GROWING PEAT

Sarah Faye Harpenslager

“Growing peat”

door

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Don’t panic

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Growing peat: an introduction

Sarah Faye Harpenslager

How to grow peat…in a nutshell

Textbox 1.1: Growing peat in a nutshell

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- Water
- Sunlight
- Peat-forming plant species
- Patience

Even the vastest peatlands covering large parts of Canada, Siberia or Indonesia (Gorham 1991; Jaenicke and others 2008) have at some point started their development on bare, mineral soil. Despite the short list of ingredients (Textbox 1.1), however, studying how peat growth initiates “from scratch” is a challenge, since the process of peatland formation occurs on a completely different timescale than the average PhD project. By definition peat consists of a layer of > 30 cm of partially decomposed material with > 60 % organic matter (Bullock and others 2012). Based on ^{14}C dating of peat cores, peat accumulation rates have been determined to range from 0.1 to 3.1 mm yr^{-1} (Wieder and others 1994; Ovenden and others 1998; Gorham and others 2003; Borren and others 2004). These rates, however, refer to accumulation in developed fens and bogs, but do not include early stages. Fully developed raised bogs of 3-10 m thick have usually taken 5,000 to 13,000 years to complete succession and build-up of peat (Gorham 1991; Waddington and Roulet 2000), while I had only four years for my PhD study. By focussing on carbon (C) fluxes instead of actual increases in thickness of the peat layer, however, it could still be determined whether a system forms a net sink of C.

Carbon dynamics of growing peatlands

Under waterlogged, anaerobic conditions, as caused by geohydrological conditions in the landscape, decomposition of plant litter is slowed down, leading to the accumulation of partially decomposed organic matter with a high C content. The reduced decomposition rates are caused by a low availability of oxygen, which is the thermodynamically most favourable terminal electron acceptor for decomposition, and related low phenol oxidase activity (Freeman and others 2001b; Reddy and Delaune 2008). In the succession of wetlands, the process of peat accumulation is the driving force behind terrestrialisation, the transition of open water to land. Although this process does not follow the same pathway in all systems and depends on geohydrological settings, transition will generally pass through an aquatic, semi-aquatic, fen and bog stage. First, open water is colonised by submerged or floating vegetation. Due to the accumulation of organic matter, the water depth is reduced and semi-aquatic species, such as rooting helophytes can enter the system. Through their air-filled roots, these species can form floating mats, where litter accumulation continues on top of and inside the root mat, thus forming thick, floating fens. Under minerotrophic conditions, when systems are influenced by groundwater or surface water, succession of mires (growing peatlands) typically starts with rich fens, which are dominated by minerotrophic plants including brown mosses. Due to the accumulation of peat, the influence of buffering groundwater or surface water decreases. Through the additional active acidification by colonising *Sphagnum* mosses, the rich fens are transformed into poor fens, with a much lower buffering capacity (Van Breemen 1995). As the accumulation of peat continues in these poor fens, the influence of groundwater further decreases and the system turns into a rainwater-fed (ombrotrophic) bog. Although some transitions have been known to occur rapidly (within a few decades; e.g. Kuhry and others (1993); Bakker and others (1997); Granath and others (2010), Crushell and others (2009)), the complete succession of open water to bog vegetation and the accumulation of thick peat layers (up to 13 m in some areas (Jaenicke and others 2008; Bos 2012)) has generally taken hundreds to thousands of years (Frolking and others 2001).

Although peatlands only cover around 3% of the total land surface, they store approximately one third of all terrestrial C and are thus very important in the global C cycle (Gorham 1991). At the same time, however, peatlands form the largest natural source of methane (CH_4) and emit -5 to 63 g C m^{-2} y^{-1} (Saarnio and others 2007; Salm and others 2009; Drewer and others 2010). Still, pristine mires generally are net C sinks (Belyea and Malmer 2004) due to high CO_2 fixation rates by peat-forming vegetation. On average, peatlands show C sequestration rates ranging from 5 to 40 g C m^{-2} yr^{-1} in fens and from 3 to 160 g C m^{-2} yr^{-1} in bogs (Lamers and others 2015). Due to the high CH_4 emissions, which has a 34 times higher global warming potential than CO_2 (IPCC 2013), they may either buffer or stimulate the greenhouse effect, with values ranging from -196 (net buffering effect) to +1540 (net stimulating effect) g CO_2-eq m^{-2} yr^{-1} (Byrne and others 2004; Salm and others 2009; Long and others 2010; Beetz and others 2013; Lamers and others 2015).

Peatland degradation and loss of services

Due to anthropogenic forcing, many peatlands throughout the world have been altered and have become either partially or completely degraded (Joosten and Clarke 2002). While pristine peatlands generally show high C sequestration rates, drained and degraded peatlands often turn into C sources, emitting 80 to 880 g C m^{-2} y^{-1} (Lamers and others 2015). The human impact on peatlands either happens directly, through peat extraction or drainage, or indirectly, by for example water pollution, global warming and habitat fragmentation. Combined, anthropogenic forcing has led to the disappearance of approximately 50% of global wetlands and 16% of global peatlands, whereas the remaining systems have been heavily degraded (Zedler and Kercher 2005; Lamers and others 2015).

Because dried peat (turf) has a high C content, it has been used as fossil fuel for many centuries. In the European lowland countries (The Netherlands, Germany and Belgium), local peat extraction started as early as the Middle Ages (van Dam 2001; Deforce and others 2007), while in other parts of the world, excavation mainly started in the second half of the 19th century and early 20th century, when machinery made it easier to start large-scale, commercial excavations (Bullock and others 2012). While small-scale extraction has actually been reported to stimulate landscape heterogeneity and biodiversity (Chapman and others 2003), large-scale peat extraction has severely altered these systems (Lamers and others 2015). Nowadays, peat is generally extracted commercially to provide peat substrates for horticultural purposes (Lavoie and Rochefort 1996), although burning of peat as a fuel still occurs (e.g. in Ireland, Figure 1.1).

Figure 1.1. Peat extraction in County Mayo, Ireland (2015)

Worldwide, approximately 15% of all peatlands have been drained to accommodate agriculture, peat extraction, forestry or urbanisation (Joosten 2009), although considerable differences exist between countries, with 10% to 85% of peatlands drained within single countries (e.g. Brock and others (1999); Zanello and others (2011); Hooijer and others (2012); Meckel and others (2006); Hoeksema (2007)). Drainage has resulted in strong degradation by oxygen intrusion, enhancing aerobic decomposition of organic matter and C emission. Although CH_4 emissions decrease after drainage, emission of CO_2 increases dramatically, thereby turning drained and degraded peatlands into net C sources (Alm and others 1999; Waddington and others 2001). Furthermore, due to compaction and consolidation (Hooijer and others 2012), drainage causes fast land subsidence (2 - 150 mm y^{-1}; Syvitski and others (2009); Figure 1.2). Given the projected sea-level rise, this continuing subsidence of peatlands - often located in densely populated coastal areas, river deltas and floodplains - poses a serious risk to public safety due to higher flooding risks (Syvitski and others 2009; Temmerman and others 2013).

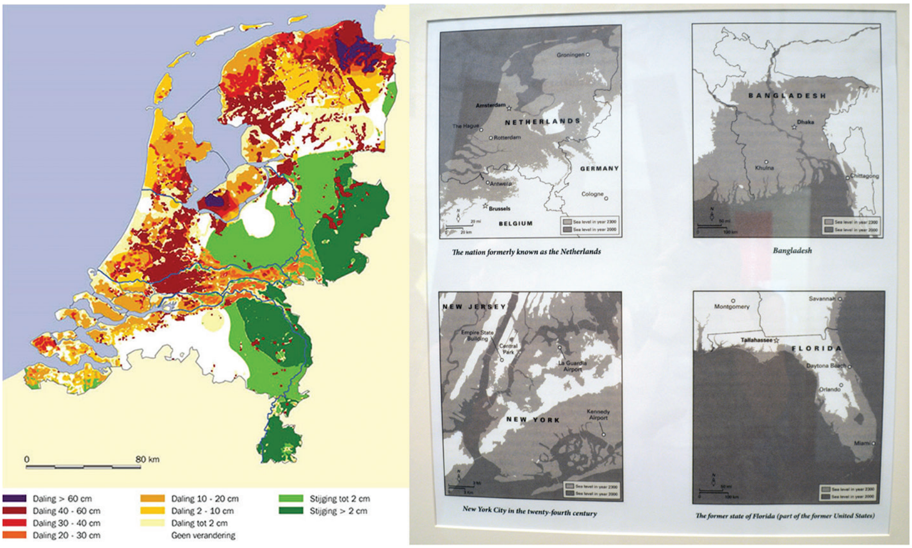

Figure 1.2. Expected land subsidence and flooding risks in low-lying areas in 2050 (left, Netherlands. Map by Rijkswaterstaat, 1997) and 2300 (right; showing the Netherlands, Bangladesh, New York City and Florida. Picture taken in the maps section of the Boston Public Library).

After drainage, peatlands are often used as pastures or arable lands. To increase production, fields are fertilised, leading to accumulation of phosphorus (P) and nitrogen (N) in the top layer and run-off of nutrients into surrounding surface water and groundwater (Britto and Kronzucker 2002; Miller and Cramer 2005). As a result of high decomposition rates (Geurts and others 2010), nutrient concentrations per unit volume increase further (Van Diggelen 2015). In combination with increased atmospheric deposition of N (Bobbink and others 1998) and nutrient loading through surface water, especially in agricultural areas, many peatlands have become eutrophic (Zedler and Kercher 2005). As a result, the species composition has changed from relatively slow-growing, peat-forming species, towards fast-growing, but also fast-decomposing species that do not add to the build-up of a peat layer (Lamers and others 2015). Furthermore, high nutrient availability often stimulates growth of a small number of fast-growing, dominant species, leading to light limitation in other, slower-growing species and to lower biodiversity (Hautier and others 2009).

Wetlands, including peatlands, are considered to provide some of the most valuable ecosystem services (Costanza and others 1997). As a result of the degradation of peatlands, however, many services that are generally provided by pristine peatlands, including C sequestration, water retention and biodiversity, are lost. It is therefore necessary to either restore degraded peatlands or create new, peat-forming wetlands to compensate for the lost services. While the former of these solutions is increasingly carried out in degraded peatlands of Europe and North America with varying results, new wetlands are still mainly constructed to filter municipal wastewater in treatment facilities. Constructed wetlands can, however, also fulfil other services, including C sequestration (De Klein and Van der Werf 2014), habitat compensation (Zedler 1996), peatland restoration (Wild and others 2001) and biodiversity (Hansson and others 2005; Hsu and others 2011). Furthermore, new applications of peatlands are currently being developed, including paludiculture and peat capping of landfills (Joosten and Clarke 2002; Egbring 2011; Gaudig and others 2013).

Abiotic conditions and peat formation

Accumulation of peat only occurs when the rate of biomass production exceeds the decomposition rate of plant litter (Figure 1.3; Saarnio and others (2007)). Both processes are strongly influenced by the biogeochemical conditions of the system (indicated by the arrows in Figure 1.3), as the availability of nutrients, carbon and toxins in soil and water will strongly determine both species composition and litter quality.

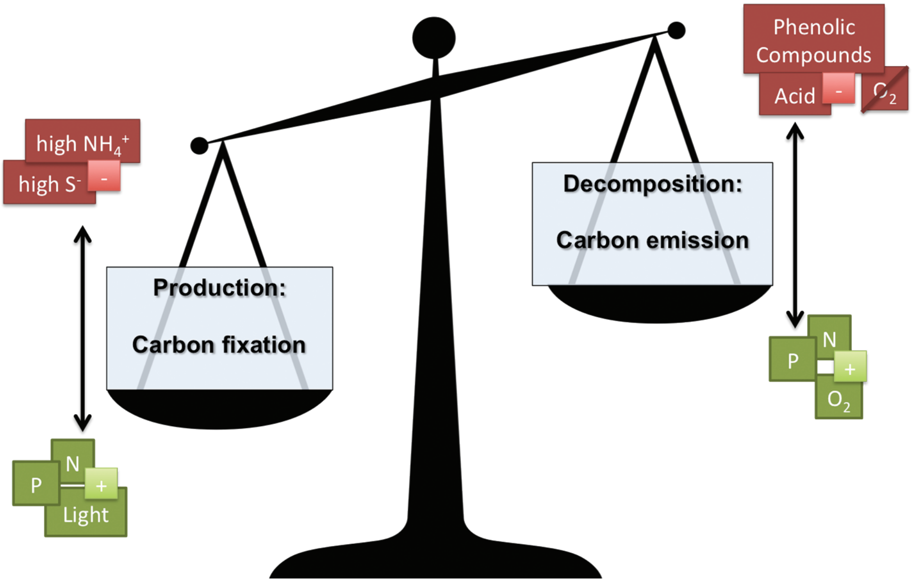

Figure 1.3. Peat formation is a matter of balance: when production and decomposition processes occur at a similar rate, the scales are balanced and there is no net increase or decrease of the peat layer. When production increases or decomposition decreases the balance tips towards production and net C fixation. Only under these circumstances organic matter accumulates and peat formation occurs. Production is stimulated by the availability of light and nutrients, whereas presence of toxins can inhibit biomass production. Decomposition processes, on the other hand, are stimulated under aerobic and eutrophic conditions, whereas high content of phenolic compounds or an acid or anaerobic environment will limit decomposition.

Nutrient availability

Although higher nutrient concentrations will, generally, increase net primary production (NPP) rates, they will also change species composition due to competitive advantages of fast growing species. In eutrophic systems, lemnoids, algae or cyanobacteria dominate the water layer, whereas terrestrial parts become dominated by graminoids such as *Glyceria* and *Juncus* spp. (Bobbink et al 1998). Furthermore, such systems are often characterised by high decomposition rates (Rejmankova and Houdkova 2006). The final balance between the high production and simultaneous high decomposition will determine whether such a system is a net sink or source of C (Figure 1.3). In more oligotrophic systems, on the other hand, vegetation growth and decomposition processes are slow. Although peatland succession usually proceeds towards an oligotrophic bog phase, where peat accumulation results from very low decomposition rates rather than high NPP (Clymo 1983; Thormann and others 1999), early-successional stages characterised by low nutrient availability will remain bare for a long time. Both in newly constructed wetlands and in degraded peatlands, soil quality will therefore strongly influence the development and composition of the vegetation and biomass production rates. While eutrophic substrates induce the risk of high nutrient mobilisation to the water layer and subsequent algal blooms, oligotrophic mineral soils will slow down plant colonisation and thus hamper the initial stage of peat formation.

Inorganic carbon

While terrestrial plant species and helophytes can readily take up C from the atmosphere, submerged aquatic vegetation relies on dissolved inorganic carbon (DIC) to fulfil its C demand. Since the diffusion of CO_2 in water is about 10,000 times lower than in air, C concentrations would be very low in aquatic systems when atmospheric CO_2 is the main C source (Smith and Walker 1980), such as in softwater lakes (Roelofs and others 2002). Through decomposition processes in the soil, CO_2 can reach supersaturated concentrations in the overlying water layer (Cole and others 1994), whereas calcareous soils or groundwater discharge will supply HCO_3^-. Due to liming of agricultural fields or the use of HCO_3^--rich riverine water to compensate for drought, however, peatlands have often been affected by alkalinisation (Roelofs 1991), especially in agricultural areas. As a result, the buffering capacity and pH increase and DIC is mainly present as bicarbonate (HCO_3^-). Not all species are able to use HCO_3^- as an alternative C source (Maberly and Madsen 1998). Furthermore, since HCO_3^- -based photosynthesis is an energetically costly process (Lucas 1983; Borowitzka 1984), most HCO_3^--using species show reduced growth. An increase in HCO_3^- concentrations therefore favours the growth of efficient HCO_3^- -using species such as different algae and submerged aquatic macrophytes such as *Chara* spp., *Potamogeton pectinatus* and *Myriophyllum spicatum* (McConnaughey 1998). Alternatively, such systems become dominated by floating species that can take up atmospheric CO_2. Moreover, high concentrations of HCO_3^- can also induce toxic responses in, for example, *Sphagnum* mosses (Clymo 1973).

Toxins

Apart from obvious, chemical contaminations resulting from, for example, discharge of industrial wastewater, high concentrations of e.g. HCO_3^- (see above), sulphide (H_2S) or ammonium (NH_4^+) can also cause toxicity in susceptible plant species. In systems with a high nitrogen (N) or sulphate (SO_4^{2-}) load, anaerobic conditions lead to the biogeochemical conversion of these substances to NH_4^+ and H_2S, respectively, which may accumulate to phytotoxic concentrations (Smolders and others 2000; Lamers and others 2013).

Biotic conditions and peat formation: Ecosystem engineers

Species that directly or indirectly alter their environment, by regulating resource availability to others or causing changes in the physical state of the environment, are called keystone species or ecosystem engineers (Jones and others 1994; Wright and Jones 2006). Beavers are the well-known icons of ecosystem engineers, since the dams they build severely impact the hydrology of their environment. In the processes of terrestrialisation and peat accumulation, several species can be considered ecosystem engineers. In this thesis, *Stratiotes aloides*, *Typha* spp. and *Sphagnum* spp. were chosen to represent ecosystem engineers occurring in the aquatic, semi-aquatic and floating mire stage of early peatland development, respectively (Figure 1.4).

Figure 1.4. The three studied ecosystem engineers in this thesis: *Stratiotes aloides* (left), *Typha* spp. (*T. latifolia*; middle) and *Sphagnum* spp. (*S. squarrosum*; right).

Aquatic phase: *Stratiotes aloides*

In the aquatic phase, fast colonising species of floating aquatic macrophytes can initiate the terrestrialisation process by forming dense floating mats that support species with air-filled rhizomes (Van Wirdum, 1991; Verhoeven & Bobbink, 2001) and are characterised by a high biodiversity (Sugier and others 2010). Furthermore, due to their high potential growth rates, they fill up the water layer with partially decomposed organic matter, thus reducing water depth and improving conditions for semi-aquatic species. Dense stands of floating aquatic macrophytes can strongly influence the oxygen concentration of open water, by limiting the atmospheric O_2 flux to the water layer (Caraco and others 2006), and decreasing turbidity of the water layer by preventing re-suspension of sediment particles (Madsen and others 2001).

In peatland areas in Western Europe, *Stratiotes aloides* dominates sheltered bays of larger fen lakes, backwater ponds, ditches and canals by forming floating mats through vigorous clonal reproduction (Figure 1.5). As a result, this aquatic macrophyte can completely fill up surface waters within a few growing seasons under meso- to eutrophic conditions (Cook and Urmi-König 1983). One of the most characteristic features of *S. aloides* is its alternating life cycle with an emergent and submerged life stage (Cook and Urmi-König 1983). During summer, plants may become buoyant and can form dense floating vegetation mats. In autumn, they sink and remain submerged during winter until they re-emerge in spring.

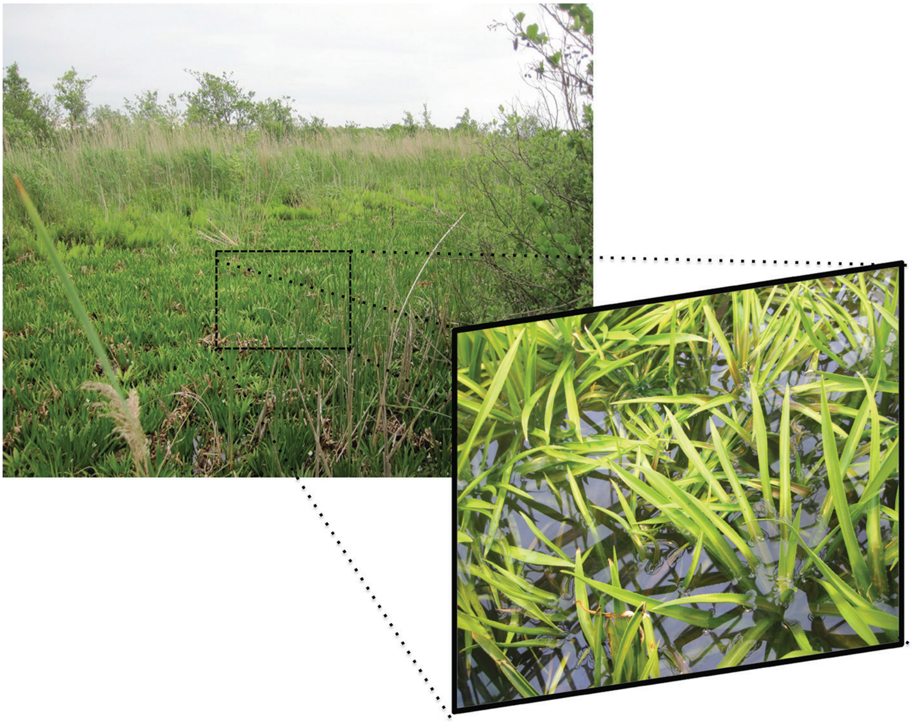

Figure 1.5. *Stratiotes aloides* forms a dense floating mat that can cover entire water bodies. This picture was taken in National Park Weerribben-Wieden, Overijssel (The Netherlands).

Semi-aquatic phase: *Typha* spp.

Under high nutrient availability, in combination with low water levels (<80 cm; Grace and Wetzel (1982)), fast growing helophytes, such as *Phragmites australis*, *Juncus* spp., *Carex* spp. or *Typha* spp. usually dominate and colonise the water from banks and shores (Timmermann *et al.*, 2006). These species can show both very high production rates (Brix *et al.*, 2001, Kirschner *et al.*, 2001 Christensen *et al.*, 2009) and relatively low decomposition rates, especially of the belowground biomass (Brinson and others 1981; Alvarez and Becares 2006). As a result, they are potentially important peat-formers. Apart from adding to the actual build-up of the peat layer, helophytes such as *Typha* spp. produce air filled rhizomes (Figure 1.6) and thus facilitate the formation of floating peat rafts (Hogg and Wein 1988; Verhoeven and Bobbink 2001; Somodi and Botta-Dukat 2004).

Figure 1.6. Formation of floating rhizomes by *Phragmites australis* (left) and *Typha latifolia* (right; picture by J.P. van Zuidam).

Floating mire phase: *Sphagnum* spp.

Sphagnum spp. strongly modify their environment and are thus important ecosystem engineers in peatlands (Van Breemen 1995). They are capable of actively acidifying their habitat by exchanging cations for protons (Clymo 1963; Hajek and Adamec 2009) and releasing organic acids (Van Breemen 1995). Furthermore, *Sphagnum* spp. keep their environment moist due to the high water holding capacity of their hyaline cells (Clymo 1973) and compact growth structure. By increasing the acidity and moisture content of their habitat, *Sphagnum* spp. also slow down decomposition rates, thus providing optimal conditions for the accumulation of organic material. Moreover, high concentrations of phenolic compounds in their tissues, including antibiotics (Verhoeven and Toth 1995), further decrease decomposition rates (Yavitt and others 2000; Freeman and others 2001b). This combination of traits results in a strong contribution of *Sphagnum* mosses to C sequestration and peat formation worldwide (Coulson and Butterfield 1978; Limpens and Berendse 2003). Still, individual species differ considerably in their growth rate, habitat requirements and tolerance to environmental stress (Figure 1.7, Clymo (1973)).

Figure 1.7. Different species of *Sphagnum*, such as *S. palustre* (left) and *S. squarrosum* (right), differ in their habitat requirements and tolerance to environmental stress. Picture by Stefan Weideveld.

Goal and hypotheses

The research presented in this thesis is embedded in the STW Programme "PeatCap: natural capping of the polluted Volgermeerpolder (The Netherlands) provides new chances for peat growth counteracting land subsidence", carried out together with the Universities of Utrecht and Amsterdam (see Textbox 1.2). Within this PeatCap project, we aimed to optimise new peat development -or rather, net C sequestration- in pioneer systems or restored peatlands. The goal of my part of the programme and of this thesis was to find the combination of soil, water and vegetation that results in the highest net C sequestration. To achieve this, a combination of laboratory, mesocosm and field experiments was carried out to study the influence of different environmental factors on growth and C sequestration rates of plants from different groups of peat-forming vegetation (*Stratiotes aloides*, *Typha* spp. and *Sphagnum* spp.). Conversely, as all discussed species can also be considered ecosystem engineers, their influence on the environment was also studied.

Textbox 1.2: Volgermeerpolder

In 2011, the construction of a new wetland, designed to function as a natural cap, was finalised on top of the heavily polluted Volgermeerpolder, near Amsterdam, the Netherlands. Thousands of years ago, this site and its surrounding area, were a continuous raised bog, with peat layers of several metres (up to 11 m (Bos 2012)) thick. Due to cultivation in the Middle Ages, the peat became oxidised and the land started to subside. After several floods, the land became unsuitable for agriculture. In the 17th century, the whole area was drained again and used as pastures until, around 1920, commercial peat excavation started at the Volgermeerpolder (Buijs and others 2005). Using heavy machinery, large amounts of peat were excavated. Due to receding demand for turf, however, the production declined and an alternative source of income was initiated: dumping household waste in the excavated area. This use of the Volgermeerpolder as a landfill started around 1927, and initially only consisted of household waste from Amsterdam. From the 1950s onwards, however, industrial waste was brought to the landfill, including toxic chemicals (Buijs and others 2005). After persistent protest from neighbouring villages - gathered together in the action group “Burgerkomitee Vuilstort Volgermeerpolder” - the landfill was finally closed in the 1980s. In 2003, a plan was presented by Vista Landscape Architects and Urban Planning (Amsterdam) to construct a new, peat-forming wetland on top of the landfill (Egbring 2011). After the construction of this wetland had been finalised in 2011, a 6 ha part of the site was designated for research by a consortium composed of ACV (a collaboration between consulting and engineering companies Tauw and Witteveen+Bos) and researchers from the University of Amsterdam, Utrecht University and the Radboud University Nijmegen. The project, titled “PeatCap: natural capping of the polluted Volgermeerpolder (The Netherlands) provides new chances for peat growth counteracting land subsidence” was financed by applied technology foundation STW and the Municipality of Amsterdam. The main aim of this project was to determine the ideal combination of soil, water quality and vegetation to initiate accumulation of organic matter and reach optimal C sequestration rates and thus obtain the fastest possible way of peat formation. The work presented in this thesis was performed as part of the PeatCap project.

Thesis outline

Chapter 2 and **3** focus on the aquatic macrophyte *Stratiotes aloides*, which can cover open water by forming floating mats that initiate terrestrialisation and support endangered species. We determined which conditions, in terms of light and CO_2 availability, are essential for this species to gain buoyancy and an optimal growth rate in order to form dense, floating vegetation mats in **Chapter 2**. In **Chapter 3**, we then study how such high-density stands differ from low-density stands regarding their tolerance to environmental stress and plant nutrition. Combined, the results from both chapters provide important information on the habitat requirements and effects of intraspecific facilitation of this species.

The open water phase of the terrestrialisation process is often followed by a floating raft stage. If this floating raft is influenced by buffered groundwater or surface water below the raft, the system is called a transitional mire. In **Chapter 4**, we study the growth of four different species of *Sphagnum* on such a transitional mire. Since *Sphagnum* mosses are sensitive to buffered conditions, we determined which species are able to grow on top of floating rafts fed by calcium-bicarbonate rich water. Furthermore, we studied the contribution of these mosses to the C budget of the transitional mire.

After studying the different habitat requirements and C sequestering abilities of different ecosystem engineers, **Chapter 5** describes year-round field measurements of net C fluxes in a newly constructed wetland, to asses which of the aforementioned species will show the highest C sequestration potential in a field setting. In the 6 ha research area at the Volgermeerpolder (See Textbox 1.2), basins were constructed using either clean sand, or sand with an added layer of clay or organic soil. On all three soil types, cover and C fluxes of helophytes (*Typha latifolia*, *Typha angustifolia*), floating aquatic macrophytes (*Stratiotes aloides*), submerged vegetation (consisting of species of *Elodea*, *Myriophyllum*, *Chara*, *Potamogeton*) and bare plots were combined to determine the performance of all species and their contribution to the C sequestration rate.

In **Chapter 6**, the restoration of peat-formation of a drained, former agricultural peatland is studied in an experimental set-up. Peat monoliths were rewetted using either artificial rainwater or surface water with enhanced nutrient (PO_4^{3-}) and/or bicarbonate (HCO_3^-) availability. Due to years of fertilisation, the top 20 cm of the peatland had been loaded with P, which may lead to nutrient mobilisation and high decomposition rates after rewetting. Therefore, half the peat monoliths were rewetted after removal of the nutrient-rich topsoil. Furthermore, *Sphagnum* mosses were grown on top, to determine whether these mosses could again initiate peat formation on top of the restored peat soils.

Finally, **Chapter 7** combines the results from the laboratory experiments, mesocosm studies and field measurements to find the environmental conditions that are necessary to initiate or restore peat formation by stimulating growth of different ecosystem engineers. I then use this information to discuss two case studies: one newly constructed wetland (Volgermeerpolder) and one degraded, former agricultural peatland. From these examples, I summarise the, sometimes counterintuitive, measures that can be taken to stimulate a process that takes place on a geological timescale, so that results can already be observed on a human lifetime scale.

Chapter 2

To float or not to float: how interactions between light and dissolved inorganic carbon species determine the buoyancy of *Stratiotes aloides*

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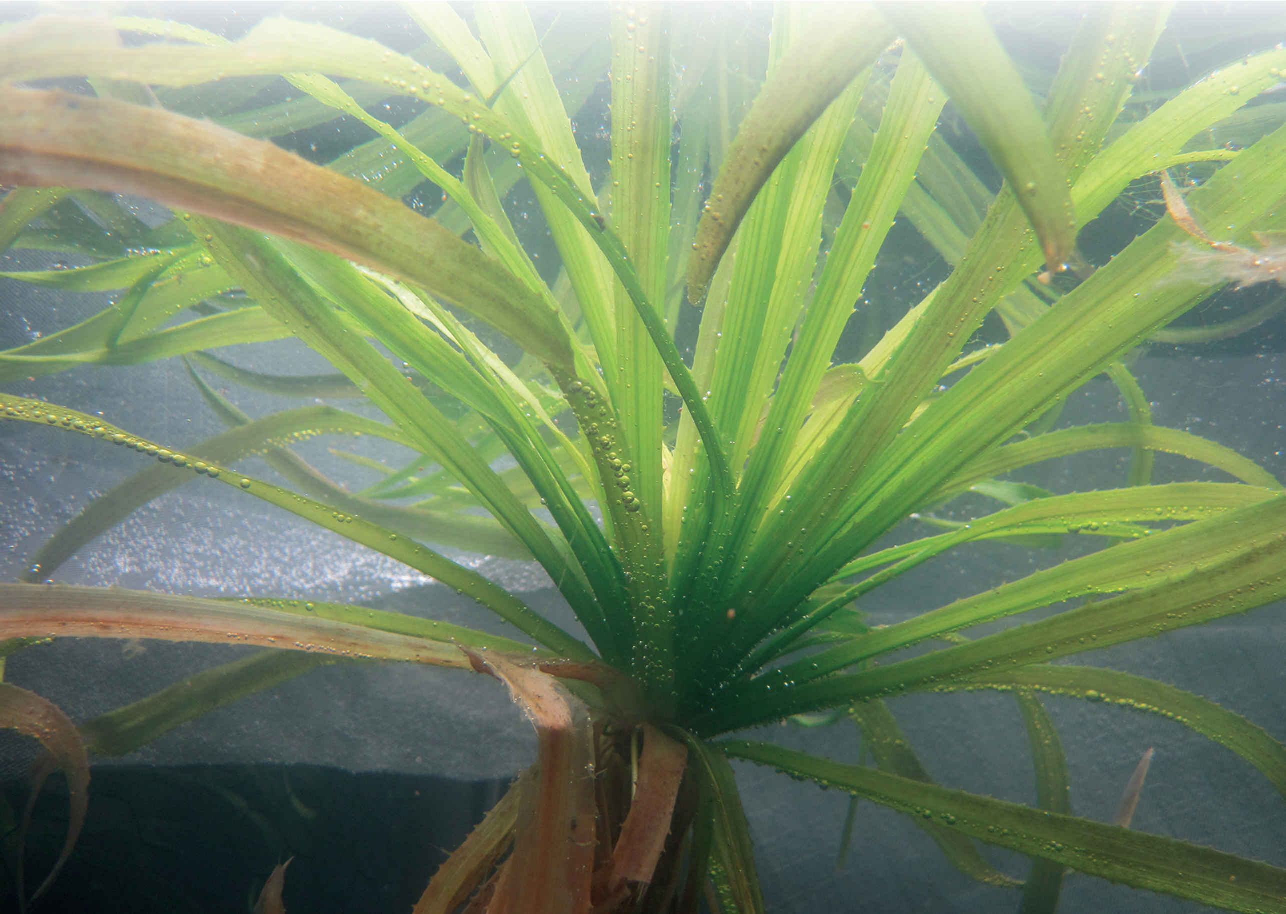

To float or not to float: how interactions between light and dissolved inorganic carbon species determine the buoyancy of *Stratiotes aloides*

Structural diversity formed by dense, floating *Stratiotes aloides* stands, generates hotspots of biodiversity of flora and fauna in wetlands. However, only part of the populations become emergent and provide this important facilitation. Since it has been hypothesised that its buoyancy depends on the rates of underwater photosynthesis, we investigated the role of dissolved CO_2 availability and PAR on photosynthesis, biomass production and buoyancy in a controlled greenhouse experiment. Photosynthesis and growth were strongly influenced by both PAR and CO_2 availability. At low PAR, plants formed less biomass and produced no emergent leaves, even when CO_2 was abundant. At low CO_2 levels, *S. aloides* switched to HCO_3^- use, resulting in lower photosynthetic O_2 production, decreased emergent leaf formation and increased $CaCO_3$ precipitation on its leaves, all of which impaired buoyancy. At high PAR, low CO_2 availability resulted in slower colonisation of the water layer, whereas CO_2 availability did not influence PAR-limited plants. Our study shows that site conditions, rather than the sole abundance of potentially facilitating species, may strongly determine whether or not they form the structure necessary to act as a facilitator for biodiversity in aquatic environments.

Introduction

In the natural succession of wetlands, the process of terrestrialisation, the transition from an aquatic to a terrestrial phase, is very important in shaping a structurally diverse habitat and supporting high biodiversity (Verhoeven and Bobbink 2001; Lamers and others 2002). However, changes in land use and water quality during the 20th century have severely influenced the functioning of wetlands in Western Europe (Roelofs 1991; Lamers and others 2002) and natural succession of open water to species-rich marshes through the formation of floating sediments or vegetation mats has become rare (Verhoeven and Bobbink 2001). Floating stands of emergent aquatic macrophytes, such as *Stratiotes aloides*, can provide structure for a wide variety of flora and fauna species, including the endangered green hawker (*Aeshna viridis*) and black tern (*Chlidonias niger*) (Rantala and others 2004; Van der Winden and others 2004; Suhonen and others 2013). Furthermore, biodiversity in these vegetation types is much higher than in similar systems where the species is absent (Strzałek and Koperski 2009; Sugier and others 2010).

S. aloides used to be very common in Europe and north-western Asia until the 1960s but has since declined severely by changes in hydrology, by eutrophication and by ammonium (NH_4^+) or sulphide (H_2S) toxicity (Roelofs 1991; Smolders and Roelofs 1996; Smolders and others 2003a). Through their high clonal growth rates, *S. aloides* populations can completely fill up surface waters within a few growing seasons under meso- to eutrophic conditions (Cook and Urmi-König 1983). Such dense vegetation also decreases turbidity of the water layer by preventing re-suspension of sediment particles (Madsen and others 2001). Furthermore, the species produces allelopathic substances that reduce algal growth (Mulderij and others 2006). As a result of these traits, the species is a strong competitor in aquatic systems and has even been reported as a noxious weed in parts of North America and Australia, where it is non-native (NRM-SA 2011; MNR 2013; NRCS 2014).

One of the most characteristic features of *S. aloides* is its alternating life cycle with an emergent and submerged life stage (Cook and Urmi-König 1983). During summer, plants may become buoyant and can form dense floating vegetation mats. In autumn, they sink and remain submerged throughout the winter until they re-emerge in spring. The increased buoyancy of *S. aloides* in spring has been speculated to be the result of enhanced underwater photosynthesis (Sculthorpe 1967; Cook and Urmi-König 1983; Smolders and others 2003a; Nielsen and Borum 2008). However, field observations and reports from literature clearly show that not all *S. aloides* stands follow this alternating life cycle and that populations may remain submerged during summer (Erixon 1979; Renman 1989; Nielsen and Borum 2008). Understanding the factors that determine whether a population remains submerged or becomes emergent is important, not only to understand the ecophysiology of floating macrophytes, but also because submerged populations show a different community composition and much lower biodiversity than emergent populations (Higler 1977) and are unable to reproduce generatively (Cook and Urmi-König 1983; Toma 2006).

So far, the mechanism behind buoyancy of *S. aloides* has not yet been unravelled under controlled experimental conditions, but as it has been hypothesised to be the direct result of photosynthesis, the absence of floating plants will most probably relate to decreased submerged photosynthetic activity. Reduced photosynthesis may not only be the result of limited nutrient (phosphorus, nitrogen) availability, but also of limited light (PAR; photosynthetically active radiation) conditions, or reduced dissolved inorganic carbon (DIC) availability. Increased turbidity and the presence of humic acids or algae can indeed limit PAR for *S. aloides* and thereby its photosynthetic rate (Bloemendaal and Roelofs 1988). C limitation, on the other hand, may not only occur in softwater lakes, typically showing low DIC concentrations, but also in alkaline waters with increased pH (>8.2) in which DIC is present as bicarbonate (HCO_3^-) instead of carbon dioxide (CO_2) (Stumm and Morgan 1996). Aquatic plant species differ considerably in their efficiency to use HCO_3^- as an alternative to CO_2 (Allen and Spence 1981; Madsen and Maberly 1991). Although *S. aloides* is able to use HCO_3^- (Prins and De Guia 1986), it may only reach approximately 15% of its maximum net photosynthesis when grown solely on HCO_3^- (Nielsen and Borum 2008).

To understand the ecophysiological traits and the potential role of *S. aloides* as a facilitating species, it is essential to understand the optimal abiotic conditions for floating mat formation. In this study, we therefore tested the interacting effects of PAR and CO_2 availability on the buoyancy of *S. aloides* in a controlled greenhouse experiment. It was hypothesised that plants that experienced either limited PAR or low CO_2 availability would survive but not become buoyant, since their photosynthetic capacity would be impaired. This process was expected to be even more profound when both CO_2 and PAR are limiting. Given their facilitating role, research into the regulation of buoyancy of *S. aloides* plants is also highly relevant for restoration projects focussing on the natural succession of wetlands and the conservation of endangered species.

Material and Methods

Experimental set-up

In total, 54 *Stratiotes aloides* plants were collected from a privately owned garden pond in the Netherlands (51°44’6”N; 6°51’49”E), in autumn 2011 and kept in artificial ponds inside the greenhouse facility of the Radboud University Nijmegen, where the experiment was carried out between April and July 2012. Nine experimental ponds of 2300 L (ø 180 cm; h 90 cm) were each divided into three equally sized compartments by nets to ensure free water movement (Figure 2.1). One compartment was covered with a double layer of shadow fabric, one with a single layer and one was kept clear of fabric to ensure full light conditions. As a result, three light intensities were created. Taking full light as 100% PAR intensity, shading led to ±40% and ±10% of full PAR intensity for the shaded and double-shaded treatments respectively. Light was mostly natural, but an artificial light regime with 200 μmol $m^{-2}s^{-1}$ lamps (Philips, Master SonT, 400W) of 16h light/8h dark was maintained to prevent large fluctuations in the light availability. Average PAR levels at the water surface level reached 130 ± 32, 50 ± 22 and 15 ± 5 μmol m^{-2} s^{-1} (mean ± SEM) for the 100%, 40% and 10% PAR treatments respectively.

Before the addition of the plants, all experimental units received 125 L of underwater peat sediment (see Table 1 for sediment characteristics) originating from a minerotrophic peatland area in the Netherlands (52°18’32”N; 4°45’42”E) and a water layer of 1500 L (60 cm) Nijmegen tap water. Since the experimental basins were dug into the greenhouse floor, water temperature remained quite constant (Median: 23.3 °C; Min: 21.1 °C; Max: 24.8 °C). As a result of the alkaline, bicarbonate (HCO_3^-) rich sediments, surface water pH values were 7.8 ± 0.1 (mean ± SEM) and CO_2 concentrations were 86 ± 53 μmol L^{-1} (mean ± SEM). Two higher CO_2 treatments were created by gently bubbling the water layer with pure CO_2 4-8 times a day using mass flow controllers (EL-FLOW select F201CV, Bronkhorst, Veenendaal, the Netherlands). These two treatments had concentrations of 228 ± 106 μmol L^{-1} (pH 7.4 ± 0.1; mean ± SEM) and 933 ± 436 μmol L^{-1} (pH 7.0 ± 0.1; mean ± SEM) CO_2. In the Results and Discussion sections, treatments are called 90, 230 and 930 μmol L^{-1} respectively. During the experiment, the CO_2 concentrations of the water layer were measured three times a week using an ABB Advance Optima Infrared Gas Analyser (ABB Analytical, Frankfurt, Germany) and treatments remained significantly different throughout the experiment (*P*=0.018; Table 1).

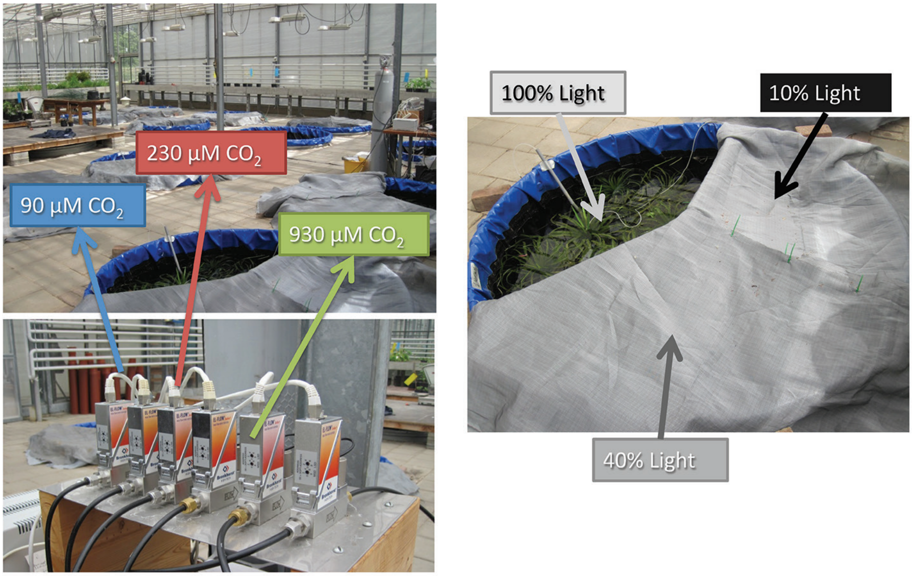

Figure 2.1. Experimental set-up in the greenhouse facility. Large mesocosms, that were dug into the greenhouse floor, were divided into three connected compartments, which received 3 different light intensities (100%, 40% and 10% light). Furthermore, flow controllers were used to bubble pure CO$_2$ through the mesocosms, creating three different CO$_2$ concentrations in these mesocosms (90, 230 and 930 μmol L^{-1}).

Chemical analyses

Water layer and pore water samples were collected every two weeks. Since the water layers of the three compartments of each mesocosm were connected and pH and CO$_2$ concentrations showed no differences between the compartments, analyses were performed on one pooled sample from the water layer of each mesocosm. Furthermore, pore water samples of all individual compartments were taken using vacuum syringes attached to ceramic cups (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) that were fixed at a depth of 10 cm in the sediment. pH was measured with a standard combined glass Ag/AgCl pH electrode (Orion, Thermo Fisher Scientific, Waltham, MA, U.S.A.) connected to a pH meter (Tim800; Radiometer Analytical, Lyon, France) and alkalinity by titrating down to pH 4.2 with 0.1 mmol L^{-1} HCl using an auto-burette (ABU901, Radiometer Analytical, Lyon, France). Concentrations of NO$_3^-$ and NH$_4^+$ were measured colourimetrically on an Auto Analyser 3 System (Bran & Luebbe, Norderstedt, Germany) using hydrazine sulphate (Kamphake and others 1967) and salicylate (Grasshof and Johannse 1972) respectively. Concentrations of Ca, Fe, K, Mg, total-P and S were analysed by inductively coupled plasma spectrometry (ICP-OES iCAP 6000; Thermo Fisher scientific, Waltham, MA, U.S.A.).

Plant parameters

While being kept in the greenhouse facility during winter 2011-2012, all plants shed their roots and started forming new ones in April 2012, just before the start of the experiment. Fresh weights, numbers of leaves and offsets, and lengths of the three largest leaves were recorded for each plant before placing two *S. aloides* plants (24.6 ± 1.6 g DW; diameter 48 ± 0.5 cm; mean ± SEM) in each compartment. The plants were allowed to grow for 4 months, during which the position of the rosette relative to the water surface (measure of buoyancy), production of emergent leaves, number of offsets and the number of roots penetrating the sediment were recorded regularly. Furthermore, plant mortality during the experiment was recorded and remaining biomass of these dead plants was harvested prematurely. Since it was not possible to record biomass during the experiment without damaging the plants, plant coverage was estimated digitally (Photoshop CS6 for Mac, Measurements Tool; Adobe Systems Incorporated, Mountain View, CA, U.S.A.) from photos that were taken every two weeks, to determine increase in plant cover. After harvest, root lengths were measured and the fresh weights of shoots, roots and offsets were determined separately. Plant material was dried at 70°C for 48h to establish final plant biomass dry weights. These values were corrected for calcium carbonate ($CaCO_3$) precipitation.

At the start and at the end of the experiment, the maximum quantum yields of photochemistry (F_v/F_M) were measured for all plants using a Pulse Amplified Modulation fluorometer (JUNIOR-PAM, Walz, Effeltrich, Germany). This method provides a relative measure of the reaction centres of photosystem II that are actively involved in photosynthesis. Also, at the start and at the end of the experiment, medium aged leaves were collected for analysis of chlorophyll content, which was extracted and measured on a spectrophotometer (Lambda 25, UV/VIS Spectrometer, PerkinElmer Instruments, Waltham, MA, U.S.A.), according to (Wintermans and De Mots 1965); Lichtenthaler and Wellburn (1983).

Photosynthetic rates of medium aged, submerged leaves were measured after 5 weeks of experimental treatments by incubating freshly cut leaves in airtight, water filled flasks. O_2 production inside the flasks was determined after 0, 2 and 4 hours of incubation using an oxygen electrode (HQ40d multi, HACH, Loveland, CO, U.S.A.). We chose to measure after 5 weeks, since this was within the crucial period where all plants were still submerged but were expected to become emergent soon. Measurements were carried out at 195 μmol m^{-2} s^{-1} PAR intensity, created by artificial light (Master SonT, 400W, Philips, The Netherlands). To maintain experimental light conditions, flasks containing leaves from 40% and 10% light treatments were covered with single and double layers of shadow fabric respectively and flasks were filled with water from the corresponding CO_2 treatments. Measurements were carried out at room temperature (22.5 °C).

$CaCO_3$ precipitation on leaves was quantified after 25, 50 and 65 days by incubating leaves in airtight bottles completely filled with 0.1 mmol L^{-1} HCl for 24 hours, after which the CO_2 concentration of the solution was measured using an ABB Advance Optima Infrared Gas Analyser (ABB Analytical, Frankfurt, Germany) and the amount of $CaCO_3$ was calculated. Homogenised dried plant material was digested with HNO_3 (65%) and H_2O_2 (30%) using a microwave oven (MLS 1200 Mega, Milestone Inc., Sorisole, Italy). Digestates were diluted and analysed for Fe, K and P by inductively coupled plasma spectrometry (ICP-OES iCAP 6000; Thermo Fisher Scientific, Waltham, MA, U.S.A.). In addition, C and N contents (%) of dried plant material were determined using an elemental analyser (Carlo Erba NA1500, Thermo Fisher Scientific, Waltham, MA, U.S.A.).

Statistical analyses

Our experimental set-up ensured a full-factorial experiment containing all possible combinations of CO_2 (90 μmol L^{-1}, 230 μmol L^{-1} and 930 μmol L^{-1}) and PAR (100%, 40% and 10%) treatments, all of which were replicated 3 times. All replicates consisted of 2 plants in the same compartment.

Normality of residuals and homogeneity of variance were checked using the Shapiro-Wilk test for normality and Levene's test of equality of error variances, respectively. Non-normal and heteroscedastic data were log transformed or square-rooted before analyses in order to meet the assumptions of parametric tests. Data on final biomass, water chemistry, chemical composition of plant material and photosynthetic parameters were analysed by two-way ANOVAs at the 0.05 confidence limit followed by a Tukey post hoc test. Experimental CO_2 concentrations, pore water NH_4^+ concentrations, plant cover, offset production, buoyancy depth and $CaCO_3$ accumulation were analysed over time using linear mixed models. Pairwise comparisons were performed using a Bonferroni adjustment for multiple comparisons to determine significant differences between all possible combinations of CO_2 or PAR treatments. For all analyses, P and F values and interaction effects are presented in Table 2.1. All statistical tests were carried out using SPSS (v21, 2012, IBM Statistics).

Table 2.1. Statistical results (*P*- and F-values) of CO_2, PAR and their interactions on plant parameters and sediment nutrient development during the experiment. Significant *P*-values ($P<0.05$) are indicated in bold.

Characteristic	**CO_2**		**PAR**		**CO_2 * PAR**	
	P	**F**	***P***	**F**	***P***	**F**
CO_2 treatment	**0.000**	233.082				
NH_4^+ decrease	**0.005**	5.485	**0.000**	15.028	**0.024**	2.912
Cover increase	**0.002**	6.682	**0.000**	40.760	**0.012**	3.386
Vegetative reproduction	**0.013**	4.617	**0.000**	79.765	**0.016**	3.265
Rosette depth	**0.000**	23.377	**0.000**	37.767	**0.005**	3.883
$CaCO_3$ accumulation	**0.001**	8.057	**0.000**	17.285	0.581	0.721
Final biomass	0.386	1.005	**0.000**	53.006	0.506	0.861
No of roots	**0.018**	5.336	**0.000**	17.160	0.566	0.763
Final length of roots	0.712	0.348	**0.001**	11.617	0.068	2.745
DW/ FW ratio	0.552	0.615	**0.005**	7.341	0.468	0.931
Leaf thickness	0.061	3.401	**0.006**	7.432	0.631	0.658
Photosynthesis	**0.003**	8.067	**0.000**	12.911	0.335	1.225
PAM	0.668	0.415	**0.002**	9.847	0.152	1.966
Chlorophyll	0.499	0.722	**0.012**	5.647	0.288	1.356
C: N ratio	0.055	3.447	**0.000**	15.307	0.509	0.856
C: P ratio	0.723	0.330	**0.163**	2.022	0.349	1.194
N: P ratio	0.180	1.898	**0.242**	1.546	0.749	0.481
C	**0.006**	6.984	**0.004**	7.937	0.202	1.677
N	**0.004**	7.882	**0.000**	22.340	0.093	2.373
P	0.764	0.274	**0.008**	6.411	0.740	0.495
K	0.893	0.113	0.507	0.706	0.376	1.128
Fe	**0.012**	5.815	0.747	0.297	0.389	1.098

Results

Biogeochemistry

Due to the HCO_3^--rich sediments (chemical properties shown in Table 2.2), characteristic for minerotrophic mires, alkalinity of the water layer in the ponds was high and ranged from 2.4 meq L^{-1} to 3.8 meq L^{-1} (mean: 2.8 meq L^{-1}). In general, nutrient concentrations in the water layer were low (Table 2.2). Pore water NH_4^+

concentrations decreased by 17-53% for 10% PAR plants and by 78-99% for plants with 40% or 100% PAR during the experiment ($P<0.001$; data not shown). CO_2 concentrations of 230 or 930 µmol L^{-1} also resulted in a stronger decrease than when plants were grown with 90 µmol L^{-1} CO_2 ($P=0.004$). An interaction effect between PAR and CO_2 ($P=0.024$) indicated that the average decrease of 98 ± 1 % observed in plants grown at ≥230 µmol L^{-1} CO_2 and ≥40% PAR (Table 2.1) was significantly stronger than the 56 ± 13 % for those grown at <230 µmol L^{-1} and <40% PAR.

Table 2.2. Chemical composition and characteristics (mean ± SEM) of the water layer, sediment pore water. For results of statistical analyses on the decline of NH_4^+ concentrations in the pore water during the experiment, see Table 2.1. Please note the different units.

Characteristic	***Unit***	***Water layer***	***Sediment***
Alkalinity	meq L^{-1}	2.85±0.11	9.82±0.25
Total Inorganic Carbon (TIC)	mmol L^{-1}	3.57±0.37	11.86±0.42
Ca^{2+}	mmol L^{-1}	3.14±0.14	11.96±0.31
Fe^{2+}	µmol L^{-1}	0.50±0.04	186.83±11.50
K^+	mmol L^{-1}	0.22±0.01	0.50±0.02
Mg^{2+}	mmol L^{-1}	0.63±0.02	2.16±0.06
SO_4^{2-}	mmol L^{-1}	2.48±0.14	9.98±0.28
NH_4^+	µmol L^{-1}	0.73±0.01	79.52±12.88*
NO_3^-	µmol L^{-1}	74.41±9.52	2.06±0.80
Total P	µmol L^{-1}	0.68±0.17	21.95±1.14
Moisture content	%		50±7
Organic matter content	%		20±6

Plant growth and photosynthesis

PAR was the most important factor determining final biomass of *Stratiotes aloides* (Table 2.3). While the biomass of PAR-limited plants decreased during the experiment due to the production of thinner leaves ($P=0.006$; Table 2.3) and shedding of large leaves, plants grown under 100% PAR had produced approximately three times their initial weight after four months of experimental treatments. Furthermore, these plants covered the water layer faster and more completely ($P<0.001$; Figure 2.2), and had a higher vegetative reproduction than PAR-limited plants ($P<0.001$; Table 2.1; Figure 2.3). Plants grown under 100% PAR produced 1.7 ± 0.2 offsets per plant, whereas plants grown under 40% or 10% PAR conditions produced only 0.4 ± 0.2 and 0.05 ± 0.05 offsets respectively.

Table 2.3. Plant growth characteristics (mean ± SEM) of *Stratiotes aloides* after 4 months of different PAR and CO_2 treatments. Significant differences among PAR treatments are indicated on the right by different capital letters (A, B, C) while significant differences among CO_2 treatments are indicated on the left by different lower case letters (a, b, c). See Table 2.1 for *P* and F values.

Characteristic	**Unit**	***100% PAR***			***40% PAR***			***10% PAR***		
		90 µmol L^{-1} CO_2	**230 µmol L^{-1} CO_2**	**930 µmol L^{-1} CO_2**	**90 µmol L^{-1} CO_2**	**230 µmol L^{-1} CO_2**	**930 µmol L^{-1} CO_2**	**90 µmol L^{-1} CO_2**	**230 µmol L^{-1} CO_2**	**930 µmol L^{-1} CO_2**
Final Plant Biomass	g DW	129.5±30.7^{C}	168.4±13.6^{C}	128.7±19.1^{C}	53±11.3^{B}	63.7±12.3^{B}	49.7±10.5^{B}	19.9±2.8^{A}	16.5±9.4^{A}	27.3±2.9^{A}
Chlorophyll a+b	mg Chl g^{-1} DW	0.79±0.04^{A}	0.68±0.18^{A}	0.65±0.09^{A}	1.24±0.26^{B}	0.95±0.15^{B}	0.82±0.08^{B}	0.93±0.12^{B}	1.14±0.1^{4} B	1.09±0.02^{B}
Photosynthetic Yield (PAM)	F_v/F_M	0.70±0.01^{A}	0.64±0.04^{A}	0.72±0.02^{A}	0.72±0.01 ^{A,B}	0.76±0.01^{A,B}	0.73±0.03^{A,B}	0.75±0.01^{B}	0.77±0.01^{B}	0.78±0.01^{B}
Final number of roots		3.3±1.6^{B}	5.8±2.6^{B}	8.7±2.0^{B}	2.0±1.2^{A,B}	3.3±0.9^{A,B}	5.3±0.8^{A,B}	1.7±1.4^{A}	0.5±0.3^{A}	1.3±0.7^{A}
Final length of roots	cm	88.2±4.0^{B}	84.3±9.2^{B}	94.3±4.4^{B}	95.0±1.4^{B}	81.3±12.1^{B}	74.3±1.3^{B}	36.5±0.4^{A}	69.3±2.2^{A}	65.7±14.8^{A}
DW/FW ratio leaves		0.25±0.06^{B}	0.24±0.03^{B}	0.20±0.02^{B}	0.17±0.02^{A}	0.13±0.03^{A}	0.16±0.003^{A}	0.14±0.01^{A}	0.13±0.01^{A}	0.14±0.01^{A}
Leaf thickness	mm	0.53±0.12^{B}	0.61±0.01^{B}	0.56±0.06^{B}	0.44±0.07 ^{A,B}	0.53±0.06^{A,B}	0.40±0.02^{A,B}	0.23±0.01^{A}	0.47±0.01^{A}	0.39±0.03^{A}
Emergent leaf formation	weeks	15	9	6	-	-	-	-	-	-

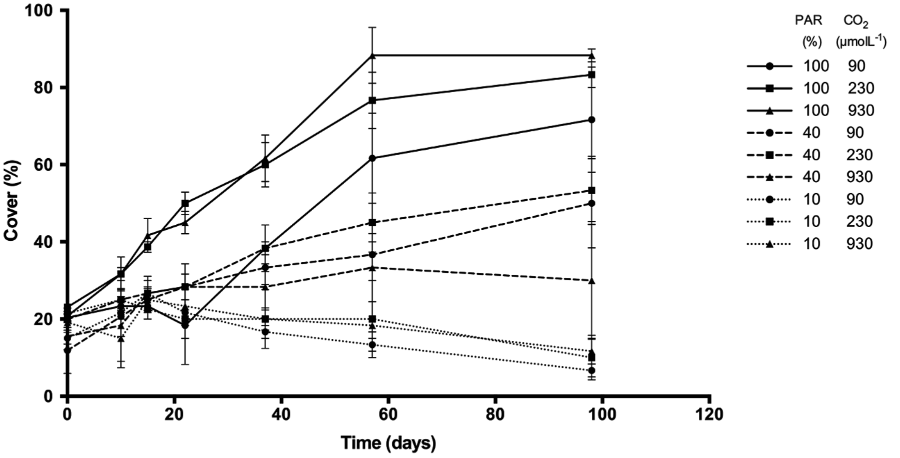

Figure 2.2. Increase in cover of the water layer by *Stratiotes aloides* (mean + SEM) subjected to different PAR levels and CO_2 availability. Low PAR significantly reduced final cover ($P<0.001$), whereas CO_2 limitation resulted in slower colonisation rates ($P=0.001$). Results of statistical tests are presented in Table 2.1.

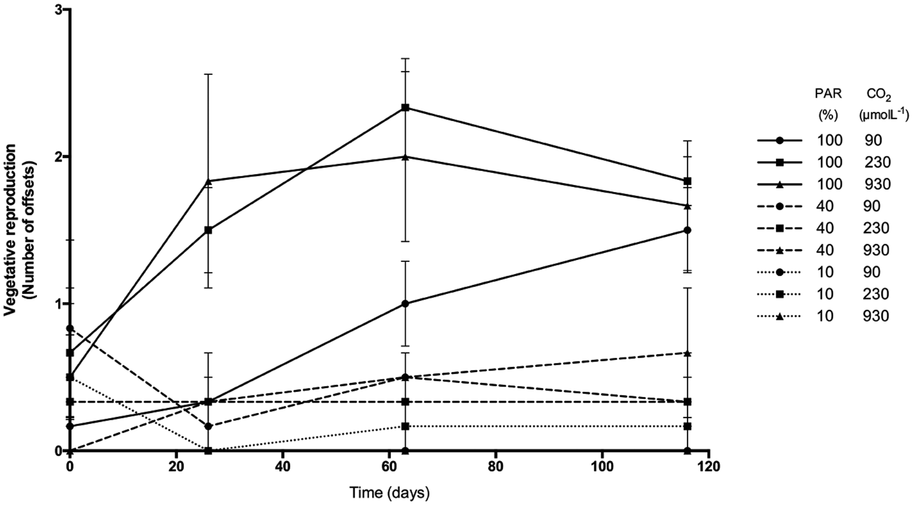

Figure 2.3. Production of offsets through vegetative reproduction in *Stratiotes aloides* (mean + SEM) subjected to different PAR levels and CO_2 availabilities. Low PAR significantly reduced the ability to produce offsets ($P<0.001$), whereas CO_2 limitation resulted in slower production rates ($P=0.021$). Results of statistical tests are presented in Table 2.1.

All plants started producing roots approximately 1 week before the start of the experiment and the plants were all firmly rooted in the sediment after 3 weeks. Plants grown at 100% PAR produced more roots (*P*<0.001; Table 2.1; Table 2.3) than those from the other light treatments, while plants from the lowest PAR treatment produced the shortest roots (*P*=0.001; Table 2.1; Table 2.3). From this 10% PAR treatment, however, a substantial part of the plants died (±40%, compared to ±20% and ±5% of the plants in the 40% and 100% PAR treatments respectively), which started with the dying off of the roots. A sufficient number of plants survived, however, for statistical analyses of plant parameters. The total length of the roots from all treatments, apart from the 10% PAR and 90 µmol L^{-1} CO_2 treatment, measured more than 60 cm and thus easily reached the sediment of the experimental basins (Table 2.3).

CO_2-limited plants initially showed much lower growth rates than plants with ample CO_2. This was illustrated by both lower increase in plant cover (*P*=0.001; Table 2.1; Figure 2.2) and slower production of offsets (*P*=0.021; Table 2.1; Figure 2.3). Plants from 90 µmol L^{-1} CO_2 treatments grown with full PAR eventually reached similar coverage and number of offsets, but took much longer to reach these values than plants from 930 µmol L^{-1} CO_2 availability.

Increase in plant cover (*P*=0.012) and vegetative production (*P*=0.016) were both also significantly influenced by interaction effects of CO_2 and PAR, since one factor enhanced the effects of the other. When plants received both high CO_2 concentrations and high PAR, the effects were stronger than the separate effects. Similarly, plants grown at the lowest CO_2 concentration and the lowest PAR did significantly worse than plants limited by only one of these factors.

The photosynthetic rates of the submerged plants were strongly influenced by both PAR (*P*<0.001; Table 2.1; Figure 2.4) and CO_2 availability (*P*=0.003; Table 2.1; Figure 2.4). Reduced PAR led lowered O_2 production in plants from the 10% PAR treatment by approximately 50% compared to plants grown at full PAR. Furthermore, reduction of the CO_2 availability from 930 µmol L^{-1} to 230 and 90 µmol L^{-1} led to 40% and 55% lower photosynthetic rates, respectively (Figure 2.4).

Maximum quantum yield values showed that all of the remaining plants had an active photosystem at the end of the experiment. These values were significantly higher for plants grown at 40% and 10% PAR than for those grown in the 100% PAR treatment (*P*=0.002; Table 2.1; Table 2.3). Furthermore, plants that were grown under darker conditions contained significantly more chlorophyll a and b than those grown in full light (*P*=0.009; Table 2.1; Table 2.3).

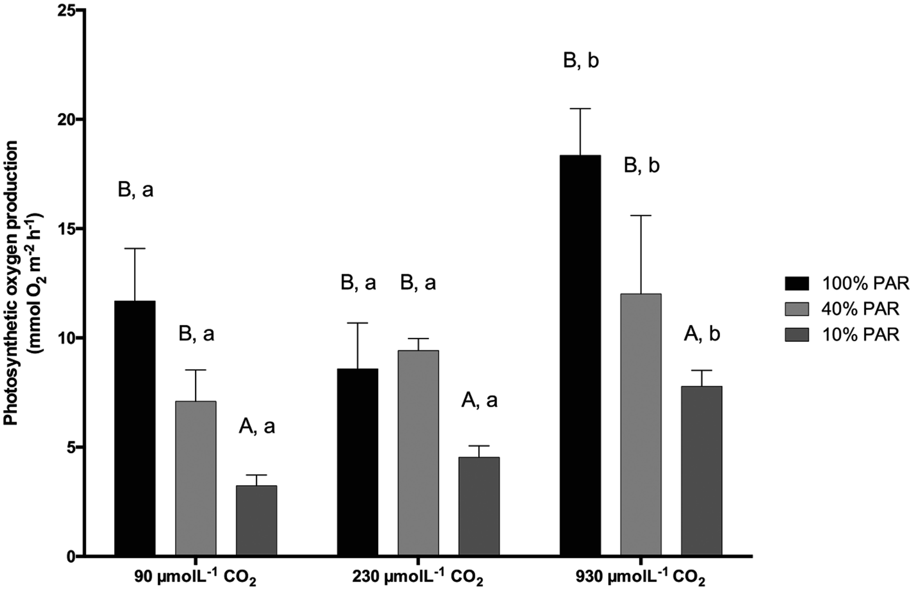

Figure 2.4. Photosynthetic oxygen production (mean + SEM) in *Stratiotes aloides* under different PAR and CO_2 availabilities. Significant differences ($P<0.01$) between different PAR and CO_2 treatments are indicated with capital and lower cased letters, respectively. Additional results of statistical tests are presented in Table 2.1.

After harvest, the C: N ratios of the plants grown at 40% and 10% PAR were lower than those of the plants grown in full PAR ($P=0.010$; Table 2.1; Table 2.4). Furthermore, plants grown at 930 µmol L^{-1} CO_2 had significantly higher N contents than those of lower CO_2 availability, which also led to lower C: N ratios. C: P and N: P ratios did not differ among treatments (Table 2.4). Plants also showed lower Fe contents ($P=0.012$; Table 2.1; Table 2.4) when CO_2 concentrations were 230 µmol L^{-1} or lower, while K contents did not differ among treatments, with all plants containing around 630±50 mmol K kg DW^{-1} (Table 2.4; mean ± SEM).

Buoyancy and calcification

Under full PAR, formation of emergent leaves started in the 930 µmol L^{-1} CO_2 treatments after approximately 6 weeks (Table 2.3). Under limited CO_2 availability, formation of emergent leaves was delayed by 3 and 9 weeks for the 230 µmol L^{-1} and 90 µmol L^{-1} CO_2 treatments respectively. When PAR was limited, plants did not form emergent leaves. Submerged leaves were significantly thinner than emergent leaves ($P=0.002$; Table 2.1; Figure 2.5).

Table 2.4. Plant chemical composition (mean ± SEM) of *Stratiotes aloides* after 4 months of different PAR and CO_2 treatments. Significant differences among PAR treatments are indicated on the right by different capital letters (A, B, C) while significant differences among CO_2 treatments are indicated on the left by different lower case letters (a, b, c). See Table 2.1 for *P* and F values. Please note a different unit for C-content (mol kg DW^{-1}).

Characteristic	***Unit***	***100% PAR***			***40% PAR***			***10% PAR***		
		90 µmol L^{-1} CO_2	**230 µmol L^{-1} CO_2**	**930 µmol L^{-1} CO_2**	**90 µmol L^{-1} CO_2**	**230 µmol L^{-1} CO_2**	**930 µmol L^{-1} CO_2**	**90 µmol L^{-1} CO_2**	**230 µmol L^{-1} CO_2**	**930 µmol L^{-1} CO_2**
C	**mol kg^{-1}DW**	^{a,b}21.77±1.76^{A}	^{a}22.53±1.92^{A}	^{b}24.06±2.51^{A}	^{a,b}23.10±1.47^{A}	^{a}21.63±1.24^{A}	^{b}24.47±1.12^{A}	^{a,b}25.85±0.24^{B}	^{a}24.24±2.07^{B}	^{b}25.44±2.44^{B}
N	**mmol kg^{-1}DW**	^{a} 788±269^{A}	^{a} 947±299^{A}	^{b}1066±390^{A}	^{a} 909±366^{B}	^{a}708±107^{B}	^{b}914±39^{B}	^{a}1204±394^{C}	^{a}958±295^{C}	^{b}1338±311^{C}
P	**mmol kg^{-1}DW**	36.09±2.82^{A}	25.54±7.13^{A}	36.42±4.71^{A}	38.65±2.98$^{A, B}$	38.28±3.76$^{A. B}$	41.06±9.64$^{A, B}$	47.66±3.80^{B}	57. 39±14.75^{B}	55.34±11.13^{B}
K	**mmol kg^{-1}DW**	675±54	412±113	567±123	819±186	618±145	566±166	520±99	814±167	743±250
Fe	**mmol kg^{-1}DW**	^{a} 3.50±1.39	^{a} 4.34±0.11	^{b}9.27±3.05	^{a}2.34±0.37	^{a}5.41±0.85	^{b}11.29±1.91	^{a}7.01±2.62	^{a}6.11±2.44	^{b}7.84±2.86
C: N	**g g^{-1}**	^{a,b}33.70±4.90^{B}	^{b}42.79±0.65^{B}	^{a}28.52±3.29^{B}	^{a,b}25.62±5.45^{A}	^{b}27.19±3.90^{A}	^{a}16.90±1.93^{A}	^{a,b}19.85±7.58^{A}	^{b}14.80±1.17^{A}	^{a}13.80±0.25^{A}
C: P	**g g^{-1}**	929±96	1453±252	1052±126	898±55	850±81	1152±126	862±58	669±45	874±135
N: P	**g g^{-1}**	30.06±5.55	33.92±11.59	38.78±6.46	37.75±2.31	33.47±1.83	72.58±4.53	54.50±6.89	44.78±2.90	64.02±7.38

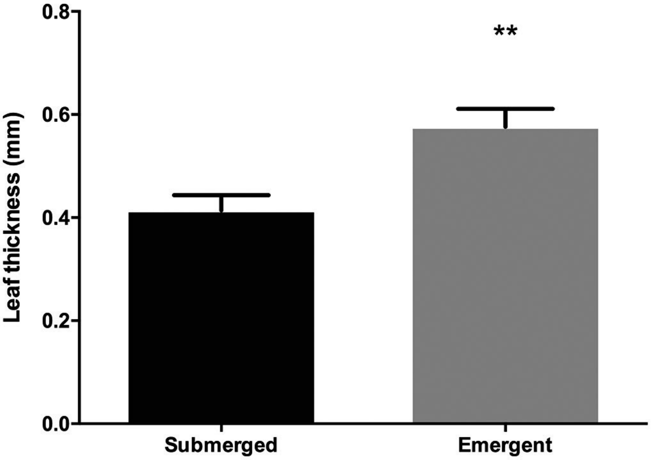

Figure 2.5. Thickness of submerged and emergent leaves (mean + SEM) formed by *Stratiotes aloides* at the end of the four-month experiment. Submerged leaves were mainly produced by PAR-limited plants and plants from 90 µmol L^{-1} treatments, whereas emergent leaves were only produced by plants grown at 100% PAR. Emergent leaves were significantly thicker than submerged leaves (P=0.002). Average values of leaf thickness per treatment are presented in Table 2.3, with statistical details in Table 2.1.

Still, even in the absence of emergent leaves, PAR-limited plants were often observed to float just below the water surface and buoyancy depth of the rosette did not differ between light regimes for plants grown at 230 µmol L^{-1} and 930 µmol L^{-1} CO_2. However, at 100% light, plants that received the lowest amount of CO_2 were lying lower in the water layer than plants with 230 or 930 µmol L^{-1} during the entire experimental period (CO_2*PAR effect; P=0.005; Table 2.1; Figure 2.6). Furthermore, plants from 230 µmol L^{-1} CO_2 treatments initially also stayed lower in the water layer (P=0.017; Table 2.1; Figure 2.6) than plants treated with 930 µmol L^{-1} CO_2.

Observations of submerged leaves showed significant accumulation of, what proved to be, $CaCO_3$ on the upper side of the leaves. While this accumulation was most apparent on plants from the 90 µmol L^{-1} CO_2 and 100% PAR treatment, eventually all plants showed some $CaCO_3$ precipitation on their leaves. This calcification of *S. aloides* leaves first became visible after approximately 3-4 weeks. Both high PAR (P=0.002; Table 2.1) and low CO_2 availability (P=0.016; Table 2.1) significantly increased the amount of $CaCO_3$ that accumulated on the leaves (Figure 2.7). The increase in $CaCO_3$ was highest during the first month of the experiment and stabilised after that to values of around 40 ± 7 g $CaCO_3$ m^{-2} (data not shown). Accumulation of $CaCO_3$ during the experiment led to an increase in leaf density of 5.7 ± 0.9% in full PAR, whereas those in 40% and 10% PAR increased 3.2 ± 1.0% and 1.8 ± 0.4% respectively (data not shown).

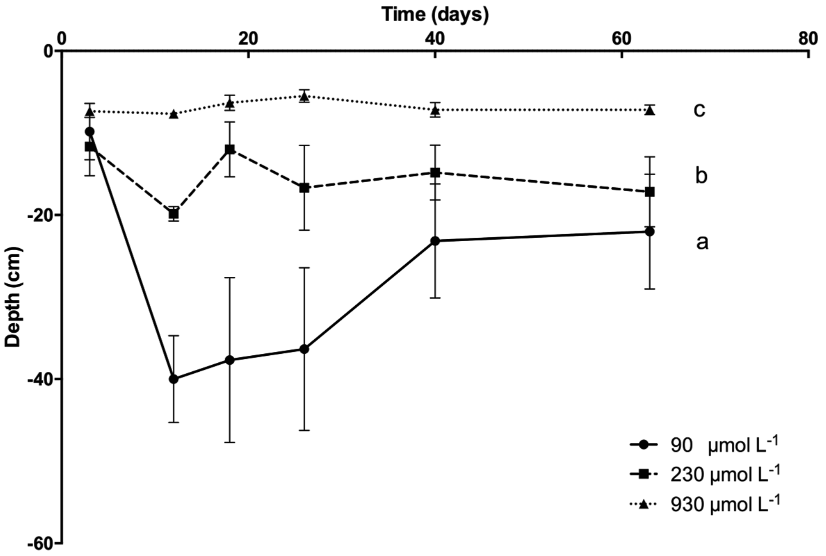

Figure 2.6. Depths of the rosettes (mean and SEM) of *Stratiotes aloides*, grown at different CO_2 availabilities and 100% PAR. Plants from 90 µmol L^{-1} CO_2 treatments sank within two weeks, after which plants remained significantly lower in the water layer than those grown at limited 230 and 930 µmol L^{-1} ($P<0.001$), as indicated by different letters. Additional results of statistical tests are presented in Table 2.1.

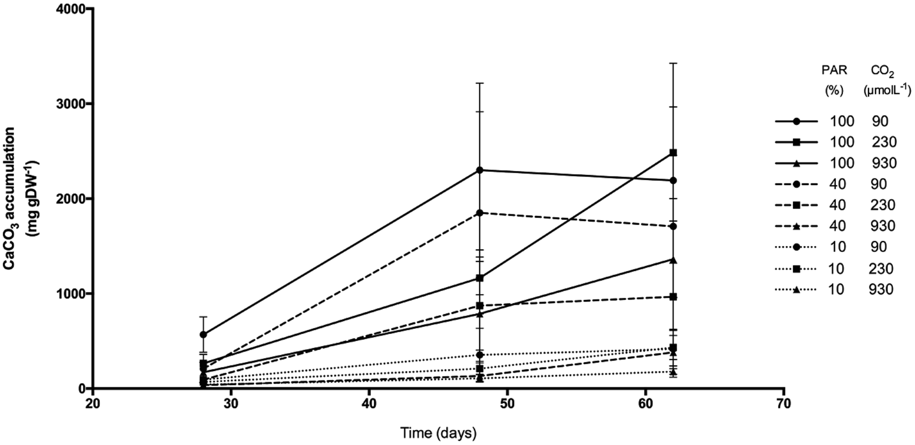

Figure 2.7. Accumulation of $CaCO_3$ (mean + SEM) on leaves of *Stratiotes aloides* under different PAR and CO_2 availabilities. $CaCO_3$ was measured from the moment the precipitation became visible (3-4 weeks after start of treatments). Low PAR significantly lowered $CaCO_3$ accumulation ($P=0.002$), while limited availability of CO_2 significantly increased the amount of $CaCO_3$ on the leaves ($P=0.016$). Additional results of statistical tests are presented in Table 2.1.

Discussion

Buoyancy and photosynthesis

We show here that both PAR and the availability of dissolved CO_2 in the water layer strongly influence photosynthetic rates, buoyancy and formation of emergent leaves for *Stratiotes aloides*. A synthesis of the most important effects of limited CO_2 or PAR on these plants is presented in Figure 2.8.

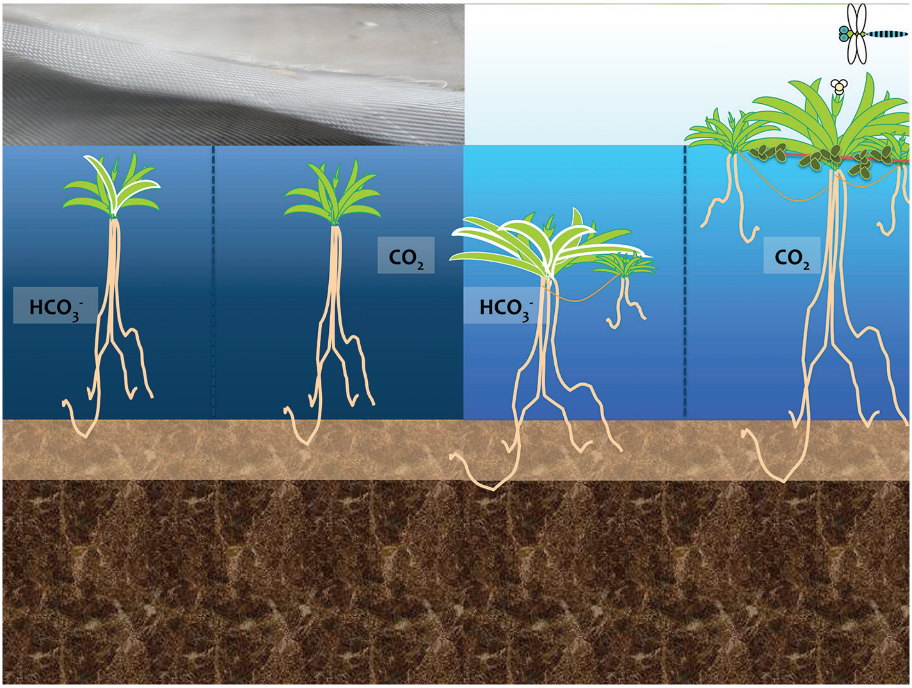

Figure 2.8. Schematic overview of the effects of limited PAR or CO_2 availability in spring on the growth rate and buoyancy of *Stratiotes aloides*. When PAR is limited (left), plants produce less biomass and do not form emergent leaves. Under limited CO_2 conditions, plants use HCO_3^- for photosynthesis (when available), which is less efficient and causes lower growth rates, slower formation of emergent leaves and reduced buoyancy. Therefore, only plants receiving ample light and CO_2 (far right) can become buoyant and reach the required growth rate necessary to fulfil their role as a facilitator for biodiversity in wetlands.

Emergent leaves did not appear at all when PAR was limiting, whereas their formation was delayed by 3-9 weeks under CO_2 limited conditions. All plants were firmly rooted in the sediment and produced roots that were long enough for the plants to become buoyant. Root growth, therefore, was not a constraint on reaching the water surface. PAR-limited plants, however, never became emergent but they floated just below the water surface (Figure 2.8). We observed that these plants show several adaptations to low light availability, including thinner leaves, a higher chlorophyll content and a higher efficiency of photosystem II. Still, the photosynthetic rate is too low to produce emergent leaves and form a dense floating mat under PAR-limiting conditions and several plants died or shed their roots during the experiment, resulting, on average, in shorter roots. We observed that PAR limited plants, which happened to have shed all of their roots still did not become buoyant. This further indicates that plants were not physically retained under water due to a limited root length. In the field, low PAR may well be caused by blooms of algae or cyanobacteria, high concentrations of humic acids or turbidity by particulate organic matter, all of which occur frequently in peatlands where *S. aloides* occurs (Lamers and others 2002).

With full PAR, however, CO_2 limitation can still cause reduced buoyancy (Figure 2.8). This can be explained by the lower photosynthetic rates of these plants and their subsequently delayed formation of emergent leaves. Even though both light and DIC availability were high enough for photosynthesis in this treatment, the lower photosynthetic rate indicates C-limitation for these plants. Emergent leaves are thicker and have a larger volume of gas-filled intercellular spaces (aerenchyma) than submerged leaves, and are able to provide buoyancy to the plants (Sculthorpe 1967; Cook and Urmi-König 1983; Efremov and Sviridenko 2012). The construction costs, in terms of energy and carbon, involved in the formation of the structurally more complex, thicker emergent leaves are much higher (Milla and Reich 2007) and this C allocation is only possible if photosynthesis is sufficiently high. Even though *S. aloides* is capable of using HCO_3^- as an alternative C-source (Prins and De Guia 1986; Nielsen and Borum 2008), photosynthetic rates are lower due to the higher costs associated with HCO_3^- use (Madsen and Sand-Jensen 1991). This would explain why, under natural conditions, this species strongly prefers systems with an organic sediment, where high microbial respiration rates ensure high CO_2 concentrations in the water layer (Smolders and others 2003a).

Furthermore, as we have shown, HCO_3^--based photosynthesis induces accumulation of $CaCO_3$ on plant leaves. Calcification of aquatic plants is a common phenomenon occurring in species that use HCO_3^- (Borowitzka 1984). When aquatic plants take up HCO_3^-, it is converted to CO_2 and OH^-. While CO_2 is used for photosynthesis, OH^- is excreted on the upper side of the leaf, thereby locally increasing the pH of the boundary layer and causing precipitation of $CaCO_3$ (Prins and others 1982; Borowitzka 1984). Accumulation of $CaCO_3$ on *S. aloides* leaves has been discovered in the last century, when Montesantos (1913) argued that $CaCO_3$ accumulation could explain the natural sinking of the species in autumn. Even though we could not find a direct link between $CaCO_3$ accumulation and limited buoyancy, we established that HCO_3^- use increases the density of plant tissue in *S. aloides*.

Biomass production and plant nutrition

Buoyancy is the key factor determining the function of *S. aloides* as a facilitator for wetland biodiversity. However, to create dense vegetation mats that can facilitate other species, the growth rate and production of sufficient new biomass are equally important (Figure 2.8). Plants grown at low PAR produced significantly lower amounts of new biomass than plants with high PAR. Plants were generally smaller and produced fewer offsets than those grown at full PAR availability (Figure 2.8). Since both PAR and CO_2 influence photosynthesis, it is not surprising that the effects of CO_2 or PAR limitation interact with each other. While plants grown at full PAR performed best when grown at high CO_2 concentrations, PAR-limited plant propagation did not respond to variations in CO_2 availability. Plants that were limited in CO_2, but received sufficient PAR for photosynthesis, used HCO_3^- as an alternative C-source. This not only reduced buoyancy, but also resulted in slower growth and reproductive rates (Figure 2.8). In wetlands, slower growth rates will easily result in plants being outcompeted by more efficient HCO_3^- using species, including both plants and algae.

While growth rates were lower under CO_2 limited conditions, there were no significant differences between the final biomasses of the lowest and highest CO_2 treatments. This can most likely be explained by space restrictions in plants grown at high CO_2, since these plants already covered over 90% of the basin after 60 days. At this point, space would limit recruitment of new offsets due to self-shading and other intra-specific competition factors.

Even though NH_4^+ availability dropped in sediments of plants with high CO_2 and high PAR, N limitation can be ruled out, since N: P ratio's of all plants were higher than 30 g g^{-1} which suggests that P, rather than N, was limiting biomass production in all treatments (Güsewell 2004). Furthermore, even though tissue of CO_2 limited plants had lower Fe contents than those grown with ample CO_2, all concentrations were within the range of healthy *S. aloides* vegetation, as was K, and Fe or K limitation can thus be ruled out (Smolders and Roelofs 1993; Marschner 1995).

Habitat quality and facilitator function

We have shown that one of the most important characteristics making *Stratiotes aloides* a facilitator species for biodiversity, buoyancy, can only be achieved when the plants are able to reach sufficiently high underwater photosynthetic rates in spring. High photosynthesis enables high colonisation rates and the formation of thicker leaves with intercellular spaces that can become filled with gas, most likely oxygen produced by photosynthesis. The resulting lower specific weight of the plants makes them emerge to the surface. This process is strongly linked to PAR availability and to the species of C, rather than the total DIC concentration, in the aquatic environment. Therefore, contrary to many other facilitators, the potential of this widespread aquatic macrophyte to facilitate other species appears to depend on the prevalent environmental conditions, rather than the mere abundance of the species.

Acknowledgements

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Chapter 3

Harnessing facilitation: Why successful re-introduction of *Stratiotes aloides* requires high densities under high nitrogen loading

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In Review

Harnessing facilitation: Why successful re-introduction of *Stratiotes aloides* requires high densities under high nitrogen loading

Dense mats of floating vegetation form complex structures that support high biodiversity in wetlands. Since the 1960s, however, high-density stands of *Stratiotes aloides* have shown strong declines throughout Europe and restoration efforts have often failed, both of which have been linked to high nitrogen (N) input rates. The low success rate of re-introduction is striking, since seemingly healthy, high-density stands are observed to grow under similar environmental conditions. We therefore hypothesise that N tolerance of *S. aloides* increases with density due to joint N uptake and detoxification. To test our hypothesis, we set up a controlled full factorial mesocosm experiment in which we manipulated N loading and *S. aloides* density, and investigated interacting effects on habitat biogeochemistry and plant performance. High-density *S. aloides* stands strongly lowered NH_4^+ availability through shared uptake, even at extremely high N loads. Furthermore, high-density stands strongly reduced water layer O_2 concentrations, which stimulated sediment P mobilisation, and increased CO_2 concentrations, which enhanced underwater photosynthesis. High-density stands thus not only detoxify NH_4^+, but also facilitate population growth through habitat manipulation. Combined, these mechanisms enabled *S. aloides* to remain viable at high loads of 800 kg N ha^{-1} y^{-1}, whereas low-density stands already collapsed at 200 kg N ha^{-1} y^{-1}. Overall, our results show that under similar environmental conditions, high-density stands can thrive, while restoration or natural recruitment may be impossible. We therefore conclude that acknowledging and harnessing intraspecific facilitation is vital for successful conservation and restoration of *S. aloides* stands.

Introduction

The floating aquatic macrophyte *Stratiotes aloides* characterises many meso- to eutrophic wetlands in Europe and Asia. This species is found mainly in sheltered bays of larger lakes, backwater ponds, ditches and canals and has a vigorous asexual reproduction, which often results in high-density, floating stands (Cook and Urmi-König 1983). Due to their diverse structure, these floating *S. aloides* stands support high biodiversity (Sugier and others 2010) and are essential for a number of associated endangered species such as the Green hawker (Rantala and others 2004; Suhonen and others 2013) and the Black tern (Van der Winden and others 2004). Healthy *S. aloides* plants show an alternating life cycle of submerged and emergent life stages, with plants becoming buoyant in spring, forming dense floating mats in summer and sinking again in autumn (Cook and Urmi-König 1983; Roelofs 1991). Since the 1960s, however, *S. aloides* populations have been in severe decline all over Europe (Mason and Bryant 1975a; Roelofs 1991; Smolders and others 2003a; Zantout and others 2011; Abeli and others 2014). This has resulted in either local extinction, or the occurrence of (often submerged) remnant populations. Although restoration efforts of wetlands in general, and *S. aloides* in particular, have increased over the last decades, the mostly small-scale and low-density reintroduction attempts have so far been largely unsuccessful (Gosling and Baker 1980; Kurstjens and others 2008; Termaat 2010; Abeli and others 2014).

Both the strong decreases in natural populations and the lack of successful re-introductions of *S. aloides* have been related to increased availability of ammonium (NH_4^+) in the environment (Zantout and others 2011; Abeli and others 2014). Laboratory experiments have shown that growth and vitality of individually grown *S. aloides* plants is already severely hampered at NH_4^+ water layer concentrations beyond 50 µmol L^{-1} (Roelofs 1991; Smolders and others 1996), and in the field the species is hardly found when concentrations exceed 30 µmol NH_4^+ L^{-1} (Smolders and Roelofs 1993). Therefore, one would expect *S. aloides* to be absent in areas with high N loads. Paradoxically, however, seemingly healthy, dense stands do still occur in agricultural systems, which are characterised by relatively high nitrogen (N)-loads of 100 to 320 kg ha^{-1} y^{-1} (Roelofs 1991; Smolders and others 2000; Saunders and Kalff 2001; Geurts and others 2010; Bakker 2012).

In this study, we investigate the hypothesis that high-density stands of *S. aloides* buffer against NH_4^+ toxicity by reducing ambient concentrations through shared uptake and manipulating habitat biogeochemistry. This would explain both the occurrence of high-density stands and the lack of restoration success in areas with high N-loads. Intraspecific, density-dependent facilitation has been shown to alleviate environmental stress in a wide range of terrestrial, marine and freshwater ecosystems (Bertness and Callaway 1994; He and others 2013). In addition, density-dependent detoxification of NH_4^+ has been shown for sea grasses (van der Heide and others 2008; van der Heide and others 2010).

To test our hypothesis, we set up a full factorial mesocosm experiment, in which we grew *S. aloides* at high or low plant density to determine density-related effects of three environmentally relevant N loads on plant growth and toxicity effects. The results of this experimental work will especially be important for future restoration efforts, as it may explain why low-density introductions of *S. aloides* and other species show low success rates under environmental stress such as high N loads.

Methods

Experimental set-up

Stratiotes aloides plants were collected from a peatland area in the Netherlands (52°44’17.02”N; 6°7’3.12”E) and transported to the greenhouse facility of Radboud University. Here, 18 large mesocosms (700 L; ø 90 cm) were filled with 100 L of organically rich soil, originating from a minerotrophic peatland in the Netherlands (52°18’32”N; 4°45’42”E) and a water layer of 500 L artificial surface water (deionised water, with addition of 700 µmol L^{-1} $CaCl_2.2H_2O$, 75 µmol L^{-1} KCl and 450 µmol L^{-1} $MgCl_2.6H_2O$). To create two densities of *S. aloides* stands, half of the basins received 3 plants (Low-Density, LD; 83.4 ± 2.3 g DW m^{-2}) and the other half 10 plants (High-density, HD; 253.2 ± 1.0 g DW m^{-2}). To maintain a low density in the LD treatment, all offsets with a diameter over 15 cm were removed. After 5 weeks of acclimatisation, both the HD and LD stands of *S. aloides* received rainwater containing 0.2, 1.2 and 6.0 g L^{-1} NH_4Cl at a rate of 800 mL $week^{-1}$, resulting in three different N loadings of 40, 200 and 800 kg N ha^{-1} y^{-1}. During the experiment, water temperatures were similar for all mesocosms, ranging from 18.1 to 23.0 °C. Light was mostly natural, but an artificial light regime (Master SonT, 400W, Philips, The Netherlands) was implemented to prevent fluctuations below 200 µmol m^{-2} s^{-1}. On average, PAR at the water level was 260±15 (mean ± SEM) in winter (January - April) and 550±50 µmol m^{-2} s^{-1} in summer (May -August).

Chemical analyses

NH_4^+ concentrations in the water layer were checked colourimetrically every week using reagents (JBL, Neuhofen/Pfalz, Germany) and a field spectrophotometer

(Spectroquant NOVA 60, MERCK, Schiphol-Rijk, The Netherlands). Every two weeks, samples were taken from the sediment pore water, using ceramic soil moisture cups (Eijkelkamp, Giesbeek, The Netherlands) fixed at -10 cm in the sediment, and from the water layer. pH was measured with a standard Ag/AgCl electrode (Orion, Thermo Fisher Scientific, Waltham, MA, U.S.A.) combined with a pH meter (Tim840 titration manager; Radiometer Analytical, Lyon, France). Total inorganic carbon (TIC) was measured by injecting 0.2 ml of sample into a compartment with 1 ml phosphoric acid (0.4 M) attached to an Infra-red Gas Analyser (IRGA; ABB Analytical, Frankfurt, Germany), after which concentrations of HCO_3^- and CO_2 were calculated based on the pH equilibrium.

Concentrations of PO_4^{3-}, NO_3^- and NH_4^+ were measured colourimetrically on an Auto Analyser 3 System (Bran & Luebbe, Norderstedt, Germany) using ammonium molybdate (Henriksen 1965), hydrazine sulphate (Kamphake and others 1967) and salicylate (Grasshof and Johannse 1972), respectively. The availability of ammonia (NH_3) was calculated using temperature, pH and NH_4^+ concentrations. Concentrations of Ca, Fe, K, Mg, total-P and SO_4 were analysed by inductively coupled plasma spectrometry (ICP-OES iCAP 6000; Thermo Fisher Scientific, Waltham, MA, U.S.A.). Every two weeks, O_2 concentrations were measured in all basins at three depths (5 cm, 20 cm and 40 cm below the water surface), using an optical oxygen probe (Hach LDO, Hach, Loveland, CO, U.S.A.).

Plant growth

Plants were emergent when they were introduced into the experimental basins, but most sank in the first month, after which their buoyancy was recorded during the experiment. Furthermore, the numbers of plants, offsets and roots were recorded at regular intervals. After 8 months of treatment, all biomass was harvested and the number of leaves and roots, fresh weight (FW) and dry weight (DW; 48h, 60°C) were determined. Plants from the LD basins treated with 800 kg N ha^{-1} y^{-1} died prematurely and the remaining biomass was harvested after 4 months of treatment.

After 3 months of experimental treatments, photosynthetic quantum yield of dark-adapted leaves was determined using Pulse Amplitude Modulated (PAM) fluorescence (Junior PAM, Heinz Walz GmbH, Effeltrich, Germany), whereas chlorophyll content (a+b) was determined by extraction on a pooled sample of three leaves per basins according to Wintermans and De Mots (1965) and Lichtenthaler and Wellburn (1983) using a Spectrophotometer (Lambda 25, UV/VIS Spectrometer, PerkinElmer Instruments, Waltham, MA, U.S.A.).

Amino acid contents were determined before the start of the experiment and after 3 and 5 months of treatment, by extraction from a representative, pooled subsamples according to (Van Dijk and Roelofs 1988), using norValine as internal standard. Freeze-dried extracts were dissolved in 0.01 N HCl and analysed by High Performance Liquid Chromatography (Varian 920-LC Analytical HPLC, Varian Inc., Palo Alto, CA, U.S.A.).

Statistical analyses

All data was checked for normality of residuals and homogeneity of variance using Shapiro Wilk's test and Levene's test for equality of variance, respectively. Non-normal or heteroscedastic data were log transformed to authorise use of parametric tests. Data on O_2, PO_4^{3-}, NH_4^+, NH_3, NO_3^- and amino acid contents were analysed over time using linear mixed models (LMM) combined with Bonferroni post hoc tests. Data on chemical composition of the water layer and pore water and final plant parameters were analysed by two-way ANOVAs with Tukey post hoc tests. All analyses were carried out with SPSS (v21, 2012, IBM Statistics).

Results

Density effects on biogeochemistry

HD stands of *S. aloides* reduced the O_2 concentration in the water layer by approximately 30% ($P<0.001$; F=141.165) at 20 cm and 50% ($P<0.001$; F=675.053) at 40 cm depth, whereas in LD treatments, O_2 concentrations in the water layer did not differ between higher and lower depths (Figure 3.1). In addition, HD stands of *S. aloides* had a higher availability of CO_2 (199.4±19.2 vs. 29.8±4.4; $P<0.001$; F=194.709) and lower pH (7.5±0.1 vs. 8.0±0.1; $P<0.001$; F=161.780) in the water layer than LD stands. There were no effects of N loading on O_2 and CO_2 concentrations.

NH_4^+ loading of 800 kg ha^{-1} y^{-1} led to accumulation of NH_4^+ ($P<0.001$; F=436.162), NO_3^- ($P<0.001$; F=350.149) and NH_3 ($P<0.001$; F=82.360) in the water layer. (Figure 3.2) For LD, NH_4 ($P<0.001$; F=118.540) and NH_3 ($P<0.001$; F=189.641) concentrations were, however, much higher. In addition, NO_3^- accumulation started earlier and reached up to 2 times higher concentrations compared to HD stands ($P<0.001$; F=109.002). Porewater NH_4^+ concentrations were low at the start of the experiment, with values of 100±5 μmol L^{-1} (data not shown). While these concentrations dropped to 50±14 μmol L^{-1} and 89±21 μmol L^{-1} under N-loadings of 40 and 200 kg N ha^{-1} y^{-1}, respectively, they increased to values of around 223.9±15.2 μmol L^{-1} at 800 kg N ha^{-1} y^{-1} (data not shown). Furthermore, HD stands showed higher net PO_4^{3-} mobilisation rates to the water layer, leading to higher PO_4^{3-} concentrations ($P<0.001$; F=89.755) during the first two months (Figure 3.2).

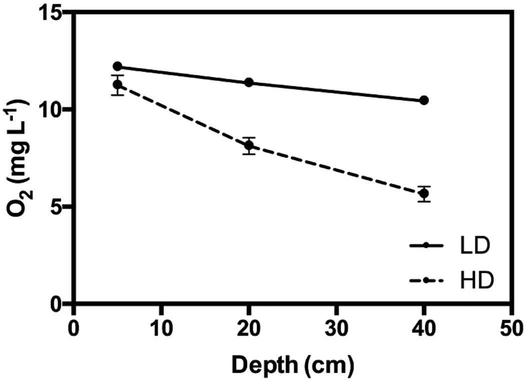

Figure 3.1. Mean O_2 concentrations (±SEM) of the water layer at 5, 20 and 40 cm depth in high-density (HD) or low-density (LD) stands of *Stratiotes aloides*.

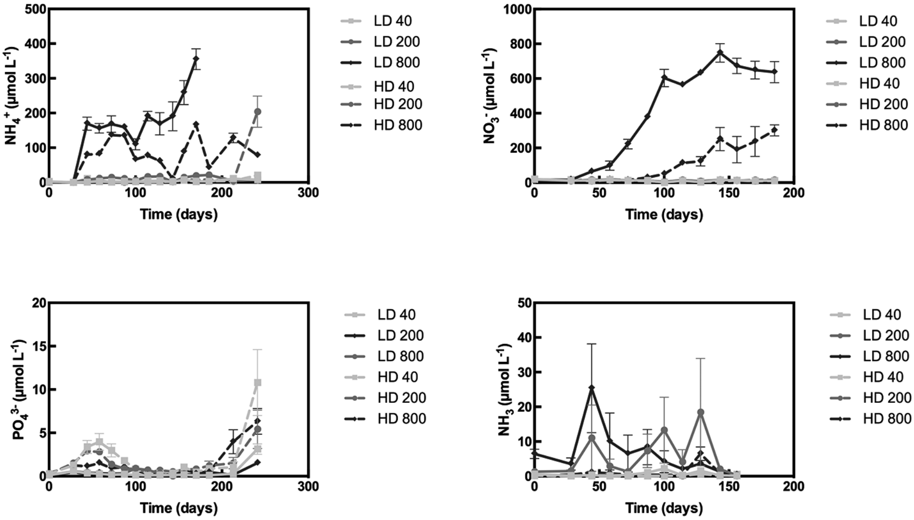

Figure 3.2. Concentrations of NH_4^+ (upper left), NO_3^- (upper right), PO_4^{3-} (lower left) and NH_3 (lower right) in the water layer of high-density (HD) and low-density (LD) stands of *Stratiotes aloides*, receiving 40, 200 or 800 kg N ha^{-1} y^{-1}.

Table 3.1. Plant parameters of *Stratiotes aloides* plants grown at high or low density, with different loadings of NH_4^+ (in kg N ha^{-1} y^{-1}). Differences ($P<0.05$) between high-density (HD) and low-density (LD) stands are indicated in Bold, whereas differences between NH_4^+ loads are represented by different superscript letters.

	LD			**HD**		
	40	**200**	**800**	**40**	**200**	**800**
Number of plants (end)	3.0±0.0	2.7±0.3*	0.0±0.0	9.7±0.9	11.7±1.7	11.7±1.7
Final Biomass per plant (g DW)	88.9±15.3 $^{A, B}$	38.0±34.5 ^{B}*	0.0±0.0 ^{A}	173.6±27.3 $^{A, B}$	338.4±10.6 ^{B}	216.4±21.5 ^{A}
Final number of roots per plant	18.33±2.55 ^{B}	8.39±4.20 ^{A}	0.00±0.00 ^{A}	13.54±3.33 ^{B}	15.43±2.17 ^{A}	1.88±0.57 ^{A}
Emergent after (days)	211±0 ^{B}	211* ^{A}	Never ^{A}	**165±24 ^{B}**	**69±39 ^{A}**	**30±0 ^{A}**
Chlorophyll a+b (mg g^{-1} DW)	0.93±0.07 ^{B}	0.78±0.13 ^{B}	0.26±0.12 ^{A}	0.85±0.06 ^{B}	1.06±0.04 ^{B}	0.71±0.10 ^{A}
PAM **	0.72±0.01	0.74±0.01	0.61±0.03	**0.75±0.00**	**0.74±0.03**	**0.79±0.01**
C (mmol kg^{-1} DW) **	26780±848 ^{A}	28580±63 ^{B}	30036±425 ^{C}	**28848±620 ^{A}**	**29930±288 ^{B}**	**31382±341 ^{C}**
N (mmol kg^{-1} DW)**	1373±184 ^{A}	2365±105 ^{B}	3808±87 ^{C}	1651±110 ^{A}	2205±189 ^{B}	3622±132 ^{C}
P (mmol kg^{-1} DW)**	110.0±9.4	119.9±13.4	179.6±71.0	134.1±0.8	156.5±12.7	160.5±14.4
K (mmol kg^{-1} DW)	1103±114	1042±45	1139±260	1140±61	1135±95	1114±11
C: N ratio g g^{-1} **	17.17±1.73 ^{C}	10.40±0.46 ^{B}	6.76±0.06 ^{A}	15.09±0.90 ^{C}	11.78±0.87 ^{B}	7.45±0.33 ^{A}
C: P ratio g g^{-1} **	189.4±41.5	244.4±27.3	283.8±42.8	215.2±4.2	193.6±15.0	199.3±20.9
N: P ratio g g^{-1} **	5.71±0.82 ^{A}	9.21±1.42 ^{B}	16.30±2.65 ^{B}	**5.56±0.33 ^{A}**	**6.37±0.21 ^{B}**	**10.38±1.12 ^{B}**

* Plants from two out of three basins in this treatment showed strong decreases in biomass and did not become emergent, whereas the third showed no reduced growth.

** Values are presented from measurements 3 months of treatment. Since plants from the LD treatments receiving 800 kg N ha^{-1} y^{-1} were still alive at this stage, effects of density and N stress can be compared between all treatments.

Plant growth

LD stands receiving 800 kg N ha^{-1} y^{-1} showed high mortality after 3 months of treatment, with no plants surviving after approximately 4 months of treatment (Table 3.1). Reduced vitality was observed in LD stands receiving NH_4^+ loadings of 200 kg N ha^{-1} y^{-1}, which is illustrated by a lower final biomass compared to the lowest NH_4^+ loading of 40 kg N ha^{-1} y^{-1} (Table 3.1). Although no mortality was observed in HD stands, the final biomass of stands receiving 800 kg N ha^{-1} y^{-1} was lower than that of stands receiving only 200 kg N ha^{-1} y^{-1} ($P=0.001$; F=12.431; Table 3.1).

Plants response to high N loads was density-dependent (*P*=0.006; F=8.250). HD stands showed 20-30% decreases in mother plant biomass under either low (40 kg ha^{-1} y^{-1}) or high (800 kg N ha^{-1} y^{-1}) N loads, while under intermediate loads (200 kg N ha^{-1} y^{-1}) plant biomass increased by approximately 20% (Figure 3.3). For LD stands, on the other hand, increasing N loads led to a strong decrease in plant biomass (*P*=0.046; F=4.009), due to increased mortality of mother plants (Figure 3.3). LD stands subjected to 200 kg N ha^{-1} y^{-1} showed a large variation in biomass increase, since plant biomass decreased by 93-98% in two basins, whereas it increased by 20% in the third basin. High NH_4^+ loads not only lowered biomass, but also resulted in a lower number of roots (*P*=0.001; F=9.580) with eventually 0 roots per plant in LD treatments receiving 800 kg N ha^{-1} y^{-1} (Table 3.1). During the experiment, 5.0±2.1, 14.0±3.6 and 2.7±0.3 offsets were produced by the LD stands receiving 40, 200 and 800 kg N ha^{-1} y^{-1} respectively, while HD plants produced 0.3±0.3, 3.7±1.8 and 3.0±2.5 offsets. HD plants had, on average, higher F_V/F_M ratios (*P*<0.001; F=22.258; Table 3.1). At both densities, plants subjected NH_4^+ loadings of 800 kg N ha^{-1} y^{-1} contained lower concentrations of chlorophyll a and b (*P*=0.013; F=6.355; Table 3.1).

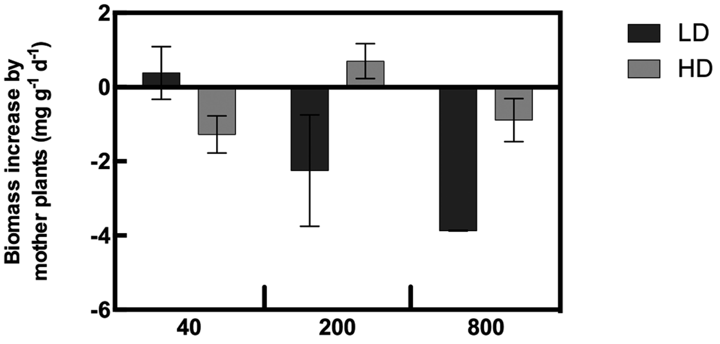

Figure 3.3. Biomass increase (in mg g^{-1} d^{-1}) of mother plants of *Stratiotes aloides* grown in high-density (HD) or low-density (LD) stands at different NH_4^+ loadings.

N accumulation in plant tissue

Increasing the NH_4^+ load from 40 to 200 and 800 kg N ha^{-1} y^{-1} resulted in a 1.5 and 2.5 times higher N contents of the leaf tissue, respectively (*P*<0.001; F=127.616; Table 3.1). HD stands showed 25% higher P content than LD stands (*P*=0.002; F=14.728). N: P ratios increased with increasing N load (*P*< 0.001; F=19.986), with values more than doubled at N loads of 800 kg ha^{-1} y^{-1} compared with 40 kg ha^{-1} y^{-1}. This effect was more profound in LD than in HD stands (*P*=0.038; 5.572).

Plants subjected to high NH_4^+ loads responded by producing high concentrations of N-rich free amino acids (FAA) (Table 3.2). LD stands produced more N-rich FAA than HD stands ($P=0.004$; F=9.986), whereas both showed increased FAA production with increasing N-load ($P<0.001$; F=28.316). With increasing N-load, the contribution of asparagine (Asn; N: C ratio 0.5) increased from 30 to 70% of total N-rich amino acids (Table 3.2). At high N loading rates, N-rich FAA made up 1.7±0.1 % of total plant N for HD plants, and 15.3±5.5 % for LD plants (Data not shown).

Table 3.2. Concentrations of N-rich amino acids (μmol g^{-1} DW) in leaves of *Stratiotes aloides* grown in high-density (HD) or low-density (LD) stands, subjected to different NH_4^+ loads. Measurements were carried out after 7, 105 and 175 days of treatment. After 175 days of treatment, plants from LD stands subjected to 800 kg N $ha^{-1}y^{-1}$ had died, indicated by †.

Amino Acid	**Date**	**LD**			**HD**		
Name (abbreviation)	**Days**	**40**	**200**	**800**	**40**	**200**	**800**
Asparagine (Asn)	7	4.7	2.9	6.2	9.8	5.7	1.8
(0.50)	105	1.1	74.8	266.7	0.3	9.3	25.0
	175	0.9	40.5	†	0.7	9.7	29.3
Glutamic Acid (Glu)	7	2.4	1.9	3.1	3.1	1.7	2.1
(0.40)	105	2.1	8.1	6.8	1.2	1.4	2.9
	175	1.4	3.2	†	2.5	2.2	3.4
Ethanolamine (Etam)	7	0.9	1.2	2.4	4.7	2.3	2.4
(0.50)	105	3.4	5.6	12.1	1.6	1.8	2.8
	175	2.1	4.7	†	1.9	2.9	3.9
Histidine (His)	7	0.3	0.2	0.4	0.5	0.6	0.2
(0.50)	105	2.2	4.0	12.3	0.0	0.4	2.9
	175	0.1	2.8	†	0.4	0.9	3.6
Arginine (Arg)	7	0.4	0.0	0.2	0.1	0.2	0.1
(0.66)	105	0.0	4.0	20.0	0.0	0.2	1.5
	175	0.0	8.3	†	0.4	1.1	7.3
N in N-rich amino	7	8.7	6.2	12.3	18.2	10.4	6.6
acids	105	8.8	96.6	317.9	3.1	13.0	35.1
	175	4.8	60.0	†	6.6	17.0	47.5

Discussion

Our results show that high-density (HD) stands of *S. aloides* facilitate their own growth and survival under elevated environmental N loading through joint uptake of NH_4^+ and alleviating adverse effects of high N loads. As a result, HD stands still thrived under extremely high NH_4^+ loads of 800 kg N ha^{-1} y^{-1}, whereas plants growing at a lower density (LD) already collapsed when receiving NH_4^+ loads of 200 kg N ha^{-1} y^{-1}. The main reason for this higher tolerance of HD stands to increasing N loads lies in the fact that NH_4^+ is taken up by a higher number of plants. This shared uptake thus functions as a detoxification mechanism under high NH_4^+ stress through intra-specific facilitation (van der Heide and others 2008; van der Heide and others 2010).

N-loading effects are density-dependent

Since NH_4^+ is taken up passively by plants (Marschner 1995) and is toxic inside plant cells (Britto and Kronzucker 2002), excess NH_4^+ is usually stored in N-rich free amino acids (Rabe 1990). Build-up of N-rich free amino acids is considered indicative for physiological stress in *S. aloides* (Smolders and others 2000). In our experiment, excess N was mainly (49-77%) stored as free asparagine, which reached concentrations of up to 267 µmol g^{-1} in LD stands receiving 800 kg N ha^{-1} y^{-1}. This level is equal to concentrations reported for *S. aloides* growing under N rich conditions in the field (Smolders and others 2000). While total N concentrations in shoots were similar for HD and LD stands, LD stands had significantly higher concentrations of free N-rich amino acids than HD, with 15% of total N present as free amino acids compared to only 1.7% in HD stands. In the field, *S. aloides* was found to contain 5.4 - 27.5 % of total N as free amino acids under high N loading (Smolders and others 2000). The strong build-up of N-rich free amino acids in LD stands subjected to 800 kg ha^{-1} y^{-1} can be explained by their nutritional status, since only these stands had an N: P ratio > 15 indicating P limitation (Güsewell 2004). Limitation of P has indeed been shown to increase accumulation of N-rich amino acids in *S. aloides* (Smolders and others 1996).

Habitat modification further reduces N stress

In HD stands, strong habitat modification provides additional benefits in terms of C and P availability. First of all, CO_2 accumulates in the water layer underneath a dense plant cover, resulting in both a lower pH, which reduces build-up of highly toxic NH_3, and a higher availability of CO_2, which stimulates underwater photosynthesis. CO_2 concentrations below 200 µmol L^{-1}, such as those observed in our LD stands, reduce underwater photosynthesis of *S. aloides* and inhibit their ability to become buoyant, due to a switch to less efficient, HCO_3^- -based photosynthesis (Cook and Urmi-König

1983; Nielsen and Borum 2008; Harpenslager and others 2015a). Due to the much higher CO_2 availability in HD stands, plants became buoyant 3 months earlier than those in LD stands. Secondly, after becoming emergent, HD stands completely closed off the water surface, thereby reducing atmospheric O_2 flux to the water layer similar to other excessively growing floating aquatic macrophytes (Caraco and others 2006). Low O_2 concentrations enhance the mobilisation of phosphorus (P) from the sediment, by weakening the bonds of iron (Fe) -P complexes under anaerobic conditions (Smolders and others 2006). Indeed, HD stands had both a higher P availability in the water layer and a higher P content in the plants than LD stands, which can be highly beneficial since *S. aloides* is known to have a high P requirement (De Lyon and Roelofs 1986; Smolders and others 1996). The absence of this facilitation that enhances C and P nutrition may provide an extra explanation for the rapid collapse of LD stands.

Implications for restoration and conservation

Although facilitation thus appears to be an important factor in both nutrition and tolerance to environmental stress in *S. aloides* stands, most restoration efforts of this, and other wetland species, are carried out on a small scale in which a small number of plants are reintroduced in seemingly suitable habitats. We here show, however, that there is a large difference in the functioning of LD and HD stands, potentially explaining the low success rate of reintroductions. Due to self-facilitating feedback mechanisms in HD stands, these plants not only improve their own growth, but also become more robust with respect to environmental stress, such as a high input of N. Although HD stands also show signs of toxicity, in terms of a higher free N-rich amino acid content and a lower growth rate, they are still able to withstand extremely high NH_4^+ loads for an entire growing season. While our N load of 800 kg ha^{-1} y^{-1} can hardly be called an ideal habitat for *S. aloides*, atmospheric deposition of 15-40 kg N ha^{-1} y^{-1} (Morris 1991), external loadings of 100 to 320 kg N ha^{-1} y^{-1} (Saunders and Kalff 2001; Bakker 2012) and internal N mobilisation rates of -13 to 188 kg ha^{-1} y^{-1} (Geurts and others 2010) illustrate that our treatment of 200 kg ha^{-1} y^{-1} can be considered a realistic N load in agricultural peatlands in Western Europe. We showed that under these conditions, HD stands still thrive, while LD stands collapse. This implies that although low-density reintroduction may still be successful under more pristine conditions, high N loading areas require introduction in high densities. The negative effects of extremely high N loads of 800 kg ha^{-1} y^{-1} on HD (e.g. reduced growth, lower root production, build-up of free N-rich amino acids), however, indicate that despite the fact that these stands were still thriving, such a system may eventually not be sustainable and loads should be severely reduced before attempting to restore *S. aloides* vegetation.

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Chapter 4

Simultaneous high C fixation and high C emissions in *Sphagnum* mires

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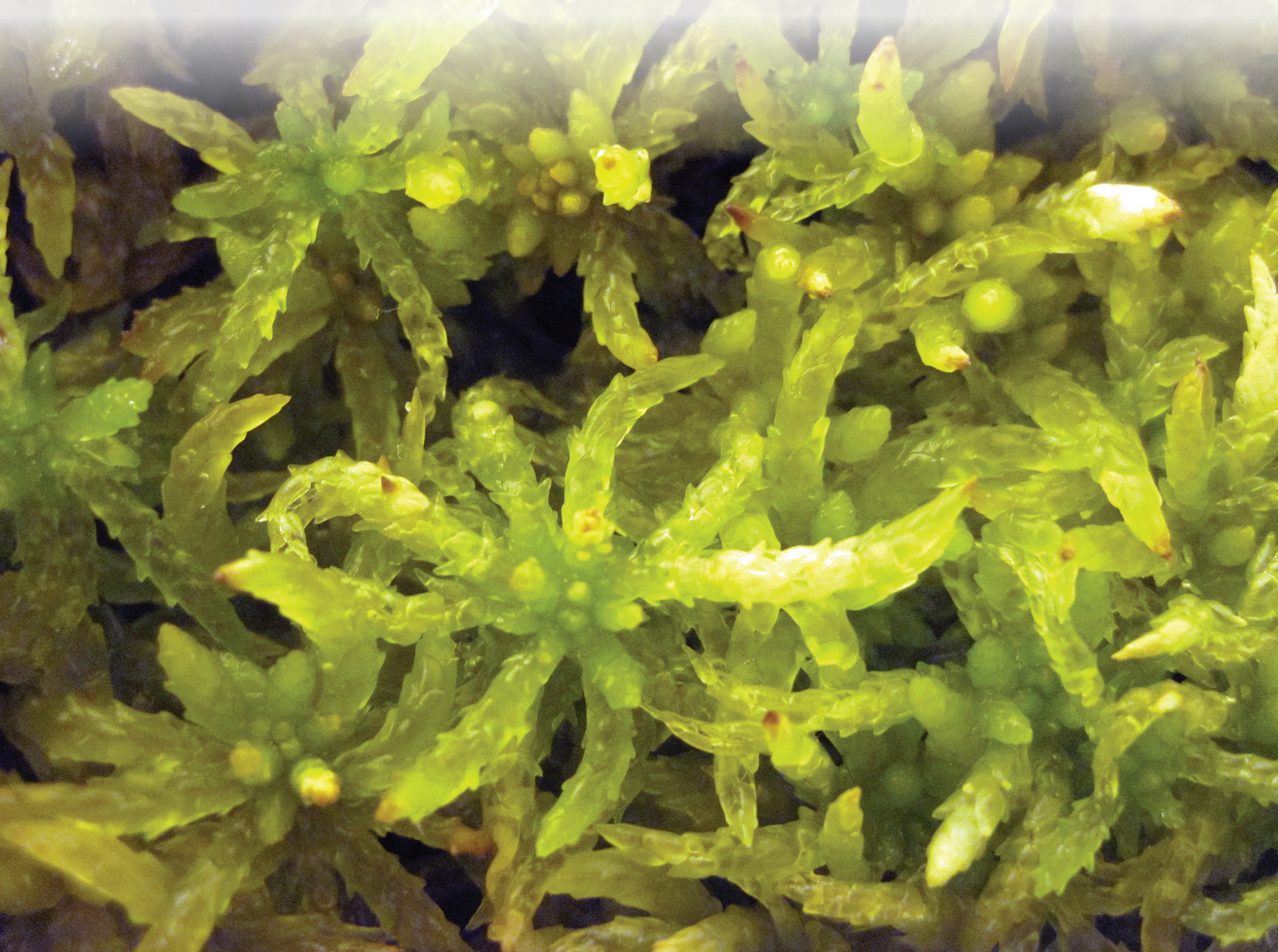

Simultaneous high C fixation and high C emissions in *Sphagnum* mires

Peatlands play an important role in the global carbon (C) cycle due to their large C storage potential. Their C sequestration rates, however, highly vary depending on climatic and geohydrological conditions. Transitional mires are often characterised by floating peat with infiltration of buffered groundwater or surface water. On top, *Sphagnum* mosses grow, producing recalcitrant organic matter and fuelling large C stocks. As *Sphagnum* species strongly differ in their tolerance to the higher pH in these mires, their species composition can be expected to influence C dynamics in transitional mires. We therefore experimentally determined growth and net C sequestration rates for four different *Sphagnum* species (*Sphagnum squarrosum*, *S. palustre*, *S. fallax* and *S. magellanicum*) in aquaria, with floating peat influenced by the infiltration of buffered water. Surprisingly, even though the first three species increased their biomass, the moss-covered peat still showed a net efflux of CO_2 that was up to three times higher than that of bare peat. This species-dependent C release could be explained by *Sphagnum*'s active lowering of the pH, which triggers the chemical release of CO_2 from bicarbonate. Our results clearly illustrate that high *Sphagnum* biomass production may still coincide with high C emission. These counterintuitive C dynamics in mire succession seem to be the result of both species- and biomass-dependent acidification and buffered water infiltration. Together, these processes can explain part of the large variation in C fluxes (ranging from C sequestration to C release) reported for pristine mires in literature.

Introduction

Since peatlands store approximately one-third of all terrestrial carbon (C), they are important in the global C cycle (Gorham 1991), and their C dynamics have been studied throughout the world (Gorham and others 2003; Bortoluzzi and others 2006; Golovatskaya and Dyukarev 2009; Rowson and others 2010). Although it is well known that degraded and drained peatlands generally are net C sources due to increased decomposition rates (Alm and others 1999; Waddington and others 2001; Moore 2002) - with net emissions ranging from +80 to +880 g C m^{-2} y^{-1} (Lamers and others (2015); for all presented values of C fluxes, positive values represent net C losses to the atmosphere, whereas negative values represent net storage of C in growing peat throughout the manuscript) - pristine, growing peatlands (mires) accumulate C and are therefore considered to be C sinks (Belyea and Malmer 2004). The full greenhouse gas budget is, however, more complex. First, almost all peatlands are sources of methane (CH_4) (Moore and Roulet 1995; Saarnio and others 2007), and second, not all pristine peatlands appear to be sinks of carbon dioxide (CO_2) (Waddington and Roulet 2000; Riutta and others 2007). For (minerotrophic) fens fed by groundwater or surface water, CO_2 fluxes generally range from -208 to +190 g C m^{-2} y^{-1} (Martikainen and others 1995; Carroll and Crill 1997; Bubier and others 2003), whereas for transitional mires, fluxes of -124 to +58 g C m^{-2} y^{-1} have been reported (Moore and Knowles 1987; Koch and others 2008b; Salm and others 2009).

Transitional mires are examples of intermediate systems that display characteristics of both minerotrophic fens and ombrotrophic bogs (Wheeler and Proctor 2000; Sjörs and Gunnarsson 2002). Other examples include edges of bog systems (lagg zones) influenced by surrounding surface water and local patches influenced by percolating water (Giller and Wheeler 1988). Transitional mires often consist of floating peat infiltrated by moderately base-rich water, which determines species composition and stimulates buoyancy, through its effect on decomposition and subsequent gas production (Lamers and others 1999; Smolders and others 2002). Since they increase habitat heterogeneity at various scales, these intermediate peatland systems often form hotspots of biodiversity (Verberk and others 2010). Transitional, floating mires are mainly characterised by *Cyperaceae* and a moss layer of different *Sphagnum* species, whose dominance strongly increase during succession (Du Rietz 1954; Vitt and Chee 1990; Wheeler and Proctor 2000). *Sphagnum* growth in transitional mires is, however, not as straightforward as in bogs, since most *Sphagnum* species are sensitive to both high pH and increased concentrations of calcium (Ca) and bicarbonate (HCO_3^-) in pore water and surface water (Clymo 1973). As *Sphagnum* spp. lack stomata, water conducting tissue and roots, their growth, nutrition and vitality depend on the chemical composition of the surrounding water (Robroek et al., 2009). Despite Ca- and HCO_3^--rich conditions, floating rafts in transitional mires may, however, still form suitable habitats for *Sphagnum* species, since they are always water-saturated and are fed by rainwater, which accumulates in the top (moss) layer and dilutes the buffered surface water (Lamers and others 1999; Smolders and others 2003b).

Sphagnum spp. strongly influence their environment and are thus important ecosystem engineers in peatlands (Van Breemen 1995). They are capable of actively acidifying their habitat by exchanging cations for protons (Clymo 1963; Hajek and Adamec 2009) and releasing organic acids (Van Breemen 1995). Furthermore, *Sphagnum* spp. keep their environment moist due to the high water holding capacity of their hyaline cells (Clymo 1973) and compact growth structure. By increasing the acidity and moisture content of their habitat, *Sphagnum* spp. also slow down decomposition rates, thus providing optimal conditions for the accumulation of organic material. Moreover, the high concentration of phenolic compounds in their tissues, including antibiotics (Verhoeven and Toth 1995), further decreases decomposition rates (Yavitt and others 2000; Freeman and others 2001b). This combination of traits results in a strong contribution of *Sphagnum* mosses to C sequestration and peat formation worldwide (Coulson and Butterfield 1978; Limpens and Berendse 2003).

Due to differences in habitat preference among *Sphagnum* species, they inhabit different successional stages in peatlands (Vitt and Chee 1990). Since biomass production (Gerdol 1995), acidification rates (Kooijman and Bakker 1994), decomposition rates (Rochefort and others 1990; Limpens and Berendse 2003) and drought-tolerance (Nijp and others 2014) are species-specific, the species composition of the *Sphagnum* layer in turn may strongly influence the biogeochemistry and C balance of their habitat. This means that the C sequestration potential of the different successional stages of peatlands may strongly depend on which *Sphagnum* species is dominant at a particular stage. In transitional mires, the species composition will strongly depend on pH, buffering components and water content. How the *Sphagnum* species composition influences the biogeochemistry and C balance in transitional mires, however, remains largely unknown.

Although a vast number of studies has presented field measurements of C dynamics in all types of peatland systems, including transitional mires, establishing the origin of the huge variation reported for both CH_4 and CO_2 fluxes in these field studies is challenging. Studies on both C dynamics and the influence of *Sphagnum* mosses using a controlled laboratory approach, however, have not yet been performed to our knowledge. The goal of this study was therefore twofold: first, to investigate the growth of different *Sphagnum* species under controlled environmental conditions characteristic for transitional mires, and second, to study C fluxes and their underlying mechanisms in these systems. Four different *Sphagnum* species, *S. squarrosum*, *S. palustre*, *S. fallax* and *S. magellanicum*, were grown on peat floating on Ca - HCO_3^- rich water. Besides growth parameters of these mosses, we studied their contribution to the net C fluxes in these potentially peat forming systems. We hypothesised that Ca - HCO_3^- rich conditions would lead to considerable differences in performance between the four *Sphagnum* species, based on differences in their tolerance to these buffering components and in their growth rates. Furthermore, we expected more tolerant *Sphagnum* species to strongly determine the C sequestration of these systems.

Material and Methods

Experimental set-up

Intact floating peat monoliths (25 x 25 cm; height 21.85 ± 2.08 cm; n=8) were cut from a floating mire in the southern part of the Netherlands (51°24’6.1” N, 6°11’10.5” E) in late March 2012. This floating mire was dominated by helophytes species *Typha latifolia* and *Calla palustris*, whereas the moss layer consisted mainly of *Sphagnum fallax*. After cutting, all vegetation was removed and the bare peat was transferred to glass aquaria (25 x 25 x 30 cm; length x width x height) in the field to minimise damage to the peat structure. The peat had an organic matter content of 92.7±0.4 % (determined by loss on ignition; 3h at 550°C) and contained 3.6±0.4 mmol kg^{-1} fresh weight (FW) of Ca (determined by digestion of 200 mg of dry soil with 4 ml of HNO_3 and 1 ml of H_2O_2 using a microwave oven (MLS 1200 Mega, Milestone Inc., Sorisole, Italy), after which diluted digestates were analysed by inductively coupled plasma spectrometry (ICP-OES iCAP 6000; Thermo Fisher scientific, Waltham, MA, U.S.A.).

In the laboratory, 6.25 l of Ca - HCO_3^--rich treatment water was added to each aquarium (Table 4.1), on which the peat floated. The underlying water layer was subsequently refreshed with treatment water at a rate of 5 L $week^{-1}$ using peristaltic pumps (Masterflex L/S, Cole-Parmer, Vernon Hills, IL, U.S.A.). All floating peat monoliths received artificial rainwater (Table 4.1) five times a week, at a rate corresponding to the Dutch annual rainfall of 800 mm. During the experiment, the aquaria were kept in a water bath maintained at 18°C (up to a maximum of 23 °C at the end of the day) using a cryostat (NESLAB, Thermoflex 1400, Breda, The Netherlands). Furthermore, a light regime of 200 µmol m^{-2} s^{-1} (PAR; 16h light/8h dark) was maintained (Master Son-T Pia Plus, Philips, Eindhoven, The Netherlands). This regime of temperature and light was chosen to mimic summer conditions.

On four floating peat monoliths, four different species of *Sphagnum* (*Sphagnum. squarrosum*, *S. fallax*, *S. palustre* and *S. magellanicum*) were planted together. *S. squarrosum* is a species of moderately rich fens and occurs in environments with pH values up to 7 (Clymo 1973). *S. fallax*, on the other hand, can be quite sensitive to high pH or drought, but is also known for its high potential growth rate under minerotrophic conditions (Buttler and others 1998). *S. palustre* is a widespread species found in habitats that are neither highly calcareous nor highly acidic (Daniels and Eddy 1990). *S. magellanicum* is a species associated with poor fens and bogs, and it is restricted to a more acidic habitat (Vitt and Chee 1990; Hajek and others 2006). The first three species were collected in a peatland area in the north-western part of the Netherlands (Ilperveld; 52°26’42.5” N, 4°55’45.1” E), while the latter species was collected in an area in the south of the Netherlands (Maasduinen; 51°34’56.3” N, 6°6’13.5” E). Of all species, a patch of 50 ± 10 g fresh material (1.6 ± 0.8 g DW; moss length 3 cm) was applied randomly to one of the corners of the aquarium. Mosses were put upright in a patch of approximately 50 cm^2. The remaining 4 floating peat monoliths were kept as non-vegetated controls.

Table 4.1. Composition of the infiltrating water and artificial rainwater used in the experimental set-up. The rainwater composition was based on the composition of Dutch rainwater. Note that all concentrations are in µmol L^{-1}, except for the sea salt addition, which is in mg L^{-1}.

	Infiltrating water	***Artificial rainwater***
HCO_3^-	3000	-
SO_4^{2-}	100	-
Cl^-	8000	54
Ca^{2+}	2000	17
Mg^{2+}	2000	-
Na^+	3000	-
K^+	200	20
NH_4^+	-	36
NO_3^-	-	36
Sea salt (mg L^{-1}) *	-	5

* Pro Reef, Tropic Marine, aQua united LTD, Telgte, Germany.

Since soils were floating and not inundated, the "surface water" will be called infiltrating water throughout this paper. This infiltrating water was sampled underneath the peat monolith, while pore water was extracted using 10 cm Soil Moisture Samplers (SMS rhizons, Eijkelkamp, Giesbeek, The Netherlands), which were inserted vertically into the soil. Per peat monolith, 2 SMS rhizons were installed and samples were taken by attaching vacuum bottles. Analyses were performed on pooled samples to reduce the effect of variation within the soil.

Chemical analyses

During the 12 weeks of the experiment, pH and total inorganic carbon (TIC) concentration of infiltrating water and pore water were measured every two weeks (7 times in total). pH was measured with a standard Ag/AgCl electrode (Orion, Thermo Fisher Scientific, Waltham, MA, U.S.A.) combined with a pH meter (Tim840 titration manager; Radiometer Analytical, Lyon, France). TIC was measured by injecting 0.2 ml of sample into a compartment with 1 ml phosphoric acid (0.4 M) in an Infra-red Gas Analyser (IRGA; ABB Analytical, Frankfurt, Germany), after which concentrations of HCO_3^- and CO_2 were calculated based on the pH equilibrium. Concentrations of PO_4^{3-}, NO_3^- and NH_4^+ were measured colourimetrically on an Auto Analyser 3 System (Bran & Luebbe, Norderstedt, Germany) using ammonium molybdate (Henriksen 1965), hydrazine sulphate (Kamphake and others 1967) and salicylate (Grasshof and Johannse 1972), respectively. Concentrations of Ca, Fe, K, Mg, total P and SO4 were analysed by inductively coupled plasma spectrometry (ICP-OES iCAP 6000; Thermo Fisher scientific, Waltham, MA, U.S.A.).

Plant data

To preserve bare control soils and monocultures of the *Sphagnum* species, all aboveground biomass of non-*Sphagnum* species was carefully removed every two weeks. This vegetation consisted mainly of *Typha latifolia* and *Juncus effusus* seedlings. Every two weeks, growth and expansion of the mosses were recorded. Mosses were allowed to grow outside of their designated quarters to include the effects of competition between species. After 8 weeks of moss growth, pH was measured within the *Sphagnum* vegetation at 0.5-1 cm above soil level, using a pH meter (HQ 40d, Hach, Loveland, CO, U.S.A.) and an Ag/AgCl pH electrode (Orion, Thermo Fisher Scientific, Waltham, MA, U.S.A.). After 12 weeks, all moss biomass was harvested and the number of capitula (top 8-10 mm of the photosynthetically active tissue of the mosses) was counted for each plot. Length of the moss fragments was measured before living plant parts and dead parts were separated and weighed. Biomass was dried for 48 hours at 70 °C to determine dry weight (DW). C and N contents (%) of dried moss material were determined using an elemental analyser (Carlo Erba NA1500, Thermo Fisher Scientific, Waltham, MA, U.S.A.).

Carbon fluxes

C fluxes were determined after 6 weeks of experimental treatments. Since the cover of *S. magellanicum* had declined severely by this time, the remaining patch was too small to cover with a closed chamber and the species was excluded from these measurements. C fluxes from soils covered with *S. squarrosum*, *S. palustre* or *S. fallax* and from bare control soils were measured in transparent and closed chambers (length, width and height of 10, 10 and 12 cm) for light and dark conditions, respectively. Dark measurements started at the end of the 8h dark period and lights remained off during measurements, so that mosses remained dark-adapted. Samples were taken from the headspace immediately after placing the chambers on the aquaria, and subsequently after 2 and 4 hours using 1 ml syringes, which were first flushed with headspace. They were analysed for CO_2 using an IRGA (ABB Analytical, Frankfurt, Germany) and for CH_4 using a gas chromatograph (5890 GC, Hewlett Packard, Wilmington, DE, U.S.A.). The slopes of the linear increases in both gasses were used to determine areal net C fluxes for each *Sphagnum* species and for bare peat. Measurements on CO_2 and CH_4 fluxes carried out under light and dark conditions were combined to calculate daily C fluxes. Under natural conditions, *Sphagnum* spp. tend to grow vertically, whereas in our experiment – due to the absence of supporting neighbouring mosses – elongated moss fragments fell over, causing an apparent horizontal growth. Our areal C fluxes measured with the closed chambers covering only part of the elongated fragments are therefore underestimates. We corrected for this by multiplying the areal C fluxes with the ratio of the area covered by elongated *Sphagnum* fragments and the area of the chamber.

Statistical analyses

All data were checked for normality of residuals and homogeneity of variance using Shapiro-Wilk's test for normality and Levene's test of equality of error variances, respectively. Differences in the chemical composition of surface water and pore water of soils with and without moss cover were analysed over time using linear mixed models. Differences between growth parameters of *Sphagnum* mosses (Figure 4.1, Figure 4.2, Table 4.3) and C fluxes were tested using one-way ANOVAs with Tukey post hoc tests. In all tables and figures, averages are presented with standard error of the mean (SEM). All statistical analyses were carried out using SPSS (V21, 2012, IBM Statistics).

Results

Chemical composition of infiltrating water and pore water

Growth of *Sphagnum* significantly lowered the pH of the infiltrating water (*P*<0.001), whereas pore water pH increased (*P*=0.007) when mosses were present (Table 4.2). Concentrations of NO_3^- (*P*=0.002) and Fe (*P*=0.018) were significantly higher in the infiltrating water of moss-covered soils, whereas concentrations of Ca (*P*=0.014) and P (*P*=0.012) were higher in pore water of bare soils (Table 4.2). Still, concentrations of NO_3^- were low, since N was predominantly present as NH_4^+ in both infiltrating water and pore water (Table 4.2). Overall, NH_4^+ concentrations in the infiltrating water were high compared to similar systems (Kooijman and Bakker 1994), with values around 40 µmol L^{-1} (Table 4.2).

Table 4.2. Infiltrating water and pore water characteristics. All nutrient concentrations are given in µmol L^{-1}. Overall averages are given, while statistical tests were carried out over time using linear mixed models (LMMs). When these LMMs showed significant differences between moss covered and bare soils, values are given in bold.

	Infiltrating water		***Pore water***	
	With moss	***Without moss***	***With moss***	***Without moss***
pH	**6.3±0.1**	**6.6±0.0**	**5.5±0.2**	**5.2±0.2**
TIC	**2014±268**	**1965±294**	**2496±160**	**2317±170**
HCO_3^-	859±318	1158±251	376±105	279±140
CO_2	1154±182	810±278	1953±300	1820±310
NO_3^-	**1.0±0.3**	**0.5±0.2**	0.3±0.2	0.5±0.2
NH_4^+	38±14	46±9	99±25	59±33
Total-P	6.2±1.4	6.5±2.2	**21±8**	**42±18**
Ca	1413±86	1366±97	**1187±129**	**1381±139**
Fe	**99±8**	**63±21**	257±58	295±68
K	284±70	228±67	310±68	298±100
Mg	1823±118	1738±123	1444±150	1574±80
SO_4	92±13	117±26	69±6	92±21

Sphagnum growth and acidification

S. squarrosum, *S. fallax* and S. *palustre* increased their biomass during the experiment (Figure 4.1). The initial biomass of these species increased by 340%, 250% and 600% respectively (*P*<0.001). While length of the green part of the mosses (living moss) remained approximately the same, the total length of the moss fragments increased by 5-6 cm (Table 4.3). In *S. fallax* and *S. palustre*, elongation appeared to be the main factor responsible for the increased biomass, since the number of capitula decreased in these species by 35% and 19% (Figure 4.2). *S. squarrosum* was the only species that increased both in biomass (*P*<0.001) and in number of capitula (*P*=0.004). This species also increased its coverage in the aquaria (*P*=0.001; Table 4.3), thereby invading the quarters of other species and proving to be a strong competitor in this experimental set-up. In contrast, *S. magellanicum* was strongly affected by the HCO_3^--rich water and showed a strong decline in both biomass (Figure 4.1) and number of capitula (Figure 4.2) during the experiment. *S. squarrosum* acidified its environment the most, with pH values down to 4.5 (*P*=0.027; Table 4.3). *S. fallax* and *S. palustre* both had pH values of around 5.2-5.4, whereas *S. magellanicum* had the highest pH with values around pH 6 (Table 4.3). The acidification rate was linearly correlated (*P*=0.005; R^2=0.43) to the net increase in biomass shown by the three species, with *S. magellanicum* showing both the lowest biomass increase and the highest pH, and *S. squarrosum* showing the lowest pH and highest growth (Supplementary Figure 4.1).

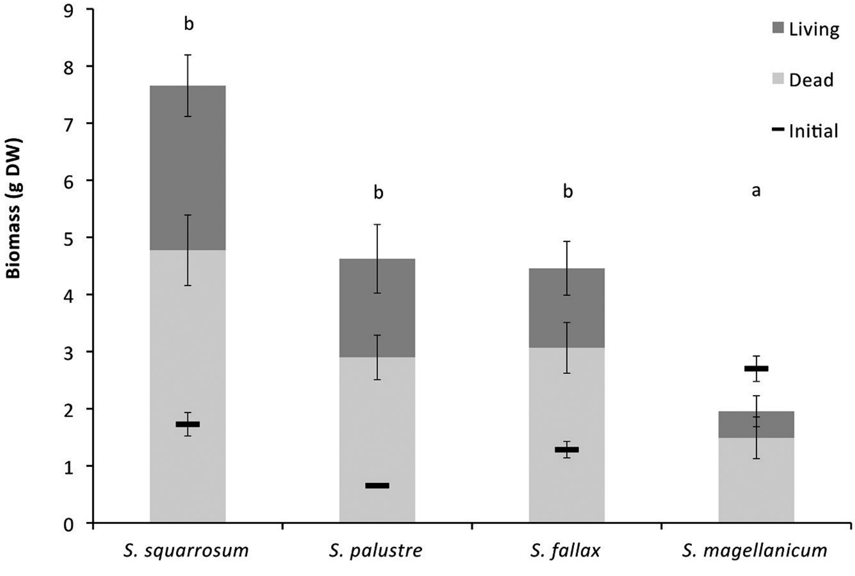

Figure 4.1. Biomass dry weight (DW, ± SEM) produced by a patch of 50 cm^2 of four different *Sphagnum* species after 12 weeks of experimental treatments, divided into living and dead tissue (dark and light bars respectively). The smaller black bars represent initial DW. Significant differences between total final biomass of the species are indicated by different letters (*P*=0.005).

Table 4.3. Characteristics of the different *Sphagnum* moss patches, including pH within the vegetation (0.5-1 cm above soil level; measured after 6 weeks of growth), and final data (after 12 weeks) on C: N ratio, length of the moss fragments and cover of the patches. For all variables, *P*-values are given (ns is not significant) and different superscript letters indicate significant differences between species.

		S. squarrosum	***S. fallax***	***S. palustre***	***S. magellanicum***	***P***
pH		4.5 ±0.2 ^{a}	5.4±0.3 ^{a,b}	5.2±0.4 ^{b}	6.1±0.4 ^{b}	0.027
Final length	mm	85.9±4.0 ^{b}	66.1±7.4 ^{a,b}	70.4±7.9 ^{b}	42.1±1.7 ^{a}	0.002
Final cover	cm^2	209±28 ^{b}	120±11 ^{a}	111±9 ^{a}	75±10 ^{a}	0.001
C: N ratio	$g\ g^{-1}$	27.0±1.3	25.3±3.6	25.2±3.2	25.2±1.7	ns

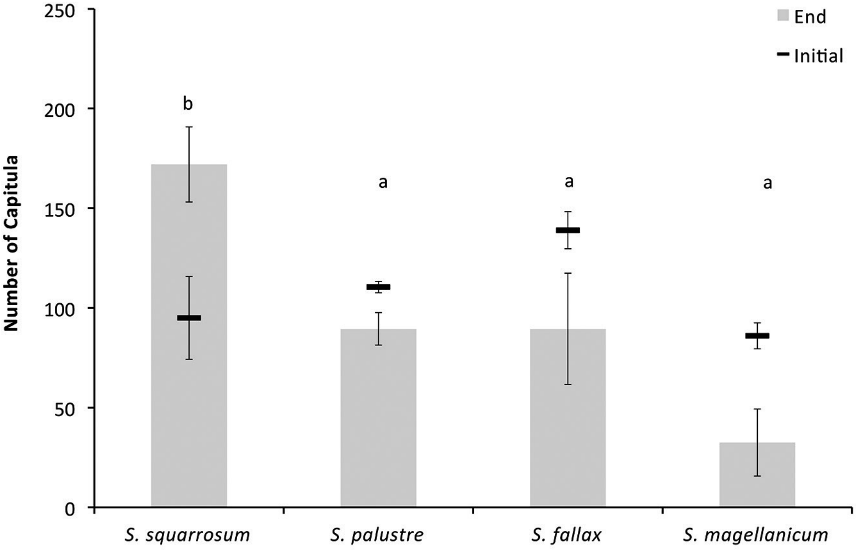

Figure 4.2. Number of capitula (± SEM) produced by a patch of 50 cm^2 of four different *Sphagnum* species after 12 weeks of experimental treatments (grey bars). The smaller black bars depict the number of capitula at the beginning of the experiment. Significant differences in the final number of capitula between the species are indicated by different letters (P=0.002).

Carbon exchange

Bare floating peat had a C emission of 0.3 ± 0.1 g C m^{-2} d^{-1} (Figure 4.3). Surprisingly, when *Sphagnum* species were grown on these soils, net C emission appeared to be similar to or even higher than that of bare peat (Figure 4.3). Soils covered with *S. squarrosum* showed the highest net emissions of 1.1 ± 0.2 g C m^{-2} d^{-1} ($P<0.001$), despite being the species with the highest biomass increase. *S. fallax* and *S. palustre* had net C effluxes similar to those of bare peat, with 0.5 ± 0.1 and 0.2 ± 0.1 g C m^{-2}

d^{-1}, respectively, even though significant increases in biomass were observed for these two species. C fluxes consisted almost exclusively of CO_2, as methane (CH_4) contributed less than 2.5% to net C emission and ranged from -0.8 to 43 mg C m^{-2} d^{-1} (data not shown). Still, due to the higher global warming potential of CH_4 compared with CO_2, CH_4 contributed 10 to 30 % to the total greenhouse gas emissions expressed in CO_2 equivalents. These greenhouse gas emissions were higher for plots covered by *S. squarrosum* (1750 ± 350 g CO_2-eq m^{-2} y^{-1}) than for plots covered by other species (580 ± 106 g CO_2-eq m^{-2} y^{-1}) or unvegetated (550 ± 180 g CO_2-eq m^{-2} y^{-1}) plots due to the higher CO_2 emission from these plots (*P*=0.002).

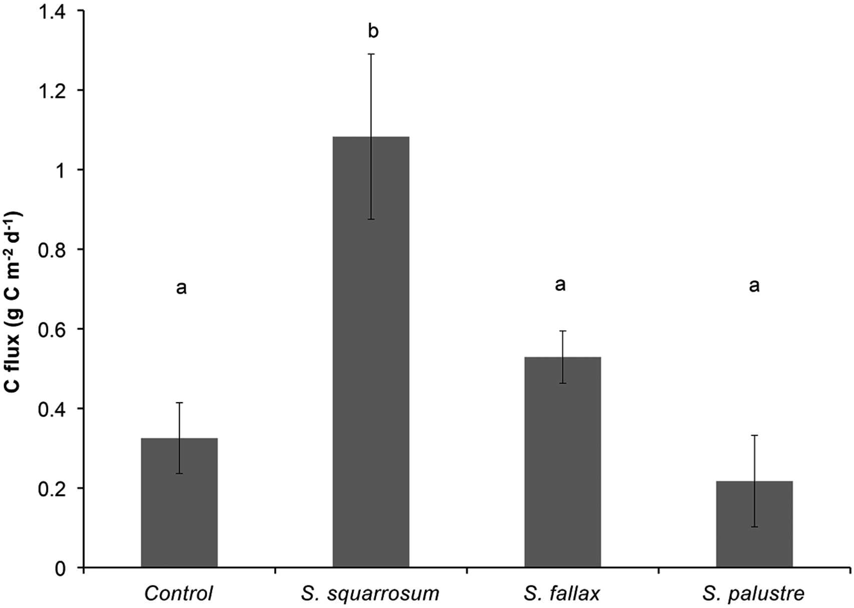

Figure 4.3. Daily net C (CO_2 + CH_4) fluxes (±SEM) for bare peat and peat covered with different *Sphagnum* vegetation, measured after 6 weeks of experimental treatments. Since *S. magellanicum* only had a few living capitula left at this moment, we excluded it from these measurements. Note that positive values represent net C emission to the atmosphere. Different letters indicate significant differences between the four species (*P*=0.012).

Discussion

For *Sphagnum* species growing on top of floating peat monoliths, the infiltration of buffered groundwater or surface water into the peat gradually lowered the influence of Ca^{2+} and HCO_3^-. As a result, even moderately sensitive species were capable of growing on top of these floating monoliths. It was remarkable to discover, however, that while some of these species strongly increased their biomass, *Sphagnum*-covered patches simultaneously showed a net C efflux.

Tolerance of *Sphagnum* species to buffered conditions

Transitional mires are *Sphagnum*-rich systems characterised by the influence of calcareous and alkaline surface water or groundwater in the subsoil and are thus partly buffered systems. These environmental conditions are, however, not limited to transitional mires and occur more widely, since local spots with higher influence of groundwater or edges in contact with calcareous surface water occur in many peatlands. The acid neutralising capacity (ANC) of peatlands is mainly based on the presence of HCO_3^- and Ca^{2+}. When protons (H^+) are released into a system, they are initially buffered by the HCO_3^- - CO_2 buffering system (Sherlock and others 1995; Lamers and others 2015). Once most of the HCO_3^- has been consumed, incoming H^+ can be buffered by the cation-exchange capacity (CEC) of the peat, where base cations bound to soil particles are exchanged for H^+ (Lamers and others 2015). Ca^{2+} usually is the main component of the CEC, since it is the dominant divalent cation in many peatlands (Bache 1984; Rippy and Nelson 2007).

Several studies have indicated that *Sphagnum* can be sensitive to calcareous groundwater and surface water due to Ca - HCO_3^- toxicity (Clymo 1973; Andrus 1986; Lamers and others 1999; Hajek and others 2006). This sensitivity to one or both of the major buffering components of groundwater and surface water is species-specific and it strongly affected the performance of the *Sphagnum* species in our study. *S. magellanicum* appeared to be the most sensitive to the tested conditions as this species decreased both in number of capitula and biomass. *S. fallax* and *S. palustre*, on the other hand, increased in biomass, even though they were obviously outcompeted by the better-adapted *S. squarrosum*. *S. squarrosum* was able to increase both horizontally, in number of capitula, and vertically, by stem elongation.

S. squarrosum is one of the few *Sphagnum* species that is still vital in systems with a higher influence of calcareous and therefore HCO_3^--rich water and is even able to tolerate (temporary) immersion in these systems (Clymo 1973; Vitt and Chee 1990). Other species, including *S. magellanicum*, are known to be highly sensitive to increased pH and buffered conditions in their habitat (Clymo 1973; Granath and others 2010), which explains why *S. magellanicum* showed a strong decrease in our study. The typical habitats of *S. fallax*, *S. palustre* and *S. magellanicum* are all characterised by low pH (4.5-4.8) and low Ca^{2+} content (35-40 µmol L^{-1}) (Vitt and Chee 1990; Hajek and others 2006), although *S. fallax* and *S. palustre* can tolerate a wider range of environmental conditions in terms of acidity and trophic level than *S.*

magellanicum (Daniels and Eddy 1990). *S. squarrosum*, on the other hand often occurs in rich to moderately rich fens (Vitt and Chee 1990; Hajek and others 2006), which are characterised by pH values of 5.1 to 6.7 and Ca^{2+} concentrations of 270-500 µmol L^{-1} (Vitt and Chee 1990; Kooijman and Bakker 1994).

Succession of *Sphagnum* species

The transition of mineral-rich fens to acidic “poor fens” to oligotrophic bogs is believed to be initiated by the acidification of pioneer *Sphagnum* species (Wilcox and Andrus 1987; Rydin and Jeglum 2006; Granath and others 2010). These pioneer species are expected to tolerate mineral-rich conditions, have a high growth rate and a high acidification capacity under more buffered conditions, which will allow them to change a mineral-rich fen into an acidic, poor fen within a few decades (Granath and others 2010). *S. squarrosum* may act as such a pioneer species and is often responsible for rapid succession in fens (Giller and Wheeler 1988; Haraguchi and others 2003), especially under nutrient-rich conditions (Kooijman and Bakker 1995).

Our data confirm that, *S. squarrosum* potentially acts as a foundation species for other *Sphagnum* spp. This species simultaneously increased its biomass considerably and acidified its environment most effectively, lowering pH to values around 4.5 despite continuous infiltration of surface water with an alkalinity of 3 meq L^{-1}, while the other three species could not lower pH below 5.2. *Sphagnum* species show differences in acidification rate, based on differences in their cation-exchange capacity (Rippy and Nelson 2007). Additionally, however, *Sphagnum* acidification rates depend on their species-specific performance under certain environmental conditions. High growth rates combined with low decomposition rates (5-35% mass loss y^{-1} (Clymo 1965; Coulson and Butterfield 1978; Verhoeven and Toth 1995; Limpens and Berendse 2003)) result in a fast build-up of the peat layer and succession in species composition, which, in floating transitional mires, will slowly reduce the influence of the underlying calcareous water.

Carbon dynamics

Increase of the thickness of the peat layer due to *Sphagnum* growth shows that these species can sequester a significant amount of C. *Sphagnum* biomass can increase by approximately 70 to 600 g DW m^{-2} y^{-1} (Gerdol 1995; Graf and Rochefort 2009; Hajek 2009; Samaritani and others 2011), which corresponds to a CO_2 fixation rate of approximately 28 to 240 g C m^{-2} y^{-1}. If we extrapolate the daily CO_2 fixation rates of the three growing species in our experiment, *S. squarrosum*, *S. fallax* and *S. palustre*, to calculate yearly production rates, based on a growing season of 8 months, we find high CO_2 fixation rates of approximately 100-450 g C m^{-2} y^{-1}. These values, however, overestimate actual field growth of these mosses, since the experiment was carried out indoors under summer conditions only. Still, even with these high CO_2 fixation rates, we found net C emissions from both bare peat and from peat covered with growing *Sphagnum* mosses.

Bare peat showed C emission rates of around 0.3 g C m^{-2} d^{-1} (Figure 4.4), which consisted of 98% CO_2 and 2% CH_4. Both bare peat and vegetated plots were a small source of CH_4, with average emission rates of 2 to 20 mg C m^{-2} d^{-1}, which fall within the range of 4 to 500 mg C m^{-2} d^{-1} usually reported for saturated peatlands (Bartlett and Harriss 1993; Byrne and others 2004; Saarnio and others 2007; Salm and others 2009). Still, the contribution of CH_4 to the greenhouse gas emission is much higher in terms of CO_2 equivalents, since the global warming potential of CH_4 is 34 times that of CO_2 (IPCC 2013). The higher greenhouse gas emissions (as CO_2 equivalents) from the plots vegetated by *S. squarrosum* were, however not due to differences in CH_4 emissions, but resulted from the much higher emissions of CO_2 from these plots.

When plots were vegetated by growing *Sphagnum* spp., CO_2 emissions increased, despite the accumulation of biomass by all three species (Figure 4.4), which indicates that the source of this CO_2 could not solely be the decomposition of *Sphagnum* litter. The only likely explanation for this remaining net CO_2 efflux is therefore the chemical reaction depicted in Eq. 1, that occurs when HCO_3^- -rich water comes into contact with the acidifying mosses (Figure 4.4). The transition of HCO_3^- to CO_2 is the first step in the ANC of aquatic systems and will occur much faster than other buffering mechanisms, such as cation-exchange of Ca^{2+} (Lamers and others 2015). Active acidification was mainly observed in *S. squarrosum*, while *S. fallax* and *S. palustre* did not significantly lower pH more than the dying *S. magellanicum*.

$$HCO_3^- + H^+ \rightarrow H_2O + CO_2 \qquad \textbf{Eq. 1}$$

To further disentangle the different CO_2 sources responsible for the net CO_2 emission from plots vegetated with different species, we used a mass approach (Eq. 2; Table 4.4). Net CO_2 fixation was estimated based on the difference between light and dark CO_2 fluxes, whereas CO_2 emission was estimated based on dark fluxes. This CO_2 emission can be further divided into separate contributors, as is shown in Eq. 2.

$$\text{Net C flux to atmosphere} = B + R + C - F \qquad \textbf{Eq. 2}$$

Here, B represents the CO_2 flux from bare peat to the atmosphere, R is the dark plant respiration, C represents the flux of chemically produced CO_2 according to Eq. 1 and

F is the gross CO_2 fixation, calculated as the light CO_2 flux minus the dark CO_2 flux. Bare peat respiration was derived from dark fluxes of non-vegetated plots. For the *Sphagnum* respiration factor R, we have used the maximum value (31%) from the range reported in literature (12 to 31 % of photosynthetic C fixation, (Haraguchi and others 2003; Laine and others 2011; Kangas and others 2014)). As a result, we obtain a conservative estimate of the C flux emitted through the chemical reaction Eq. 1, driven by the acid production of the *Sphagnum* mosses (C). Although the likely higher respiration rates during the light period and the use of estimated *Sphagnum* respiration impede an exact quantification of factor C, the fact that we find CO_2 emissions during the light period in growing *Sphagnum* patches clearly points out that there is a considerable chemical CO_2 source.

Table 4.4 shows the different sources of the C fluxes as presented in Eq.2. Furthermore, the implications that these values have on a landscape scale are depicted in a schematic overview of a floating transitional mire that is being fed by HCO_3^- rich water (Figure 4.4). Here, we show simultaneous C fixation and C emission of the three growing *Sphagnum* species from our experiment, with their different growth and acidification rates.

	Net C flux (B+C-F)	***Gross C fixation (F)***	***Gross C emission (B+R+C)***	***Bare peat (B)***	***Autotrophic respiration (R)***	***Additional HCO_3^- derived CO_2 (C)***
S. squarrosum	1.1±0.2	1.9±05	3.0±0.7	0.3±0.1	0.6	2.1±0.7
S. fallax	0.5±0.1	0.9±0.2	1.5±0.2	0.3±0.1	0.3	0.9±0.2
S. palustre	0.2±0.1	0.4±0.1	0.6±0.3	0.3±0.1	0.1	0.2±0.3

Table 4.4. Origin and rates of C fluxes (in g C m^{-2} d^{-1}) of peat covered with different species of *Sphagnum*. Net C fluxes, gross C fixation rates and gross C emissions are based on closed chamber measurements carried out under light and dark conditions. Other fluxes are calculated using Eq. 2, with the fraction of autotrophic respiration based on the maximum value found for *Sphagnum* respiration in literature (31%, Laine and others (2011)).

The production of HCO_3^- -derived CO_2 will occur in any situation where HCO_3^- -rich water comes into contact with an acidic environment, such as in the highly acidic lower layers of floating bog systems influenced by HCO_3^- -rich water (Lamers and others 1999; Smolders and others 2003b). Therefore, CO_2 effluxes measured from the slightly acidic bare peat in our experiment, are likely at least partially derived from acid-driven CO_2 production from HCO_3^-, as is illustrated in Figure 4.4. Our finding that the most strongly acidifying and fastest growing mosses such as *Sphagnum squarrosum* show the highest C effluxes strongly suggests that active acidification enhances the production of HCO_3^--derived CO_2.

This leads to the apparent contradiction that while growth of *Sphagnum* will lead to accumulation of organic matter and thus contributes to the build-up of a peat layer, it is accompanied by a large net efflux of CO_2 ranging from 0.2 to 1.1 g C m^{-2} d^{-1} (Table 4.4, Figure 4.4). While we show this phenomenon here in a controlled laboratory setting, net CO_2 effluxes have indeed been reported for transitional mires, with rates ranging from -0.34 to +0.16 g C m^{-2} d^{-1} (Moore and Knowles 1987; Koch and others 2008b; Salm and others 2009). As mentioned before, however, this phenomenon may not be limited to transitional mires. For example, bogs typically show an outflow of acidic water (H^+ and organic acids) and therefore *Sphagnum*-produced acids may also cause chemical CO_2 production outside the peatland system, thereby counteracting at least a part of the C sequestration realised by peat growth.

Conclusion

To provide insight into the processes driving the highly variable C fluxes measured in *Sphagnum*-dominated mires, we used an experimental approach, which revealed a novel, overlooked mechanism, explaining part of the variation in CO_2 fluxes. Our results clearly show that high biomass production in mires can coincide with a net emission of C, due to a combination of biological and chemical processes. We feel that the acidification-driven CO_2 production is an underestimated factor that plays a significant role in C fluxes in transitional mires and other systems where calcareous (Ca and HCO_3^--rich) groundwater or surface water comes into contact with growing and acidifying *Sphagnum* mosses. Our results suggest that, under these conditions, for every gram of C that is fixed by *Sphagnum*, there is an emission of 0.8 to 1.4 g C through chemical processes, depending on *Sphagnum* acidification potential. We hypothesise that this phenomenon can specifically play an important role in early succession from minerotrophic to ombrotrophic conditions, when the influence of calcareous water in combination with *Sphagnum* growth is greatest. Due to the continuous build-up in these systems, the thickness of floating rafts will increase during succession and the lateral influence of the calcareous water will decline, leading to strong spatial and temporal variation in C fluxes in these systems. This finding may therefore help explain part of the strong variation in C balances measured in seemingly similar peatland systems.

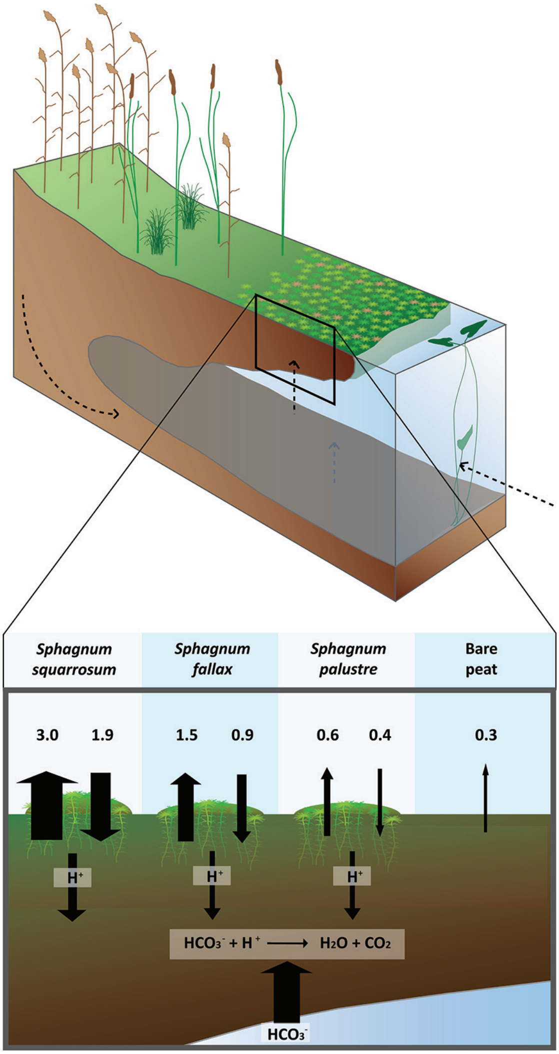

Figure 4.4. Schematic overview of a transitional floating mire influenced by HCO_3^- -rich groundwater or surface water, illustrated by dashed arrows in the figure above. Due to differences in the thickness of the floating peat or the origin and composition of the HCO_3^- -rich water, there is a high heterogeneity within these systems. Part of the floating raft is shown in more detail below. Here, peat soils are covered with different *Sphagnum* species. Rates of C fixation in peat (downward arrow) and C emission to the atmosphere (upward arrows) are both derived from C-flux measurements and presented in g C m^{-2} d^{-1}. As the mosses showed differences in final biomass, higher or lower amounts of biomass are depicted in the figure. Furthermore, the mosses differ in acidification rate, with significantly higher amounts of acids produced by *Sphagnum squarrosum* (left) than the other species. Since *Sphagnum magellanicum* declined severely in biomass due to its sensitivity to the calcareous water, its C-fluxes could not be measured and the species was excluded from this figure. [Illustration by Gijs van Dijk]

Acknowledgements

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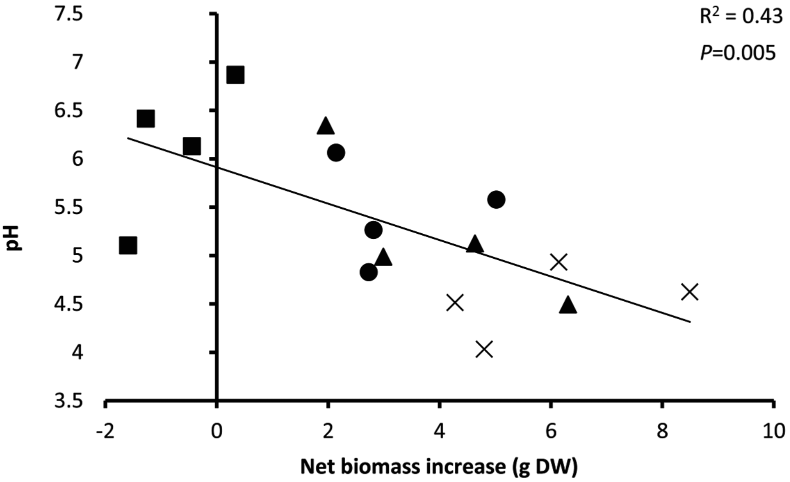
Supplementary information



Supplementary Figure 4.1. A linear relationship (R^2=0.43; *P*=0.005) exists between biomass production of *Sphagnum* mosses and acidification of the environment. Here, ■ = *S. magellanicum*, ● = *S. fallax*, ▲ = *S. palustre* and x = *S. squarrosum*. *Sphagnum* species that show the highest biomass increases also have a higher acidification rate, leading to lower pH in their environment than slower growing species.

Chapter 5

Peat capping: natural capping of wet landfills by peat formation

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Submitted

Peat capping: natural capping of wet landfills by peat formation

Given the bioremediation potential of peat, natural capping of landfills in wetlands with a "peat cap" could provide a sustainable addition to regular capping methods, which have limited lifespans. It is still unknown, however, which initial conditions optimise growth of a capping peat layer. We therefore tested the combined effects of topsoil addition (using clay or organic soil) and vegetation type (*Typha latifolia*, *T. angustifolia*, *Stratiotes aloides* and submerged spp.) on net ecosystem exchange and water quality in 18 sandy basins situated in a constructed wetland on top of a former landfill. Highest C sequestration rates occurred in *Typha* stands. In sandy basins, however, *Typha* covered only 15%, whereas in clay or organic soil basins, *Typha* covered 40 and 70%, respectively. Simultaneously, however, topsoil addition increased net C loss through decomposition. Despite higher C sequestration rates of vegetated sand, however, substantial unvegetated parts impede the build-up of a uniform peat layer. We therefore conclude that when quick results are important (i.e. when capping a highly contaminated landfill), applying nutrient-rich soil - preferably recycled - is necessary for sufficient biomass production to accumulate organic material. By recycling local soils, the accompanying initial C loss becomes negligible on a landscape scale.

Introduction

Wetlands near urban or industrial areas, including drained and excavated peatlands, have been extensively used as landfills throughout the world, e.g. in Germany (Vielhaber and Weiss 2014), the Netherlands (Heida 1986; Buijs and others 2005), Finland (Assmuth and Strandberg 1993), Canada (Wreford and others 2000) and the United States (Ewing 2002). After closure, landfills are usually capped with a technical barrier, consisting of a basal liner (often made of high-density polyethylene; HDPE) and clean sand, and subsequently transformed into dry grasslands with a source-monitoring programme (Ewing 2002; Simon and Muller 2004). Meanwhile the creation of new wetlands generally remains limited to wetlands constructed for treatment of landfill leachate or municipal wastewater (Bulc 2006; Vymazal and Kropfelova 2009). Given the high potential of peat to sequester contaminants such as heavy metals in a similar way to activated carbon (Mclellan and Rock 1988) and to stimulate the bioremediation of organic pollutants (Couillard 1994), the construction of new, peat-forming wetlands on top of former landfills could provide a sustainable addition to regular capping methods, which have a limited durability (Allen 2001; Rowe and Sangam 2002). The realisation of such a "peat cap" would not only serve as an efficient capping method for landfills, but may also provide additional services including carbon (C) sequestration, water retention, recreation and biodiversity (Knight 1997; Wild and others 2001; De Klein and Van der Werf 2014).

To effectively utilise these novel wetlands as natural caps, optimal conditions for C sequestration have to be created. Maximising C sequestration requires simultaneously high biomass production and low decomposition rates, both of which are strongly linked to a suite of biogeochemical processes (Aerts and Toet 1997; Lamers and others 2015). It is therefore essential to define the optimal water and soil quality for wetland construction. When using soils low in organic matter, such as sand, both the recruitment and growth of peat-forming vegetation can be expected to progress slowly. Therefore, it may be necessary to add a layer of richer soil, such as organic soil or clay, to stimulate the development of fast growing, rooting wetland macrophytes. For example, soil that has been removed during construction works or dredged from waterways may be recycled to provide additional nutrients in constructed wetlands and stimulate vegetation development, especially when they already contain propagules of target vegetation. Now, these previously anaerobic soils are usually stored under aerobic conditions, which stimulates decomposition and results in high carbon dioxide (CO_2) and methane (CH_4) emissions (Gebert and others 2006).

Due to the higher availability of labile organic matter, addition of clay or organic soil to stimulate vegetation development is expected to lead to higher CO_2 and CH_4 production, thereby counteracting the C sequestration potential of the capping system. Furthermore, application of such soils may increase the risk of surface water eutrophication through nutrient mobilisation, leading to unwanted blooms of phytoplankton and suppressing vegetation growth. It has yet to be tested whether the benefits of the addition of top layers of clay or organic soil, in terms of increased biomass production, outweigh negative side effects of additional nutrient mobilisation and C emission.

Highly productive target species include *Typha latifolia* and *Phragmites australis*, which are often applied in constructed wetlands for the extraction of nutrients from wastewater because of their high net primary production (NPP) and nutrient uptake rates (Tanner 1996; Wild and others 2001). The high NPP rates (Brinson and others 1981; Brix and others 2001) and relatively low decomposition rates of -mainly belowground- biomass (Hartmann 1999; Alvarez and Becares 2006) that characterise these species may result in a considerable contribution to the C sequestration function of the newly constructed wetland. Next to helophytes, floating aquatic macrophytes, such as *Stratiotes aloides*, may also reach high NPP rates in peatland systems. Through their vigorous vegetative growth (Cook and Urmi-König 1983; Smolders and others 1995), *S. aloides* forms dense mats that may cover entire water bodies in only a few growing seasons (Cook and Urmi-König 1983). As a result, this species can reduce the area of open water faster than helophytes, which mainly colonise from the shores (Van Geest and others 2003). Furthermore, floating macrophytes facilitate high biodiversity by serving as foundation species (Rantala and others 2004; Van der Winden and others 2004; Sugier and others 2010). They may thus not only help to stimulate peat accumulation and C sequestration, but also promote biodiversity, thereby additionally enhancing the attractiveness of the wetland as a recreational and educational area.

Given the limited lifespan of basal liners used to cover landfills (Allen 2001; Rowe and Sangam 2002), a sustainable “peat cap” can be created on top of traditional capping methods to take over the capping function when basal liners degrade. Furthermore, while traditional capping methods generally only aim at immobilising contaminants, the peat layer could add to the bioremediation of (organic) pollutants. Since peatland formation is a novel approach to cap former landfills, however, it still has to be determined which initial conditions, in terms of soil and vegetation type, result in high C sequestration rates and the build-up of a peat layer. The main question that needs to be answered is therefore: How to obtain high biomass production and low decomposition rates simultaneously, in order to have a fast transformation from sand to growing peat?

A newly constructed wetland (2011) on top of a former landfill close to Amsterdam (The Netherlands) provided the optimal test location to find an answer to this question. At this location, a 4 ha research area was provided to test the effect of soil type and vegetation on net ecosystem exchange (NEE) of C and surface water quality. Our experimental set-up comprised 18, rainwater-filled sand basins of which 6 received an additional layer of organic soil and another 6 clay soil. Vegetation was allowed to develop spontaneously over the course of 3 years, resulting in 4 dominant vegetation types: *Typha latifolia*, *T. angustifolia*, *Stratiotes aloides* (the only species that required active introduction as it mainly reproduces clonally (Smolders and others 1995)) and submerged species. We analysed vegetation development, CO_2 sequestration rates and CH_4 fluxes of all combinations of vegetation type and soil. Based on the outcome of this study, we discuss the optimisation of the construction as well as the possibility of recycling local clay or organic soils to stimulate vegetation development.

Material and Methods

Site description

In 2011, the first phase of the construction of a “natural cap” was completed on top of the former landfill Volgermeerpolder (52°25’21.03” N, 4°59’32.23” E) in the Netherlands, which had been used as a waste dump between 1927 and 1981 (Buijs and others 2005). After sealing off the contaminated landfill with sheets of high-density polyethylene (HDPE, 2 mm; life expectancy 80-100 years) and a 50 cm layer of clean sand, a new wetland of 100 ha was created on top, consisting of several basins enclosed by clay dykes (designed by Vista Landscape Architecture and Urban Planning, Amsterdam, the Netherlands and developed by ACV (Egbring 2011); See Supplementary Figure 5.1 for an aerial photograph of the research area). In 18 of these basins, ranging in size from 550 to 1600 m^2, we compared basins without (controls; n=6) or with the application of an additional layer of 30 cm of clay (n=6; originating from a freshwater wetland in the north-western part of the Netherlands; 52°40’15” N, 5°7’2” E) or organic soil (n=6; originating from a nearby peatland area; 52°17’13” N, 4°46’12” E) on top of the clean layer of sand. Soil characteristics are displayed in Table 5.1. Basins were naturally filled with rainwater and a water depth of 40 to 80 cm (minimum depth of 20 cm during droughts) was maintained by pumping in water from large rainwater basins (with characteristics similar to sand soils, see Table 5.1) during dry periods in spring and summer.

Table 5.1. Soil characteristics of sand, organic soil and clay used in the construction of the Volgermeerpolder (mean ± SEM, n=6). Different superscript letters indicate statistically significant differences between soils ($P<0.05$).

		Organic	Clay	Sand
Water content	%	64.2 ± 0.8^{c}	41.2 ± 1.7^{b}	19.3 ± 0.7^{a}
Bulk density	kg DW L^{-1} FW	1.35 ± 0.02^{a}	1.62 ± 0.03^{b}	2.01 ± 0.03^{c}
Organic matter	%	21.5 ± 0.8^{c}	5.0 ± 0.5^{b}	0.5 ± 0.2^{a}
NO_3^-	µmol L^{-1} FW	22.6 ± 15.2	2.0 ± 1.4	13.0 ± 5.2
NH_4^+	µmol L^{-1} FW	$251\pm57^{a,b}$	353 ± 71^{b}	91 ± 23^{a}
Olsen-P	µmol L^{-1} FW	475 ± 20^{a}	792 ± 107^{b}	354 ± 39^{a}
C	%	9.3 ± 0.4^{c}	2.7 ± 0.2^{b}	0.7 ± 0.1^{a}
N	%	0.46 ± 0.02^{c}	0.16 ± 0.01^{b}	0.04 ± 0.00^{a}

Vegetation in all basins developed naturally, with the exception of *Stratiotes aloides*, which was introduced (50 plants per basin, originating from a nearby pond; 52°23’10.1” N, 4°56’55.0” E) in the north-eastern corner of all basins (most suitable given the predominant south-western winds) in summer 2011. Natural development resulted in dominant vegetation of helophytes (*Typha latifolia*, *T. angustifolia*, *Phragmites australis*, *Glyceria maxima*) and submerged vegetation (*Potamogeton pusillus*, *P. pectinatus*, *Myriophyllum spicatum*, *Characeae*, *Elodea nuttallii*). In September 2013, we allocated one plot of 1 m^2 per basin in representative patches (when present) of (*Typha latifolia* (n=16), *T. angustifolia* (n= 8), *S. aloides* (n= 15) and bare soil (n=17), totalling 56 plots. *Phragmites australis* and *Glyceria maxima* were excluded, as they were only present in two basins. While the patches of bare soil were unvegetated in September 2013, submerged plant species invaded the plots in most basins, starting in spring 2014 and eventually reached 169±66 g DW m^{-2} in August 2014. Total plant cover of all dominant vegetation types was determined at the peak of the growing season, in August 2014.

Soil and water analyses

Surface water and pore water samples were taken in November 2013 and February, May and July 2014. Pore water was collected by attaching vacuum syringes to ceramic soil moisture cups (Eijkelkamp, Giesbeek, The Netherlands), fixed in the soil at 15 cm depth. pH was measured using a standard Ag/AgCl electrode (Orion, Thermo Fisher Scientific, Waltham, MA, USA) combined with a pH meter (Tim840 titration manager; Radiometer Analytical, Lyon, France), whereas alkalinity was determined by titrating down to pH 4.2 with 0.1 N HCl using an auto-burette (ABU901, Radiometer, Copenhagen, Denmark). Total inorganic carbon (TIC) was measured by injecting 0.1 ml of sample into an N_2 flushed chamber filled with 1 ml phosphoric acid (0.4 M), connected to an infrared gas analyser (IRGA; ABB Analytical, Frankfurt, Germany), whereas total organic carbon (TOC) was measured on a TOC-L CPH/CPN analyser (Shimadzu, Kyoto, Japan). Concentrations of phosphate (PO_4^{3-}), ammonium (NH_4^+) and nitrate (NO_3^-) were measured colourimetrically on an Auto Analyser 3 System (Bran & Luebbe, Norderstedt, Germany) using ammonium molybdate (Henriksen 1965), hydrazine sulphate (Kamphake and others 1967) and salicylate (Grasshof and Johannse 1972), respectively. Concentrations of Ca, Fe, Mg, Total-P and S were measured by inductively coupled plasma spectrometry (ICP-OES iCAP 6000; Thermo Fisher Scientific, Waltham, MA, U.S.A.). Data were combined to calculate yearly averages of surface water and pore water pH, alkalinity, TIC, TOC and nutrients.

In February 2014, soil samples were collected in airtight bags and kept at 4 °C until analyses. Per basin, 5 subsamples of the top 10 cm were pooled for analyses. Samples of known volume were weighed and dried for 48 hours at 70 °C to determine dry weight (DW) and bulk density. Organic matter content was determined through loss on ignition (3h 550 °C). Plant available P was extracted according to Olsen and others (1954), whereas a salt extraction was performed by incubating 17.5 g of homogenised fresh soil overnight with 50 ml of 0.2 M NaCl. Extracts were analysed for PO_4^{3-}, NH_4^+ and NO_3^- as described above. Total concentrations of Ca, Fe, Mg, Total-P and S were determined by digestion of 200 mg of dried soil with 4 ml HNO3 (65%) and 1 ml H_2O_2 (30%) using a microwave oven (MLS 1200 Mega, Milestone Inc., Sorisole, Italy), after which digestates were measured by ICP as described above. Carbon (C) and (nitrogen) N contents of the soil were determined using an elemental analyser (Carlo Erba NA1500, Thermo Fisher Scientific, Waltham, MA, U.S.A.).

C flux measurements

Diffusive fluxes of CO_2 and CH_4 were measured in November (2013), January, April, May, July, August and September (2014) by placing a gastight transparent closed chamber over the 56 plots (Figure 5.1). For helophyte vegetation, a large closed chamber (ø 50 cm, 140 cm, 270 L; Figure 5.1) was pressed into the soil with three thin pins to fix the chamber but prevent large disturbance to the soil. A smaller, floating closed chamber (ø 30 cm; 20 cm; 14.5 L; Figure 5.1) was used in plots with submerged, floating or no vegetation. Chambers were placed on the plots 5 minutes before the start of the measurement and the internal air was subsequently analysed during 6 minutes for linear increase or decrease in CO_2 and CH_4 concentrations using cavity ring-down spectrometry by connecting the chambers in a closed loop to a greenhouse gas analyser (GGA-LGR, Los Gatos Research, CA, U.S.A.). C-fluxes were measured under both light and dark conditions (Figure 5.1). For dark measurements, plots were dark-adapted by covering the closed chambers with opaque fabric before measurements. Hourly data on daytime temperature and radiation were collected from a nearby weather station (KNMI, Schiphol; 52°18’03.6” N, 4°46’26.4” E) and are presented in Supplementary Figure 5.2.

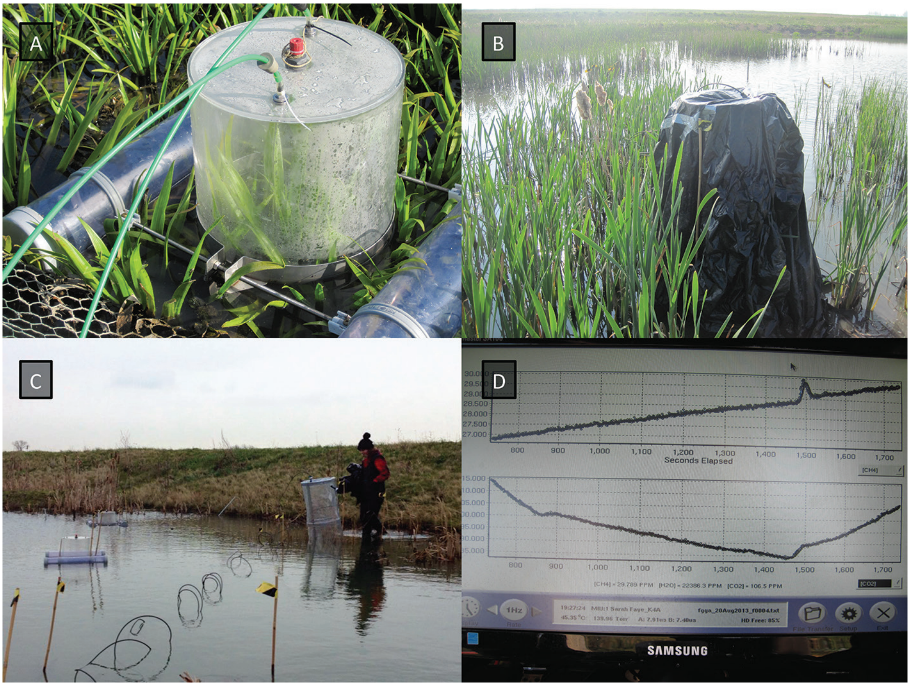

Figure 5.1. Measuring C fluxes at the Volgermeerpolder. Fluxes from plots vegetated by floating or submerged plants were measured using a small floating chamber (A), whereas taller vegetation was measured using a 1.5 m high chamber that was fixed in the sediment (B). Chambers were attached to the greenhouse gas analyser using gastight tubing (C) after which the fluxes could be derived from the linear increase or decrease of CH_4 and CO_2 (D). Here, the CH_4 concentration (above) increased linearly during both light and dark measurements. CO_2 fluxes, on the other hand, showed a net decrease (fixation) during the light measurements, whereas CO_2 was observed to increase after the chamber was covered and the dark measurement started (D).

Net ecosystem exchange of CO_2 (NEE_{CO2}; g C m^{-2} d^{-1}) comprised the net CO_2 flux from plots vegetated by *T. latifolia*, *T. angustifolia* and *S. aloides* including soil and water respiration (Eq. 1). By integrating the separate NEE_{CO2} values, the annual CO_2 sequestration of these three species was calculated. Combined light and dark fluxes of CO_2 and CH_4 from bare plots (which included some submerged vegetation during summer) and of plots vegetated by *T. latifolia*, *T. angustifolia* and *S. aloides* were used to calculate the net ecosystem exchange of carbon (NEE_C; Eq. 2). As with

NEE_{CO2}, these NEE_C values were extrapolated to annual values (g C m^{-2} y^{-1}) by integration of separate measurements. In all analyses, plots that were measured less than 4 times during the year were excluded (n=2). Missing data were estimated using the average value of replicates when available (n=7). Otherwise, consecutive data points were used to cover for the missing values (n=5).

The following equations were used, with negative fluxes indicating C-sequestration and positive fluxes indicating loss to the atmosphere:

$$NEE_{CO2} = (h_{light} * CO_{2\text{-}light}) + (h_{dark} * CO_{2\text{-}dark}) \quad \textbf{Eq. 1}$$

$$NEE_C = (h_{light} * (CO_{2\text{-}light} + CH_{4\text{-}light})) + (h_{dark} * (CO_{2\text{-}dark} + CH_{4\text{-}dark})) \quad \textbf{Eq. 2}$$

with h_{light} and h_{dark} being the hours of light and dark during that time of year, $CO_{2\text{-}light}$ and $CH_{4\text{-}light}$ the fluxes measured under light conditions and $CO_{2\text{-}dark}$ and $CH_{4\text{-}dark}$ the fluxes measured under dark conditions.

Statistical analyses

Data on soil characteristics and annual NEE rates were tested for normality of residuals and homogeneity of variance using Shapiro Wilk's test and Levene's test, respectively. Non-normal or heteroscedastic data were log transformed to authorise use of parametric tests. Data were analysed using two-way ANOVAs with "Soil" and "Species" as fixed variables, followed by Tukey post-hoc tests. Monthly NEE rates were tested for normality of residuals using Levene's test, after which they were analysed using repeated measures followed by Bonferroni post-hoc tests. All analyses were carried out in SPSS (V21, 2012, IBM Statistics).

Results

Nutrient characteristics

Although all soils were characterised by high pH and alkalinity, the applied top layers of clay and organic soil had an even higher alkalinity than the original sand soil (P=0.017; F=5.479), due to a higher HCO_3^- (P=0.007; F=7.285) availability. Calcium (Ca; P=0.009; F=6.792) and, in organic soils, magnesium (Mg; P=0.025; F=4.841) also showed higher concentrations than in sand (Table 5.2). Mobilisation of HCO_3^- to the water layer caused a high surface water pH and alkalinity (Table 5.2), which was again higher for basins with an organic or clay top layer (P=0.016; F=5.519). Concentrations of NO_3^- and NH_4^+ were low in both the water layer and the pore water (Table 5.2), and did not differ between soil types. Organic soils had a higher availability of PO_4^{3-} in the pore water than clay and sand soils ($P<0.001$; F=27.147; Table 5.2). Pore water Fe: PO_4^{3-} ratios were high for all soils, but lower ($P=0.004$; F=8.351) for organic and sand soils (16.9±4.4, and 57.5±16.9, respectively) than for clay soil (217.2±79.9). Surface water concentrations of PO_4^{3-} ($P=0.028$; F=4.597) and Total-P ($P=0.013$; F=5.877) were higher for organic soils than sand, but PO_4^{3-} concentrations remained below 3 µmol L^{-1} in all treatments throughout the year (Table 5.2).

Table 5.2. Chemical characteristics of the water layer and pore water (mean ± SEM, n=6) of basins with organic, clay or sand soils. Values represent averages between November 2013 and September 2014. All concentrations are given in µmol L^{-1}, whereas alkalinity is presented in meq L^{-1}. Different superscript letters indicate significant differences between soil types ($P<0.05$).

	Water layer			**Pore water**		
	Organic	**Clay**	**Sand**	**Organic**	**Clay**	**Sand**
pH	7.8±0.2	7.8±0.1	7.8±0.1	7.0±0.1	7.1±0.0	6.9±0.1
Alkalinity	3.8±0.2 ^{b}	3.7±0.5 ^{b}	2.4±0.2 ^{a}	19.1±2.8 ^{b}	19.0±2.5 ^{b}	8.4±0.9 ^{a}
CO_2	244.5±68.6	262.8±97.8	112.0±17.0	3669±240 ^{b}	2974±295 ^{a,b}	2373±425 ^{a}
HCO_3^-	3322±208 ^{b}	3162±589 ^{a,b}	1975±96 ^{a}	14303±1659 ^{b}	14659±1613 ^{b}	7757±880 ^{a}
NO_3^-	0.40±0.11	0.82±0.28	0.47±0.08	4.51±0.74	4.51±1.16	4.56±0.41
NH_4^+	2.91±0.46	6.27±2.41	2.13±0.09	34.19±17.51	20.93±8.58	49.46±12.82
o-PO_4^{3-}	2.07±0.64 ^{b}	1.29±0.63 ^{a,b}	0.27±0.05 ^{a}	13.31±1.63 ^{b}	1.98±0.48 ^{a}	4.36±0.86 ^{a}
Total-P	5.07±1.62 ^{b}	4.27±1.60 ^{a,b}	1.12±0.19 ^{a}	189.7±39.3 ^{b}	43.5±10.3 ^{a}	67.9±5.7 ^{a}
Ca	2480±269	2179±57	2508±112	7603±989 ^{b}	7544±991 ^{b}	3813±351 ^{a}
Fe	1.70±0.11	14.20±8.84	4.48±0.46	204.6±49.4	316.6±103.9	184.0±19.3
Mg	655±84	602±25	730±43	1961±286 ^{b}	1528±196 ^{a,b}	1046±92 ^{a}
S	1680±472	1379±298	2462±321	988±567	1888±700	963±311

Vegetation development

Addition of clay or organic soil stimulated the cover by helophytes (Figure 5.2; $P<0.001$; F=17.184). While control basins showed a helophyte cover of only 14.1±2.5% after 3 years, the addition of clay and organic topsoils resulted in an increase to 39.5±9.6% and even 70.6±6.4%, respectively. Furthermore, while basins with sand soils only showed colonisation by helophytes from the shore, development of the helophyte vegetation in basins with an applied layer of clay or organic soils occurred both from the shores and by germination or vegetative growth throughout the basin. In organic soils, this led to an explosive development of *Typha latifolia*, which eventually covered around 65% of the basins. On clay soils, *Typha angustifolia* was the dominant species, covering around 30% of the basins (Figure 5.2). Sand soils showed the lowest cover by emergent species, with 77.3±2.7% of the basin remaining unvegetated or covered by submerged species (Figure 5.2). Other species occurring were *Eleocharis palustris*, *Bolboschoenus maritimus*, *Alisma plantago-aquatica* and *Phragmites australis*, none of which covered more than 10% of a basin. Only in two basins with added clay soil, a significant area (15 and 60%) was covered by *Glyceria maxima*. Introduced *Stratiotes aloides* persisted in most basins during subsequent years, but its low colonisation rate resulted in the lowest coverage (less than 5%) of the three measured species (Figure 5.2).

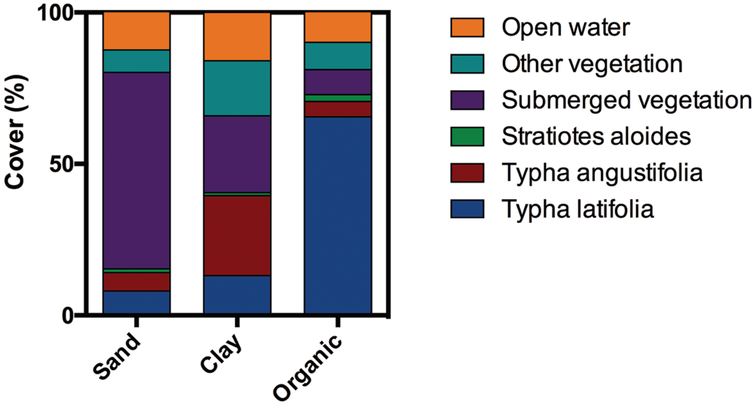

Figure 5.2. Vegetation cover (%) of *Typha latifolia*, *T. angustifolia*, *Stratiotes aloides*, other emergent vegetation, submerged vegetation and bare soil after three years of development on sand soils, or soils with an additional layer of clay or organic soil.

Net ecosystem exchange of bare plots

Combined net ecosystem exchange (NEE) rates of CO_2 and CH_4 of bare soil, overlying water and submerged vegetation (present between spring and autumn) were determined throughout the year. Overall, values ranged from +0.07 to +1.11 g C m^{-2} d^{-1} (loss to the atmosphere) for most of the year. During autumn, emission of CO_2 and CH_4 increased to rates of up to +4.2 and +3.8 g C m^{-2} d^{-1} respectively (Figure 5.3). CO_2 respiration rates did not differ among the different soil types, whereas the relative contribution of CH_4 to the NEE_C was higher for organic soils than for sand soils (P=0.021; F=5.192). None of the bare plots showed net CO_2 fixation (negative

NEE), indicating that the contribution of submerged species to the net C sequestration was negligible (Figure 5.3).

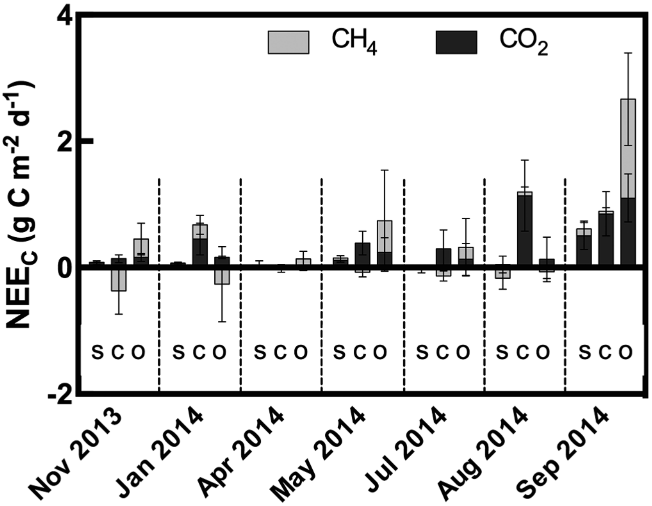

Figure 5.3. NEE$_C$ rates of plots without emergent vegetation on sand soils (s), or soils with an additional layer of clay (c) or organic soil (o) between November 2013 and September 2014 (Mean ± SEM), separated into CO_2 and CH_4 fluxes. Rates result from processes in soil, water and submerged vegetation (occurring on these plots between spring and autumn 2014). Note that negative values indicate C sequestration, whereas positive values represent a loss of C to the atmosphere.

Net ecosystem exchange of vegetation

The CO_2 exchange rates of vegetated plots (NEE$_{CO2}$) closely followed seasonal variation in temperature and light availability (Supplementary Figure 5.2), with the highest CO_2 fixation rates (indicated by negative NEE$_{CO2}$ rates) occurring in July and net emissions (positive NEE$_{CO2}$ flux) of CO_2 in autumn and winter (Figure 5.4). Both *Typha* species had a significantly higher NEE$_{CO2}$ than *S. aloides* (*P*=0.003; F=7.719; Figure 5.4 and 5.5A). There appeared to be differences, however, among annual NEE$_{CO2}$ rates by *Typha* spp. for the three different soil types (Figure 5.5A), with 4 times higher net fixation on sand than on organic soils (-590 vs. -140 g C m^{-2} y^{-1}; *P*=0.058). The lower average *Typha* NEE$_{CO2}$ rates on organic soil resulted from high variation, due to two basins showing net effluxes of C, while the other four showed net fixation rates. However, when including CH_4 fluxes (NEE$_C$), 4 out of 6 plots of *Typha* on organic soil became net C sources, resulting in an average NEE of +300 g C m^{-2} *Typha* y^{-1}, whereas negative NEE$_C$ rates of *Typha* vegetated clay and sand soils indicated that these remained net sinks, with NEE$_C$ rates of -10 to -100 and -450 to -600 g C m^{-2} *Typha* y^{-1}, respectively (Figure 5.5B).

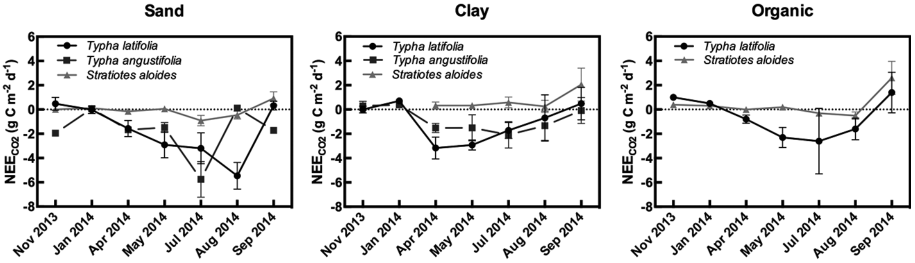

Figure 5.4. Net ecosystem exchange of CO_2 of *Typha latifolia*, *T. angustifolia* and *Stratiotes aloides* in Organic, Clay or Sand soils between November 2013 and September 2014 (Mean ± SEM). Note that negative values indicate C sequestration, whereas positive values represent a loss of C to the atmosphere.

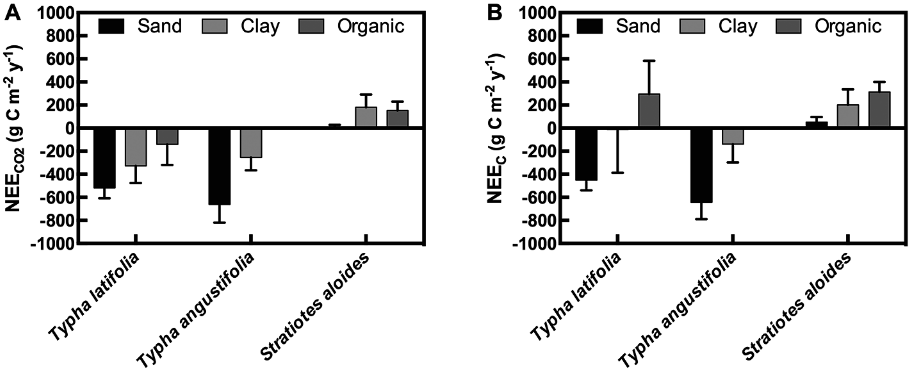

Figure 5.5. Cumulative yearly net ecosystem exchange (NEE) rates of CO_2 (A) and of CO_2 and CH_4 (B) from plots vegetated by *T. latifolia*, *T. angustifolia* (not present on organic topsoils) or *S. aloides* (Mean ± SEM). Note that negative values indicate C sequestration, whereas positive values represent a loss of C to the atmosphere.

Discussion

For the development of a constructed wetland designed for landfill peat capping, fast accumulation of organic matter by highly productive vegetation is very important. By varying soil and water characteristics as well as emergent, submerged or floating macrophyte species, our large-scale field experiment showed that it is possible to gain a high coverage of highly productive macrophytes within a time-span of 3 years. There is, however, a trade-off between confined high CO_2 sequestration rates (up to 590 g C m^{-2} y^{-1}) on the one hand and high colonisation rates (up to 80%) on the other.

CO_2 fixation by vegetation

In our field experiment, the *Typha* species *T. latifolia* and *T. angustifolia*, showed the highest CO_2 sequestration rates. These species showed NEE rates ranging from -140 to -590 g C m^{-2} y^{-1}, which are comparable to, or even higher than, those reported for *Typha*-dominated marshes (-100 to -270 g C m^{-2} y^{-1}) (Reddy and others 1993; Bernal and Mitsch 2012) and those reported for temperate, minerotrophic fens (-208 to +190 g C m^{-2} y^{-1}) (Martikainen and others 1995; Bubier and others 2003; Saarnio and others 2007; Koch and others 2008a). Both submerged vegetation and *S. aloides* had a negligible contribution to the C sequestration potential of the system. While submerged vegetation showed relatively high production rates, these species are generally also characterised by high decomposition rates (Wetzel 1983; Kirschner and others 2001), which results in a low contribution to the total C budget. The poor development of the population of *S. aloides*, on the other hand, can be explained by the high alkalinity and low CO_2 availability in the basins, which lowers underwater photosynthesis and thus prevents vegetative reproduction (Harpenslager and others 2015a).

We found that when basins were constructed with sand, only 15% became covered by helophytes over the course of 3 years, while the remaining 85% consisted of submerged species or bare soil. The next question therefore is how to speed up the colonisation by highly productive species in newly constructed wetlands and thereby maximise net biomass accumulation. The application of a layer of clay or organic soil strongly increased the cover by helophyte species, resulting in an average cover of 40% and 70% after 3 years, respectively. This strongly enhanced colonisation most likely resulted from the higher nutrient availability in the applied soils, including higher P availability in both pore water and surface water of, especially, organic soils. The higher nutrient availability in the clay and organic soil layers enabled colonisation by *Typha* spp. throughout the basins, instead of being restricted to the shores, as was the case in basins with only sand. In these basins, the shores most likely had a higher nutrient availability than the rest of the basin, due to run-off from the surrounding clay dykes.

Carbon balance

Increasing organic matter content of the applied soil led to decreases in the CO_2 fixation rates of soils vegetated by *Typha*. On sand soils, *Typha* spp. reached NEE_{CO2} rates of approximately -590 g C m^{-2} y^{-1}, whereas plots of *Typha* growing on clay and organic soil had NEE_{CO2} rates of -290 g C m^{-2} y^{-1} and -140 g C m^{-2} y^{-1}, respectively. Since biomass production of *Typha* stands growing on clay and organic was approximately 1.5 times higher on clay and organic soils than on sandy soils (Overbeek et al., in prep.; Figure 7.3), the lower net fixation can only have resulted from a higher emission of CO_2 from clay and organic soils through decomposition of labile organic matter. Moreover, the organic soils generally showed higher emissions of CH_4 than sand and clay soils, especially from plots with *T. latifolia* vegetation, which may largely result from plant-mediated CH_4 transport (Bubier 1995; Nykänen and others 1995; Bellisario and others 1999; Bastviken and others 2011). Since contributions of ebullition events could not be included in the measurements, these CH_4 fluxes most likely underestimate actual emissions.

Eutrophication risks

Apart from influencing C dynamics, there is a risk that higher nutrient concentrations in the soil may lead to enhanced nutrient mobilisation to the water layer (Zak and Gelbrecht 2007), which may stimulate phytoplankton blooms resulting in lower production by peat-forming vegetation. Although clay soils contained more P than the other two soils, the very high Fe: PO_4^{3-} ratios (much higher than the threshold value of 1 mol mol^{-1}; Geurts and others (2010)) prevented mobilisation of P to the water layer in all soils. For helophytes, however, a large part of the P in the soil will still be available through root uptake, which is supported by the higher plant-available (Olsen-extracted) P levels. Despite the high Fe: PO_4^{3-} ratio, PO_4^{3-} availability was somewhat higher in both pore water and the water layer in soils with an organic layer than in soils with only sand or an added layer of clay. Still, since surface water PO_4^{3-} concentrations remained mainly below 3 μmol L^{-1} and no problems with algal blooms were observed, eutrophication problems did not occur.

Balancing pros and cons of topsoil addition

Although the application of clay and organic topsoils strongly increased the cover, and thus the net primary production, by helophyte species, the strong variation observed in C fluxes and nutrient availability of these soils implies a “hit-or-miss” outcome in terms of other services, such as net C sequestration. Potential negative side effects in terms of nutrient mobilisation or CH_4 emission can, however, be estimated beforehand. Geurts and others (2010) offered simple calculations based on pore water PO_4^{3-}, Fe and NH_4^+ concentrations to determine whether there is a risk of high mobilisation of P or N to the overlying water layer. Expected C loss, as either CO_2 or CH_4, from the applied soils, on the other hand, can be derived from simple laboratory incubations (Moore and Dalva 1993; van de Riet and others 2013).

By combining information on colonisation rates and C sequestration rates, we set up a budget for constructed wetlands with a sand soil or with an applied layer of clay or organic soil (Table 5.3). These budgets indicate that sand soils with an average cover of 15% of helophytes formed a net sink of CO_2, with an NEE_{CO2} of -51.1 g C m^{-2} y^{-1}. After the addition of a clay soil the cover of helophytes increased to 40% and although CO_2 emissions from breakdown of organic matter increases, the system still formed a net CO_2 sink with an NEE_{CO2} of -20 g C m^{-2} y^{-1}. Finally, the implementation of a layer of organic matter increased helophyte cover to 70%, but reduced the net fixation in the helophyte stands due to CO_2 emission by decomposition processes. As a result, these organic soils had an NEE_{CO2} of -67.4 g C m^{-2} y^{-1} of CO_2 and form the largest CO_2 sink of the three soil types (Table 5.3). Due to high CH_4 emissions in some basins with clay or organic soil and stands of *Typha latifolia*, some of these stands turned into net C sources emitting 670±270 g C m^{-2} y^{-1}, whereas others remained C sinks with net exchange rates of -370±50 g C m^{-2} y^{-1}. The high variation in NEE may have resulted from differences in availability of nutrients and readily decomposable organic matter in the sediment, which causes variability in the availability of substrates for microbial activity. Higher availability of PO_4^{3-}, for example, has been reported to stimulate decomposition processes (Rejmankova and Houdkova 2006), leading to higher C emission.

Management implications

Because regular covers of landfills, usually constructed of HDPE, have limited lifespans (Allen 2001; Rowe and Sangam 2002), there is a need to develop more sustainable capping methods for highly contaminated landfills, especially those occurring in wetlands. The realisation of a newly constructed, peat-forming wetland would not only provide such a sustainable method of bioremediation, but would also provide additional biodiversity, recreational and educational services. When such a wetland is created using only sand as a substrate, development will be slow and although patches of vegetation will form a net C sink, terrestrialisation and the uniform build-up of a peat layer throughout the entire wetland will take a long time. By applying nutrient rich soils, such as clay or organic soils, the development of the vegetation is strongly stimulated, especially when these soils already contain propagules of target species. The application of these soils will, however, almost always be accompanied by an initial C loss. When recycling clay or organic soils to stimulate vegetation development, however, the effect of C emission will be negligible on a landscape scale because although initially, CO_2 and CH_4 emission due to decomposition of labile organic matter would result in a decrease of the thickness of the cap, this material would otherwise decompose when left exposed to the atmosphere (Gebert and others 2006; Vermaat and Hellmann 2010; Schrier-Uijl and others 2011), either submerged or on the shore. In general, we therefore conclude that when quick results are important, for instance when capping a highly contaminated landfill, the application of -preferably recycled- clay or organic soil is necessary to obtain a biomass production high enough for the build-up of an organic layer within a short period.

Table 5.3. C budget of peat capping using sand soils with and without an added layer of clay or organic soil.

Factor	***Unit***	***Sand***	***Clay***	***Organic***
***Typha* spp.**[*]				
Cover *Typha*	**%**	15	40	70
NEE CO_2	**g C m^{-2} *Typha* y^{-1}**	-590	-290	-140
NEE CO_2	**g C m^{-2} basin y^{-1}**	-88.5	-116	-98
NEE CH_4	**g C m^{-2} basin y^{-1}**	7.6	90	305
Bare soil				
Cover bare soil[**]	**%**	85	60	30
NEE CO_2	**g C m^{-2} bare plot y^{-1}**	44	160	102
NEE CO_2	**g C m^{-2} basin y^{-1}**	37.4	96	30.6
NEE CH_4	**g C m^{-2} basin y^{-1}**	0.8	-0.7	32
Total				
NEE CO_2	**g C m^{-2} basin y^{-1}**	-51.1	-20	-67.4
NEE CH_4	**g C m^{-2} basin y^{-1}**	8.4	89.3	337
NEE C	**g C m^{-2} basin y^{-1}**	-42.7	69.3	269

* Fluxes from plots of *Typha latifolia* and *Typha angustifolia* were averaged to obtain NEE CO_2 and CH_4 rates.

** The area not covered by *Typha* is assumed to be bare soil, which may cause a slight underestimation of the net C fixation, due to presence of some other emergent species (<10% cover).

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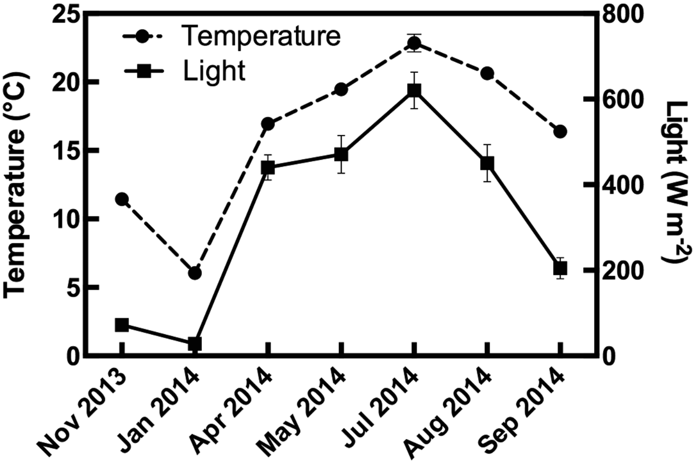
Supplementary information

Supplementary Figure 5.1. Aerial photograph of a newly constructed wetland north of Amsterdam, the Netherlands (Volgermeerpolder; 52°25’21.03” N, 4°59’32.23” E). The area used in this study has been indicated in red and comprises small experimental basins and two large, rainwater storage basins.



Supplementary Figure 5.2. Seasonal variation in daytime temperature (dashed line) and light (continous line) in the immediate area of the field location during the measurements performed between November 2013 and September 2014 (Mean ± SEM). Hourly data from the Royal Dutch Meteorological Institute (KNMI, Schiphol, 52°18’03.6” N, 4°46’26.4” E).

Chapter 6

Rewetting former agricultural peatlands: topsoil removal as a prerequisite to avoid strong nutrient and greenhouse gas emissions

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Picture by Eva van den Elzen

Rewetting former agricultural peatlands: topsoil removal as a prerequisite to avoid strong nutrient and greenhouse gas emissions

Globally 15%, and in Europe over 50%, of all peatlands have been drained for agricultural use leading to high carbon (C) losses, severe land subsidence and increased flooding risks. For the restoration of C sequestration and peat formation, abandoned peatlands are being rewetted at a large scale, but this transforms them into strong methane (CH_4) sources. Furthermore, due to the high topsoil nutrient contents and/or high buffering capacities of water used for rewetting, this will inevitably result in eutrophication of restored peatlands and downstream areas, which may compromise the regrowth of peat forming vegetation including *Sphagnum* spp. To experimentally determine the extent of these negative side effects in relation to water quality, and to test topsoil removal as an abatement strategy, we used a controlled laboratory approach in which topsoil and subsoil monoliths of a former agricultural peatland were rewetted with water of different qualities (+P, $+HCO_3^-$, $+P/+HCO_3^-$ and Control), mimicking rainwater vs. surface water storage. In addition, two different *Sphagnum* moss species (*S. squarrosum* and *S. palustre*) were compared. Without topsoil removal, rewetting led to high P and N mobilisation, algal blooms, and high CH_4, carbon dioxide (CO_2) and dissolved organic carbon (DOC) emissions. P-rich water resulted in further eutrophication. Bicarbonate (HCO_3^-) enrichment by surface water not only stimulated P release and CO_2 emission, but also strongly reduced *Sphagnum* vitality. We conclude that topsoil removal will, at least in initial stages of rewetting, strongly reduce eutrophication problems (by 80-90 %), CH_4 emission (99 %), DOC loss (60 %) and global warming potential (50-70 %) of rewetted former agricultural peatlands. Furthermore, to reduce mineralisation rates and enable *Sphagnum* growth, storage of rainwater rather than surface water is preferred. Finally, removed topsoils can be reused in adjacent subsiding agricultural areas, and thereby optimise the overall C balance and allow higher water levels in rewetted peatlands.

Introduction

Land use change, hydrological operations and other forms of anthropogenic forcing have severely compromised the functioning of global wetlands and their services including flood protection, water purification, biodiversity and C sequestration (Foley and others 2005; Zedler and Kercher 2005). Approximately 15% of peatlands worldwide have been drained to accommodate agriculture, peat extraction, forestry or urbanisation (Joosten 2009), although considerable differences exist between countries, with 10% to 85% of peatlands drained within a single country (e.g. Brock and others (1999); Zanello and others (2011); Hooijer and others (2012); Meckel and others (2006); Hoeksema (2007)). While the accumulation of thick peat layers has generally taken thousands of years (~1.1 mm y^{-1}; Ovenden and others (1998)), drainage of these systems has resulted in strong degradation by oxygen intrusion, enhancing aerobic decomposition of organic matter and carbon (C) emission. Together with compaction and consolidation (Hooijer and others 2012), this has caused fast land subsidence (2 to 150 mm y^{-1}; Syvitski and others (2009)). Given the projected sea-level rise, this continuing subsidence of peatlands - often located in heavily populated coastal areas, river deltas and floodplains - poses a serious risk to public safety due to higher flooding risks (Syvitski and others 2009; Temmerman and others 2013).

Pristine, growing peatlands (mires) generally form net C sinks, in which the fixation of carbon dioxide (CO_2) into layers of organic matter exceeds the emission of methane (CH_4) and CO_2, leading to net ecosystem exchange (NEE) rates ranging from -5 to -40 g C m^{-2} y^{-1} (Gorham 1991; Belyea and Malmer 2004; Saarnio and others 2007; Lamers and others 2015). Drained and degraded peatlands, on the other hand, are almost always net C sources (Alm and others 1999; Waddington and others 2001), with NEE rates ranging from +80 to +880 g C m^{-2} y^{-1} (Lamers and others 2015) and a yearly global emission of 30 to 370 Mt C y^{-1} (Armentano 1980). These high C emission rates from drained peatlands are the result of strongly increased aerobic ecosystem respiration rates and therefore mainly consist of CO_2 (Nykänen and others 1995; Silvola and others 1996; Waddington and Day 2007). Emissions of CH_4, on the other hand, are much lower for drained peatlands than for pristine or rewetted peatlands (Moore and Knowles 1989; Salm and others 2009), as a result of the inhibition of CH_4 production and stimulation of CH_4 oxidation under aerobic conditions (Lai 2009; Maljanen and others 2010). Since CH_4 is a much more potent greenhouse gas than CO_2, its enhanced emission after rewetting strongly increases the global warming potential (GWP) of these restored systems. Due to the high availability of easily degradable organic matter (and therefore of acetate, CO_2 and H_2), and nutrients for methanogens (Aerts and Toet 1997; Fiedler and Sommer 2000), CH_4 emissions from rewetted, former agricultural peatlands may be considerable (Lamers and others 2015). So far, however, only few studies have been published on CH_4 fluxes in these systems (e.g. Hendriks and others (2007); van de Riet and others (2013)).

Since over 85% of drained peatlands have been used for agriculture (Joosten 2009), they have been heavily fertilised and often also limed, resulting in an extremely nutrient-rich, buffered top layer of the soil. Especially phosphate (PO_4^{3-}) has accumulated in these soils, since it is strongly bound in iron (Fe) complexes and to organic matter under aerobic conditions (Smolders and others 2006; Smolders and others 2008; Lamers and others 2015). Upon rewetting, however, there is a considerable risk of PO_4^{3-} mobilisation and eutrophication of the peatland and downstream areas (Patrick and Khalid 1974; Rupp and others 2004; van Dijk and others 2007). Furthermore, heavily fertilised soils often also turn into sources of N after rewetting (Van Dijk and others 2004; Zak and Gelbrecht 2007; van de Riet and others 2013). Thus, although rewetting of drained peatlands may counteract land subsidence and CO_2 losses by restoring the anaerobic soil conditions and inhibiting complete organic matter oxidation, it may have several negative side effects in former agricultural systems. Therefore, despite seeming counterintuitive, removal of the easily degradable eutrophic topsoil may be a useful abatement strategy to prevent strong greenhouse gas (GHG) emission and nutrient mobilisation after rewetting and thus restore peat formation (Emsens and others 2015).

The actual effect of rewetting a drained peatland will also strongly depend on the quality of the water used. Instead of conserving rainwater by building dams to counteract desiccation in peatlands, many areas have been rewetted by flooding them with surface water (Roelofs 1991; Grootjans and others 2002; Lamers and others 2002). Especially in agricultural areas, the quality of this surface water may be compromised, with high nutrient concentrations and/or high buffering capacity (alkalinity). Both factors can be expected to have a strong influence on the peatlands' biogeochemistry and other services, including C sequestration (Lamers and others 2015).

To fully restore the C sequestering function of a system and promote regrowth of peat, restoration of the original peat-forming vegetation is essential. Limitations in seed or spore dispersal, absence of viable seeds or spores in the soil or unfavourable habitat conditions may hamper natural return of peat-forming vegetation, such as *Phragmites*, *Carex* or *Sphagnum* species (Campeau and Rochefort 1996; Aggenbach and others 2013). Of these species, *Sphagnum* mosses produce more recalcitrant organic matter than other peat-forming species due to the unique characteristics of these ecosystem engineers (Van Breemen 1995), including habitat acidification (Clymo 1963; Van Breemen 1995; Hajek and Adamec 2009), production of organic matter with high phenolic contents (Yavitt and others 2000), and high water retention, keeping the environment moist and anaerobic (Clymo 1973). While *Sphagnum* can be reintroduced successfully on oligotrophic, acidic cut-over peatlands (Campeau and Rochefort 1996; Smolders and others 2003b; Robroek and others 2009), revegetation of eutrophic or alkaline soils may pose a serious problem to these mosses, since they may easily be out-competed by vascular plants (Berendse and others 2001; Smolders and others 2008; Aggenbach and others 2013) or suffer from high pH (Clymo 1973; Andrus 1986; Lamers and others 1999; Hajek and others 2006).

Increased CH_4 production and nutrient mobilisation after rewetting of former agricultural peatlands have already been shown in the field (e.g. Hendriks and others (2007); Zak and Gelbrecht (2007)). In field studies, however, determining the extent and impact of these two processes is difficult due to multiple biogeochemical interactions, complex hydrology and large variations in climatic parameters. Therefore, we chose a controlled, experimental approach to determine the extent of the GHG emission and eutrophication after rewetting of a former agricultural peatland using water of different qualities, mimicking rainwater, or surface water with high P and/or HCO_3^- availability. This controlled mesocosm approach also allowed us to quantify the effect of topsoil removal on the restoration of ecosystem services, by using both topsoils (5-20 cm) and subsoils (25-45 cm). Furthermore, to test whether the original fen vegetation, characterised by *Phragmites australis* and *Sphagnum* spp., could be restored after rewetting and topsoil removal, we introduced two species of *Sphagnum* (*S. palustre* and *S. squarrosum*) that are typical of this vegetation, and studied their growth potential under the different water and soil conditions and their contribution to C sequestration.

Material and Methods

Experimental set-up

In autumn 2013, 32 peat monoliths (25 x 12 x 20 cm; length x width x height) were randomly collected from a drained and fertilised, agricultural peatland managed as a pasture in the north-western part of the Netherlands (Ilperveld; 52°44’075” N; 4°94’960” E). Cores were taken from two depths, 5-25 cm (topsoil; n=16) and 25-45 cm (subsoil; n=16), immediately transferred into glass aquaria (25 x 12 x 30 cm; l x w x h) and transported to the lab. Both layers consisted of *Sphagnum/Carex* peat, but as a result of drainage, the top layer was decomposed further, as illustrated by a higher bulk density (0.41±0.03 vs. 0.19±0.01 kg DW L^{-1} FW), lower organic matter content (47.9±9.3 vs. 78.4±1.8) and lower content of porewater phenolic compounds (2.75±0.64 vs 4.14±0.85 mg L^{-1}). The two chosen depths were based on measurements of the Olsen-P (Olsen and others 1954) profile showing much higher P availability (951±71 vs. 153±22 mg P kg DW^{-1}) in the top layer due to fertilisation and decomposition.

In the lab, demineralised water was added to 2 cm above soil level (0.6 L per aquarium), after which the aquaria were left for 2 weeks to acclimatise in a water bath at 15°C (NESLAB cryostat, Thermoflex 1400, Breda, The Netherlands) with a light regime of 16h light (400W, Philips, Master Son-T PiaPlus, Belgium; 150 μmol m^{-2} s^{-1} PAR), mimicking Dutch summer conditions. All soils received artificial rainwater at a rate of 750 mm y^{-1} corresponding to Dutch annual rainfall (250 mL; three times a week), and with a composition equal to Dutch rainwater (5 mg L^{-1} sea salt (Tropic Marine, aQua united LTD, Wartenberg), 19 μmol L^{-1} KCl, 10 μmol L^{-1} $CaCl_2$, 10 μmol L^{-1} Fe-EDTA, 1 μmol L^{-1} KH_2PO_4, 0.7 μmol L^{-1} $ZnSO_4$, 0.8 μmol L^{-1} $MnCl_2$, 0.2 μmol L^{-1} $CuSO_4$, 0.8 μmol L^{-1} H_3BO_3, 8 nmol L^{-1} $(NH_4)_6Mo_7O_{24}$ and 91 μmol L^{-1} NH_4NO_3).

After two weeks, the demineralised water was replaced with treatment solutions containing 5 mg L^{-1} of sea salt (see above) and either 0 or 5 μmol L^{-1} $Na_4P_2O_7.10H_2O$ and 0 or 3 mmol L^{-1} $NaHCO_3$ creating four treatments: Control, +P, +HCO_3^- and +P/+HCO_3^- (randomly applied; n=4 for each treatment). The addition of HCO_3^- and PO_4^{3-} simulates the use of alkaline or nutrient-rich surface water for the rewetting of peatlands. Treatment solutions were flowing through the aquaria at a rate of 5.44 L $week^{-1}$ using peristaltic pumps (Masterflex L/S tubing pump; Cole-Palmer, Chicago, IL, U.S.A.), which, together with a fixed outflow, ensured a stable water layer of 2 cm above soil surface. After the first period of 2.5 months, patches of *Sphagnum palustre* (2.37±0.11 g DW; 67±1.3 capitula; mean ± SEM) and *S. squarrosum* (1.84±0.07 g DW; 84±1.4 capitula; mean ± SEM) were applied to each half of the aquaria. These two species are still abundant in natural, undisturbed parts of the peatland area where the soils were collected and are typical fen species in the Netherlands. They differ, however, in their habitat preference, with *S. squarrosum* being more tolerant to nutrient-rich and alkaline conditions than *S. palustre* (Clymo 1973). The upper parts of the mosses were cut at equal lengths (4 cm) and placed upright to avoid submergence of the capitula (top 8-10 mm of the photosynthetically active tissue of the mosses).

Chemical analyses

Two soil moisture samplers of 10 cm length (Rhizon SMS-10; Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) were inserted into the sediments to allow collection of pore water using vacuum bottles. While peat was bare, both samples were pooled, whereas samples were analysed separately after application of the *Sphagnum* mosses. Surface water was sampled simultaneously with pore water.

pH was measured with a standard Ag/AgCl electrode (Orion, Thermo Fisher Scientific, Waltham, MA, U.S.A.) combined with a pH meter (Tim840 titration manager; Radiometer Analytical, Lyon, France), after which alkalinity was determined by titrating down to pH 4.2 using 0.1 M HCl using an auto-burette (Tim840 titration manager; Radiometer Analytical, Lyon, France). Total inorganic carbon (TIC) was measured by injecting 0.2 mL of sample into an N_2-flushed compartment with 1 mL phosphoric acid (0.4 M) in an Infra-red Gas Analyser (IRGA; ABB Analytical, Frankfurt, Germany), after which concentrations of HCO_3^- and CO_2 were calculated based on the pH equilibrium. Total organic carbon (TOC), dissolved organic carbon (DOC) and total nitrogen (TN) were measured in unfiltered water layer samples and filtered (Ø 0.45 μm) pore water samples, using a TOC-L CPH/CPN analyser (Shimadzu, Kyoto, Japan). Net mobilisation rates of nutrients (P, N and Fe) and TOC were calculated based on their concentrations in the water layer and the flow rate of treatment water.

At the end of the experiment, homogenised soil samples were taken, volume weighed and dried (48h 60 °C) to determine bulk density. Organic matter content was determined through loss on ignition (3h, 550 °C). Dried soils were digested with 4 mL HNO_3 (65%) and 1 mL H_2O_2 (30%) using a microwave oven (MLS 1200 Mega, Milestone Inc., Sorisole, Italy). Plant available P was extracted according to Olsen and others (1954), whereas a salt extraction was performed by incubating 17.5 g of homogenised fresh soil overnight with 50 mL of 0.2 M NaCl. Furthermore, total phenol concentrations of soil pore water were determined colourimetrically on a spectrophotometer (750 nm; Lambda 25, UV/VIS Spectrometer, PerkinElmer Instruments), according to Box (1983) and Carter and Gregorich (2007).

Soil extracts and water samples were analysed colourimetrically for PO_4^{3-}, NH_4^+ and NO_3^-, on an Auto Analyser 3 System (Bran & Luebbe, Norderstedt, Germany) using ammonium molybdate (Henriksen 1965), hydrazine sulphate (Kamphake and others 1967) and salicylate (Grasshof and Johannse 1972) respectively. Concentrations of Ca, Fe, K, Mg and total-P in water samples and digestates were analysed by inductively coupled plasma spectrometry (ICP-OES iCAP 6000; Thermo Fisher Scientific, Waltham, MA, U.S.A.).

Greenhouse gas flux measurements

CO_2 and CH_4-fluxes were measured on bare peat (4 weeks after start of the treatments) and on *Sphagnum*-covered peat (15 weeks; i.e. 6 weeks after placing *Sphagnum*) under light and dark conditions using transparent and dark closed chambers (10x10x12 cm) respectively, connected to a Greenhouse Gas Analyser (GGA-24EP, Los Gatos Research, Mountain View, CA, U.S.A.). Dark measurements were carried out after the night-period to ensure dark-adaptation of plants. Measured fluxes were used to estimate GWPs, expressed (on a mass basis) in CO_2-eq m^{-2} d^{-1}, with CH_4 corresponding to 34 CO_2-eq over a 100 year period including climate-carbon feedbacks (IPCC 2013). Estimates of year-round fluxes of GWP are based on an 8-month growing season to prevent overestimation during winter months.

Methane Production Potential (MPP) was determined by incubating 3 g of homogenised soil samples (2-6 cm depth) in 60 mL serum bottles with 3 mL of demineralised H_2O. An anaerobic headspace was ensured by 5 cycles of evacuation and gassing with N_2. For controls and acetate (5 mmol L^{-1} final concentration) treatments the headspace consisted of N_2, whereas the headspace of the hydrogen treatment consisted of 80% H_2/ 20% CO_2. Bottles were incubated on a horizontal shaker (100 rpm) at room temperature. CH_4 concentrations in the headspace were measured 7 times during one week on a HP 5890 gas chromatograph (Hewlett Packard, Wilmington, DE, USA) according to e.g. Ettwig and others (2008). Linear increases were used to calculate MPP.

Plant parameters

At the beginning and at the end of the experiment, the fresh weight (FW) and number of capitula of the mosses were determined. At the end, DW was determined (48 h, 60 °C), while initial moss DW was calculated using the DW/FW ratio determined by weighing and drying extra mosses (n=5 per species) at the start of the experiment. At the end of the experiment, photosynthetic rates were determined by measuring CO_2 consumption of capitula (top 0 to 3 cm) from both species and all aquaria in a closed glass chamber (100 mL) connected to a greenhouse gas analyser (GGA-24EP, Los Gatos Research, Mountain View, CA, U.S.A.) at a light intensity of 200 μmol m^{-2} s^{-1}. Furthermore, health of photosystem II (F_V/F_M) was determined using pulse-amplified modulation (JUNIOR-PAM, Waltz, Effeltrich, Germany). During the experiment, the concentrations of unicellular algae in the water layer were determined three times using a PhytoPAM (Phytoplankton Analyser System, Waltz, Effeltrich, Germany).

Statistical analyses

Normality of residuals and homogeneity of variance were checked using Shapiro-Wilk's test of normality and Levene's test of equality of error variances, respectively. Non-normal and heteroscedastic data were log transformed before analyses to authorise use of parametric tests. All data were subsequently analysed by two-way ANOVAs at the 0.05 confidence limit followed by a Tukey post hoc test. For all analyses, *P* and F values are presented. All statistical tests were carried out using SPSS (V21, 2012, IBM Statistics).

Results

Soil characteristics

Topsoils were characterised by a much higher P availability than subsoils, with significantly higher Olsen-P ($P<0.001$; F=127.870) and total P (TP; $P<0.001$; F=118.299) concentrations in soil and pore water extracts respectively (Table 6.1). Furthermore, topsoils had much higher salt-extractable NH_4^+ ($P<0.001$; F=19.533) and NO_3^- ($P<0.001$; F=20.288) concentrations and contained more total N (TN; $P<0.001$; F=46.309) than subsoils (Table 6.1). Topsoils were also characterised by higher concentrations of Ca ($P<0.001$; F=20.612, data not shown), Mg ($P=0.004$; F=9.914, data not shown) and HCO_3^- ($P<0.001$; F=105.565; Table 6.1). While Fe concentrations did not differ between topsoils and subsoils, the much higher TP content of topsoils resulted in much lower Fe: TP ratios (4.5±0.4 mol mol^{-1}) compared with subsoils (11.8±0.9 mol mol^{-1}; data not shown; $P<0.001$; F=49.124). Furthermore, subsoils had a 30% lower bulk density ($P<0.001$; F=39.340), 20% higher OM content ($P=0.007$; F=8.860) and 50% higher concentration of phenolic compounds ($P=0.020$; F=6.249) than topsoils (Table 6.1).

Water and pore water quality

The water layer above topsoils was characterised by a higher pH ($P=0.008$; F=8.496; Table 6.2) and alkalinity ($P<0.001$; F=29.608; Table 6.2) than the water layer of subsoils. Similar differences were observed in the soil pore water (data not shown). Rewetting with HCO_3^- -rich water increased both pH ($P<0.001$; F=231.050) and alkalinity ($P<0.001$; F=1956.640) in the water layer (Table 6.2) and pore water (data not shown), while use of P-rich water resulted in a higher PO_4^{3-} availability in the water layer ($P<0.001$; F=11.234; Table 6.2). Furthermore, a combination of P and HCO_3^- in the inlet water resulted in an even higher pH than sole addition of HCO_3^- ($P<0.001$; F=231.050; Table 6.2).

Table 6.1. Sediment characteristics of topsoil and subsoil of a former agricultural peatland, after 15 weeks of rewetting. Olsen P, NH_4^+ and NO_3^- were derived from Olsen- and salt extractions, respectively, and are presented as µmol L^{-1} FW. Other nutrient concentrations and concentrations of phenolic compounds and bicarbonate (HCO_3^-) were present in collected pore waters and presented per L pore water. Significant differences between topsoils and subsoils are indicated with asterisks, with * representing $P \leq 0.05$ and *** $P \leq 0.001$.

		Topsoil	**Subsoil**
Bulk density * **	kg DW L^{-1} FW	0.38±0.02	0.27±0.01
Moisture Content * **	%	66.5±1.1	76.3±0.8
Organic Matter * **	%	46.8±1.3	57.3±2.9
**Total Phenolic Compounds * **	mg L^{-1}	3.26±0.36	4.91±0.51
C: N * **	g g^{-1}	11.44±0.11	16.29±0.47
Olsen P * **	µmol L^{-1} FW	434.1±70.0	144.5±7.4
NH_4^+ * **	µmol L^{-1} FW	1651±398	720±81
NO_3^- * **	µmol L^{-1} FW	841.1±475.0	5.6±1.9
TP * **	µmol L^{-1}	268.4±39.8	7.0±0.9
TN * **	mmol L^{-1}	4.43±0.31	1.95±0.15
DOC * **	mmol L^{-1}	76.6±7.9	38.0±3.2
HCO_3^- * **	µmol L^{-1}	381.6±123.2	17.5±1.0

The higher nutrient content of topsoils also resulted in a higher availability of P and N in the overlying water layers (Table 6.2). P and N were mobilised from topsoils to the water layer at rates of 413±81 and 152±21 µmol m^{-2} d^{-1} respectively, whereas mobilisation from subsoils was limited to 27±7 µmol P and 34±5 µmol N m^{-2} d^{-1} ($P<$ 0.001 for both; data not shown). The mobilisation of P concurred with the mobilisation of Fe, at rates of 915±202 and 141±39 µmol m^{-2} d^{-1} from topsoils and subsoils respectively ($P=0.003$; data not shown). The water layer above topsoils contained significantly higher concentrations of algae during the experiment ($P<0.001$; F=45.842; Figure 6.1), whereas there was a trend ($P=0.089$; F=2.441; Figure 6.1) indicating a stimulating effect of P, HCO_3^- or a combination of P and HCO_3^- on algal growth in the water layer.

Table 6.2. Chemical composition of the water layer overlying topsoils or subsoils, with or without addition of P and/or HCO_3^-. Significant differences between topsoil and subsoil are indicated with asterisks, with ** representing *P*≤0.01 and *** *P*≤0.001. Significant differences between water treatments are indicated with different letters (a, b, c).

Water layer	Soil	Control	+P	$+HCO_3^-$	$+P/+HCO_3^-$
pH	Topsoil**	5.3±0.2 ^{a}	5.4±0.1 ^{a}	7.6±0.2 ^{b}	8.0±0.2 ^{c}
	Subsoil	4.6±0.1 ^{a}	4.6±0.0 ^{a}	7.6±0.1 ^{b}	8.2±0.2 ^{c}
Alkalinity	Topsoil***	0.25±0.04 ^{a}	0.26±0.07 ^{a}	2.92±0.03 ^{b}	2.97±0.06 ^{b}
(meq L^{-1})	Subsoil	0.10±0.02 ^{a}	0.09±0.01 ^{a}	2.69±0.06 ^{b}	2.77±0.06 ^{b}
PO_4^{3-}	Topsoil***	6.86±0.95 ^{a}	14.57±4.77 ^{b}	10.23±2.70 ^{b}	11.72±1.20 ^{b}
(µmol L^{-1})	Subsoil	1.08±0.07 ^{a}	3.01±0.41 ^{b}	2.66±0.65 ^{b}	5.31±0.21 ^{b}
Total P	Topsoil***	15.93±2.71 ^{a}	27.62±6.94 ^{b}	17.78±9.08 ^{a}	15.68±3.80 ^{b}
(µmol L^{-1})	Subsoil	1.03±0.26 ^{a}	7.47±0.56 ^{b}	2.80±0.19 ^{a}	8.05±0.94 ^{b}
NH_4^+	Topsoil**	5.87±0.81	7.39±1.84	10.96±2.59	8.57±2.13
(µmol L^{-1})	Subsoil	1.32±0.19	1.34±0.54	4.99±2.36	7.49±2.27
Ca	Topsoil	64±9	128±64	66±12	54±8
(µmol L^{-1})	Subsoil	71±11	64±6	79±14	57±10
TOC	Topsoil***	3.7±1.1	3.9±1.0	2.5±1.2	3.0±1.1
(mmol L^{-1})	Subsoil	0.7±0.1	1.4±0.4	1.9±0.2	1.3±0.1
TN	Topsoil***	0.29±0.09	0.29±0.07	0.17±0.09	0.22±0.08
(mmol L^{-1})	Subsoil	0.05±0.01	0.09±0.03	0.11±0.01	0.07±0.01

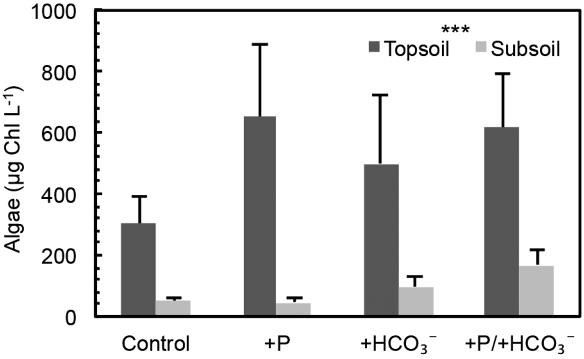

Figure 6.1. Average (unicellular) algal concentrations (±SEM), expressed as µg Chl L^{-1} for topsoils and subsoils treated with a water layer with or without addition of P and/or HCO_3^-. The significant difference between topsoil and subsoils is indicated using an asterisk, with *** representing *P*≤0.001.

Sphagnum growth

Both *Sphagnum* species were similarly affected by soil type and water quality, although *S. squarrosum* generally showed a higher relative growth rate (RGR) (*P*<0.001; F=21.810; Figure 6.2) and a higher photosynthetic rate (*P*=0.001; F=13.787; Supplementary table 6.1) than *S. palustre*. Both moss species had higher growth rates (*P*=0.007; F=8.041; Figure 6.2) on topsoils than on subsoils and a similar trend was observed for the photosynthetic rates of both species (*P*=0.073; F=3.368). Due to the higher availability of both N and P on topsoils, mosses grown on these soils also had higher N (*P*=0.027; F=5.179) and P (*P*<0.001; F=17.279) contents than mosses grown on subsoils (Supplementary table 6.1).

Both *S. palustre* and *S. squarrosum* species were negatively affected by addition of HCO_3^-. RGR (*P*=0.001; F=6.732; Figure 6.2), number of capitula (*P*<0.001; F=42.084), moss length (*P*<0.001; F=174.041), photosynthesis (*P*<0.001; F=8.266) and PAM (*P*<0.001; F=16.560) were lower in mosses from +HCO_3^- or +P / +HCO_3^- treatments than in mosses from control or +P treatments (Supplementary table 6.1). For *Sphagnum* grown without HCO_3^-, the number of capitula increased by approximately 15-20% during the experiment, while mosses that grew under HCO_3^--rich conditions had a 50-67 % lower number of capitula at the end of the experiment than at the beginning (data not shown). Addition of HCO_3^- resulted in a higher N-content (*P*<0.001; F=13.805) and a lower K-content (*P*<0.001; F=116.846) in the mosses, whereas addition of HCO_3^- together with P resulted in a higher P- (*P*=0.001; F=6.402) and N-content (*P*<0.001; F=22.872) in the moss tissue (Supplementary table 6.1).

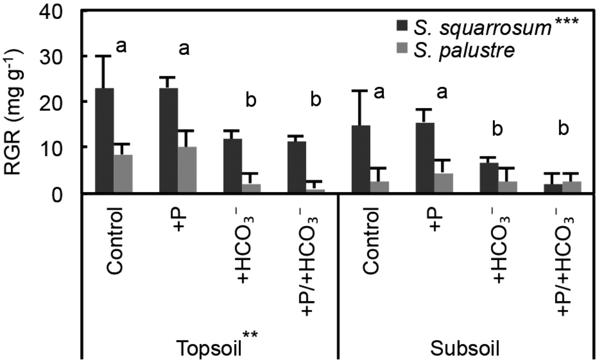

Figure 6.2. Relative Growth Rate (RGR; mean ± SEM) of *S. squarrosum* and S. *palustre* growing on topsoils or subsoils treated with a water layer with or without addition of P and/or HCO_3^-. Significant differences between topsoil and subsoils, and between *Sphagnum* species are indicated using asterisks, with ** representing *P*≤0.01 and *** *P*≤0.001. Significant differences between water treatments are indicated using different letters (a, b).

C-dynamics

After four weeks of rewetting, all bare sediments showed a net emission of both CH_4 (Figure 6.3A) and CO_2 (Figure 6.3B). While CO_2 emissions were similar for topsoils and subsoils, emission of CH_4 was more than 100 times higher for topsoils ($P<0.001$; F=32.191). This difference was still observed after 15 weeks of rewetting (6 of which with *Sphagnum* cover; $P<0.001$; F=38.848; Figure 6.3C), with CH_4 emissions for topsoils reaching average emissions of 0.2 to 1.1 g C m^{-2} d^{-1}, whereas subsoils showed much lower emissions, with rates of $1.5 \cdot 10^{-4}$ to $2.5 \cdot 10^{-3}$ g C m^{-2} d^{-1}. After 15 weeks of rewetting (6 of which with *Sphagnum* cover), CO_2 emissions increased more for topsoils than for subsoils ($P<0.001$; F=31.499; Figure 6.3D). While a few subsoils covered with *S. palustre* showed net CO_2-fixation, most subsoils and all topsoils showed a net release of CO_2, even when *Sphagnum* species grew on top.

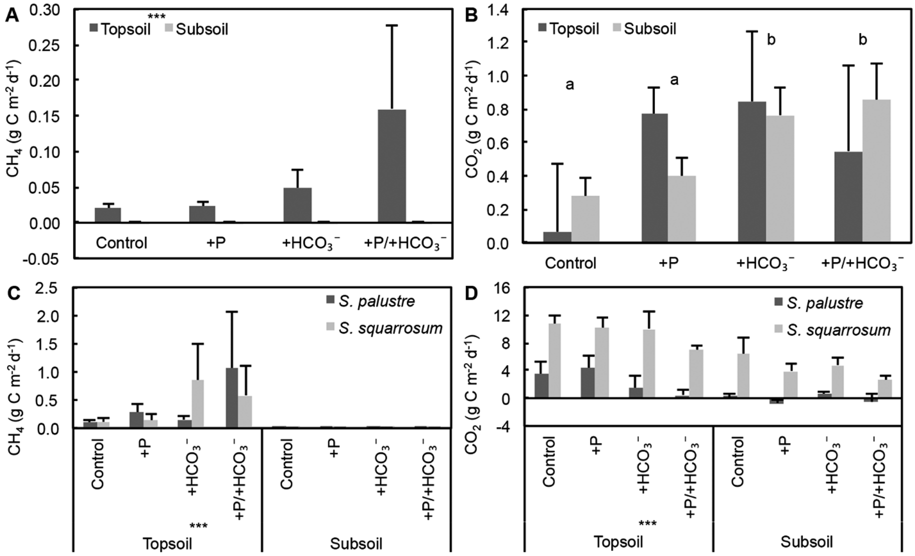

Figure 6.3. Fluxes (± SEM) of CH_4 (left) and CO_2 (right) from bare soils (A and B) and soils covered by *Sphagnum* mosses (C and D). Soils are either topsoils or subsoils, treated with a water layer with or without addition of P and/or HCO_3^-. Note different scales for the y-axes. Some treatments show high variation in CH_4 diffusion, resulting in large SEMs. Significant differences between topsoil and subsoils are indicated using asterisks, with *** representing $P \leq 0.001$. Significant differences between water treatments are indicated using different letters (a, b).

GWPs of bare soils did not differ significantly, but a trend ($P=0.097$; F=3.020) was observed indicating higher GWPs (5.73±1.91 CO_2-eq m^{-2} d^{-1}) for topsoils than subsoils (2.18±0.34 CO_2-eq m^{-2} d^{-1}; Figure 6.4A). After 15 weeks of treatment and introduction of *Sphagnum*, topsoils had a much higher GWP than subsoils, with average values of 26.94±11.62 and 53.76±9.40 CO_2-eq m^{-2} d^{-1} for topsoils covered with *S. palustre* (*P*=0.034; F=4.884) and *S. squarrosum* (*P*=0.001; F=13.578) respectively, compared to only -0.40±1.35 and 16.30±2.66 CO_2-eq m^{-2} d^{-1} for subsoils covered by the same species (Figure 6.4B).

Topsoils contained higher amounts of organic carbon, with significantly higher concentrations of TOC in the water layer above topsoils (*P*<0.001; F=17.619; Table 6.2) and of DOC in the pore water of topsoils (*P*<0.001; F=28.263; Table 6.1). Based on TOC measurements from the water layer, we could calculate that organic carbon fluxes from the sediments were around 1.01 ± 0.16 and 0.41 ± 0.05 g C m^{-2} d^{-1} for topsoils and subsoils respectively (*P*=0.002; F=12.033; data not shown).

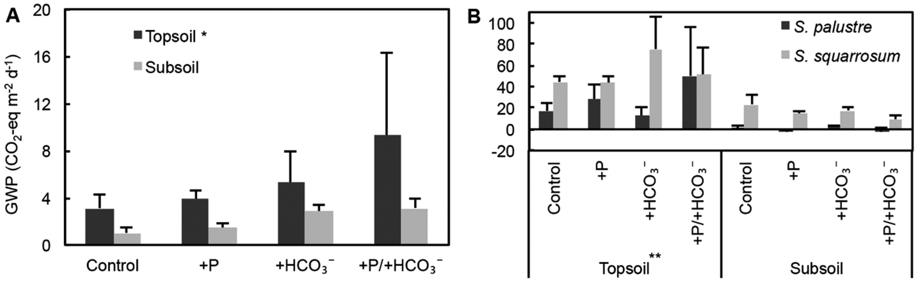

Figure 6.4. Global warming potential (GWP; mean ± SEM) of bare peat soils (A) and soils covered by *S. palustre* or *S. squarrosum* (B). Values for bare peat were measured after 6 weeks of experimental treatments, whereas rates for *Sphagnum* covered soils were measured after 15 weeks of experimental treatments (6 weeks after introduction of *Sphagnum*). Soils were either topsoils (5-25 cm depth) or subsoils (25-45 cm depth), treated with a water layer with or without addition of P and/or HCO_3^-. GWP is expressed in CO_2-equivalents on a mass basis, with CH_4 representing 34 CO_2-equivalents over a 100-year period (IPCC 2013). Significant differences between topsoil and subsoil are indicated with asterisks, with * representing *P*≤0.05 and ** *P*≤0.01.

Methane production potential (MPP)

In line with the overall higher C-fluxes from intact sediments, MPP rates were up to 99% higher in topsoils than subsoils (*P*<0.001; F=31.646; Figure 6.5A and 6.5B). Incubations of homogenised soils without substrate addition (Control headspace) showed a 10-fold increase in CH_4 production rates when soils were treated with HCO_3^- (*P*=0.033). While addition of acetate increased MPP (*P*=0.014; F=4.559) in all topsoil treatments, it had no stimulating effect on the MPP of subsoils. Furthermore, combined addition of H_2 and CO_2 did not stimulate the potential CH_4 production for either soil depth.

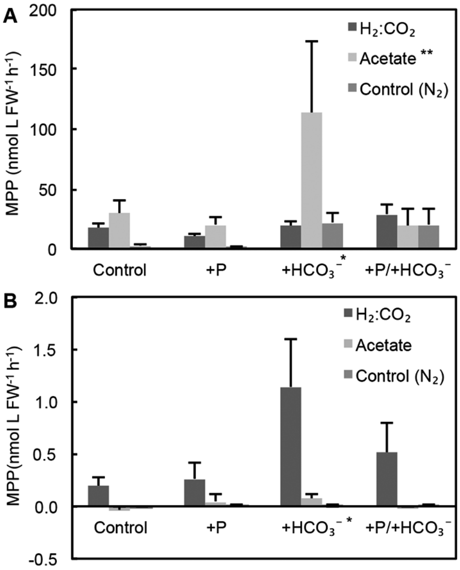

Figure 6.5. Potential soil CH_4 production (MPP) rates of topsoils (A) and subsoils (B) treated with different water layer compositions (with or without added P and/or HCO_3^-), after addition of different substrates: H_2/CO_2 (potential CH_4 production), Acetate (potential acetoclastic/ fermentative CH_4 production) or N_2 (headspace control). Note different scales of the *y*-axis. Significant differences between MPP substrates or different water treatments are indicated with asterisks, with * representing $P \leq 0.05$ and ** $P \leq 0.01$.

Discussion

Challenges of rewetting former agricultural peatlands

Peatlands used as arable lands or pastures for a long time have severely been altered through long-term drainage and fertilisation (Meyer and Turner 1992). In order to halt the high C and nutrient losses associated with these changes, rewetting programs are being carried out or planned for a growing number of drained peatlands. Projects include both the restoration of natural wetlands (Zak and Gelbrecht 2007; Tanneberger and Wichtmann 2011) and the change to alternative, wet agricultural use of peatlands e.g. by *Sphagnum* farming (Joosten and Clarke 2002; Verhoeven and Setter 2010; Gaudig and others 2013). The rewetting of these areas causes a fast shift from aerobic to anaerobic soil processes in which alternative terminal electron acceptors are used (Knorr and Blodau 2009). Although CO_2 and N_2O emissions are generally lowered after rewetting (Salm and others 2009), CH_4 production is strongly increased. While drained peatlands show CH_4 emission rates of -0.014 to 0.012 g C m^{-2} d^{-1} (Salm and others 2009; Maljanen and others 2010; Hatala and others 2012; Mander and others 2012), pristine "wet" peatlands produce 0.0 to 0.2 g C m^{-2} d^{-1}, with some 'hotspots' even emitting up to 1.5 g C m^{-2} d^{-1} (Bartlett and Harriss 1993; Saarnio and others 2007; Drewer and others 2010). So far, however, most studies have focused on oligotrophic bogs and pristine fens, reporting CH_4 fluxes up to 0.07 g C m^{-2} d^{-1} (Moore and Knowles 1989; Mander and others 2010; Beetz and others 2013; Wilson and others 2013; Green and others 2014), while few have studied the effects of rewetting for eutrophic peatlands. The CH_4 fluxes we showed for rewetted, former agricultural soils, ranging from 0.05 to 4.03 (median 0.16) g C m^{-2} d^{-1}, are comparable to rates of 0.05 to 0.30 g C m^{-2} d^{-1} found in other fertilised peatlands (Hendriks and others 2007; Schrier-Uijl and others 2010; van de Riet and others 2013).

Since CH_4 is a much more potent GHG than CO_2, it will strongly influence the global warming potential (GWP) of rewetted areas. GWPs of drained and degraded peatlands range from 188 to 3,230 CO_2-eq m^{-2} y^{-1} (Salm and others 2009; Teh and others 2011; Beetz and others 2013; Lamers and others 2015), whereas pristine systems generally show lower GWPs of -196 to 685 CO_2-eq m^{-2} y^{-1} (Byrne and others 2004; Long and others 2010; Olson and others 2013). We here show high GWP rates of 760 to 2253 CO_2-eq m^{-2} y^{-1} (based on an 8-month growing season) after rewetting of a former agricultural peatland, due to high CH_4 emission. In addition, C losses through DOC production and mobilisation accounted for 35-55% of the total C loss in our system and showed high average rates of around 1 g C m^{-2} d^{-1}. These rates are on par with the daily CO_2 fluxes from these soils, indicating a substantial, but often overlooked role of DOC loss in the total C emissions in these systems (Kalbitz and others 2000; Freeman and others 2001a; Fenner and others 2007).

In addition to high C losses and a high GWP, we showed a strong increase in plant-available N and P after rewetting as a result of mobilisation from the nutrient loaded soils, which is in accordance with observations in similar rewetted soils (Zak and Gelbrecht 2007; van de Riet and others 2013). While N can easily be mobilised under aerobic conditions during agricultural use, P is usually immobilised in Fe-P complexes. In the absence of O_2 after rewetting, however, Fe^{3+} is reduced and previously Fe-bound P is mobilised to the water layer (Smolders and others 2006), especially when Fe: P ratios of the sediment are below 10 mol mol^{-1} (Geurts and others 2008), as was indeed the case for the topsoils in our experiment. This mobilisation of P and N resulted in a higher occurrence of algae and may, in a field situation, also favour the growth of fast-growing vascular plants, such as *Juncus* spp. (Berendse and others 2001; Smolders and others 2008; Aggenbach and others 2013). As expected, eutrophication-related problems were further stimulated when P-rich water was used for rewetting, whereas an alkalinity characteristic of minerotrophic surface water stimulated additional P release and simultaneously doubled CO_2 emission rates. The latter effect may be caused by competition for anion binding sites (Roelofs 1991; Smolders and others 2006) and enhanced decomposition rates (Lamers and others 2015).

Topsoil removal strongly reduces C and nutrient emissions after rewetting

Topsoil removal resulted in 99% reduction in net CH_4 emission rates. While topsoils showed very high potential CH_4 production rates, with values up to 325 µmol CH_4 m^{-3} s^{-1} for fermentative CH_4 production, these rates decreased to 0.03 and 4.10 µmol CH_4 m^{-3} s^{-1} after topsoil removal and thus fall within the range of 0.01 to 10 µmol CH_4 m^{-3} s^{-1} reported in literature (Segers 1998). This strong reduction results from limited organic substrate (acetate) and P availability for methanogen communities due to the lower availability of easily degradable organic matter and nutrients in the subsoil (Updegraff and others 1995; Yavitt and others 1997; Tomassen and others 2003).

In addition to the strong reduction in CH_4 emission, GWPs and DOC fluxes were also reduced by 50-70% and 60% respectively. Furthermore, topsoil removal was shown to reduce the mobilisation of dissolved N and P by 80% and 93%, respectively. This will prevent algal blooms and dominance of highly competitive vascular vegetation, which makes the environment more suitable for growth of peat-forming *Sphagnum* spp. (Emsens and others 2015).

Water quality and *Sphagnum* growth: bicarbonate toxicity

Although we show net effluxes of both CH_4 and CO_2 from soils with dense *Sphagnum* cover, these mosses are potentially strong peat-forming species that can sequester large amounts of C as recalcitrant organic matter. Based on the biomass increase in our experiment, the net primary production (NPP) of both *Sphagnum* species was around 90-500 g DW m^{-2} y^{-1}. Although the NPP of other peat-forming species, such as *Phragmites australis*, may be higher, with rates around 300-1300 g DW m^{-2} y^{-1} (Brix and others 2001; Christensen and others 2009), litter of these species also decomposes much faster and more completely, with an average annual mass loss of around 60% y^{-1} (Kirschner and others 2001; Christensen and others 2009). *Sphagnum* mosses, on the other hand, have annual mass loss rates that only range from 5-20% y^{-1} (Clymo 1965; Coulson and Butterfield 1978; Verhoeven and Toth 1995; Limpens and Berendse 2003). Furthermore, they actively slow down decomposition processes by the production of acids. Under these circumstances, average C sequestration rates for *Sphagnum* range from 28-240 g C m^{-2} y^{-1} (Gerdol 1995; Graf and Rochefort 2009; Hajek 2009; Samaritani and others 2011), whereas those for P*hragmites* *australis* range from 30-160 g C m^{-2} y^{-1} (assuming an average C-content of 40% for both species; Kirschner and others (2001); Longhi and others (2008)). In our study, C fixation rates of 386-663 and 121-349 g C m^{-2} y^{-1} were reached by *S. squarrosum* and *S. palustre*, respectively, based on the increase in DW biomass and assuming a growing season of 8 months. Despite these high C fixation rates, the acidification by *Sphagnum* spp. has been shown to initially result in a net C-efflux, due to the transformation of HCO_3^- into CO_2 (Harpenslager and others 2015b).

Both moss species grew better on topsoils than subsoils, but only when rewetted with water without HCO_3^-. This was most likely due to the higher availability of nutrients on topsoils. *S. squarrosum* grew 2-5 times faster and fixed 2-3 times more C than *S. palustre*, especially on topsoils. The high nutrient availability may have favoured the growth of *S. squarrosum* (Clymo 1973; Kooijman and Bakker 1995), but this may also have been related to higher HCO_3^- availability (20 times higher HCO_3^- concentrations) in topsoils. *S. squarrosum* is more resistant to higher pH than *S. palustre* (Clymo 1973), and its high acidification potential (Giller and Wheeler 1988; Kooijman and Bakker 1995) may counteract negative effects of HCO_3^- by stimulating the conversion into CO_2 (Harpenslager and others 2015b). This may also explain the higher CO_2 fluxes observed for soils covered with *S. squarrosum*.

After 3-4 weeks, both *S. squarrosum* and *S. palustre* showed reduced growth and vitality upon increasing HCO_3^- levels, resulting in dying, algae covered mosses In a field situation, the huge nutrient stocks will also stimulate development of dense stands of fast growing vascular vegetation, which will eventually out-compete *Sphagnum* mosses (Berendse and others 2001; Smolders and others 2008; Aggenbach and others 2013) and thereby hamper C sequestration. This implies that *Sphagnum* growth will also benefit from topsoil removal.

Conclusion

Rewetting former agricultural peatlands halts land subsidence, but simultaneously results in strongly enhanced emissions of GHG and nutrients. Removal of the nutrient-rich topsoil before rewetting strongly improves the prospects of restoring the C balance in these soils by strongly reducing eutrophication (by 80-95%), DOC mobilisation (60%), CH_4 emission (99%) and GWP (50-70%). This not only results in lower C losses, but also prevents algal blooms and monocultures of fast-growing plants, improving the regrowth of peat forming *Sphagnum* vegetation and subsequent restoration of C sequestration. Although removal of the topsoil of an already subsiding system may appear undesirable, we here show that the remaining subsoil will provide more suitable conditions for peat regrowth than the nutrient rich topsoils. The removed topsoils should subsequently be used in drained, subsiding peatlands that are still being used for agriculture, since this may help to maintain traditional agriculture on these fields by increasing the surface level. This will also allow the establishment of higher water levels in restored peatlands without increasing flooding risks of the surrounding agricultural area.

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Supplementary information

Supplementary Table 6.1. Growth characteristics and mineral contents (mean ± SEM) of *S. palustre* (PAL) and *S. squarrosum* (SQU) grown on topsoils or subsoils, with a water layer with and without added P and/or HCO_3^-. Significant differences between topsoil and subsoil are indicated with asterisks, with * representing $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$. Significant differences between water treatments are indicated with different letters (a, b, c). Differences between *Sphagnum* species are indicated in the first column by asterisks, similar to soil effects.

Characteristic	***Unit***	***Soil***	***Control***		***+P***		***+HCO$_3^-$***		***+P/+HCO$_3^-$***	
			PAL	***SQU***	***PAL***	***SQU***	***PAL***	***SQU***	***PAL***	***SQU***
Photosynthetic rate	**µmol O$_2$**	**Topsoil**	86.9^{b}	91.7^{b}	52.3^{b}	99.6^{b}	39.8^{a}	83.4^{a}	33.8^{a}	48.6^{a}
(SQU>PAL)**	**gDW^{-1} h^{-1}**		±12.8	±27.0	±5.6	±22.9	±9.6	±23.1	±3.9	±13.2
		Subsoil	40.6^{b}	69.8^{b}	54.3^{b}	82.2^{b}	42.3^{a}	31.6^{a}	26.5^{a}	53.7^{a}
			±12.4	±7.0	±8.4	±16.6	±15.3	±9.1	±20.7	±21.8
Final No of Capitula		**Topsoil*****	81^{b}	104^{b}	77^{b}	108^{b}	22^{a}	35^{a}	20^{a}	45^{a}
(SQU>PAL*)**			±20	±9	±13	±14	±2	±4	±4	±22
		Subsoil	71^{b}	89^{b}	76^{b}	94^{b}	22^{a}	50^{a}	27^{a}	39^{a}
			±9	±6	±10	±9	±4	±13	±8	±13
Moss length	**mm**	**Topsoil*****	74.8^{b}	77.2^{b}	74.0^{b}	72.8^{b}	39.7^{a}	33.7^{a}	36.6^{a}	34.9^{a}
			±5.7	±4.4	±3.4	±3.5	±2.9	±1.1	±1.2	±3.9
		Subsoil	70.4^{b}	68.9^{b}	71.2^{b}	69.5^{b}	35.7^{a}	36.0^{a}	31.1^{a}	32.6^{a}
			±3.0	±4.0	±3.6	±3.1	±1.2	±3.6	±1.7	±7.7
pH vegetation		**Topsoil**	5.7^{a}	5.3^{a}	5.1^{a}	5.2^{a}	6.2^{b}	6.2^{b}	6.9^{b}	6.6^{b}
			±0.6	±0.1	±0.1	±0.1	±0.2	±0.3	±0.5	±0.2
		Subsoil	4.6^{a}	5.0^{a}	4.8^{a}	4.7^{a}	6.3^{b}	6.8^{b}	7.1^{b}	7.2^{b}
			±0.1	±0.3	±0.2	±0.2	±0.6	±0.6	±0.9	±1.1
N: P	**g g^{-1}**	**Topsoil**	4.55^{a}	1.68^{a}	1.19^{a}	1.34^{a}	4.24^{b}	6.23^{b}	8.47^{b}	8.12^{b}
			±1.81	±0.18	±0.11	±0.17	±1.63	±0.83	±1.10	±0.84
		Subsoil	4.78^{a}	1.38^{a}	1.65^{a}	1.32^{a}	4.58^{b}	5.61^{b}	6.72^{b}	7.65^{b}
			±1.31	±0.11	±0.26	±0.26	±2.04	±1.03	±1.18	±0.34
N: K	**g g^{-1}**	**Topsoil**	6.55	5.95	5.10	5.27	5.39	4.48	7.64	5.56
			±1.27	±0.78	±1.31	±0.89	±0.44	±0.36	±1.86	±0.46
		Subsoil**	10.00	7.91	8.30	5.77	6.93	7.09	7.50	5.75
			±2.99	±1.12	±0.96	±1.28	±0.58	±2.04	±1.18	±0.34
K: P	**g g^{-1}**	**Topsoil**	2.66^{c}	3.53^{c}	4.54^{c}	3.89^{c}	2.42^{b}	0.76^{b}	0.88^{a}	0.69^{a}
			±1.07	±0.23	±1.46	±0.20	±1.09	±0.12	±0.11	±0.03
		Subsoil**	3.25^{c}	5.92^{c}	5.20^{c}	4.32^{c}	2.62^{b}	1.52^{b}	1.24^{a}	0.76^{a}
			±1.51	±1.08	±0.61	±0.40	±1.10	±0.49	±0.40	±0.07
N	**µmol**	**Topsoil****	1020^{a}	845^{a}	962^{a,b}	969^{a,b}	1099^{b}	1177^{b}	1230^{c}	1340^{c}
	gDW^{-1}		±112	±26	±49	±57	±77	±47	±89	±115
		Subsoil	1157^{a}	777^{a}	942^{a,b}	780^{a,b}	1098^{b}	922^{b}	998^{c}	1109^{c}
			±149	±54	±77	±48	±123	±132	±188	±85
P	**µmol**	**Topsoil*****	78.0^{a}	68.5^{a}	100.7^{a,b}	90.8^{a,b}	94.3^{a,b}	120.2^{a,b}	83.6^{b}	109.6^{b}
	gDW^{-1}		±13.9	±11.4	±21.5	±15.7	±9.3	±9.8	±15.2	±7.6
		Subsoil	79.5^{a}	49.8^{a}	55.0^{a,b}	73.4^{a,b}	80.0^{a,b}	61.1^{a,b}	77.9^{b}	86.9^{b}
			±30.4	±13.0	±11.5	±20.0	±13.5	±11.9	±26.7	±2.62
K	**µmol**	**Topsoil**	144.0^{b}	188.5^{b}	294.3^{b}	273.4^{b}	166.0^{a}	71.4^{a}	54.3^{a}	60.7^{a}
	gDW^{-1}		±61.0	±25.9	±18.3	±35.8	±63.4	±9.4	±6.2	±6.7
		Subsoil	99.0^{b}	204.7^{b}	220.7^{b}	237.3^{b}	172.0^{a}	63.2^{a}	53.9^{a}	52.5^{a}
			±15.6	±15.3	±35.6	±44.8	±70.0	±9.0	±3.9	±5.3
PAM		**Topsoil**	0.542^{b}	0.627^{b}	0.565^{b}	0.648^{b}	0.626^{a}	0.503^{a}	0.493^{a}	0.467^{a}
			±0.039	±0.008	±0.015	±0.019	±0.046	±0.062	±0.069	±0.058
		Subsoil	0.606^{b}	0.634^{b}	0.609^{b}	0.644^{b}	0.554^{a}	0.414^{a}	0.529	0.397^{a}
			±0.034	±0.025	±0.027	±0.016	±0.050	±0.082	±0.031	±0.109

Chapter 7

Synthesis: Growing peat…the extended edition

Sarah Faye Harpenslager

How to grow peat…the extended edition

Anthropogenic forcing has severely changed the biogeochemistry, ecology and functioning of wetlands all over the world (Foley and others 2005; Zedler and Kercher 2005). As a result of drainage, fertilisation, peat extraction and other activities, wetlands have degraded and peatlands have turned from C sinks into C sources (Alm and others 1999; Waddington and others 2001; Lamers and others 2015). As a result, multiple services that are provided by pristine, growing peatlands, including C sequestration, water retention, biodiversity and water purification, have been lost. Simultaneously, drainage of peatlands has often led to land subsidence, thereby increasing flooding risks in low-lying, coastal areas (Syvitski and others 2009; Temmerman and others 2013). To counteract degradation, and restore the ecosystem services of peatlands, it is important that degraded systems are restored and new, peat-forming wetlands are created. In this thesis, I experimentally tried to find the optimal environmental conditions necessary to initiate or restore peat formation by stimulating growth of different ecosystem engineers.

The build-up of a peat layer will commence when the net primary production exceeds the rate of decomposition (Saarnio and others 2007). Although this may sound simple at a first glance, the initiation or restoration of peat formation is not an easy task. While the ingredient list from Chapter 1 (Text box 1.1) implied that peat formation only requires water, substrate and vegetation (and a lot of patience and sunshine), our lab and field experiments have shown that there are other strings attached. Especially when peat formation is a necessity instead of merely a preference in conservation and restoration - for instance when peat is supposed to act as a natural cap of a highly contaminated landfill (Textbox 1.2) - long lasting patience (thousand of years) is something one cannot afford. We have shown that a bare system, consisting of a sandy sediment with a rainwater layer on top, develops very slowly into a peat forming system and that even small ponds will largely remain open water for a long time (Chapter 5). Even with growth of highly productive submerged species (e.g. *Myriophyllum spicatum*, *Chara* spp or *Elodea nutalii*), open water hardly contributes to C sequestration (Chapter 5; Stefanik and Mitsch (2014)). Thus, when the fast build-up of a peat layer (i.e. within a time-span that is generally required for management practice and policy) is necessary, merely rewetting a bare system and hoping for the best will not suffice. Therefore, actively facilitating the growth of ecosystem engineers that can initiate the process of terrestrialisation is required to speed up succession.

With a little help… from ecosystem engineers

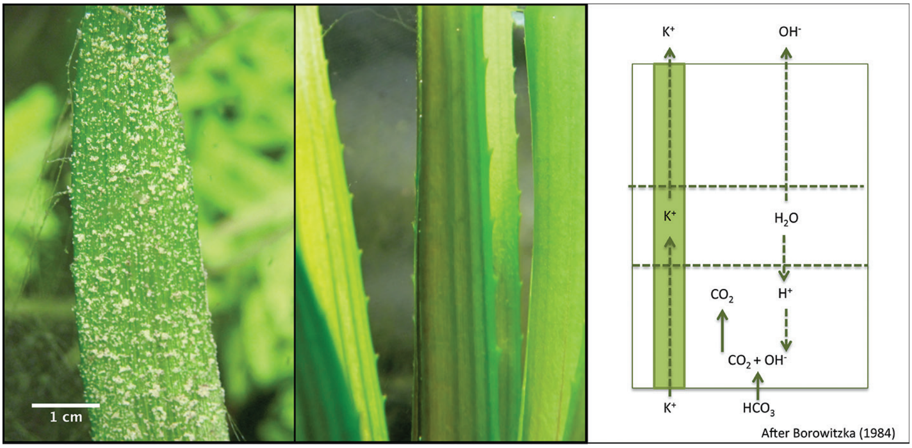
Different ecosystem engineers are associated with different successional stages of peat formation. In this thesis, three different types of ecosystem engineers were studied: *Stratiotes aloides*, characteristic of the aquatic phase, *Typha* spp., occurring in the semi-aquatic phase, and *Sphagnum* mosses, which grow in different mire systems including the floating fen stage. Each of these ecosystem engineers has its own specific requirements, which must be met in order to fulfil its role as keystone species in the ecosystem and initiate and drive succession. Apart from habitat requirements, it is becoming clear that positive species interactions also strongly influence growth of species, especially under pioneer conditions or elevated environmental stress (Bertness and Callaway 1994; Bruno and others 2003; He and others 2013). Below, I discuss how species interactions and environmental conditions influence growth of the three groups of ecosystem engineers and their peat forming potential (these characteristics are also summarised in Table 1).

Stratiotes aloides

The aquatic macrophyte *Stratiotes aloides* can be an important ecosystem engineer in peatlands by forming dense floating mats that strongly alter the biogeochemistry of their habitat. Plants usually gain buoyancy in spring, remain floating in summer and then sink again in autumn, after which they hibernate on the sediment surface (Cook and Urmi-König 1983). Due to the alternating life cycle of emergent and submerged life stages, the species uses atmospheric CO_2 when buoyant, whereas it uses CO_2 or HCO_3^- from the water layer when submerged. To become buoyant, however, submerged plants require minimum water layer CO_2 concentrations of 200 μmol L^{-1}, roughly 15 times higher than atmospheric equilibrium concentrations, to gain sufficient photosynthetic capacity to form energetically costly emergent leaves and become buoyant (Chapter 2; Milla and Reich 2007).

In strongly buffered systems (pH > 7.5 and high TIC), dissolved inorganic carbon (DIC) is often mainly available as HCO_3^-, while CO_2 concentrations are low (Stumm and Morgan 1996). HCO_3^- can be used by many but not all aquatic plants as an alternative C source (Allen and Spence 1981; Maberly and Madsen 1998). The photosynthetic pathway that uses HCO_3^- is, however, energetically costly (Figure 7.1; Lucas (1983); Borowitzka (1984)). Although *S. aloides* is able to use HCO_3^- as an alternative C source, this photosynthetic pathway is much less efficient due to the high costs (Prins and De Guia 1986; Nielsen and Borum 2008). Furthermore, use of HCO_3^- results in the accumulation of calcium carbonate ($CaCO_3$) on the upper side of the leaves (Borowitzka 1984). While this calcification occurs in almost all submerged

HCO_3^--using plants, a floating plant, such as *S. aloides*, might be weighed down by the extra mass of $CaCO_3$ on its thin submerged leaves (Figure 7.1; Chapter 2; McConnaughey (1998)). Combined, both the lower efficiency of HCO_3^- based photosynthesis and the additional weight of $CaCO_3$, delays buoyancy by 3 to 9 weeks compared to systems where plants can acquire ample CO_2 (Chapter 2). During this delay, *S. aloides* might be outcompeted by efficient HCO_3^- using species, including *Chara* spp., *Potamogeton pectinatus* and *Myriophyllum spicatum*, or floating species, such as *Lemna* spp. (McConnaughey 1998).



After Borowitzka (1984)

Figure 7.1. Submerged leaves of *Stratiotes aloides* growing with either low (left panel) or high (middle panel) availability of CO_2. Plants growing with low CO_2 concentrations switched to HCO_3^- based photosynthesis (right), which is not only less efficient, but also leads to calcification. HCO_3^- is taken up on the lower side of the leaf, where acidification results in the conversion to CO_2. The calcification occurs due to an excretion of OH^- on the upper side of the leave, which compensates for the excretion of H^+ on the lower side of the leaf. As a result, the pH of the upper boundary layer increases, leading to precipitation of $CaCO_3$.

With sufficiently high CO_2, nutrient and light levels, dense floating stands of *S. aloides* can quickly cover entire shallow water bodies through vegetative growth (Cook and Urmi-König 1983; Smolders and others 1995). Such high-density stands strongly differ from stands with scattered submerged or emergent individuals in both their impact on the habitat and their resistance against toxicity (Chapter 3). A dense, floating mat causes accumulation of CO_2 in the water layer underneath, which, as we showed in Chapter 2, can determine whether a stand becomes buoyant or not. Like other floating aquatic species (Caraco and others 2006), dense stands of buoyant *S. aloides* lower the diffusion of oxygen into the water layer and may cause anoxia of the sediment-water interface, which stimulates the mobilisation of iron-bound phosphorus (P) to the water layer (Chapter 3; Smolders and others (2006)).

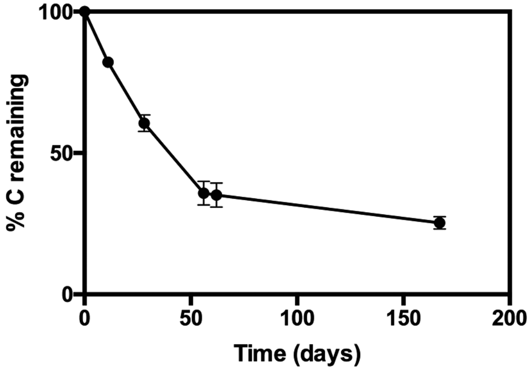


Figure 7.2. Decomposition rate of leaf litter of *S. aloides*, expressed as % of C remaining. Results are derived from an incubation experiment, using plant litter of plants grown with ≥200 µmol L^{-1} CO_2 and full light in the mesocosm experiment presented in Chapter 2. After 6 months around 25% of the initial C remains (Harpenslager, unpublished data).

As this species mainly reproduces clonally, it requires active introduction in newly constructed wetlands and in restored peatlands without remnant populations. Although input of a low number of plants may succeed under pristine conditions, (re) introduction in high numbers will form a more robust stand, which can facilitate itself in terms of C and P nutrition (Chapter 3). Furthermore, dense stands of this species can withstand higher levels of environmental stress, such as high ammonium ($NH4^+$) loading, than low-density stands (Chapter 3). This intra-specific facilitation (Chapter 3), combined with high light and CO_2 availability (Chapter 2) can result in the fast build-up of organic matter due to a high net primary production (NPP). Although literature data on NPP of *S. aloides* are scarce, general field production rates range from 159 to 235 g DW m^{-2} (Brammer 1979; Krolikowska 1997). Healthy plants growing in our experiments weighed approximately 20 to 30 g DW each (Chapter 2 and 3). Based on an average density of 15 plants m^{-2} (including offsets; high-density treatment Chapter 3), NPP could therefore reach approximately 330 to 500 g DW m^{-2} y^{-1} (Table 7.1) under optimal conditions. Decomposition of *S. aloides*, on the other hand, could be estimated from an incubation experiment, which showed that approximately 25% of the original leaf material remained after 6 months (Figure 7.2; Table 7.1). Combined, these species could thus theoretically sequester approximately 23 to 44 g C m^{-2} y^{-1} (Table 7.1). Although pollen of this species are often found in paleo-ecological records (Bennike and Hoek 1999), the almost complete absence of macrofossils implies that the role of *S. aloides* in long-term peat formation is probably more an indirect and facilitating one, by providing substrate (sapropel) and structure for peat-forming species (Sarneel and others 2011), rather than a direct one.

Table 7.1. Habitat requirements and potential net primary production (NPP) and decomposition, indicated by annual mass loss (%) of litter produced by the three studied ecosystem engineers: *Stratiotes aloides*, *Typha* spp. and *Sphagnum* spp. In the final column, the C sequestration potentials of the three different ecosystem engineers are estimated, based on NPP, C content and decomposition. Values are either derived from experimental data or from literature. References are numbered [1] to [27] and listed below.

Species	**Phase**	**Role**	**Water quality requirements**	**Soil quality requirements**	**NPP (g DW m^{-2})**	**Decomposition (% mass loss y^{-1})**	**C content (%)**	**C sequestration (g C m^{-2} y^{-1})**
Stratiotes aloides	Aquatic	Structure	Low NH_4^+ [1], high CO_2 [2]	Rich in P [3-4]	330 - 500 [1-2, 10-11]	≥75 * [20]	28-35 [1-2]	23-44
***Typha* spp.**	Semi-aquatic	Floating mat; Accumulation		Rich in N and P [5-6]	1100-1800 [12-15]	60 [21-22]	48 [15]	140-590
***Sphagnum* spp.**	(Floating) mire	Acidification; Peat formation	Low HCO_3^- [7-9]		70-1000 [8, 16-19]	5-20 [23-26]	40 [27]	28-450

[1] Chapter 3
[2] Chapter 2
[3] De Lyon and Roelofs (1986)
[4] Smolders and others (1996)
[5] Tanner (1996)
[6] Wild and others (2001)
[7] Clymo (1973)
[8] Chapter 4
[9] Chapter 6
[10] Brammer (1979)
[11] Krolikowska (1997)
[12] Mason and Bryant (1975b)
[13] Brinson and others (1981)
[14] Zerbe and others (2013)
[15] Overbeek and others (in prep.)
[16] Gerdol (1995)
[17] Graf and Rochefort (2009)
[18] Hajek (2009)
[19] Samaritani and others (2011)
[20] Harpenslager et al., (unpublished; Figure 7.2)
[21] Kirschner and others (2001)
[22] Christensen and others (2009)
[23] Clymo (1965)
[24] Coulson and Butterfield (1978)
[25] Verhoeven and Toth (1995)
[26] Limpens and Berendse (2003)
[27] Longhi and others (2008)

* Annual mass loss may be underestimated, since this value is based on a 6-month incubation experiment

Typha spp.

Some of the species that may be facilitated by *S. aloides*, are helophytes, such as *Typha* spp. The air-filled rhizomes of *Typha* species are thought to find structure within a floating vegetation stand (Sarneel and others 2011) and subsequently increase the buoyancy of these mats (Hogg and Wein 1988). Furthermore, the organic layer, or sapropel, created by *S. aloides* reduces water depth and provides a nutrient-rich substrate for rooting helophytes.

Typha latifolia and *T. angustifolia*, are often used in constructed wetlands because of their high nutrient uptake rates (Tanner 1996; Wild and others 2001) and high primary production (Brinson and others 1981). Under nutrient-rich conditions, these species can reach very high growth rates and produce 300 to 1800 g DW m^{-2} y^{-1} (Overbeek et al., in prep.; Mason and Bryant (1975b); Brinson and others (1981); Zerbe and others (2013)). These species can, however, only expand when they have either support to form a floating root mat or a nutrient rich soil in combination with a low water level. As the colonisation by these species generally occurs from the shore, covering open water can take a long time when relying solely on rhizomal growth. Applying soils containing propagules, such as plant fragments or a viable seed bank (Chapter 5), can strongly enhance colonisation by *Typha latifolia* or *T. angustifolia*, which are able to germinate under water (Keddy & Ellis, 1984; Coops & Van der Velden, 1995). Although they appear to be quite similar, *T. latifolia* and *T. angustifolia* have slightly different habitat preferences. While *T. latifolia* usually dominates shallow zones, where its broad but shorter leaves can catch maximum light, the narrower leaves of *T. angustifolia* and its large rhizomal storage area allow it to grow in deeper water (Grace and Wetzel 1981).

Although both *Typha* spp. have similar NPP and decomposition rates (approximately 60% mass loss y-*1*; Kirschner and others (2001); Christensen and others (2009); Table 7.1) to, for example, *Phragmites australis* (Mason and Bryant 1975b; Brinson and others 1981; Webster and Benfield 1986; Alvarez and Becares 2006), this latter species is generally accepted to be a peat-forming species, whereas the peat-forming ability of *Typha* spp. is subject of debate (Lambert 1951; Campbell and Kennedy 1981; Moore 1989; Zerbe and others 2013). This difference is mainly based on paleo-ecological records, which show macrofossils of *P. australis*, but not of *Typha* species (Van Geel and others 1981; Bakker and Van Smeerdijk 1982). *Typha* pollen are, however, commonly found in layers of peat (Van Geel and others 1981; Bakker and Van Smeerdijk 1982), showing that the species was often present in early successional stages of these systems. There are several possible explanations for the absence of thick deposits of *Typha* peat. First of all, *Typha* litter may break down further than other species and become less recognisable. This degraded material can, however still be present in the fully degraded humus component of peat layers (Figure 7.3; Reddy and others (1993); Wichtmann and Joosten (2007)). Secondly, *Typha* is a species preferring higher nutrient concentrations, which can now be found in many peatland areas, but since paleo-ecological records comprise of peat that has been formed thousands of years ago, it is by no means certain that conditions were comparable to the current situation. It is therefore not unthinkable that historical conditions favoured growth of other species, such as *Phragmites australis* or *Carex* spp., explaining why these are found more often in old peat layers. Finally, even if suitable habitat conditions existed, the continued build-up of organic matter by *Typha* would decrease the water depth, which favours growth of other helophytes such as *Phragmites australis* (Asaeda and others 2005; Bellavance and Brisson 2010). The time period during which *Typha* could effectively produce litter to be turned into peat could thus be too short to find any remains after hundreds of years of subsequent peat formation by *Phragmites*, *Carex* or *Sphagnum* species. Still, it is also highly possible that, like *Stratiotes aloides*, *Typha* plays a more important role in the facilitation of peat formation than the actual build-up of the peat layer.

Figure 7.3. Core taken within a dense *Typha* stand. The top 10 cm in this core represents partially decomposed *Typha* litter, illustrating that this species can contribute to the build up of an organic layer. Picture taken in the Anklamer Stadtbruch (Germany) by C. Fritz.

Sphagnum spp.

While both *S. aloides* and *Typha* spp. may be more important as facilitating species in the process of peat formation than as actual contributors to the build-up of the peat layer, the high peat-forming potential of *Sphagnum* mosses has been widely demonstrated. Most of the 400-550 Pg C that is reportedly stored in peatlands (Lamers and others 2015) has been fixed by *Sphagnum* (Clymo and Hayward 1982; Van Breemen 1995). These mosses strongly alter the biogeochemistry of their habitat and as a result create the optimal conditions for the build-up of a peat layer (Clymo 1973; Van Breemen 1995; Hajek and Adamec 2009; Adkinson and Humphreys 2011). Furthermore, due to the high content of phenolic compounds (Yavitt and others 2000; Freeman and others 2001b), including antibiotics (Verhoeven and Toth 1995), *Sphagnum* litter is not easily decomposed. While annually 75% and 60% of *S. aloides* and *Typha* litter is decomposed, respectively, *Sphagnum* annual decomposition rates only range from 5 to 20% mass loss (Clymo 1965; Verhoeven and Toth 1995; Limpens and Berendse 2003) Table 7.1). These mosses are, however, restricted to more acidic habitats than the aforementioned species (Clymo 1973), indicating that they are rarely found in the early, minerotrophic stages of peatland development. Still, differences exist in the habitat requirements, acidification potential and growth rate of the 100-odd species of *Sphagnum* that have been described (Clymo 1973; Daniels and Eddy 1990). While some species, such as *S. magellanicum*, *c*annot grow in minerotrophic systems, others, such as *S. squarrosum*, are more tolerant to buffered conditions (Chapter 4; Clymo (1973)).

During succession, peatlands generally move from high to low nutrient availability and from high to low buffering (Kuhry 1994; Granath and others 2010). Due to its high acidification rate, especially under more nutrient rich conditions (Kooijman and Bakker 1994), *S. squarrosum* is considered one of the pioneer *Sphagnum* species that will initiate succession towards a more acidic, peat-forming system (Giller and Wheeler 1988; Haraguchi and others 2003). Although direct contact with HCO_3^--rich water hampers growth and eventually results in mortality in this species (Chapter 6), they can thrive on top of floating rafts fed by HCO_3^- rich water (Chapter 4), as long as they grow in dense patches. Like *S. aloides* (Chapter 3), *Sphagnum* species strongly facilitate their own growth when present in a high density. In our experiments, *S. squarrosum* could only lower pH down to 4.5 when grown together with other *Sphagnum* species. When grown in a similar set-up, together with *Caliergonella cuspidata* and *Scorpidium scorpioides*, however, the pH did not drop below pH 6, and *S. squarrosum* had a 12 times lower growth rate (0.15 vs. 1.9 g m^{-2} d^{-1}) than when grown with other *Sphagnum* species (Chapter 4 and unpublished data). This indicates that although *S. squarrosum* can still grow in a suboptimal environment, it requires a certain critical mass to outcompete other moss species and initiate the succession towards and acidic, *Sphagnum* dominated system.

Although *Sphagnum* mosses can profit from enhanced nutrient availability (Chapter 6), eutrophic soils favour fast growing vascular plants, which subsequently outcompete *Sphagnum* spp. (Berendse and others 2001; Smolders and others 2008; Aggenbach and others 2013). Under favourable conditions, *Sphagnum* mosses can reach high biomass production rates, ranging from 70-600 g DW m^{-2} y^{-1} (Chapter 4; Gerdol (1995); Graf and Rochefort (2009); Hajek (2009); Samaritani and others (2011)). In combination with the low decomposition rate of these mosses, they may reach a net C sequestration rate of 28 to 450 g C m^{-2} y^{-1} (Table 7.1).

Application of ecosystem engineers: initiation and restoration of peat formation

The ecosystem engineers *Stratiotes aloides*, *Typha* spp. and *Sphagnum*, that are associated with different phases of peat formation, all have specific requirements regarding their habitat, which may limit their occurrence and efficiency as keystone species. Furthermore, the ability of these species to act as ecosystem engineers and strongly modify their habitat to their own benefit is usually density-dependent. *Stratiotes aloides*, for example, improves its own C and P nutrition and buffers against NH_4^+ toxicity by growing in dense stands (Chapter 3), whereas a critical mass of *Sphagnum* appears to be required to acidify its environment and start succession towards a *Sphagnum*-dominated, peat forming wetland (Chapter 4). Although *Sphagnum* also appears to be the only species, out of the three studied ecosystem engineers, that significantly contributes to the build-up of the peat layer, its growth cannot occur without facilitation of earlier stages by the other two groups of ecosystem engineers. These processes of intra- and interspecific facilitation are, however, hardly recognised within projects aimed at peat formation. Although the importance of facilitation has recently been identified in high-stress environments, such as intertidal habitats and salt marshes (van der Heide and others 2010; He and others 2013; Silliman and others In prep.), it has remained largely overlooked in wetland management. In the next section, I will use two examples of applied projects, the construction of a new peat-forming wetland and the restoration of a degraded, former agricultural peatland, where ecosystem engineers are used to initiate and restore peat formation, respectively, to show the importance of harnessing both habitat requirements and facilitation.

Initiation of primary peat formation: construction of new peatlands

Although the creation of new wetlands has increased in recent decades, these systems are almost exclusively used for water purification (Bulc 2006; Vymazal and Kropfelova 2009). Constructed wetlands can, however, provide similar services to natural wetlands, including C sequestration, recreation, biodiversity and water retention (Knight 1997; Wild and others 2001; De Klein and Van der Werf 2014). At the Volgermeerpolder, near Amsterdam (The Netherlands), a newly constructed wetland was created to function as natural capping for a highly contaminated landfill. Due to the high potential of peat to bind contaminants (Mclellan and Rock 1988) and stimulate bioremediation of organic pollutants (Couillard 1994), such a newly constructed, peat-forming wetland can provide a sustainable addition to regular capping methods. Since basal liners, which are usually implemented in capping of landfills, have a limited lifespan (Allen 2001; Rowe and Sangam 2002), the newly formed peat layer can take over when the technical layer degrades.

For the construction of this wetland, landscape architects designed a wetland inspired by rice-paddies, with small basins surrounded by clay banks. All basins were rainwater-fed, with a water level of 20-80 cm throughout the year (median 60 cm). Due to the limited size of the basins, there was a low influence of wind and erosion and a relatively large shore area, which can stimulate colonisation by emergent vegetation. Still, when only bare sand and rainwater were used for construction, colonisation was slow and 85% of the basin area remained unvegetated or was colonised by submerged species (Chapter 5).

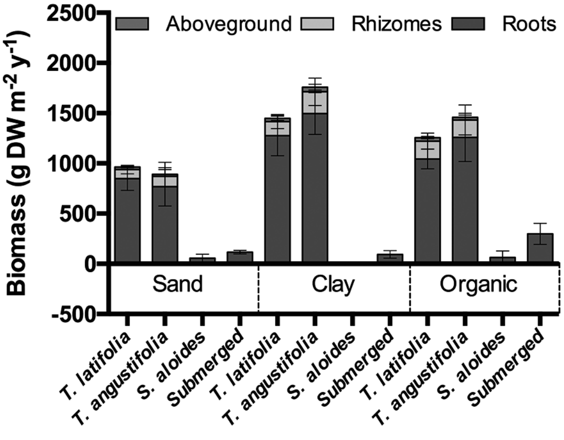


Figure 7.4. Biomass production in stands of *Typha latifolia*, *T. angustifolia*, *S. aloides* and submerged species growing in the newly constructed Volgermeerpolder. Plots of these vegetation types were harvested in 2014, after 3 years of development (Overbeek and others, in prep.).

In basins where an additional layer of clay or organically rich soil was applied, the nutrient availability was much higher. Application of these soils increased the P availability (Olsen-P) approximately 1.5 and 2 times and the N availability (Salt-extracted N) 3 and 4 times for clay and organic soils, respectively, which strongly stimulated the development of *Typha latifolia* and *T. angustifolia*. After 3 years, these species covered 40 and 70% on applied clay and organic soils, respectively (Chapter 5). Apart from strongly stimulating the vegetation cover, addition of these soils also increased plant biomass production within growing *Typha* stands. As a result of the higher nutrient availability, 1 m^2 of *Typha angustifolia*, for example, produced a total net dry weight of 900 ± 240 g DW m^{-2} y^{-1}, whereas 1 m^2 of this same species growing on organic soil or clay produced 1460 ± 290 and 1690 ± 260 g DW m^{-2} y^{-1} (Overbeek and others, in prep.; Figure 7.4; P=0.016).

Since the soils used for construction were all derived from former marine areas, they contained high amounts of sulphate (SO_4^{2-}), calcium (Ca) and HCO_3^-. Due to mobilisation of these substances, the water layer was characterised by a high pH and DIC was mainly available as HCO_3^-. Due to this combination of high HCO_3^- (median: 2400 µmol L^{-1}) and low CO_2 (median: 70 µmol L^{-1}) concentrations in the water layer, the area did not form a suitable habitat for *S. aloides* (Chapter 2). Still, in 2011, 50 plants were introduced in enclosures (to ensure high density) in all 27 basins. After 3 years of development, only 5 of these basins contained populations with over 100 plants, and average biomass production over 2014 was around 80 g DW m^{-2} y^{-1} (Figure 7.4). Although no single biogeochemical factor could explain why these 5 basins had a healthy stand of *S. aloides*, whereas the other 22 did not, the fact remains that after these stands had reached sufficient density, they continued to thrive the following years, whereas low-density stands slowly disappeared. These observations can be explained by the strong effect that dense stands of this species have on their environment, as shown in Chapter 3.

Due to the high availability of HCO_3^-, habitat conditions were also unsuitable for *Sphagnum* growth. In 2014, a few artificial floating rafts with *S. squarrosum*, *S. fallax* and *S. palustre* were introduced at the Volgermeerpolder, but none of these survived until the end of the growing season due to flooding with HCO_3^- rich water and eutrophication by bird droppings (Tomassen and others 2005), which stimulated development of fast-growing vascular plants on the floating mats (Figure 7.5).

Figure 7.5. Patches of *Sphagnum squarrosum* on artificial floating rafts at the Volgermeerpolder in February (left), May (middle) and August (right) 2014

Thus, the only species that could colonise this newly constructed wetland and form enough biomass to contribute significantly to the terrestrialisation process and the build-up of organic material were *Typha latifolia* and *T. angustifolia* (Chapter 5). Although patches of *Typha* spp. growing on sand showed a high net C sequestration rate of 590 g C m^{-2} y^{-1}, these species only covered 15% of a basin after 3 years, whereas the remaining area was either bare or covered by submerged macrophytes. Since bare soil or submerged species do not contribute to the accumulation of organic matter (Chapter 5; Stefanik and Mitsch (2014)), the formation of a uniform peat layer throughout the wetland is impossible until *Typha* or other ecosystem engineers colonise the remaining 85%. To stimulate both the colonisation and the production of *Typha*, clay or organic soil should be applied. The application of material with a higher nutrient and labile organic matter content than sand is, however, accompanied by risks of eutrophication and C effluxes. While risks of nutrient mobilisation can be predicted beforehand (Geurts and others 2010) and subsequently avoided by only using low-risk soils, initial C losses seem unavoidable (Chapter 5).

Initiation of secondary peat formation: restoration of degraded peatlands

Globally, around 15% of all wetlands have been drained (Joosten 2009). This percentage is even higher in Western Europe, where drained wetlands form up to 85% of the total wetland surface (e.g. Brock and others (1999); Zanello and others (2011); Hooijer and others (2012); Meckel and others (2006); Hoeksema (2007)). Most of these areas (>85%; Joosten (2009)) have been drained for agricultural use and have therefore been limed and/or fertilised (Meyer and Turner 1992), leading to a topsoil that is loaded with P and rich in HCO_3^-. In recent years, several attempts have been made to restore the peat-forming function of these areas through rewetting. While some of these projects have a nature conservation goal (Zak and Gelbrecht 2007; Tanneberger and Wichtmann 2011), others focus on transforming terrestrial agriculture into aquatic agriculture, with cultivation of *Sphagnum* or helophytes (Joosten and Clarke 2002; Verhoeven and Setter 2010; Gaudig and others 2013).

When a system with a eutrophic topsoil is rewetted, nutrients and organic carbon are mobilised to the water layer (Van de Riet, 2013; Zak and Gelbrecht, 2007; Chapter 6; Figure 7.6). As a result, the water layer may become dominated by phytoplankton (Chapter 6), whereas fast growing vascular vegetation starts rooting in the nutrient rich topsoil (Berendse and others 2001; Smolders and others 2008; Aggenbach and others 2013). Peat-forming vegetation, such as *Sphagnum* species, are often introduced in restoration projects (Campeau and Rochefort 1996; Chirino and others 2006; Robroek and others 2009), but their reintroduction will most likely be unsuccessful in eutrophic systems due to competition with dense stands of fast growing vascular vegetation and phytoplankton (Berendse and others 2001; Smolders and others 2008; Aggenbach and others 2013). After removal of the nutrient-rich topsoil, however, the 80-90% lower nutrient mobilisation rates make the system much more suitable for reintroduction of *Sphagnum* mosses and restoring the peat accumulation function of the peatland (Chapter 6; Figure 7.6). Furthermore, topsoil removal strongly limits the high emission of CO_2, CH_4 and dissolved organic carbon (DOC), thereby improving conditions for net C sequestration (Chapter 6). Furthermore, by implementing the removed topsoil in nearby agricultural fields, water levels can be raised without risks of flooding neighbouring pastures or croplands (Figure 7.6).

Since topsoil removal is a costly and highly invasive measure, an alternative approach would be to stimulate growth of fast-growing helophytes, such as *Typha* species. As we have seen in Chapter 5, *Typha* spp. can reach very high growth rates on nutrient-rich soils. In water purification treatments, *Typha* species and other helophytes are often used to filter nutrients, due to their high nutrient uptake rates (Tanner 1996; Wild and others 2001), and regular harvest and removal of biomass can remove nutrients from the system. Although it is still estimated to take 20 to 50 years for P-loaded soils to become depleted (Zak et al 2014), these highly productive systems can in the meantime be used for so-called paludiculture, or wet agriculture (Joosten and Clarke 2002; Verhoeven and Setter 2010). *Typha* material can be used for building material or fodder and biofuel production, whereas *Phragmites* has been used as roof material for centuries (Kobbing and others 2013). With more intensive management, however, even *Sphagnum* mosses are being grown in aquatic agriculture (Gaudig and others 2013).

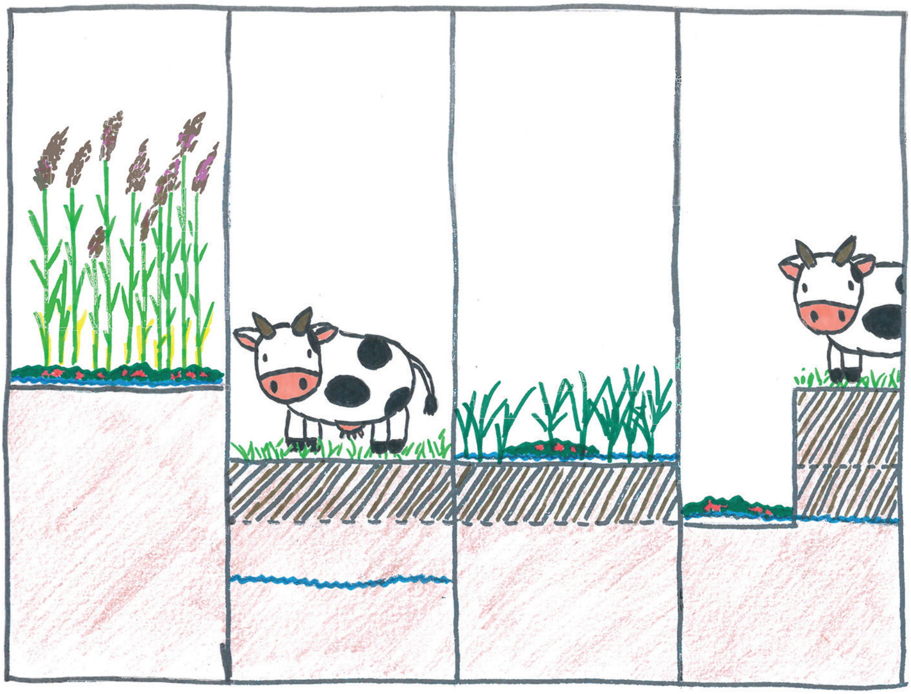


Figure 7.6. Schematic overview of the impact of drainage and agricultural use on peatlands, and how to restore such systems. In the first column, a peatland is represented that is dominated by *Phragmites australis* and *Sphagnum* mosses. High water levels ensure moist and anaerobic conditions in the topsoil, which reduces decomposition and stimulates the build-up of a peat layer. Such systems have, however, often been drained. Due to lowered water levels, consolidation and decomposition of the peat led to land subsidence. Furthermore, since most of these drained systems have been used for agriculture, the topsoil is loaded with nutrients (mainly P) and labile organic matter. When such a system is rewetted and *Sphagnum* mosses are reintroduced, nutrients will be mobilised, leading to algal dominance in the water layer and growth of graminoids on the organic soils, both of which may outcompete *Sphagnum*. Furthermore, the high labile organic matter content will lead to a high emission of carbon dioxide (CO_2), methane (CH_4) and dissolved organic carbon (DOC). If the topsoil is removed, before rewetting, however, both eutrophication and C emission risks are severely reduced, making the system more suitable for restoration of *Sphagnum* growth. Also, by implementing the removed soil on neighbouring agricultural fields, there is no risk of high water levels on pastures when rewetting degraded peatlands for nature restoration.

Constraints on peat formation

So far, I have shown that the three ecosystem engineers *Stratiotes aloides*, *Typha* and *Sphagnum* may all contribute to the process of terrestrialisation and peat formation when their habitat requirements are met (Figure 7.7). Through their impact on the environment, these species will stimulate the transition in the early stages of peat formation. In the aquatic phase, self-facilitation in dense stands of *S. aloides* will maximise production and stimulate the build-up of an organic layer, whereas the diverse structure of the floating mats will not only support a high biodiversity, but also provide substrate for e.g. air-filled rhizomes of *Typha* (Figure 7.7). In the subsequent semi-aquatic phase, *Typha* species can reach very high NPP rates, while simultaneously giving buoyancy to floating root mats (Figure 7.7). By increased litter production, these floating mats are first transformed into minerotrophic floating mires. By colonising pioneer *Sphagnum* mosses, such as *S. squarrosum*, moist, acid conditions are created that lower decomposition processes and strongly stimulate the build-up of a peat layer (Figure 7.7). Under natural conditions, however, the complete succession from an unvegetated sandy pond to a *Sphagnum*-dominated mire takes a long time and every stage requires optimal habitat conditions for the corresponding ecosystem engineer.

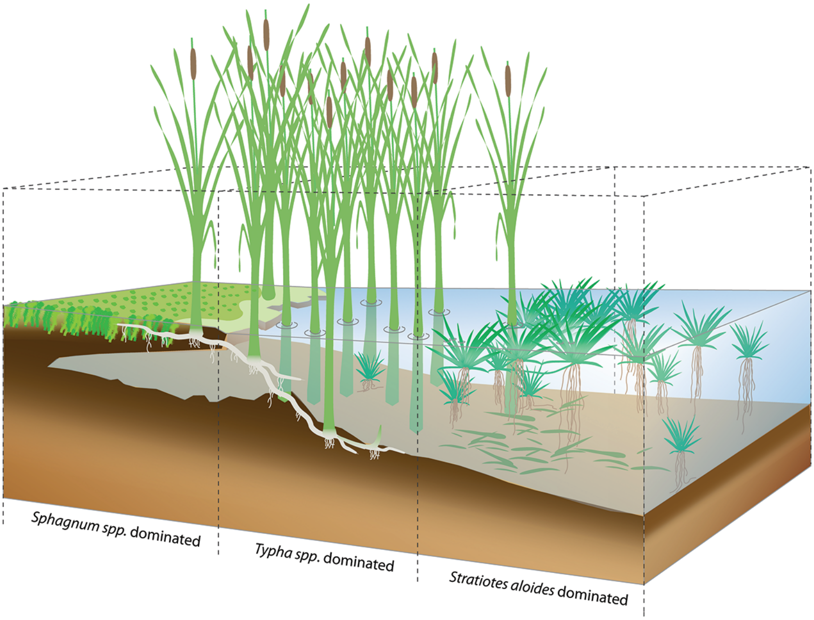


Figure 7.7. Schematic overview of one pathway of succession in the initial stages of peatland development. First, open water will be colonised by submerged plants (not shown). When these species have accumulated a thin organic layer, the ecosystem engineer *Stratiotes aloides* can enter the system. In newly constructed systems, this species will have to be introduced, since it mainly reproduces by vegetative growth and natural colonisation will be impossible without the presence of a source population nearby. Although a few scattered individuals will mainly remain submerged, intraspecific facilitation allows dense stands to become buoyant and form dense floating mats that can cover the entire water body. This mat supports high biodiversity, but also provides structure for floating rhizomes of helophytes, such as *Typha* spp. These fast growing helophytes can enter the system once the water layer is lowered below 80 cm, and support the formation of floating mats through their air-filled rhizomes. Due to the continued build-up of organic material, the influence of the surface water decreases and pioneer *Sphagnum* mosses, such as *S. squarrosum*, may colonise the mat. Due to active acidification and production of recalcitrant organic matter, *Sphagnum* species significantly contribute to the build-up of a peat layer. [Illustration by Gijs van Dijk].

Habitat constraints

The two case studies of the constructed wetland at the Volgermeerpolder and the rewetted agricultural peatland illustrate that environmental conditions strongly limit the choice of ecosystem engineers that can be used to facilitate or initiate peat formation. By taking habitat requirements of plant species into account, unsuccessful introductions (e.g. *Stratiotes aloides* in a low CO_2 environment or *Sphagnum* in a system flooded by HCO_3^--rich water) can be avoided. Alternatively, conditions can be modified beforehand (e.g. lowering NH_4^+ loads, applying organic or clay soils) to make an environment more suitable for target species. Still, often concessions have to be made and newly constructed wetlands that were designed to hold dense *Phragmites australis* stands will sometimes (initially) have to settle for highly productive *Typha*-dominated marshes.

Time constraints

The thick peat layers that exist throughout the world have been formed during thousands of years. The initial conditions, that stimulated the build-up of these peat layers in the first place most likely differed from modern climate conditions. Most modern peatlands have started to form during the early Holocene, when conditions were wetter and warmer than they are today (Jones and Yu 2010). Although ^{14}C dating can give insight into the age of peat layers and can thus estimate the accumulation rates, the duration of the initial phase of peat-formation is hard to assess, because thousands of years of compaction, decomposition and consolidation implies that a very thin layer will be left to represent this initial phase.

On the other hand, it has been shown that transitions from the aquatic to the terrestrial phase - and thus peat formation - can occur rapidly in peatlands (Bakker and others 1994; Crushell and others 2009; Granath and others 2010). For example, Bakker and others (1994) used aerial photographs taken between 1937 and 1989 to show that turbaries (peat extraction pits) could terrestrialise within 20 to 40 years. In this situation, however, the initial conditions provided a peat layer 0.8 to 1.5 m thick as a substrate for peat formation. They are, therefore, only comparable to restoration projects, such as the topsoil removal experiment that was discussed in Chapter 6. Here, *Sphagnum* species were shown to thrive on former agricultural peat soils after rewetting as long as the water quality was suitable. The contrasting examples of a new (Chapter 5) and a restored (Chapter 6) peatland imply that there is a huge difference between primary and secondary peat formation. While peat formation “from scratch” (on nutrient-poor sand) takes a long time when no additional measures are taken, restoration of peat formation in degraded systems could theoretically be achieved much faster. Still, both starting points have to be optimised to make the environment suitable for ecosystem engineers.

Everything has its price: investing in peat formation

Primary peat formation: adding substrate

To enhance the potential C sequestration rate of a new system, it is necessary to provide enough nutrients for optimal NPP and colonisation. In this thesis, it was shown that vegetation development can be strongly stimulated by applying an additional layer of organically rich soil (Chapter 5). This benefit may, however, be accompanied by enhanced nutrient or bicarbonate mobilisation into the water layer, thereby risking phytoplankton blooms (Chapter 5; Aerts and Toet (1997); Fiedler and Sommer (2000)) or the creation of an unsuitable habitat for HCO_3^--sensitive target species. Furthermore, introducing soils that are rich in labile organic matter may enhance CH_4 emission, because additional substrates are provided for methanogens (Updegraff and others 1995; Yavitt and others 1997; Rejmankova and Houdkova 2006), while the higher cover by *Typha* vegetation provides an additional pathway for CH_4 emission (Bubier 1995; Nykänen and others 1995; Bellisario and others 1999; Bastviken and others 2011). Thus, although the application of additional, richer soil layers strongly increases the litter production, the accompanying C efflux simultaneously leads to an initial decrease in the thickness of the organic matter layer. Furthermore, since CH_4 is a much stronger greenhouse gas than CO_2 (IPCC 2013), this enhanced CH_4 emission stimulates global warming.

Preferably, new wetlands should therefore receive soils that enhance nutrient availability to rooting plants, while having a low risk of both nutrient mobilisation (e.g. by having high Fe or Ca contents to bind P) and net C emission (e.g. by having a low labile organic matter content). For the construction of wetlands, however, large amounts of soil are required and the optimal soil may be unavailable or too expensive. It is therefore advisable to determine the quality of soils that are readily available for wetland construction, such as dredged soils or soils that become available during construction works. Although the application of such soils, with a higher nutrient and labile organic matter content, will initially lead to higher C emission, the organic matter in a thinly applied layer will deplete over time and such a system will eventually turn into a C sink (Brix and others 2001; Mitsch and others 2014). Furthermore, C emission may be decreased on a landscape scale when soils are recycled to stimulate vegetation development in constructed wetlands instead of being exposed to the atmosphere during storage (Chapter 5).

Secondary peat formation: removing nutrients

The majority of drained peatlands has been used for agriculture (Joosten 2009). Due to years of fertilisation, the topsoil of these systems has become loaded with P (Barberis and others 1996), which is immobilised in iron (Fe-) complexes under aerobic conditions (Smolders and others 2006; Smolders and others 2008; Lamers and others 2015). When the system is rewetted, oxygen intrusion into the soil is lowered and the bonds in Fe-P complexes are weakened, leading to mobilisation of P to the porewater and overlying surface water (Smolders and others 2006). Since such a system will be quickly dominated by phytoplankton in the aquatic zones (Chapter 6) and graminoids in the terrestrial zones (Berendse and others 2001; Smolders and others 2008; Aggenbach and others 2013), peat-forming vegetation, such as *Sphagnum* mosses will be out-competed. Therefore, the removal of the nutrient-rich topsoil should be considered before rewetting a system to restart peat-formation (Chapter 6). Although it seems counterintuitive to remove soil from an already subsiding system (Syvitski and others 2009) in order to restore peat formation, the lower nutrient conditions will prevent graminoids outcompeting *Sphagnum* mosses and thus stimulate new peat formation. Furthermore, since topsoil removal lowers the emission of CH_4 by 99%, much lower NPPs are necessary to tip the balance and turn the system into a net C sink. Since topsoil removal is a costly restoration measure, alternative approaches, such as paludiculture (Joosten and Clarke 2002; Verhoeven and Setter 2010), could also be pursued.

Stimulating production or lowering decomposition?

Peat formation occurs when the net primary production outweighs decomposition and partially decomposed litter accumulates (Saarnio and others 2007). To effectively manage systems in which peat formation should be (re)initiated, it is important to know whether measures should be aimed at maximising production or minimising decomposition. Although *Sphagnum* spp. are the most efficient peat-forming species, they will not grow on bare sand. First, either succession or human interference has to supply them with a substrate, such as a floating fen (Chapter 4; Tomassen and others (2004)). The main goal of initiating peat formation is to speed up the transition from pioneer to *Sphagnum*-dominated system. From our experiments, it can be derived that when a system still consists of bare sand and rainwater, the most important step is to increase vegetation cover, because open water or submerged vegetation do not contribute significantly to the fast build-up of an organic layer. Here, the application of a nutrient-rich layer would improve conditions for plant growth and stimulate the growth of e.g. *Typha* spp.

Subsequently, however, the decomposition rates determine whether the system will form a net C source or a net C sink. While stimulation of vegetation development requires nutrients, application of nutrient-rich soil is usually accompanied by the introduction of high labile organic matter contents, which stimulates decomposition. The subsequent increased CH_4 and CO_2 emission counteracts peat formation by decreasing the thickness of the layer of organic matter. Therefore, it appears that, after an initial period where a high NPP is important to reduce the area of open water, decomposition determines whether a system will be a net sink or source of C. This is also illustrated by the fact that systems with the highest build-up rates generally are ombrotrophic bogs on the northern hemispheres (Clymo 1983; Aerts and Toet 1997). Along the bog-fen-marsh gradient, decomposition and production rates increase, whereas actual peat build-up decreases (Thormann and others 1999).

Facilitating facilitators

Since the goal of this thesis was to study the initiation of peat formation, it aimed to do something that is virtually impossible. Namely, to study a process that occurs on a geological timescale, while working on a PhD timescale. This thesis shows, however, that by taking some -initially counterproductive- measures it is possible to facilitate ecosystem engineers and thereby speed up succession. Once their habitat requirements are met, these ecosystem engineers can have high production rates due to self-facilitating processes. Harnessing this facilitation is therefore highly important in both the construction of new wetlands and the reintroduction of species in restoration projects. As plant growth in general, and growth of peat-forming ecosystem engineers in particular, is difficult under the nutrient-poor, highly buffered conditions of a constructed wetland with sandy soils and rainwater, modifying habitat conditions to suit the requirements of target species and harnessing inter- and intraspecific facilitation is essential to transform such a system into a net C sink without having to wait a thousand years.

Chapter 8

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Chapter 9

Nederlandse samenvatting

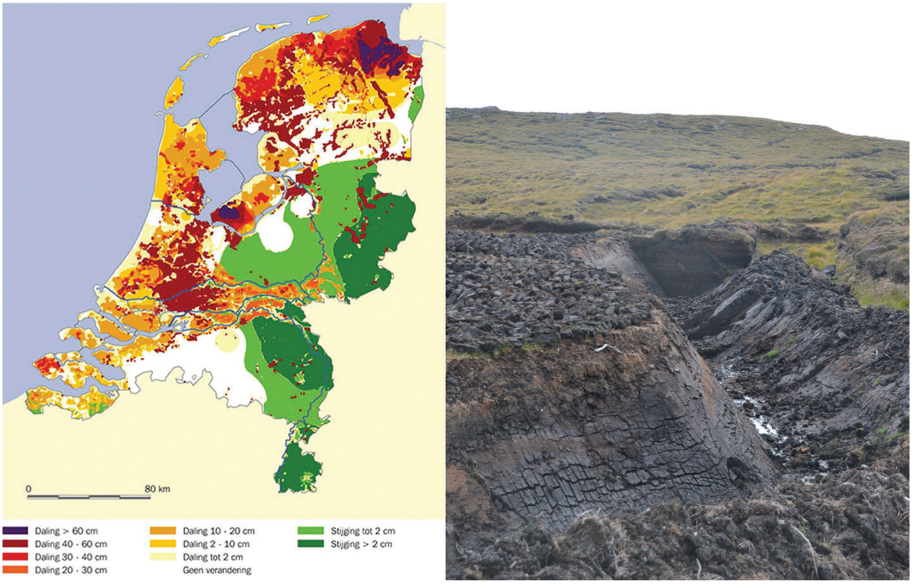
Veengroei

De titel van dit proefschrift luidt “Growing peat”, oftewel het (laten) groeien van veen. Veen is opgebouwd uit dode plantenresten en “groeit” dus als er meer plantenmateriaal geproduceerd wordt dan er door micro-organismen of schimmels afgebroken wordt. Dit proces verloopt echter erg langzaam en de dikte van veenpakketten neemt gemiddeld slechts toe met 1 tot 3 mm per jaar. De metersdikke veenpakketten die bijvoorbeeld grote delen van Canada, Siberië en Indonesië bedekken zijn dan ook over een periode van honderden of duizenden jaren gevormd. Vergeleken met de tijd die een onderzoeker gemiddeld heeft voor zijn of haar onderzoek (zo’n 4 jaar in dit geval), verloopt veenvorming dus uitermate traag.

Achteruitgang

Net als andere wetlands zijn veengebieden over de hele wereld aangetast door menselijke activiteiten. Veel veengebieden zijn bijvoorbeeld drooggelegd om landbouw te faciliteren. Verder is, vooral in vorige eeuwen, veel veen afgegraven om te gebruiken als brandstof (turf). Deze beide praktijken hebben grote invloed gehad op het functioneren van natuurlijke systemen, zoals te zien is in Figuur 9.1.

Bovendien worden de meeste systemen ook beïnvloed door vervuiling, vermesting en versnippering (habitat fragmentatie). Deze menselijke impact op veengebieden zorgt voor verminderde waarde van het landschap, aangezien natuurlijke functies wegvallen. Zo zorgt het droogleggen van veengebieden ervoor dat de bodem versneld afgebroken wordt door het binnendringen van zuurstof. Als gevolg daarvan komen er veel broeikasgassen vrij, wat de opwarming van de aarde stimuleert, en begint het land te zinken. Grote delen van Nederland liggen al ruim onder zeeniveau, wat een direct gevolg is van het droogleggen van veengebieden. Het gebruik van voormalig veengebieden als landbouwgrond, zorgt bovendien voor extra bemesting van deze gebieden, waardoor er veel nutriënten (voedingsstoffen) uitspoelen naar naastgelegen natuurgebieden. Hier profiteren meestal slechts enkele soorten van terwijl de rest weggeconcurreerd wordt, waardoor de biodiversiteit achteruit gaat.



Figuur 9.1. Droogleggen van veengebieden leidt tot bodemdaling. In Noord en West Nederland zakt de bodem gemiddeld met zo'n 30 tot 60 cm tussen 2000 en 2050 (links; Figuur van Rijkswaterstaat (1997)). Naast droogleggen, heeft ook het afgraven van veen, zoals hier in Ierland (rechts) duidelijk impact op het gebied.

Doordat natuurlijke veengebieden groeien door ophoping van organisch materiaal gaan ze niet alleen bodemdaling tegen, maar vormen ze ook een belangrijke "sink" voor koolstof aangezien de veenvormende planten over het algemeen meer koolstof vastleggen dan er vrijkomt door afbraak van organisch materiaal. Hoewel veengebieden slechts 3% van het landoppervlakte op aarde bedekken, bevatten ze ongeveer een derde van de totale hoeveelheid koolstof. Door deze belangrijke ecosysteem diensten die door veengebieden geleverd kunnen worden, is het van belang om veengebieden die achteruit gegaan zijn te herstellen of de ontwikkeling van nieuwe veengebieden te stimuleren. Het doel van dit onderzoek was daarom ook te onderzoeken welke milieuomstandigheden deze nieuwe of hernieuwde veenvorming stimuleren. Dit heb ik onderzocht door experimenten uit te voeren in het lab en op onze veldlocatie: de Volgermeerpolder (zie kader).

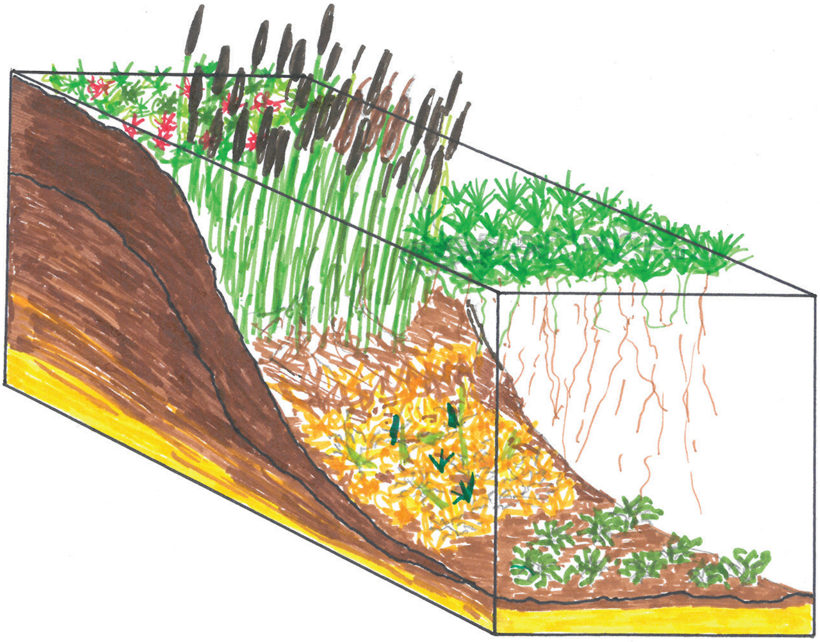
Onderzoeksgebied de Volgermeerpolder

De Volgermeerpolder, vlakbij Amsterdam, is een gebied met een roerig verleden. Zoals het grootste deel van Noord Holland, was dit gebied vroeger onderdeel van een aaneengesloten hoogveengebied, waar tot wel 10 meter veen opgestapeld lag. Dit veen is, eerst kleinschalig maar vervolgens op commerciële schaal, afgegraven om te gebruiken als turf. Op de Volgermeer begon de veenderij Zunderdorp met het grootschalig winnen van turf in 1927. De vraag naar turf liep echter snel terug en na de Tweede Wereldoorlog was de veenderij niet rendabel meer. Vandaar dat gezocht werd naar een nieuwe bron van inkomsten. Deze werd gevonden in het beschikbaar stellen van de Volgermeer als stortplaats. Vanaf de jaren ’50 werd met boten huisafval uit Amsterdam en omgeving naar de Volgermeer gebracht. Al snel werd er echter niet alleen huisafval, maar ook zwaar chemisch afval gestort. Na lang protest van omwonenden, georganiseerd in het Burgerkomitee Volgermeer, werd de stort gesloten in de jaren ’80. Het duurde echter tot 2003 tot daadwerkelijk werd overgegaan tot sanering van het gebied. In het uiteindelijke ontwerp werd de afvallaag afgedekt met synthetische folie en schoon zand, waarna een wetland gecreëerd werd in de stijl van Aziatische rijstvelden. In dit nieuwe wetland worden vervolgens de optimale condities onderzocht die moeten leiden tot veenvorming. Het uiteindelijke doel is een veenlaag te creëren waardoor de afvallaag ingekapseld wordt in veen, aangezien veen de eigenschap heeft om chemische verbindingen te binden.

Ecosystem Engineers

Aangezien veen is opgebouwd uit dode plantenresten, is het stimuleren van plantengroei de eerste stap in de overgang van open water naar een veenvormend gebied. Niet alle plantensoorten zijn even geschikte veenvormers. In dit proefschrift heb ik 3 soorten gekozen als modelsoort voor mijn experimenten. Deze soorten (krabbenscheer, lisdodde en veenmos) komen achtereenvolgens voor in de openwater fase, de moerasfase en de drijvend veen of verlandingsfase (zie Figuur

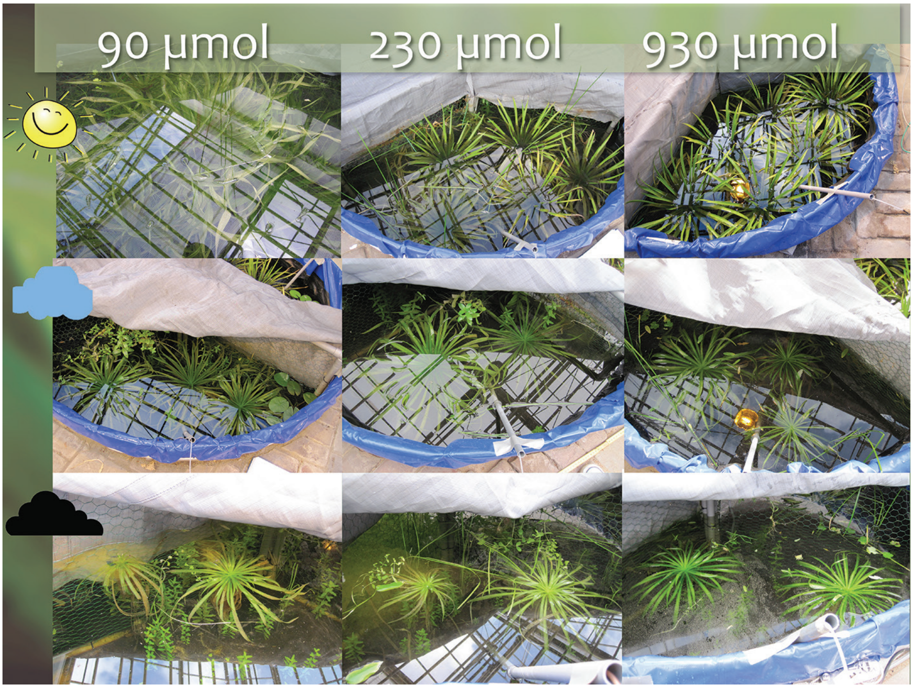
9.2). Zij hebben met elkaar gemeen, dat ze allen een belangrijke invloed uitoefenen op hun omgeving (het zijn zogenaamde "ecosystem engineers" of "biobouwers"), maar verschillen in hun habitat voorkeur.



Figuur 9.2. Overgang van open water naar land, met achtereenvolgens vegetatie van krabbenscheer (*Stratiotes aloides*), grote lisdodde (*Typha latifolia*) en veenmos (*Sphagnum*). Verlanding treedt op door ophoping van organisch materiaal van bijvoorbeeld krabbenscheer of lisdodde. Pas als de waterlaag vrijwel geheel opgevuld is of de bodem is komen opdrijven (drijftilvorming), kan het systeem gekoloniseerd worden door veenmossen. [Illustratie door R.D.A. Harpenslager]

Verlanding van open water begint met de kolonisatie door ondergedoken waterplanten, of drijvende planten zoals krabbenscheer. Deze soort heeft een afwisselende levenscyclus van ondergedoken en drijvende stadia: de planten beginnen te drijven in de lente, waarna ze een dichte, drijvende mat vormen in de zomer. In de herfst zinken alle planten weer naar de bodem, waar ze overwinteren voordat ze in de lente weer naar boven komen. Het vormen van een drijvende mat is een belangrijke stap in het verlandingsproces omdat in deze fase veel organisch materiaal wordt gevormd dat kan ophopen en omdat de mat structuur biedt voor verschillende andere planten- en diersoorten. Niet alle krabbenscheerpopulaties komen echter jaarlijks opdrijven. In hoofdstuk 2 en 3 laat ik enkele factoren zien die hiermee te maken hebben.

In de lente komen krabbenscheerplanten normaal gesproken opdrijven, omdat de zuurstof die door de planten geproduceerd wordt tijdens fotosynthese (het proces waarbij water en kooldioxide (CO_2) worden omgezet in zuurstof en suikers onder invloed van licht) wordt opgeslagen in bladeren met luchtholtes. Hierdoor worden de planten lichter dan water en gaan ze drijven. Als planten eenmaal drijven, kunnen ze het kooldioxide dat ze nodig hebben voor fotosynthese opnemen uit de lucht en hebben ze voldoende licht. Onder water is de beschikbaarheid van kooldioxide echter vaak beperkt en kunnen andere planten of algen licht wegnemen waardoor de fotosynthese afneemt. In hoofdstuk 2 heb ik laten zien dat planten daardoor niet of minder snel op komen drijven (zie Figuur 9.3). Bovendien nemen ze in plaats van kooldioxide een andere koolstofbron op (bicarbonaat; HCO_3), wat zorgt voor kalkneerslag op de bladeren van de plant.



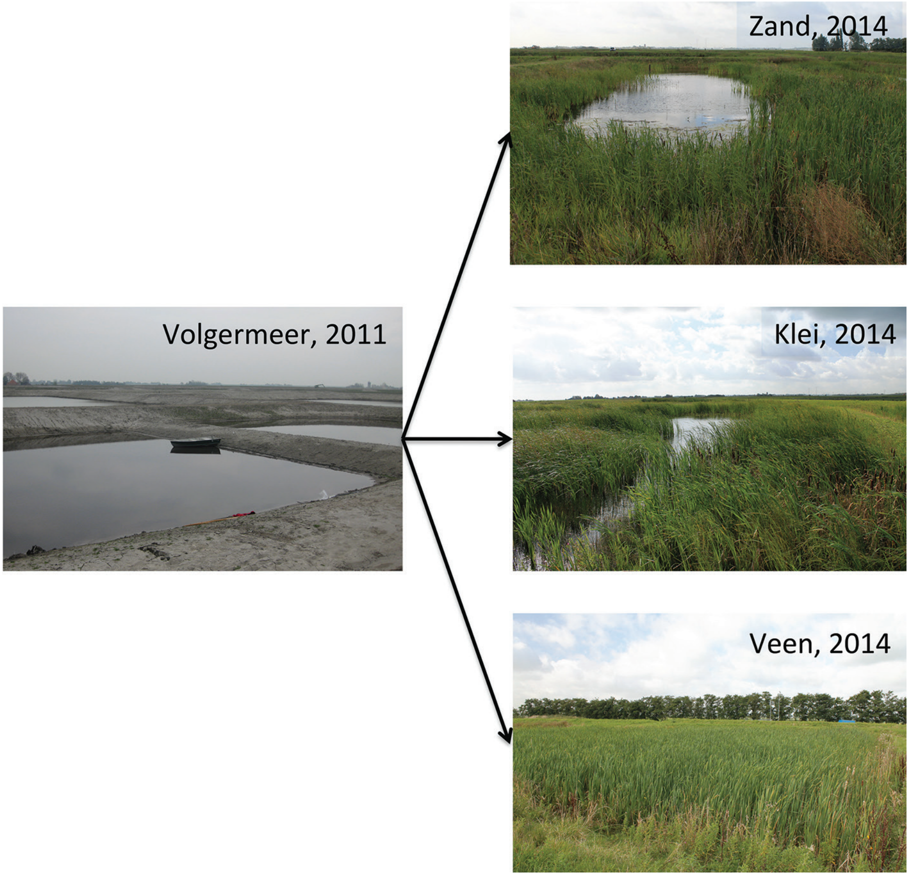
Figuur 9.3. Deze foto's laten zien dat krabbenscheer veel licht en kooldioxide nodig heeft om te gaan drijven. Planten op de bovenste rij foto's groeiden bij volledig licht, en zijn daardoor veel groter dan planten die groeiden bij 40% (middelste rij) of 10% (onderste rij) beschikbaar licht. De getallen boven de 3 kolommen met foto's geven weer hoeveel kooldioxide beschikbaar was voor de planten. Als planten minder dan 230 µmol L^{-1} kregen, groeiden ze langzamer en kwamen ze niet, of pas 6 tot 9 weken later opdrijven dan planten met voldoende kooldioxide.

Krabbenscheer kan extra kooldioxide tot zijn beschikking hebben, als er veel planten dicht op elkaar groeien. Onder deze dichte vegetatie hoopt kooldioxide op, zodat de planten makkelijker kunnen gaan drijven. Zo helpen ze zichzelf; een proces dat zelf-facilitatie heet. In hoofdstuk 3 wordt een experiment beschreven dat zelf-facilitatie bij krabbenscheer nader onderzocht. Hier vond ik niet alleen het positieve effect van ophoping van kooldioxide, maar ook dat de dichte vegetatie zorgde voor extra beschikbaarheid van fosfaat, een belangrijk nutriënt. Bovendien zijn dichte vegetaties robuuster wanneer er zich toxische stoffen in het milieu bevinden. Krabbenscheer is bijvoorbeeld erg gevoelig voor hoge beschikbaarheid van sulfide (wat een rotte eierenlucht veroorzaakt) of ammonium (dat veel uitgestoten wordt in landbouwgebieden). De zelf-facilitatie van deze soort geeft aan dat het belangrijk is om bijvoorbeeld bij introductie van deze soort in een nieuw aangelegd veengebied of een restoratie van een verslechterd gebied rekening te houden met dichtheid. Zo hebben wij deze soort op de Volgermeer aangebracht in kooien, zodat de planten een dichte vegetatie konden vormen en zo hun eigen groei konden faciliteren onder deze moeilijke omstandigheden.

In het laboratorium heb ik ook proeven uitgevoerd met de plantensoort die vooral bij latere stadia van veenvorming een rol speelt: veenmos of *Sphagnum*. Deze mossoorten zijn zeer efficiënte veenvormers, aangezien ze hun omgeving zodanig veranderen dat veenvorming gestimuleerd wordt. Zo houden ze hun omgeving vochtig door als een soort spons te fungeren, waardoor de invloed van zuurstof afneemt en de afbraak van organisch materiaal geremd wordt. Deze afbraak wordt nog verder geremd door het actief verzuren van de omgeving en het produceren van slecht afbreekbaar materiaal. Het nadeel is alleen dat veenmos niet zomaar in een pioniersgebied zal groeien, omdat het slecht tegen gebufferde en kalkrijke omstandigheden kan. Wij hebben daarom onderzocht of deze mossen wel in zo'n milieu kunnen groeien als ze aangeplant worden op een zogenaamde drijftil: een stuk drijvend veen. Dit veen kan drijven door de aanwezigheid van lucht-gevulde plantenwortels of de productie van methaan (bubbels). Door de mossen op zulke drijftillen te plaatsen vermindert de invloed van het harde, kalkrijke water. Daardoor kunnen sommige, tolerantere veenmossoorten (zoals haakveenmos) wel in een dergelijk milieu groeien, terwijl gevoelige soorten (zoals hoogveenveenmos) hier niet tegen kunnen (zie Figuur 9.4). Wel vindt er, onder invloed van het kalkrijke water, een chemische reactie plaats tussen bicarbonaat en het zuur dat door de mossen geproduceerd wordt, wat resulteert in de netto uitstoot van kooldioxide. Terwijl een "groeiend" veen normaal gesproken een netto vastlegging van koolstof laat zien, laat dit systeem dus een uitstoot zien, terwijl er wel ophoping van veenmosveen plaatsvindt.

Figuur 9.4. Deze foto laat zien hoe vier verschillende veenmossen op een drijvende bodem groeien. Terwijl hoogveenveenmos (linksboven) te gevoelig is voor het kalkrijke water en langzaam verdwijnt, groeien de andere drie veenmos soorten goed (met de klok mee: haakveenmos, gewoon veenmos en fraai veenmos). Haakveenmos (rechtsboven) breidt zich zelfs uit naar de compartimenten van de andere soorten en wint duidelijk de competitie onder deze milieuomstandigheden.

Zowel krabbenscheer als veenmos zijn, om verschillende redenen, gevoelig voor kalkrijke omstandigheden. Omdat de net aangelegde Volgermeerpolder enorm kalkrijk is, doen beide soorten het hier (nog) niet goed. Behalve de introductie van 50 krabbenscheerplanten per sawa (waarvan slechts enkele nog een gezonde krabbenscheer populatie bevatten), hebben we dan ook vooral de natuur haar gang laten gaan en gedurende 3 jaar de spontane ontwikkeling van planten gevolgd. Na 3 jaar blijkt dat sawa's, waarvan de bodem compleet bestaat uit zand, voor ongeveer 15% bedekt zijn door lisdodde en andere emergente waterplanten terwijl de overige 85% bedekt is met ondergedoken waterplanten (zie Figuur 9.5). Ondergedoken waterplanten worden snel afgebroken en dragen daarom weinig bij aan de veenvorming. Om het proces van verlanding te versnellen zijn er bij de aanleg van de Volgermeerpolder ook sawa's ingericht met een toplaag bestaande uit klei of veen. Omdat deze bodems meer voedingsstoffen voor planten bevatten, waren de sawa's met deze bodems na 3 jaar veel verder begroeid met emergente planten dan de sawa's met een zandbodem. Aanbrengen van een kleilaag leidde tot 40% bedekking door veenvormende planten na 3 jaar, terwijl veensawa's wel voor 70% bedekt waren na 3 jaar. Ondanks dat er uit deze aangebrachte bodems in eerste instantie meer koolstof vrijkomt dan er wordt opgeslagen door de afbraak van het aanwezige organische materiaal, is de stimulans voor de vegetatieontwikkeling van essentieel voor de uiteindelijke veenvorming.



Figuur 9.5. Vegetatieontwikkeling in de Volgermeerpolder. In maart 2011 bestond het gebied uit weinig meer dan kleidijkjes en water. In augustus 2014 waren er echter grote verschillen in bedekking tussen de sawa's met een zandbodem en sawa's met een extra laag klei of veen.

In een volledig leeg gebied, zoals de Volgermeerpolder, is het vooral erg moeilijk om plantengroei van de gewenste soorten op gang te brengen. In een veensysteem dat achteruit gegaan is door menselijke activiteiten spelen echter hele andere problemen. Een van deze problemen heb ik behandeld in hoofdstuk 6. Hier heb ik gekeken naar een verdroogd veensysteem dat opnieuw vernat werd zodat hier opnieuw een veenmosrietland kon ontstaan. Doordat het drooggelegde land lange tijd als landbouwgebied is gebruikt, is de 20 centimeter van de bodem verzadigd met voedingsstoffen. Na vernatting kunnen deze voedingsstoffen beschikbaar komen in het water en in de bodem en hier voor problemen zorgen zoals algengroei of vergrassing. Bovendien treedt er na vernatting een hoge uitstoot op van broeikasgassen, zoals methaan. Door vóór het vernatten eerst de voedingsrijke toplaag te verwijderen, kunnen deze problemen voorkomen worden.

Hoewel in dit laatste geval snelle veenvorming verwacht kan worden, omdat het gebied direct geschikt is voor efficiënte veenvormers zoals veenmossen, is het starten van veengroei op een kale ondergrond een behoorlijke uitdaging. Doordat het hele proces van veenvorming op hele andere tijdschaal plaatsvindt, is het onmogelijk om tijdens een promotieonderzoek al iets zinnigs te zeggen over de snelheid van veengroei en de verwachte veendikte op een locatie als de Volgermeerpolder. Wel heb ik laten zien dat, als er rekening gehouden wordt met de habitatvereisten van veenvormende planten en er wat simpele aanpassingen aan het milieu gedaan worden (zoals het inbrengen van voedselrijker materiaal), de ontwikkeling van een gebied versneld kan worden, zodat het niet nodig zal zijn om duizenden jaren te wachten.

Chapter 10

Dankwoord

Dankwoord

Ondanks dat er maar één naam op de omslag van dit boekje staat, was er nooit een pagina van geschreven zonder de voorbereidende, begeleidende, ondersteunende én afleidende rol van een grote en diverse groep mensen, die ik hier allemaal graag wil bedanken.

Allereerst wil ik mijn (co-)promotoren bedanken dat ze mij de kans hebben gegeven om dit leuke en afwisselende onderzoek te mogen uitvoeren én hierop te promoveren. Leon, jouw enthousiasme voor onderzoek is aanstekelijk! Na onze gesprekken was ik altijd dubbel gemotiveerd om weer aan de slag te gaan, ook al zag ik het daarvóór allemaal even niet meer zitten. Ik heb echt enorm veel van je geleerd in de afgelopen jaren. Bedankt voor het vertrouwen dat je in me hebt gehad! Jan (ook mijn promotor, wat de universiteit er ook van denkt), wil ik graag bedanken voor zijn deskundigheid en betrokkenheid bij het hele project, maar zeker ook voor de gezelligheid! Ook Fons wil ik graag bedanken voor zijn input in vrijwel alle experimenten die in dit proefschrift beschreven staan. Door jouw originele (en vaak kritische) blik ontstonden nuttige discussies, die de kwaliteit van zowel de experimenten als de papers sterk verbeterd hebben. Sarian, jij schoof wat later aan als co-promotor bij mijn onderzoek, maar juist jouw frisse “Wageningse”-blik was een welkome aanvulling op die van de Nijmegenaren. Ik heb veel van je geleerd over (het meten van) C fluxen. Bovendien vond ik het erg gezellig om mee te gaan naar het Shallow Lakes symposium (ook al was het in zo’n verschrikkelijk all-inclusive resort…).

Graag bedank ik ook de rest van het PeatCap team voor de fijne samenwerking. Ik wil Jos Verhoeven, Merel Soons, Wim Admiraal en Harm van der Geest hartelijk danken voor hun nuttige bijdragen aan de PeatCap meetings. Jos en Wim, bedankt dat jullie in mijn manuscriptcommissie wilden plaatsnemen. Ciska en Jeroen, ondanks onze slechte timing telkens (wie verzint het nou om waterdiepte te meten als het vriest…twee keer op rij!), heb ik (m.u.v. mijn vingertoppen) genoten van het gezamenlijke (veld)werk. De foto’s die gemaakt zijn tijdens de oogst spreken boekdelen! Jeroen, veel plezier met je verdere carrière bij Floron en Ciska, heel veel succes met jouw boekje!

Mijn dank gaat ook uit naar STW, de gemeente Amsterdam en het Burgerkomitee. Piet-Jan Westendorp van Witteveen & Bos vormde vaak een essentiële schakel tussen de onderzoekers en de gemeente en directe omgeving. Hartelijk dank daarvoor en veel succes in je nieuwe directeursfunctie bij B-Ware! Cees Loogman en Jan Melman dank ik voor hun praktische ondersteuning op de Volgermeerpolder.

Verder wil ik de leden van de gebruikerscommissie bedanken voor hun betrokkenheid bij het project gedurende de afgelopen jaren.

Als ik al het werk dat hier in dit boekje beschreven is alleen had moeten uitvoeren, dan zat ik nu nog ergens bedolven onder dozen gedroogd plantenmateriaal of flesjes bodemvocht. Gelukkig had ik een reeks enthousiaste studenten, die het aandurfden om bij mij hun stage te lopen. Olympe, Laury, Ariët, Tim, Mirte, Johan, en Stefan, bedankt (et merci) voor jullie hulp en gezelligheid in het veld en in het lab. Voor verdere hulp bij analyses (en ongehoorzame auto-analyzers) moest ik vaak terugvallen op de deskundigheid van Jelle Eygensteyn, Paul van der Ven en Sebastian Krosse. Hartelijk dank voor het meten van de grote hoeveelheden monsters en voor jullie geduldige uitleg. Ook de analisten Roy Peters, Martin Versteegh, Germa Verheggen, Ankie de Vries-Brock en Peter Cruijsen wil ik enorm bedanken voor al hun hulp bij veldwerk, het opzetten of afbreken van experimenten en verdere analyses. Hoofdstuk 2 en 3 van dit proefschrift beschrijven grote experimenten die zijn uitgevoerd in de (oude) kassen. Gerard, Harry, Walter en Yvette, ik wil jullie niet alleen bedanken voor het beschikbaar stellen van de faciliteiten van de kas, maar zeker ook voor de interesse die jullie toonden voor mijn onderzoek en voor de vele gezellige ~~koffie~~ theepauzes.

Ondanks dat ik niet zo'n fan ben van kanovaren (en kinderachtig genoeg ben om dat te laten merken), bedacht ik me tijdens ons recente uitje dat ik enorm bof met de leuke afdeling waar ik mijn onderzoek mocht doen. Het spreekt al boekdelen dat het me niet eens zo veel moeite kostte om de halve afdeling mee te laten helpen met de eindoogst op de Volgermeerpolder. Ik dank Marlous (kopje thee buurvrouw!?), Eva, Yingying, Ernandes, Ralph, Ralf, Annieke, Tjisse, Jeroen, Christian, Leon, Gijs (bedankt voor de fancy 3D plaatjes!), Moni, José, Laura, Monique, Valérie en de eerder genoemde analisten overigens zeker niet alleen voor hun hulp bij mijn (veld) werk, maar ook voor alle gezamenlijke lunchpauzes (en lunch walks), wetenschappelijke/ maatschappelijke/ onzinnige discussies, vrijdagmiddagborrels en spelletjesavonden. Bovendien werd het alleen maar gezelliger met (voormalig) collega-PhD's van andere afdelingen: Natan, Anne, Maartje, Dina, Nils, Qian, Marjolein, Onno, Caspar, Marco, Isabella, Marloes en Janneke. Bovendien dank ik José B. voor alle administratieve ondersteuning en haar luisterend oor op de vroege ochtend.

De afgelopen 5 jaar was ik niet alleen hard aan het werk op de afdeling ecologie, maar ook actief als vrijwilliger bij filmhuis Lux. Bij deze wil ik alle collega's daar bedanken voor de gezelligheid en broodnodige afleiding. Bovendien waren de “vaste” vrijdagavonden van de filmclub - in wisselende samenstelling - altijd erg gezellig! De groep “biologen” - Steven, Suzanne, Sanne, Karin, Karin en Tina - is ook altijd een belangrijke afleiding geweest, ook al woont inmiddels iedereen iets te ver uit elkaar voor regelmatige etentjes. Tina, ik vind het heel leuk dat je mijn paranimf wilt zijn!

Simone en Marianne: ik vind het geweldig dat wij al zo veel jaar vriendinnen zijn! Bedankt voor alle gezelligheid, steun (waar nodig) en uiteraard voor de jaarlijkse vakanties! Al onze mooie reizen waren voor mij essentieel om te ontstressen (dat dwangmatige plannen van mij is geen stress, maar enthousiasme!), en tegelijkertijd mijn geschiedeniskennis op te halen (Hoekse en Kabeljauwse twisten waren van 1345 tot 1500; maar is dat vóór of na het uitvinden van het haringkaken?). En Marianne, heel erg leuk dat ook jij mijn paranimf wilt zijn! Tot slot dank ik mijn familie. Thomas en Hana, papa en mama: bedankt dat jullie er altijd voor me zijn!

Chapter 11

Curriculum vitae

Curriculum vitae

On the 7th of May 1987, I was born in Arnhem, the Netherlands. I grew up there and attended primary school and pre-university secondary education. After receiving my gymnasium diploma in 2005, I decided to study biology at Radboud University in Nijmegen. During the final year of my bachelor's, I did an internship at the department of Aquatic Ecology and Environmental Biology, in which I studied the effects of high-density growth of submerged macrophytes on P mobilisation from peat sediments. I received my bachelor degree in 2008 and, after spending three months as a teaching assistant, I continued with the master's programme. During this time I did two, very different internships. During my first MSc. internship, I was part of the re-introduction programme of the Common Hamster (*Cricetus cricetus*) in the Netherlands. Apart from studying breeding success of wild and captive-bred common hamsters in the Netherlands, I successfully followed a course on laboratory animal science (receiving a certificate to legally perform animal experiments in the Netherlands) and presented the results of my internship at an international conference (2009; Ranis, Germany) for the first time. For my second internship, I went to the University of Copenhagen, where I studied the effects of desiccation and/or organic matter removal on sediment and water chemistry in two Danish lakes at the Freshwater Biological Laboratory in Hillerød. After returning to the Netherlands, I started working as a Junior Researcher at B-Ware Research Centre for three months, where I worked on an experiment studying the effect of liming on sediments from Norwegian softwater lakes. In March 2011, I started working as a PhD candidate on the PeatCap project, the result of which you are reading right now. From June 2015 onwards, I have been working as a researcher on the Rich Water World project, which focuses on the use of alternative constructed wetlands for nutrient removal and recycling.

Publications and symposia

Publications

- **Harpenslager, S.F.**, Van den Elzen, E., Kox, M.A.R., Smolders, A.J.P., Ettwig, K.F. and Lamers, L.P.M. “Topsoil removal as a prerequisite to avoid strong nutrient and greenhouse gas emissions”, 2015, Ecological Engineering 84, p. 159-168
- **Harpenslager, S.F.**, Van Dijk, G., Kosten, S., Roelofs, J.G.M., Smolders, A.J.P and Lamers, L.P.M. “Simultaneous high C fixation and high C emissions in *Sphagnum* mires”, 2015, Biogeosciences 12, p. 4739-4749
- **Harpenslager, S.F.**, Smolders, A.J.P., Kieskamp, A.M., Roelofs, J.G.M. and Lamers, L.P.M. “To float or not to float: how interactions between light and dissolved inorganic carbon species determine the buoyancy of *Stratiotes aloides*”, 2015, PLOSONE 10, issue 4
- **Harpenslager, S.F.**, Lamers, L.P.M., Van der Heide, T., Roelofs, J.G.M. and Smolders, A.J.P. “Harnessing facilitation: Why successful re-introduction of *Stratiotes aloides* requires high densities under high nitrogen loading”, *under review*
- **Harpenslager, S.F.**, Overbeek, C.C., van Zuidam, J.P., Roelofs, J.G.M., Kosten, S. and Lamers, L.P.M. “Peat capping: natural capping of wet landfills by peat formation”, *submitted*

International symposia

- Temmink, R.J.M., Van Kempen, M.M.L., Tang, Y., **Harpenslager, S.F.**, Smolders, A.J.P., Roelofs, J.G.M. and Lamers, L.P.M., 2015, “Rich Water World: constructing a novel type of wetland to support self-sustaining regions”, Poster presentation, Wetlands in agricultural landscapes: present state and perspectives in Europe’, 11 - 16 October 2015, České Budějovice, Czech Republic
- **Harpenslager, S.F.**, Van den Elzen, E., Kox, M.A.R., Smolders, A.J.P., Ettwig, K.F. and Lamers, L.P.M., 2015, “Topsoil removal as a prerequisite to avoid strong nutrient and greenhouse gas emissions”, Oral presentation, Society for Wetland Scientists Symposium, 31 May - 4 June 2015, Providence, RI, USA
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- **Harpenslager, S.F.**, Smolders, A.J.P., Kieskamp, A.M., Roelofs, J.G.M. and Lamers, L.P.M. “To Float or Not to Float; How biogeochemical factors influence the buoyancy of foundation species *Stratiotes aloides*, Oral presentation, Shallow Lakes Symposium, 12-17 October 2014, Antalya, Turkey
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