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

S. F. Harpenslager[1], **G. van Dijk**[1,2], **S. Kosten**[1], **J. G. M. Roelofs**[1],
A. J. P. Smolders[1,2], **and L. P. M. Lamers**[1]

[1]Department of Aquatic Ecology and Environmental Biology, Institute for Water and Wetland Research, Radboud University, Heyendaalseweg 135, 6525AJ, Nijmegen, the Netherlands
[2]B-Ware Research Centre, Toernooiveld 1, 6525ED, Nijmegen, the Netherlands

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Correspondence to: S. F. Harpenslager (s.harpenslager@science.ru.nl)

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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 $\mathrm{g\,C\,m^{-2}\,y^{-1}}$ (Lamers et al., 2015) – 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 $\mathrm{g\,C\,m^{-2}\,y^{-1}}$ (Martikainen et al., 1995; Carroll and Crill, 1997; Bubier et al., 2003), whereas for transitional mires fluxes of −124 to +58 $\mathrm{g\,C\,m^{-2}\,y^{-1}}$ 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 (HCO_3^-) in pore water and surface water (Clymo, 1973). As *Sphagnum* spp. lack stomata, water conducting tissue and roots, they are strongly influenced by 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 but never completely flooded by buffered water (Lamers et al., 1999; Smolders et al., 2003) and the direct influence of buffered surface water in the moss layer is therefore relatively low.

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 C sequestration of these systems.

2 Material and methods

2.1 Experimental set-up

Intact floating peat monoliths (25 cm × 25 cm; height 21.85 ± 2.08 cm) 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 ($n = 8$). 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 cm × 25 cm × 30 cm; length × width × height) in the field to minimize damage to the peat structure. The peat had an organic matter content of 92.7 ± 0.4 % and contained 3.6 ± 0.4 mmol kg^{-1} fresh weight (FW) of Ca.

In the laboratory, 6.25 L of Ca-HCO_3^--rich treatment water was added to each aquarium (Table 1), on which the peat floated. The underlying water layer was subsequently flushed through the aquaria at a rate of 5 L week^{-1} using peristaltic pumps (Masterflex L/S, Cole-Parmer, Vernon Hills, Il, 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 using a cryostat (NESLAB, Thermoflex 1400, Breda, the Netherlands). Furthermore, a light regime of 200 µmol m^{-2} s^{-1} (PAR; 16 h light/8 h dark) was maintained (Master Son-T Pia Plus, Philips, Eindhoven, the Netherlands).

On four floating peat monoliths, four different species of *Sphagnum* (*Sphagnum. squarrosum*, *S. fallax*, *S. palustre* and *S. magellanicum*) were planted. *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; 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.

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 + Luebbe, 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 h 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, USA).

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, 10 and 12 cm for length, width and height) respectively. Dark measurements started at the end of the 8 h 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 h 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, 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 expand vertically, whereas in our experiment – due to absence of supporting neighbouring mosses – expansion occurred partly horizontally. Our areal C fluxes measured with the closed chambers are therefore underestimates. We corrected for this by multiplying the areal C fluxes with the actual area to original area ratio.

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 (Figs. 1 and 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 $40\,\mu mol\,L^{-1}$ (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 ($P = 0.001$; 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).

3.3 Carbon exchange

Bare floating peat had a C emission of $0.3 \pm 0.1\,g\,C\,m^{-2}\,d^{-1}$ (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 $1.1 \pm 0.2\,g\,C\,m^{-2}\,d^{-1}$ ($P < 0.001$), 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 $0.2 \pm 0.1\,g\,C\,m^{-2}\,d^{-1}$ 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 2.3 % to net C emission.

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 $\mu mol\,L^{-1}$) (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 $\mu mol\,L^{-1}$ (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* can potentially act as a foundation species for other *Sphagnum* spp., since it 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 70–600 g DW m^{-2} yr^{-1} (Gerdol, 1995; Graf and Rochefort, 2009; Hajek, 2009; Samaritani et al., 2011), which corresponds to a C fixation rate of approximately 28–240 g C m^{-2} yr^{-1}. If we extrapolate the daily C 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 C-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 C-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). When vegetated, however, C emissions increased, despite the accumulation of biomass by all three species (Fig. 4), which indicates that the source of this C could not solely be the decomposition of *Sphagnum* litter. The only likely explanation for this remaining net C efflux is therefore the chemical Reaction (R1) 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*.

$$HCO_3^- + H^+ \rightarrow H_2O + CO_2 \qquad \text{(R1)}$$

To further disentangle the different C sources responsible for the net C emission from plots vegetated with different species, we used a mass approach (Eq. 1; Table 4). C fixation could be determined from the difference between day and night C fluxes, whereas night fluxes determined C emission. This emission could then be divided into the known factor of the bare peat respiration (*B*) and an unknown factor. The unknown factor in this equation is the CO_2 production through the conversion of HCO_3^- (Reaction R1), driven by the acid production of the *Sphagnum* mosses (C).

$$\text{Net C flux to atmosphere} = B + C - F \qquad (1)$$

Here, *B* represents the C flux from bare peat to the atmosphere, *C* is the flux of chemically produced CO_2 according to Reaction (R1) and *F* is the net fixation, calculated as the night-time C flux minus the day-time C flux.

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, C 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^-. 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). 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). So far, however, only few field measurements of C fluxes in transitional mires have been published, mostly without sufficient biogeochemical and/or hydrological information providing a mechanistic explanation for the observed fluxes.

5 Conclusion

To obtain insight into the processes driving the highly variable C-fluxes measured in *Sphagnum* 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 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 S. F. Harpenslager, G. van Dijk and L. P. M. Lamers and carried out by S. F. Harpenslager and G. van Dijk. Figure 4 was designed by G. van Dijk, based on input provided by S. F. Harpenslager. The manuscript was prepared by S. F. Harpenslager, with contributions of all co-authors.

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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}.

	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.

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.

	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**
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

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 (A < B).

	S. squarrosum	*S. fallax*	*S. palustre*	*S. magellanicum*	*P*
pH	4.5 ± 0.2^{A}	$5.4 \pm 0.3^{A,B}$	5.2 ± 0.4^{B}	6.1 ± 0.4^{B}	0.027
Final length (mm)	$85.9 \pm 4.^{B}$	$66.1 \pm 7.4^{A,B}$	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

Table 4. Origin and rates of C fluxes (in $\mathrm{g\,C\,m^{-2}\,d^{-1}}$) of peat covered with different species of *Sphagnum*. Net C fluxes, 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).

	Net C flux ($B+C-F$)	C Fixation (F)	Gross C emission ($B+C$)	Bare peat (B)	Additional $\mathrm{HCO_3^-}$ derived $\mathrm{CO_2}$ (C)
S. squarrosum	1.1 ± 0.2	1.9 ± 05	3.0 ± 0.7	0.3 ± 0.1	2.7 ± 0.7
S. fallax	0.5 ± 0.1	0.9 ± 0.2	1.5 ± 0.2	0.3 ± 0.1	1.2 ± 0.2
S. palustre	0.2 ± 0.1	0.4 ± 0.1	0.6 ± 0.3	0.3 ± 0.1	0.3 ± 0.3

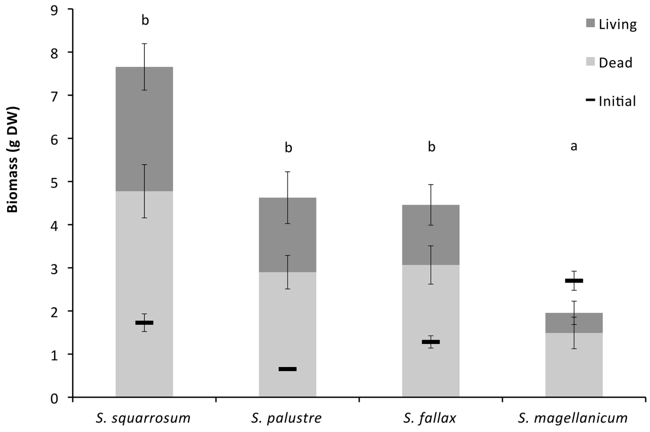

Figure 1. The biomass dry weight (DW, ± SEM) produced by a patch of 50 $\mathrm{cm^2}$ of four different *Sphagnum* species after 12 weeks of experimental treatments, divided into living and dead tissue (dark and light bars respectively). The smaller black bars represent initial DW. Significant differences between total final biomass of the species are indicated by different letters (P = 0.005).

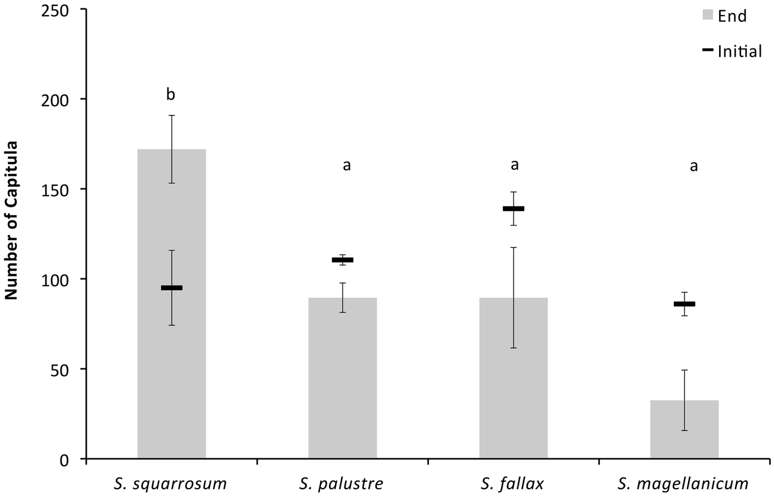

Figure 2. Number of capitula (± SEM) produced by a patch of 50 cm^2 of four different *Sphagnum* species after 12 weeks of experimental treatments (grey bars). The smaller black markers depict the number of capitula at the beginning of the experiment. Significant differences in the final number of capitula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

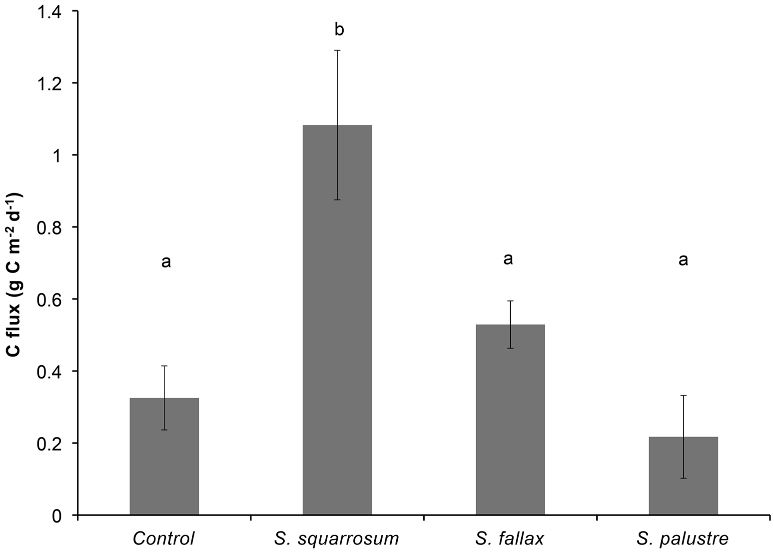

Figure 3. Daily net C ($CO_2 + CH_4$) fluxes (± SEM) for bare peat and peat covered with different *Sphagnum* vegetation, measured after 6 weeks of experimental treatments. Since *S. magellanicum* only had a few living capitula left at this moment, we excluded it from these measurements. Note that positive values represent net C emission. Different letters indicate significant differences between the four species ($P = 0.012$).

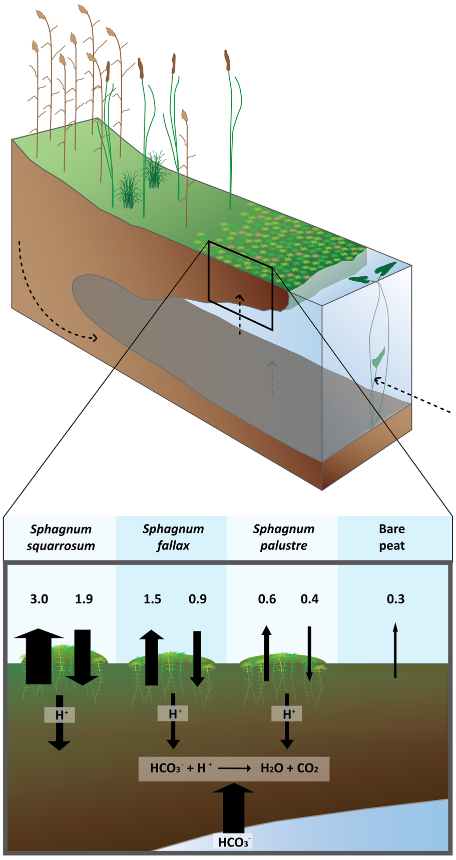

Figure 4. Schematic overview of a transitional floating mire influenced by HCO_3^--rich groundwater or surface water, illustrated by dashed arrows in the figure above. Due to differences in the thickness of the floating peat or the origin and composition of the HCO_3^--rich water, there is a high heterogeneity 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 (downward arrow) and gross C emission (upward arrows) are both derived from C-flux measurements and presented in $\mathrm{g\,C\,m^{-2}\,d^{-1}}$. 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.