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

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Abstract

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 the 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.
1. 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 et al., 2003; Bortoluzzi et al., 2006; Golovatskaya and Dyukarev, 2009; Rowson et al., 2010). Although it is well known that degraded and drained peatlands generally are net C sources due to increased decomposition rates (Alm et al., 1999; Waddington et al., 2001; Moore, 2002) - with net emissions ranging from +80 to +880 g C m$^{-2}$ y$^{-1}$ (Lamers et al., 2015); With 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 et al., 2007), and second, not all pristine peatlands appear to be sinks of carbon dioxide (CO$_2$) (Waddington and Roulet, 2000; Riutta et al., 2007). For groundwater or surface-water fed (minerotrophic) fens, CO$_2$ fluxes have been reported to range from -208 to +190 g C m$^{-2}$ y$^{-1}$ (Martikainen et al., 1995; Carroll and Crill, 1997; Bubier et al., 2003), whereas for transitional mires fluxes of -124 to +58 g C m$^{-2}$ y$^{-1}$ have been reported (Moore and Knowles, 1987; Koch et al., 2008; Salm et al., 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 et al., 1999; Smolders et al., 2002). Since they increase habitat heterogeneity at various scales, these intermediate peatland systems often form hotspots of biodiversity (Verberk et al., 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 (HCO₃⁻) 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 HCO₃⁻-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 et al., 1999; Smolders et al., 2003)

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 et al., 2000; Freeman et al., 2001). 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 et al., 1990; Limpens and Berendse, 2003) and drought-tolerance (Nijp et al., 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 that 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 amount 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.

2. Material and Methods

2.1 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 (N 51°24′6.1″ E 6°11′10.5″) 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 minimize 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 HNO₃ and 1 ml of H₂O₂ 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 Fischer scientific)).

In the laboratory, 6.25 l of Ca - HCO₃⁻-rich treatment water was added to each aquarium (Table 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, II, USA). All floating peat monoliths received artificial rainwater (Table 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 pH 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 et al., 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 et al., 2006). The first three species were collected in a peatland area in the north-western part of the Netherlands (Ilperveld; N52°26'42.5", E4°55’45.1”), while the latter species was collected in an area in the south of the Netherlands (Maasduinen; N51°34’56.3", E6°6’13.5”).

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 cm². The remaining 4 floating peat monoliths were kept as non-vegetated controls.

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.
2.2 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 Research, Beverly, CA, USA) 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&Lubbe, Norderstedt, Germany) using ammonium molybdate (Henriksen, 1965), hydrazine sulphate (Kamphake et al., 1967) and salicylate (Grasshof and Johannse, 1972) respectively. Concentrations of Ca, Fe, K, Mg, total-P and SO$_4$ were analysed by inductively coupled plasma spectrometry (ICP-OES icap 6000; Thermo Fischer scientific).

2.3 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, USA) and Ag/AgCl pH electrode (Orion 9156BNPW, Thermo Fisher Scientific, Waltham, MA, USA). 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.).

2.4 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 under both light and dark conditions were therefore only measured from soils covered with *S. squarrosum, S. palustre* or *S. fallax* and from bare control soils, using transparent and dark closed chambers (10 cm, 10 cm and 12 cm for length, width and height) 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 CO₂ using an IRGA (ABB Analytical, Frankfurt, Germany) and for CH₄ using a
Gas Chromatograph (5890 GC, Hewlett Packard, Wilmington, DE, USA). 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 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.

### 2.5 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 (Fig. 1, Fig. 2, Table 3) and C fluxes were tested using one-way ANOVAs with Tukey Post Hoc. In all tables and figures, averages are presented with standard error of the mean (SEM). All statistical analyses were carried out using SPSS for Mac (V21, IBM Statistics).
3. Results

3.1 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. 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 2). Still, concentrations of NO\(_3^-\) were low, since N was predominantly present as NH\(_4^+\) in both infiltrating water and pore water (Table 2). Overall, NH\(_4^+\) concentrations in the infiltrating water were high compared to similar systems (e.g. Kooijman and Bakker (1994)), with values around 40 µmol l\(^{-1}\) (Table 2).

3.2 Sphagnum growth and acidification

*S. squarrosum*, *S. fallax* and *S. palustre* increased their biomass during the experiment (Fig. 1). Initial biomass of these species was 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 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\% (Fig. 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 \((P=0.001; \text{Table 3})\), 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 (Fig. 1) and number of capitula (Fig. 2) during the experiment. *S. squarrosum* acidified its environment most, with pH values down to 4.5 (*P* = 0.027; Table 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 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 1).

### 3.3 Carbon exchange

Bare floating peat had a C emission of 0.3±0.1 g C m$^{-2}$ d$^{-1}$ (Fig. 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 (Fig. 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 gCO$_2$-eq m$^{-2}$ y$^{-1}$) than for plots covered by other species (580±106 gCO$_2$-eq m$^{-2}$ y$^{-1}$) or unvegetated (550±180 gCO$_2$-eq m$^{-2}$ y$^{-1}$) plots due to the higher CO$_2$ emission from these plots ($P=0.002$; data not shown).

4. Discussion

For $Sphagnum$ species growing on top of floating peat monoliths, the influence of infiltration of groundwater or surface water, buffered by Ca$^{2+}$ - HCO$_3^-$, into the peat was shown to be reduced, and even moderately sensitive species were capable of growing under these conditions. It was remarkable, however, to discover that while some of these species strongly increased their biomass, $Sphagnum$-covered patches simultaneously showed a net C efflux.

4.1 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 are released into a system, they are initially buffered by the HCO$_3^-$ - CO$_2$ buffering system (Sherlock et al., 1995; Lamers et al., 2015). Once most of the HCO$_3^-$ has been consumed, protons (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 et al., 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 or surface water due to Ca - HCO\(_3^-\) toxicity (Clymo, 1973; Andrus, 1986; Lamers et al., 1999; Hajek et al., 2006). This sensitivity to one or both of the major buffering components of groundwater or surface water is species-specific and it strongly affected the performance of the *Sphagnum* species in our study. *S. magellanicum* appeared to be 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 et al., 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 et al., 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 et al., 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).

### 4.2 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 et al., 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 acid, poor fen within a few decades (Granath et al., 2010). *S. squarrosum* may act as such a pioneer species and is often responsible for rapid succession in fens (Giller and Wheeler, 1988; Haraguchi et al., 2003), especially under nutrient rich conditions (Kooijman and Bakker, 1995).

Our data confirms 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 yr\(^{-1}\) (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.

### 4.3 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\(^{-2}\) yr\(^{-1}\) (Gerdol, 1995; Graf and Rochefort, 2009; Hajek, 2009; Samaritani et al., 2011), which corresponds to a CO\(_2\) fixation rate of approximately 28 to 240 g C m\(^{-2}\) yr\(^{-1}\).

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\(^{-2}\) yr\(^{-1}\). 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\(^{-1}\) (Fig. 4), which consisted for 98% of CO\(_2\) and 2% of 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 (e.g. Salm, Barlett & Harris, Saarnio, Byrne 2004). 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 (Fig. 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 R. (1) that occurs when HCO\(_3^-\)-rich water comes into contact with the acidifying mosses (Fig. 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 et al., 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.

\[
\text{HCO}_3^- + \text{H}^+ \rightarrow \text{H}_2\text{O} + \text{CO}_2 \quad \text{R. 1}
\]

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. 1; Table 4). Net CO\(_2\) fixation was estimated based on the difference between light and dark CO\(_2\) fluxes, whereas
CO₂ emission was estimated based on dark fluxes. This CO₂ emission can be further divided into separate contributors, as is shown in Eq. 1.

\[
\text{Net C flux to atmosphere} = B + R + C - F \quad \text{Eq. 1}
\]

Here, B represents the CO₂ flux from bare peat to the atmosphere, \( R \) is the dark plant respiration. \( C \) represents the flux of chemically produced CO₂ according to R. 1 and F is the gross CO₂ fixation, calculated as the light CO₂ flux minus the dark CO₂ flux. Bare peat respiration was derived from dark fluxes of non-vegetated plots. For the \( \text{Sphagnum} \) respiration factor R, we have used the maximum value (31%) from the range reported in literature (12 to 31% of photosynthetic C fixation, (Haraguchi et al., 2003; Laine et al., 2011; Kangas et al., 2014)). As a result, we obtain a conservative estimate of the C flux emitted through the chemical reaction R. 1, driven by the acid production of the \( \text{Sphagnum} \) mosses (C). Although the likely higher respiration rates during the light period and the use of estimated \( \text{Sphagnum} \) respiration impede an exact quantification of factor C, the fact that we find CO₂ emissions during the light period in growing \( \text{Sphagnum} \) patches clearly points out that there is a considerable chemical CO₂ source.

Table 4 shows the different sources of the C fluxes as presented in Eq.1. 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 HCO₃⁻ rich water (Figure 4). Here, we show simultaneous C fixation and C emission of the three growing \( \text{Sphagnum} \) species from our experiment, with their different growth and acidification rates.
The production of HCO$_3^-$-derived CO$_2$ will occur in any situation where HCO$_3^-$-rich water comes into contact with an acid environment, such as in the highly acidic lower layers of floating bog systems influenced by HCO$_3^-$-rich water (Lamers et al., 1999; Smolders et al., 2003). 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. 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 0.2-1.1 g C m$^{-2}$ d$^{-1}$ (Table 4, Figure 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 et al., 2008; Salm et al., 2009). As mentioned before, however, this phenomenon may not be limited to transitional mires. For example, bogs typically show an outflow of acid 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.
5. Conclusion

To obtain 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 concur with a net emission of carbon, 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 0.8-1.4 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.
Author Contributions

The experiment presented in this paper was designed by SH, GvD and LL and carried out by SH and GvD. Figure 4 was designed by GvD, based on input provided by SH. The manuscript was prepared by SH, with contributions of all co-authors.
Acknowledgements

The authors would like to thank Paul van der Ven and Roy Peters for their help with the chemical analyses and Laury Loeffen, Tim ten Broeke and Ariët Kieskamp for their help with setting up the experiment, sampling and daily maintenance. We thank the owners of the field locations, Stichting het Limburgs Landschap and Stichting Landschap Noord-Holland, for their kind permission to collect floating peat and Sphagnum mosses. This experiment was part of a project financed by the Dutch Technology Foundation STW (PeatCap; grant number 11264) and the Municipality of Amsterdam. SK was supported by NWO-VENI grant 86312012.
References


**Table 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\(^{-1}\).

<table>
<thead>
<tr>
<th></th>
<th>Infiltrating water</th>
<th>Artificial rainwater</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCO(_3)(^-)</td>
<td>3000</td>
<td>-</td>
</tr>
<tr>
<td>SO(_4)(^2-)</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>8000</td>
<td>54</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>2000</td>
<td>17</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>2000</td>
<td>-</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>3000</td>
<td>-</td>
</tr>
<tr>
<td>K(^+)</td>
<td>200</td>
<td>20</td>
</tr>
<tr>
<td>NH(_4)(^+)</td>
<td>-</td>
<td>36</td>
</tr>
<tr>
<td>NO(_3)(^-)</td>
<td>-</td>
<td>36</td>
</tr>
<tr>
<td>Sea salt (mg l(^{-1}))(^1)</td>
<td>-</td>
<td>5</td>
</tr>
</tbody>
</table>

\(^1\) Pro Reef, Tropic Marine, aQua united LTD, Telgte, Germany.
Table 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 (LMM). When these LMM showed significant differences between moss covered and bare soils, values are given in bold.

<table>
<thead>
<tr>
<th></th>
<th>Infiltrating water</th>
<th>Pore water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With moss</td>
<td>Without moss</td>
</tr>
<tr>
<td>pH</td>
<td>6.3±0.1</td>
<td>6.6±0.0</td>
</tr>
<tr>
<td>TIC</td>
<td>2014±268</td>
<td>1965±294</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>859±318</td>
<td>1158±251</td>
</tr>
<tr>
<td>CO₂</td>
<td>1154±182</td>
<td>810±278</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>1.0±0.3</td>
<td>0.5±0.2</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>38±14</td>
<td>46±9</td>
</tr>
<tr>
<td>Total-P</td>
<td>6.2±1.4</td>
<td>6.5±2.2</td>
</tr>
<tr>
<td>Ca</td>
<td>1413±86</td>
<td>1366±97</td>
</tr>
<tr>
<td>Fe</td>
<td>99±8</td>
<td>63±21</td>
</tr>
<tr>
<td>K</td>
<td>284±70</td>
<td>228±67</td>
</tr>
<tr>
<td>Mg</td>
<td>1823±118</td>
<td>1738±123</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>92±13</td>
<td>117±26</td>
</tr>
</tbody>
</table>
Table 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 and significant differences between species are represented by different capital letters.

<table>
<thead>
<tr>
<th></th>
<th><em>S. squarrosum</em></th>
<th><em>S. fallax</em></th>
<th><em>S. palustre</em></th>
<th><em>S. magellanicum</em></th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH</strong></td>
<td>4.5±0.2&lt;sup&gt;A&lt;/sup&gt;</td>
<td>5.4±0.3&lt;sup&gt;A,B&lt;/sup&gt;</td>
<td>5.2±0.4&lt;sup&gt;B&lt;/sup&gt;</td>
<td>6.1±0.4&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.027</td>
</tr>
<tr>
<td><strong>Final length (mm)</strong></td>
<td>85.9±4.0&lt;sup&gt;H&lt;/sup&gt;</td>
<td>66.1±7.4&lt;sup&gt;A,B&lt;/sup&gt;</td>
<td>70.4±7.9&lt;sup&gt;H&lt;/sup&gt;</td>
<td>42.1±1.7&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Final cover (cm&lt;sup&gt;2&lt;/sup&gt;)</strong></td>
<td>209±28&lt;sup&gt;H&lt;/sup&gt;</td>
<td>120±11&lt;sup&gt;A&lt;/sup&gt;</td>
<td>111±9&lt;sup&gt;A&lt;/sup&gt;</td>
<td>75±10&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>C: N ratio (g g&lt;sup&gt;-1&lt;/sup&gt;)</strong></td>
<td>27.0±1.3</td>
<td>25.3±3.6</td>
<td>25.2±3.2</td>
<td>25.2±1.7</td>
<td>ns</td>
</tr>
</tbody>
</table>
**Table 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. 1, with the fraction of autotrophic respiration based on the maximum value found for *Sphagnum* respiration in literature (31%, Laine et al. (2011)).

<table>
<thead>
<tr>
<th>Species</th>
<th>Net C flux (B+C-F)</th>
<th>Gross C Fixation (F)</th>
<th>Gross C emission (B+R+C)</th>
<th>Bare Peat (B)</th>
<th>Autotrophic respiration (R)</th>
<th>Additional HCO$_3^-$ derived CO$_2$ (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. squarrosum</td>
<td>1.1±0.2</td>
<td>1.9±0.5</td>
<td>3.0±0.7</td>
<td>0.3±0.1</td>
<td>0.6</td>
<td>2.1±0.7</td>
</tr>
<tr>
<td>S. fallax</td>
<td>0.5±0.1</td>
<td>0.9±0.2</td>
<td>1.5±0.2</td>
<td>0.3±0.1</td>
<td>0.3</td>
<td>0.9±0.2</td>
</tr>
<tr>
<td>S. palustre</td>
<td>0.2±0.1</td>
<td>0.4±0.1</td>
<td>0.6±0.3</td>
<td>0.3±0.1</td>
<td>0.1</td>
<td>0.2±0.3</td>
</tr>
</tbody>
</table>
Captions to Figures

**Fig. 1**: The 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 ($P=0.005$).

**Fig. 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 markers 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 ($P=0.002$).

**Fig. 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 ($P=0.012$).

**Fig. 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.
Supplementary Fig. 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 ● = *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.