrés no disminuyó al adicionar fósforo junto al nitrógeno. Contrariamente, la adición de nutrientes estimuló la desecación del *Sphagnum* al disminuir la densidad de musgos (y por lo tanto, la capacidad de retener agua). Concluimos que el exceso de nutrientes puede perjudicar el crecimiento del *Sphagnum*, especialmente en Patagonia, donde el musgo está expuesto a desecación por viento (capítulos 3 y 7).

La desecación también limita el crecimiento del *Sphagnum* en climas cálido-templados de Nueva Zelanda y Tasmania. Sequías de la superficie son frecuentemente causadas por pérdidas por evaporación: los niveles de agua pueden disminuir 40 cm durante el verano (capítulo 4). Niveles bajos de agua en las turberas mejoran las condiciones de crecimiento para las plantas vasculares como *Empodisma minus*. Durante las inundaciones, el crecimiento es limitado ya que las raíces no disponen de oxígeno por quedar sumergidas en el agua (anegadas). Identificamos dos mecanismos que protegen a las raíces de *Empodisma* del estrés por anegamiento (capítulo 4). En primer lugar, la superficie de la turba de *Empodisma* asciende cuando el nivel de agua aumenta. Segundo, grupos de raíces de *Empodisma* tienen la habilidad de crecer hacia arriba, reduciendo el riesgo de quedar anegados. Nuestra hipótesis es que las plantas de *Empodisma* proveen ellas mismas el mecanismo (como un ingeniero) de movimiento hacia arriba y abajo de la superficie, por ejemplo, llevando raíces llenas de aire a la matriz de turba. La primera evidencia podría encontrarse en la estrecha relación entre la densidad de plantas y la eficiencia en la mitigación de los efectos de la inundación (capítulo 4).

El capítulo 5 se centra en el transporte gaseoso y la producción de metano. Encontramos que una gran cantidad de raíces llenas de aire de las plantas en cojín dominan los suelos anegados de las turberas patagónicas. Las raíces de estas plantas alcanzan los 200 cm de longitud. Transportan y liberan el oxígeno tan efectivamente que el suelo se vuelve aeróbico, aún debajo del nivel del agua. Concluimos que las plantas en cojín crean por sí mismas condiciones de aerobiosis (como ingenieros), mejorando la disponibilidad de nutrientes y el crecimiento de las raíces. Como resultado, las plantas invierten una gran fracción de sus recursos en producir biomasa radicular. En su parte aérea, ellas continúan ganando en su competencia con el *Sphagnum* (capítulos 5–7). Las condiciones aeróbicas generadas por las raíces provocan cambios substanciales en la biogeoquímica del suelo por estimular procesos oxidativos. La oxigenación completa del suelo suprime la producción y emisión de metano (capítulo 5). Al cortar los tallos, impedimos el transporte de oxígeno y este patrón se revierte. Sorprendentemente, las plantas en cojín son capaces de almacenar dióxido de carbono (como turba) sin potenciar las emisiones de metano. –

En las turbera patagónicas, los musgos de *Sphagnum* viven en asociación con las bacterias metanotróficas (p. ej., la especie *Methylocystis*; capítulo 6). Las bacterias metanotróficas oxidan el metano a dióxido de carbono, el cuál es utilizado en la fotosíntesis de los musgos. Los capítulos 5 y 6 tratan de la baja actividad de las bacterias metanotróficas en turberas que quedaron agotadas en metano (es decir, en lugares con *Sphagnum* rodeados por plantas en cojín). Nuestra hipótesis es que las plantas en cojín mejorarían su ventaja competitiva sobre los musgos al reducir la disponibilidad de metano, lo cual reduce el dióxido de carbono que proveen los metanotrofos, un compuesto necesario para la fotosíntesis de los musgos.

Los capítulos 3–7 aportan evidencias de las condiciones sub-óptimas para el crecimiento del *Sphagnum* en las turberas australes. Sugerimos que el crecimiento del *Sphagnum* empeora por la desecación por vientos. Los escasos aportes atmosféricos de nutrientes también limitan el crecimiento del *Sphagnum*. Contrariamente, las plantas en cojín han desarrollado diversas adaptaciones para hacer frente a las condiciones adversas de crecimiento (capítulo 7); un ejemplo son las hojas duras y gruesas que crecen cerca de la superficie, protegidas de los vientos fríos y desecadores. Experimentos a campo y de laboratorio demostraron que las raíces movilizan nutrientes, los cuales se extienden en toda la zona de raíces (capítulos 5 y 7). Al crecer en grupos densos, las plantas en cojín son capaces de conservar y reciclar los nutrientes. La mejor nutrición por las largas raíces facilita una mayor fotosíntesis y producción de biomasa, necesaria para mantener cojines densos. Por otro lado, la gran inversión en biomasa radicular, deja vulnerable a las plantas al sombreado (por ej., por plantas que crecen mas rápido) y disturbios (por ej., inundaciones) (capítulo 7). Sin embargo, al formar una vegetación densa, el establecimiento de plantas vasculares competitivas es fuertemente limitado.

El éxito de las plantas vasculares en el Hemisferio Sur está determinado por dos factores principales: En primer lugar, los musgos de *Sphagnum* pierden su habilidad competitiva debido a las condiciones adversas de crecimiento, como desecación por vientos y bajo aporte de nutrientes. Bajo estas condiciones, el *Sphagnum* pierde en la competencia con las plantas vasculares, que tienen suficiente acceso a los nutrientes. Como los nutrientes son escasos en los ecosistemas, se requiere una biomasa de raíces particularmente densa. En segundo lugar, la gran masa de raíces es beneficiosa para las plantas vasculares solamente si las raíces están protegidas de la anoxia en condiciones de anegamiento. Las plantas en cojín (por. ej., *Astelia pumila*) se mantienen en el agua al crear condiciones extensas de oxigenación, facilitadas por el bajo consumo de oxígeno a bajas temperaturas. Del mismo modo, el *Empodisma minus* escapa del agua en las turberas de Nueva Zelanda por el crecimiento y movimiento ascendente de las raíces, siguiendo los movimientos del agua. Pero, ¿qué impide que estos especialistas de turberas australes no tomen el control de las turberas del Hemisferio Norte? Darwin podría haberlo sabido …

Samenvatting

Veenlandschappen vervullen waardevolle functies. Ten eerste kunnen deze landschappen koolstofdioxide vanuit de afmosfeer opslaan doordat veen zich ophoopt (oude planten worden niet volledig afgebroken). Daarnaast vormen ze belangrijke zoet water reservoirs. Een klassiek voorbeeld van veenvormende ecosystemen zijn hoogvenen. Gespecialiseerde *Sphagnum* soorten, ook wel veenmossen genoemd, zijn daar uitgebreid aanwezig.

Op het noordelijk halfrond winnen *Sphagnum* mossen gewoonlijk de competitie van vaatplanten, ofwel hogere planten. De mossen houden schaarse voedingsstoffen en water efficiënter vast dan hogere planten. Bovendien kunnen hogere planten in natte omstandigheden slechts beperkt wortels vormen omdat deze onder natte condities aan zuurstof gebrek leiden. Als de omstandigheden voor de wortelgroei van hogere planten verbeteren (lagere waterstanden en hogere concentraties voedingsstofffen) kan de biomassa productie van deze planten toenemen en kunnen zij dominant worden. In veel hoogvenen in Europa, Noord-Amerika en China heeft zo'n verandering naar de overheersende aanwezigheid van hogere planten plaatsgevonden. In de meeste gevallen zijn de hogere planten hier dominant geworden door een kunstmatig laag waterpeil en door van mensen verhoogde (atmosferische) stikstofdeposities.

Op het zuidelijk halfrond zijn er ook veel hoogvenen waar hogere planten (kussenplanten) domineren over *Sphagnum* mossen. De dominante rol van de hogere planten in de zuidelijke venen is echter al duizenden jaren oud en kan dus niet worden toegeschreven aan antropogene invloeden. Hoogvenen die gedomineerd worden door kussenplanten, ofwel kussenvenen, zijn in gematigde streken over het algemeen wijder verspreid dan *Sphagnum* venen. Kussenvenen zijn rijkelijk aanwezig langs de kust van Patagonië en komen ook voor in de bergen van Nieuw Zeeland en Tasmanië, waar de ophoping van veen wordt bevorderd als er voldoende regen valt. De meest voorkomende soorten kussenplanten in Patagonië zijn *Astelia pumila* en *Donatia fasciculares*. *Empodisma minus* hoort bij een andere belangrijke groep veenplanten ('restiads' van de Restionaceae familie) die in Nieuw Zeeland 'restiad' venen vormen. *Deze thesis richt zich met name op de kussenvenen in Patagonië, maar ook op de restiad venen in Nieuw Zeeland, als model ecosystemen, om te onderzoeken welke factoren sturend zijn in de competitie tussen Sphagnum mossen en hogere planten.*

De hoogvenen die worden beschouwd in dit proefschrift waren niet aangetast door ecohydrologische veranderingen zoals drainage**.** Om te onderzoeken welke factoren sturend zijn in de competitie tussen mossen en hogere planten in hoogvenen heb ik de groei-omstandigheden van *Sphagnum* mossen onderzocht. Daarbij heb ik me vooral gericht op de beschikbaarheid van voedingsstoffen, het hydro-klimaat en gaswisseling ten bate van fotosynthese. Verder heb ik onderzocht hoe de hogere planten zich hebben aangepast aan de beperkte groeimogelijkheden in venen.

Sphagnum magellanicum mossen, die in Patagonië groeien, hebben zich goed aangepast aan de voedselarme omgeving (hoofdstuk 2). Ecofysiologische experimenten lieten zien dat de opnamecapaciteit van stikstof door *Sphagnum magellanicum* uitzondelijk hoog is. De opname efficiëncy verminderde bij hogere stikstofconcentraties en langer durende blootstelling aan stikstof. Wanneer deze fysiologische opnamesnelheid geëxtrapoleerd wordt naar het veld, betekent dit dat de stikstof vanuit regen in enkele minuten opgenomen zou worden door *Sphagnum magellanicum*. De snelle opnamecapaciteit zou de *Sphagnum* mossen zo een monopolie positie geven over natuurlijke atmosferische voedingsbronnen (hoofdstuk 2 & 3). Alleen wanneer de stikstofconcentraties hoog zouden zijn (bijvoorbeeld vanuit regen in gebieden waar stikstofvervuiling is) zouden de *Sphagnum* mossen de stikstof niet volledig op kunnen nemen. De stikstof zou dan beschikbaar komen voor hogere planten die dieper geworteld zijn.

Het monopolie effect, waar aanwijzingen voor werden gevonden in de lab experimenten, werd ook gevonden in een Patagonisch hoogveen gedomineerd door *Sphagnum magellanicum.* Hoewel de effectieve beschikbaarheid van voedingsstoffen voor hogere planten zeer laag is in Patagonische venen, had 3 jaar flinke bemesting weinig effect. De biomassa productie en de concentraties van bladnutrienten in de hogere planten bleef laag. De groei van *Sphagnum magellanicum* daarentegen, verdubbelde nadat belangrijke voedingsstoffen zoals stikstof en fosfor in overdaad werden toegevoegd. Een overdaad aan stikstof veroorzaakte echter ook fysiologische stress. Dit zagen we aan fysiologische stress parameters zoals de ophoping van aminozuren en chlorofyl. We hadden verwacht dat deze stress minder zou worden door de toevoeging van fosfor, maar dat bleek niet te gebeuren. De *Sphagnum* mossen droogden uit door de toevoeging van voedingsstoffen. Dit werd veroorzaakt doordat de toevoeging van voedingsstoffen de dichtheid van de mossen verminderde en daarmee de capaciteit van de mossen om water vast te houden evenzo verminderde. Een tekort aan water, bijvoorbeeld door uitdrogende wind, is een beperkende factor voor *Sphagnum* groei in Patagonië (hoofdstuk 3 en 7). Extra voedingsstoffen kunnen het tekort aan water potentieel versterken.

Ook in de gematigde warme klimaten van Nieuw Zeeland en Tasmanië wordt de groei van *Sphagnum* mossen beperkt door uitdroging. Droogte wordt daar vaak veroorzaakt door verdamping waardoor het waterpeil in de zomer wel 40 cm kan zakken (hoofdstuk 4). Een laag waterpeil verbetert de groei-omstandigheden voor hogere planten, wat resulteert in de dominantie van hogere planten over *Sphagnum.* In gematigde warme klimaten en voedselarme omstandigheden worden venen voornamelijk gevormd door *Empodisma minus.* De grote wortelmassa van *Empodisma* speelt, behalve in de voorziening van voedingstoffen en water aan de plant zelf, ook een centrale rol in de vorming van veen. Een te hoog waterpeil (overstroming) zorgt voor een gebrek aan zuurstof in wortels in hogere planten. We hebben 2 mechanismen ontdekt die wortels van *Empodisma* soorten tegen overstroming beschermen (hoofdstuk 4). Ten eerste bewegen patches van *Empodisma* mee met het waterpeil. Ten tweede kunnen de klusterwortels van *Empodisma* omhoog groeien, waarmee zij het risico op overstroming op de lange termijn verminderen. We hebben een nauwe relatie gevonden tussen de dichteid van *Empodisma* planten en de efficiëncy waarmee ze de gevolgen van overstroming voorkomen. Er wordt bediscussieerd in welke mate *Empodisma* planten zelf de op en neergaande beweging verbeteren, danwel ontwikkelen, door gasgevulde wortels te groeien in de veengrond.

De focus van hoofdstuk 5 is gericht op gas transport en de productie en emissie van methaan in kussenvenen. De hoogvenen in Patagonië bevatten grote hoeveelheden luchtgevulde wortels van kussenplanten. Deze wortels kunnen zuurstof effectief transporteren en afzetten in de wortelzone, waardoor de veen gronden, zelfs onder water, geoxideerd worden. Kussenplanten verzorgen op deze manier zelf de geoxideerde bodemconditieswaardoor de wortels beter kunnen groeien en de beschikbaarheid van voedingsstoffen verbeterd. Hoewel de investering in de wortelbiomassa aanzienlijk is, kunnen de kussenplanten het ook bovengronds nog steeds van *Sphagnum* mossen winnen (hoofdstuk 5-7). De wortels van kussenplanten worden tot wel 200cm lang. De grote hoeveelheden wortels van kussenplanten veroorzaken een substantiële verandering in de biogeochemie van het veen door de stimulering van oxideringsproces tot diep in het veen. De methaan productie in het veen en methaan emissie uit het veen werden geremd als gevolg van het uitgebreide zuurstof verlies van de wortels in de wortelzone. Het onderdrukken van zuurstoftransport in de planten door het afknippen van de scheuten keerde dit patroon. Interessant is dat kussenplanten de mogelijkheid hebben koolstofdioxide op te slaan in de vorm van veen, zonder methaan emissie te stimuleren.

Hoofdstuk 6 toont de samenleving van *Sphagnum* mossen met methanotrofe bacteriën aan in de hoogvenen van Patagonië. Door methaan te oxideren leveren methanotrofe bacterien koolstofdioxide, wat wordt gebruikt voor de fotosynthese in de mossen. De remmende werking van van kussenplanten op de productie van methaan zoals gevonden in hoofdstuk 5 hebben we ook kunnen meten in naastgelegen patches van *Sphagnum* mossen. In deze *Sphagnum* patches, waar weinig methaan aanwezig was (bijvoorbeeld wanneer *Sphagnum* mossen omringd waren door kussenplanten), hebben we een lagere activiteit van methanotrofe bacteriën gevonden. Daarom denken we dat kussenplanten zo hun competitieve voordeel ten opzichte van mossen verder verbeteren door de methaanbeschikbaarheid te verminderen. De verminderde methaanbeschikbaarheid verminderd ten slotte via de samenleving uiteindelijk de beschikbaarheid van koolstofdioxide voor de fotosynthese van mossen.

Hoofdstukken 3 tot 7 laten zien dat de condities in zuidelijke veengebieden sub-optimaal zijn voor de groei van *Sphagnum*. De belangrijkste beperkende factor voor *Sphagnum* groei is waarschijnlijk de lage vochtigheid aan het mosoppervlak, veroorzaakt door uitdrogende winden. Daarnaast verlaagt de beperkte inbreng van voedingsstoffen via natuurlijke atmosfersche deposities de groei van *Sphagnum*. De kussenplanten in zuidelijke veengebieden hebben zich daarentegen aangepast aan deze moeilijke groei-omstandigheden (hoofdstuk 7). Kussenplanten beschermen zich tegen de koude en droge wind door dikke, leerachtige bladeren die dicht aan de oppervlakte groeien. Veld- en laboratoriumexperimenten bewezen dat de lange wortels van kussenplanten voedingsstoffen mobiliseren, die over het gehele worteltraject opgenomen kunnen worden (hoofdstuk 5 & 7). Door dicht bij elkaar ('kussens') te groeien kunnen kussenplanten nog meer voedingsstoffen vast houden en recyclen. Een verbeterde voedingstoestand verhoogt het vermogen van fotosynthese en biomassaproductie, wat nodig is om dichte kussens te vormen. Aan de andere kant, een grote investering in wortelbiomassa maakt kussenplanten kwetsbaar voor schaduw van andere planten en verstoringen zoals lange overstromingen en vorstperiodes (hoofsdstuk 7). Echter door het vormen van een dichte clonale vegetatie wordt de vestiging van andere competitieve hogere planten beperkt.

Het succes van hogere planten in de hoogvenen van het zuidelijk halfrond wordt voornamelijk bepaald door de volgende twee factoren: Ten eerste, *Sphagnum* mossen missen de competitieve kracht door de "moeilijke" groeiomstandigheden zoals uitdrogende wind, de beperkte aanwezigheid van voedingsstoffen en lage zomer temperaturen. Als kussenplanten over voldoende voedingsstoffen beschikken, kunnen ze onder deze omstandigheden de *Sphagnum* mossen gemakkelijk wegconcurreren. Maar alleen hogere planten met een heel dichte wortelmassa zijn hiertoe in staat omdat de voedingsstoffen schaars zijn in hoogvenen. Een grotere wortelmassa is alleen voordelig voor de hogere planten als hun wortels beschermd blijven tegen het gebrek aan zuurstof onder natte omstandigheden. Kussenplanten voegen zelf voldoende zuurstof toe aan het water en gebruiken weinig door de lage temperatuur in de zomer. Restiad planten, bij voorbeeld *Empodisma minus*, ontsnappen als het ware aan het water door opwaartse groei van wortels en het meebewegen met het waterpeil. Waarom overheersen deze zuidelijke hoogveen specialisten niet in de veengebieden op het noordelijk halfrond? Daar wist Darwin misschien een antwoord op …

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Curriculum vitae

Christian Fritz was born in Güstrow on 15 August 1980 and grew up in the former GDR. Already during high school Christian became involved in projects concerning water management and restoration of peatlands that are characteristic for the lowlands surrounding Güstrow. He graduated from high school at the John-Brinckman-Gymnasium in Güstrow in 1999. Before going to university in 2000 Christian worked for the government fulfilling the compulsory civil service. At university he followed a diverse range of Master courses, i.e. hydrology, plant science, peatland ecology and environmental ethics and economics at Greifswald University (Germany) and Waikato University (New Zealand).

In November 2005 Christian went for the first time to Tierra del Fuego (Argentina) to take field measurements. At that time the idea for the present thesis emerged together with other participants of the international field symposium of the IMCG held in Tierra del Fuego. This first visit to Argentina formed base of his PhD research that was conducted in a formal co-operation with the universities of Groningen and Nijmegen (the Netherlands), the CADIC and Recursos Hídricos in Argentina. The PhD research lasted from 2007 to 2012 and included a total of 2 years of field campaigns in Argentina. During his PhD research Christian enjoyed the collaboration with research groups from Greifswald University and Münster University (Germany). Supervision of 12 master student projects and the organisation of 2 field courses in Ecohydrology rendered valuable teaching experiences.

At present Christian is employed as a post-doctoral researcher by the Experimental Plant Ecology group at Nijmegen University. His post-doctoral research focuses on roots and root plasticity when roots cope with water and nutrient limitations.

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2012