

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

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11 **Abstract**

12 Peatlands play an important role in the global carbon (C) cycle due to their large C storage  
13 potential. Their C sequestration rates, however, highly vary depending on climatic and  
14 geohydrological conditions. Transitional mires are often characterised by floating peat with  
15 infiltration of buffered groundwater or surface water. On top, *Sphagnum* mosses grow,  
16 producing recalcitrant organic matter and fuelling the large C stocks. As *Sphagnum* species  
17 strongly differ in their tolerance to the higher pH in these mires, their species composition  
18 can be expected to influence C dynamics in transitional mires.

19 We therefore experimentally determined growth and net C sequestration rates for four  
20 different *Sphagnum* species (*Sphagnum squarrosum*, *S. palustre*, *S. fallax* and *S.*

21 *magellanicum*) in aquaria, with floating peat influenced by the infiltration of buffered water.  
22 Surprisingly, even though the first three species increased their biomass, the moss-covered  
23 peat still showed a net efflux of CO<sub>2</sub> that was up to three times higher than that of bare peat.  
24 This species-dependent C release could be explained by *Sphagnum*'s active lowering of the  
25 pH, which triggers the chemical release of CO<sub>2</sub> from bicarbonate.

26 Our results clearly illustrate that high *Sphagnum* biomass production may still coincide with  
27 high C emission. These counterintuitive C dynamics in mire succession seem to be the result  
28 of both species- and biomass-dependent acidification and buffered water infiltration.  
29 Together, these processes can explain part of the large variation in C fluxes (ranging from C  
30 sequestration to C release) reported for pristine mires in literature.

31

## 32 **1. Introduction**

33 Since peatlands store approximately one third of all terrestrial carbon (C), they are important  
34 in the global C cycle (Gorham, 1991), and their C dynamics have been studied throughout the  
35 world (Gorham et al., 2003; Bortoluzzi et al., 2006; Golovatskaya and Dyukarev, 2009;  
36 Rowson et al., 2010). Although it is well known that degraded and drained peatlands  
37 generally are net C sources due to increased decomposition rates (Alm et al., 1999;  
38 Waddington et al., 2001; Moore, 2002) - with net emissions ranging from +80 to +880 g C m<sup>-2</sup>  
39 y<sup>-1</sup> (Lamers et al. (2015); With all presented values of C fluxes, positive values represent net  
40 C losses to the atmosphere, whereas negative values represent net storage of C in growing  
41 peat throughout the manuscript) - pristine, growing peatlands (mires) accumulate C and are  
42 therefore considered to be C sinks (Belyea and Malmer, 2004). The full greenhouse gas  
43 budget is, however, more complex. First, almost all peatlands are sources of methane (CH<sub>4</sub>)  
44 (Moore and Roulet, 1995; Saarnio et al., 2007), and second, not all pristine peatlands appear  
45 to be sinks of carbon dioxide (CO<sub>2</sub>) (Waddington and Roulet, 2000; Riutta et al., 2007). For  
46 groundwater or surface-water fed (minerotrophic) fens, CO<sub>2</sub> fluxes have been reported to  
47 range from -208 to +190 g C m<sup>-2</sup> y<sup>-1</sup> (Martikainen et al., 1995; Carroll and Crill, 1997; Bubier  
48 et al., 2003), whereas for transitional mires fluxes of -124 to +58 g C m<sup>-2</sup> y<sup>-1</sup> have been  
49 reported (Moore and Knowles, 1987; Koch et al., 2008; Salm et al., 2009).

50 Transitional mires are examples of intermediate systems that display characteristics of both  
51 minerotrophic fens and ombrotrophic bogs (Wheeler and Proctor, 2000; Sjörs and  
52 Gunnarsson, 2002). Other examples include edges of bog systems (lagg zones) influenced by  
53 surrounding surface water and local patches influenced by percolating water (Giller and

54 Wheeler, 1988). Transitional mires often consist of floating peat infiltrated by moderately  
55 base-rich water, which determines species composition and stimulates buoyancy, through its  
56 effect on decomposition and subsequent gas production (Lamers et al., 1999; Smolders et al.,  
57 2002). Since they increase habitat heterogeneity at various scales, these intermediate peatland  
58 systems often form hotspots of biodiversity (Verberk et al., 2010). Transitional, floating  
59 mires are mainly characterised by *Cyperaceae* and a moss layer of different *Sphagnum*  
60 species, whose dominance strongly increase during succession (Du Rietz, 1954; Vitt and  
61 Chee, 1990; Wheeler and Proctor, 2000). *Sphagnum* growth in transitional mires is, however,  
62 not as straightforward as in bogs, since most *Sphagnum* species are sensitive to both high pH  
63 and increased concentrations of calcium (Ca) and bicarbonate ( $\text{HCO}_3^-$ ) in pore water and  
64 surface water (Clymo, 1973). *As Sphagnum spp. lack stomata, water conducting tissue and*  
65 *roots, their growth, nutrition and vitality depend on the chemical composition of the*  
66 *surrounding water (Robroek et al., 2009).* Despite Ca and  $\text{HCO}_3^-$ -rich conditions, floating  
67 rafts in transitional mires may, however, still form suitable habitats for *Sphagnum* species,  
68 since they are always water-saturated and are fed by rainwater, which accumulates in the top  
69 (moss) layer and dilutes the buffered surface water (Lamers et al., 1999; Smolders et al.,  
70 2003)

71 *Sphagnum* spp. strongly influence their environment and are thus important ecosystem  
72 engineers in peatlands (Van Breemen, 1995). They are capable of actively acidifying their  
73 habitat by exchanging cations for protons (Clymo, 1963; Hajek and Adamec, 2009) and  
74 releasing organic acids (Van Breemen, 1995). Furthermore, *Sphagnum* spp. keep their  
75 environment moist due to the high water holding capacity of their hyaline cells (Clymo,

76 1973) and compact growth structure. By increasing the acidity and moisture content of their  
77 habitat, *Sphagnum* spp. also slow down decomposition rates, thus providing optimal  
78 conditions for the accumulation of organic material. Moreover, the high concentration of  
79 phenolic compounds in their tissues, including antibiotics (Verhoeven and Toth, 1995),  
80 further decreases decomposition rates (Yavitt et al., 2000; Freeman et al., 2001). This  
81 combination of traits results in a strong contribution of *Sphagnum* mosses to C sequestration  
82 and peat formation worldwide (Coulson and Butterfield, 1978; Limpens and Berendse, 2003).  
83 Due to differences in habitat preference among *Sphagnum* species, they inhabit different  
84 successional stages in peatlands (Vitt and Chee, 1990). Since biomass production (Gerdol,  
85 1995), acidification rates (Kooijman and Bakker, 1994), decomposition rates (Rochefort et  
86 al., 1990; Limpens and Berendse, 2003) and drought-tolerance (Nijp et al., 2014) are species-  
87 specific, the species composition of the *Sphagnum* layer in turn may strongly influence the  
88 biogeochemistry and C balance of their habitat. This means that the C sequestration potential  
89 of the different successional stages of peatlands may strongly depend on which *Sphagnum*  
90 species is dominant at that stage. In transitional mires, the species composition will strongly  
91 depend on pH, buffering components and water content. How the *Sphagnum* species  
92 composition influences the biogeochemistry and C balance in transitional mires, however,  
93 remains largely unknown.

94 Although a vast amount of studies has presented field measurements of C dynamics in all  
95 types of peatland systems, including transitional mires, establishing the origin of the huge  
96 variation reported for both CH<sub>4</sub> and CO<sub>2</sub> fluxes in these field studies is challenging. Studies  
97 on both C dynamics and the influence of *Sphagnum* mosses using a controlled laboratory

98 approach, however, have not yet been performed to our knowledge. The goal of this study  
99 was therefore twofold: first, to investigate the growth of different *Sphagnum* species under  
100 controlled environmental conditions characteristic for transitional mires, and second, to study  
101 C fluxes and their underlying mechanisms in these systems. Four different *Sphagnum* species,  
102 *S. squarrosum*, *S. palustre*, *S. fallax* and *S. magellanicum*, were grown on peat floating on Ca  
103 - HCO<sub>3</sub><sup>-</sup> rich water. Besides growth parameters of these mosses, we studied their contribution  
104 to the net C fluxes in these potentially peat forming systems. We hypothesised that Ca -  
105 HCO<sub>3</sub><sup>-</sup> rich conditions would lead to considerable differences in performance between the  
106 four *Sphagnum* species, based on differences in their tolerance to these buffering components  
107 and in their growth rates. Furthermore, we expected more tolerant *Sphagnum* species to  
108 strongly determine the C sequestration of these systems.

109

## 110 **2. Material and Methods**

### 111 **2.1 Experimental set-up**

112 Intact floating peat monoliths (25 x 25 cm; height 21.85 ± 2.08 cm; n=8) were cut from a  
113 floating mire in the southern part of the Netherlands (N 51°24'6.1" E 6°11'10.5") in late  
114 March 2012. This floating mire was dominated by helophytes species *Typha latifolia* and  
115 *Calla palustris*, whereas the moss layer consisted mainly of *Sphagnum fallax*. After cutting,  
116 all vegetation was removed and the bare peat was transferred to glass aquaria (25 x 25 x 30  
117 cm; length x width x height) in the field to minimize damage to the peat structure. The peat  
118 had an organic matter content of 92.7±0.4 % (determined by loss on ignition; 3h at 550°C)

119 and contained  $3.6 \pm 0.4$  mmol  $\text{kg}^{-1}$  fresh weight (FW) of Ca (determined by digestion of 200  
120 mg of dry soil with 4 ml of  $\text{HNO}_3$  and 1 ml of  $\text{H}_2\text{O}_2$  using a microwave oven (mks 1200  
121 Mega, Milestone Inc., Sorisole, Italy), after which diluted digestates were analysed by  
122 inductively-coupled plasma spectrometry (ICP-OES icap 6000; Thermo Fischer scientific)).

123 In the laboratory, 6.25 l of Ca -  $\text{HCO}_3^-$ -rich treatment water was added to each aquarium  
124 (Table 1), on which the peat floated. The underlying water layer was subsequently refreshed  
125 with treatment water at a rate of 5 L  $\text{week}^{-1}$  using peristaltic pumps (Masterflex L/S, Cole-  
126 Parmer, Vernon Hills, IL, USA). All floating peat monoliths received artificial rainwater  
127 (Table 1) five times a week, at a rate corresponding to the Dutch annual rainfall of 800 mm.  
128 During the experiment, the aquaria were kept in a water bath maintained at  $18^\circ\text{C}$  (up to a  
129 maximum of  $23^\circ\text{C}$  at the end of the day) using a cryostat (NESLAB, Thermoflex 1400,  
130 Breda, The Netherlands). Furthermore, a light regime of  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$  (PAR; 16h light/8h  
131 dark) was maintained (Master Son-T Pia Plus, Philips, Eindhoven, The Netherlands). This  
132 regime of temperature and light was chosen to mimic summer conditions.

133 On four floating peat monoliths, four different species of *Sphagnum* (*Sphagnum. squarrosum*,  
134 *S. fallax*, *S. palustre* and *S. magellanicum*) were planted together. *S. squarrosum* is a species  
135 of moderately rich fens and occurs in environments with pH values up to pH 7 (Clymo,  
136 1973). *S. fallax*, on the other hand, can be quite sensitive to high pH or drought, but is also  
137 known for its high potential growth rate under minerotrophic conditions (Buttler et al., 1998).  
138 *S. palustre* is a widespread species found in habitats that are neither highly calcareous nor  
139 highly acidic (Daniels and Eddy, 1990). *S. magellanicum* is a species associated with poor  
140 fens and bogs, and it is restricted to a more acidic habitat (Vitt and Chee, 1990; Hajek et al.,

141 2006). The first three species were collected in a peatland area in the north-western part of  
142 the Netherlands (Ilperveld; N52°26'42.5," E4°55'45.1"), while the latter species was  
143 collected in an area in the south of the Netherlands (Maasduinen; N51°34'56.3", E6°6'13.5").  
144 Of all species, a patch of 50±10 g fresh material (1.6±0.8 g DW; moss length 3 cm) was  
145 applied randomly to one of the corners of the aquarium. Mosses were put upright in a patch  
146 of approximately 50 cm<sup>2</sup>. The remaining 4 floating peat monoliths were kept as non-  
147 vegetated controls.

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

## 155 **2.2 Chemical analyses**

156 During the 12 weeks of the experiment, pH and total inorganic carbon (TIC) concentration of  
157 infiltrating water and pore water were measured every two weeks (7 times in total). pH was  
158 measured with a standard Ag/AgCl electrode (Orion Research, Beverly, CA, USA) combined  
159 with a pH meter (Tim840 titration manager; Radiometer analytical, Lyon, France). TIC was  
160 measured by injecting 0.2 ml of sample into a compartment with 1 ml phosphoric acid (0.4  
161 M) in an Infra-red Gas Analyser (IRGA; ABB Analytical, Frankfurt, Germany), after which



162 concentrations of  $\text{HCO}_3^-$  and  $\text{CO}_2$  were calculated based on the pH equilibrium.  
163 Concentrations of  $\text{PO}_4^{3-}$ ,  $\text{NO}_3^-$  and  $\text{NH}_4^+$  were measured colourimetrically on an auto analyser  
164 3 system (Bran&Lubbe, Norderstedt, Germany) using ammonium molybdate (Henriksen,  
165 1965), hydrazine sulphate (Kamphake et al., 1967) and salicylate (Grasshof and Johannse,  
166 1972) respectively. Concentrations of Ca, Fe, K, Mg, total-P and  $\text{SO}_4$  were analysed by  
167 inductively coupled plasma spectrometry (ICP-OES icap 6000; Thermo Fischer scientific).

### 168 **2.3 Plant data**

169 To preserve bare control soils and monocultures of the *Sphagnum* species, all aboveground  
170 biomass of non-*Sphagnum* species was carefully removed every two weeks. This vegetation  
171 consisted mainly of *Typha latifolia* and *Juncus effusus* seedlings. Every two weeks, growth  
172 and expansion of the mosses were recorded. Mosses were allowed to grow outside of their  
173 designated quarters to include the effects of competition between species. After 8 weeks of  
174 moss growth, pH was measured within the *Sphagnum* vegetation at 0.5-1 cm above soil level,  
175 using a pH meter (HQ 40d, Hach, Loveland, CO, USA) and Ag/AgCl pH electrode (Orion  
176 9156BNPW, Thermo Fisher Scientific, Waltham, MA, USA). After 12 weeks, all moss  
177 biomass was harvested and the number of capitula (top 8-10 mm of the photosynthetically  
178 active tissue of the mosses) was counted for each plot. Length of the moss fragments was  
179 measured before living plant parts and dead parts were separated and weighed. Biomass was  
180 dried for 48 hours at 70 °C to determine dry weight (DW). C and N contents (%) of dried  
181 moss material were determined using an elemental analyser (Carlo Erba NA1500, Thermo  
182 Fisher Scientific, Waltham, MA, U.S.A.).

## 183 **2.4 Carbon fluxes**

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

## 205 **2.5 Statistical analyses**

206 All data were checked for normality of residuals and homogeneity of variance using Shapiro-  
207 Wilk's Test for Normality and Levene's Test of Equality of Error Variances, respectively.  
208 Differences in the chemical composition of surface water and pore water of soils with and  
209 without moss cover were analysed over time using Linear Mixed Models. Differences  
210 between growth parameters of *Sphagnum* mosses (Fig. 1, Fig. 2, Table 3) and C fluxes were  
211 tested using one-way ANOVAs with Tukey Post Hoc. In all tables and figures, averages are  
212 presented with standard error of the mean (SEM). All statistical analyses were carried out  
213 using SPSS for Mac (V21, IBM Statistics).

214

## 215 **3. Results**

### 216 **3.1 Chemical composition of infiltrating water and pore water**

217 Growth of *Sphagnum* significantly lowered the pH of the infiltrating water ( $P<0.001$ ),  
218 whereas pore water pH increased ( $P=0.007$ ) when mosses were present (Table 2).  
219 Concentrations of  $\text{NO}_3^-$  ( $P=0.002$ ) and Fe ( $P=0.018$ ) were significantly higher in the  
220 infiltrating water of moss-covered soils, whereas concentrations of Ca ( $P=0.014$ ) and P  
221 ( $P=0.012$ ) were higher in pore water of bare soils (Table 2). Still, concentrations of  $\text{NO}_3^-$   
222 were low, since N was predominantly present as  $\text{NH}_4^+$  in both infiltrating water and pore  
223 water (Table 2). Overall,  $\text{NH}_4^+$  concentrations in the infiltrating water were high compared to  
224 similar systems (e.g. Kooijman and Bakker (1994)), with values around  $40 \mu\text{mol l}^{-1}$  (Table 2).

### 225 **3.2 *Sphagnum* growth and acidification**

226 *S. squarrosum*, *S. fallax* and *S. palustre* increased their biomass during the experiment (Fig.  
227 1). Initial biomass of these species was increased by 340%, 250% and 600% respectively  
228 ( $P<0.001$ ). While length of the green part of the mosses (living moss) remained  
229 approximately the same, the total length of the moss fragments increased by 5-6 cm (Table  
230 3). In *S. fallax* and *S. palustre*, elongation appeared to be the main factor responsible for the  
231 increased biomass, since the number of capitula decreased in these species by 35% and 19%  
232 (Fig. 2). *S. squarrosum* was the only species that increased both in biomass ( $P<0.001$ ) and in  
233 number of capitula ( $P=0.004$ ). This species also increased its coverage in the aquaria  
234 ( $P=0.001$ ; Table 3), thereby invading the quarters of other species and proving to be a strong  
235 competitor in this experimental set-up. In contrast, *S. magellanicum* was strongly affected by  
236 the  $\text{HCO}_3^-$ -rich water and showed a strong decline in both biomass (Fig. 1) and number of  
237 capitula (Fig. 2) during the experiment. *S. squarrosum* acidified its environment most, with  
238 pH values down to 4.5 ( $P=0.027$ ; Table 3). *S. fallax* and *S. palustre* both had pH values of  
239 around 5.2-5.4, whereas *S. magellanicum* had the highest pH with values around pH 6 (Table  
240 3). The acidification rate was linearly correlated ( $P=0.005$ ;  $R^2=0.43$ ) to the net increase in  
241 biomass shown by the three species, with *S. magellanicum* showing both the lowest biomass  
242 increase and the highest pH and *S. squarrosum* showing the lowest pH and highest growth  
243 (Supplementary Figure 1).

### 244 3.3 Carbon exchange

245 Bare floating peat had a C emission of  $0.3 \pm 0.1 \text{ g C m}^{-2} \text{ d}^{-1}$  (Fig. 3). Surprisingly, when  
246 *Sphagnum* species were grown on these soils, net C emission appeared to be similar to or  
247 even higher than that of bare peat (Fig. 3). Soils covered with *S. squarrosum* showed the  
248 highest net emissions of  $1.1 \pm 0.2 \text{ g C m}^{-2} \text{ d}^{-1}$  ( $P < 0.001$ ), despite being the species with the  
249 highest biomass increase. *S. fallax* and *S. palustre* had net C effluxes similar to those of bare  
250 peat, with  $0.5 \pm 0.1$  and  $0.2 \pm 0.1 \text{ g C m}^{-2} \text{ d}^{-1}$  respectively, even though significant increases in  
251 biomass were observed for these two species. C fluxes consisted almost exclusively of  $\text{CO}_2$ ,  
252 as methane ( $\text{CH}_4$ ) contributed less than 2.5% to net C emission and ranged from  $-0.8$  to  $43$   
253  $\text{mg C m}^{-2} \text{ d}^{-1}$  (data not shown). Still, due to the higher global warming potential of  $\text{CH}_4$   
254 compared with  $\text{CO}_2$ ,  $\text{CH}_4$  contributed 10 to 30 % to the total greenhouse gas emissions  
255 expressed in  $\text{CO}_2$  equivalents. These greenhouse gas emissions were higher for plots covered  
256 by *S. squarrosum* ( $1750 \pm 350 \text{ gCO}_2\text{-eq m}^{-2} \text{ y}^{-1}$ ) than for plots covered by other species  
257 ( $580 \pm 106 \text{ gCO}_2\text{-eq m}^{-2} \text{ y}^{-1}$ ) or unvegetated ( $550 \pm 180 \text{ gCO}_2\text{-eq m}^{-2} \text{ y}^{-1}$ ) plots due to the higher  
258  $\text{CO}_2$  emission from these plots ( $P = 0.002$ ; data not shown).

259

### 260 4. Discussion

261 For *Sphagnum* species growing on top of floating peat monoliths, the influence of infiltration  
262 of groundwater or surface water, buffered by  $\text{Ca}^{2+} - \text{HCO}_3^-$ , into the peat was shown to be  
263 reduced, and even moderately sensitive species were capable of growing under these

264 conditions. It was remarkable, however, to discover that while some of these species strongly  
265 increased their biomass, *Sphagnum*-covered patches simultaneously showed a net C efflux.

#### 266 **4.1 Tolerance of *Sphagnum* species to buffered conditions**

267 Transitional mires are *Sphagnum* rich systems characterised by the influence of calcareous  
268 and alkaline surface water or groundwater in the subsoil and are thus partly buffered systems.  
269 These environmental conditions are, however, not limited to transitional mires and occur  
270 more widely, since local spots with higher influence of groundwater or edges in contact with  
271 calcareous surface water occur in many peatlands. The acid neutralising capacity (ANC) of  
272 peatlands is mainly based on the presence of  $\text{HCO}_3^-$  and  $\text{Ca}^{2+}$ . When protons are released into  
273 a system, they are initially buffered by the  $\text{HCO}_3^-$  -  $\text{CO}_2$  buffering system (Sherlock et al.,  
274 1995; Lamers et al., 2015). Once most of the  $\text{HCO}_3^-$  has been consumed, protons ( $\text{H}^+$ ) can be  
275 buffered by the cation-exchange capacity (CEC) of the peat, where base cations bound to soil  
276 particles are exchanged for  $\text{H}^+$  (Lamers et al., 2015).  $\text{Ca}^{2+}$  usually is the main component of  
277 the CEC, since it is the dominant divalent cation in many peatlands (Bache, 1984; Rippey and  
278 Nelson, 2007).

279 Several studies have indicated that *Sphagnum* can be sensitive to calcareous groundwater or  
280 surface water due to Ca -  $\text{HCO}_3^-$  toxicity (Clymo, 1973; Andrus, 1986; Lamers et al., 1999;  
281 Hajek et al., 2006). This sensitivity to one or both of the major buffering components of  
282 groundwater or surface water is species-specific and it strongly affected the performance of  
283 the *Sphagnum* species in our study. *S. magellanicum* appeared to be most sensitive to the  
284 tested conditions, as this species decreased both in number of capitula and biomass. *S. fallax*

285 and *S. palustre*, on the other hand, increased in biomass, even though they were obviously  
286 outcompeted by the better-adapted *S. squarrosum*. *S. squarrosum* was able to increase both  
287 horizontally, in number of capitula, and vertically, by stem elongation.

288 *S. squarrosum* is one of the few *Sphagnum* species that is still vital in systems with a higher  
289 influence of calcareous, and therefore HCO<sub>3</sub><sup>-</sup>-rich, water and is even able to tolerate  
290 (temporary) immersion in these systems (Clymo, 1973; Vitt and Chee, 1990). Other species,  
291 including *S. magellanicum*, are known to be highly sensitive to increased pH and buffered  
292 conditions in their habitat (Clymo, 1973; Granath et al., 2010), which explains why *S.*  
293 *magellanicum* showed a strong decrease in our study. The typical habitats of *S. fallax*, *S.*  
294 *palustre* and *S. magellanicum* are all characterised by low pH (4.5-4.8) and low Ca<sup>2+</sup> content  
295 (35-40 µmol L<sup>-1</sup>) (Vitt and Chee, 1990; Hajek et al., 2006), although *S. fallax* and *S. palustre*  
296 can tolerate a wider range of environmental conditions in terms of acidity and trophic level  
297 than *S. magellanicum* (Daniels and Eddy, 1990). *S. squarrosum*, on the other hand often  
298 occurs in rich to moderately rich fens (Vitt and Chee, 1990; Hajek et al., 2006), which are  
299 characterised by pH values of 5.1 to 6.7 and Ca<sup>2+</sup> concentrations of 270-500 µmol L<sup>-1</sup> (Vitt  
300 and Chee, 1990; Kooijman and Bakker, 1994).

## 301 **4.2 Succession of *Sphagnum* species**

302 The transition of mineral-rich fens to acidic “poor fens” to oligotrophic bogs is believed to be  
303 initiated by the acidification of pioneer *Sphagnum* species (Wilcox and Andrus, 1987; Rydin  
304 and Jeglum, 2006; Granath et al., 2010). These pioneer species are expected to tolerate  
305 mineral-rich conditions, have a high growth rate and a high acidification capacity under more

306 buffered conditions, which will allow them to change a mineral-rich fen into an acid, poor fen  
307 within a few decades (Granath et al., 2010). *S. squarrosum* may act as such a pioneer species  
308 and is often responsible for rapid succession in fens (Giller and Wheeler, 1988; Haraguchi et  
309 al., 2003), especially under nutrient rich conditions (Kooijman and Bakker, 1995).

310 Our data confirms that, *S. squarrosum* potentially acts as a foundation species for other  
311 *Sphagnum* spp. This species simultaneously increased its biomass considerably and acidified  
312 its environment most effectively, lowering pH to values around 4.5 despite continuous  
313 infiltration of surface water with an alkalinity of 3 meq l<sup>-1</sup>, while the other three species could  
314 not lower pH below 5.2. *Sphagnum* species show differences in acidification rate, based on  
315 differences in their cation-exchange capacity (Rippy and Nelson, 2007). Additionally,  
316 however, *Sphagnum* acidification rates depend on their species-specific performance under  
317 certain environmental conditions. High growth rates combined with low decomposition rates  
318 (5-35% mass loss yr<sup>-1</sup> (Clymo, 1965; Coulson and Butterfield, 1978; Verhoeven and Toth,  
319 1995; Limpens and Berendse, 2003)) result in a fast build-up of the peat layer and succession  
320 in species composition, which, in floating transitional mires, will slowly reduce the influence  
321 of the underlying calcareous water.

### 322 **4.3 Carbon dynamics**

323 Increase of the thickness of the peat layer due to *Sphagnum* growth shows that these species  
324 can sequester a significant amount of C. *Sphagnum* biomass can increase by approximately  
325 70 to 600 g DW m<sup>-2</sup> yr<sup>-1</sup> (Gerdol, 1995; Graf and Rochefort, 2009; Hajek, 2009; Samaritani et  
326 al., 2011), which corresponds to a CO<sub>2</sub> fixation rate of approximately 28 to 240 g C m<sup>-2</sup> yr<sup>-1</sup>.



327 If we extrapolate the daily CO<sub>2</sub> fixation rates of the three growing species in our experiment,  
328 *S. squarrosum*, *S. fallax* and *S. palustre*, to calculate yearly production rates, based on a  
329 growing season of 8 months, we find high CO<sub>2</sub> fixation rates of approximately 100-450 g C  
330 m<sup>-2</sup> y<sup>-1</sup>. These values, however, overestimate actual field growth of these mosses, since the  
331 experiment was carried out indoors under summer conditions only. Still, even with these high  
332 CO<sub>2</sub> fixation rates, we found net C emissions from both bare peat and from peat covered with  
333 growing *Sphagnum* mosses.

334 Bare peat showed C emission rates of around 0.3 g C m<sup>-2</sup> d<sup>-1</sup> (Fig. 4), which consisted for  
335 98% of CO<sub>2</sub> and 2% of CH<sub>4</sub>. Both bare peat and vegetated plots were a small source of CH<sub>4</sub>,  
336 with average emission rates of 2 to 20 mg C m<sup>-2</sup> d<sup>-1</sup>, which fall within the range of 4 to 500  
337 mg C m<sup>-2</sup> d<sup>-1</sup> usually reported for saturated peatlands (e.g. Salm, Barlett & Harris, Saarnio,  
338 Byrne 2004). Still, the contribution of CH<sub>4</sub> to the greenhouse gas emission is much higher in  
339 terms of CO<sub>2</sub> equivalents, since the global warming potential of CH<sub>4</sub> is 34 times that of CO<sub>2</sub>  
340 (IPCC, 2013). The higher greenhouse gas emissions (as CO<sub>2</sub> equivalents) from the plots  
341 vegetated by *S. squarrosum* were, however not due to differences in CH<sub>4</sub> emissions, but  
342 resulted from the much higher emissions of CO<sub>2</sub> from these plots.

343 When plots were vegetated by growing *Sphagnum* spp., CO<sub>2</sub> emissions increased, despite the  
344 accumulation of biomass by all three species (Fig. 4), which indicates that the source of this  
345 CO<sub>2</sub> could not solely be the decomposition of *Sphagnum* litter. The only likely explanation  
346 for this remaining net CO<sub>2</sub> efflux is therefore the chemical reaction R. (1) that occurs when  
347 HCO<sub>3</sub><sup>-</sup>-rich water comes into contact with the acidifying mosses (Fig. 4). The transition of  
348 HCO<sub>3</sub><sup>-</sup> to CO<sub>2</sub> is the first step in the ANC of aquatic systems and will occur much faster than

349 other buffering mechanisms, such as cation-exchange of  $\text{Ca}^{2+}$  (Lamers et al., 2015). Active  
350 acidification was mainly observed in *S. squarrosum*, while *S. fallax* and *S. palustre* did not  
351 significantly lower pH more than the dying *S. magellanicum*.



353 To further disentangle the different  $\text{CO}_2$  sources responsible for the net  $\text{CO}_2$  emission from  
354 plots vegetated with different species, we used a mass approach (Eq. 1; Table 4). Net  $\text{CO}_2$   
355 fixation was estimated based on the difference between light and dark  $\text{CO}_2$  fluxes, whereas  
356  $\text{CO}_2$  emission was estimated based on dark fluxes. This  $\text{CO}_2$  emission can be further divided  
357 into separate contributors, as is shown in Eq. 1.

$$358 \quad \text{Net C flux to atmosphere} = \text{B} + \text{R} + \text{C} - \text{F} \quad \text{Eq. 1}$$

359 Here, B represents the  $\text{CO}_2$  flux from bare peat to the atmosphere, R is the dark plant  
360 respiration, C represents the flux of chemically produced  $\text{CO}_2$  according to R. 1 and F is the  
361 gross  $\text{CO}_2$  fixation, calculated as the light  $\text{CO}_2$  flux minus the dark  $\text{CO}_2$  flux. Bare peat  
362 respiration was derived from dark fluxes of non-vegetated plots. For the *Sphagnum*  
363 respiration factor R, we have used the maximum value (31%) from the range reported in  
364 literature (12 to 31 % of photosynthetic C fixation, (Haraguchi et al., 2003; Laine et al., 2011;  
365 Kangas et al., 2014)). As a result, we obtain a conservative estimate of the C flux emitted  
366 through the chemical reaction R. 1, driven by the acid production of the *Sphagnum* mosses  
367 (C). Although the likely higher respiration rates during the light period and the use of  
368 estimated *Sphagnum* respiration impede an exact quantification of factor C, the fact that we

369 find CO<sub>2</sub> emissions during the light period in growing *Sphagnum* patches clearly points out  
370 that there is a considerable chemical CO<sub>2</sub> source.

371 Table 4 shows the different sources of the C fluxes as presented in Eq.1. Furthermore, the  
372 implications that these values have on a landscape scale are depicted in a schematic overview  
373 of a floating transitional mire that is being fed by HCO<sub>3</sub><sup>-</sup> rich water (Figure 4). Here, we show  
374 simultaneous C fixation and C emission of the three growing *Sphagnum* species from our  
375 experiment, with their different growth and acidification rates.

376 The production of HCO<sub>3</sub><sup>-</sup> -derived CO<sub>2</sub> will occur in any situation where HCO<sub>3</sub><sup>-</sup> -rich water  
377 comes into contact with an acid environment, such as in the highly acidic lower layers of  
378 floating bog systems influenced by HCO<sub>3</sub><sup>-</sup> -rich water (Lamers et al., 1999; Smolders et al.,  
379 2003). Therefore, CO<sub>2</sub> effluxes measured from the slightly acidic bare peat in our experiment,  
380 are likely at least partially derived from acid-driven CO<sub>2</sub> production from HCO<sub>3</sub><sup>-</sup>, as is  
381 illustrated in Figure 4. Our finding that the most strongly acidifying and fastest growing  
382 mosses such as *Sphagnum squarrosum* show the highest C effluxes strongly suggests that  
383 active acidification enhances the production of HCO<sub>3</sub><sup>-</sup> -derived CO<sub>2</sub>.

384 This leads to the apparent contradiction that while growth of *Sphagnum* will lead to  
385 accumulation of organic matter and thus contributes to the build-up of a peat layer, it is  
386 accompanied by a large net efflux of CO<sub>2</sub> ranging from 0.2-1.1 g C m<sup>-2</sup> d<sup>-1</sup> (Table 4, Figure  
387 4). While we show this phenomenon here in a controlled laboratory setting, net CO<sub>2</sub> effluxes  
388 have indeed been reported for transitional mires, with rates ranging from -0.34 to +0.16 g C  
389 m<sup>-2</sup> d<sup>-1</sup> (Moore and Knowles, 1987; Koch et al., 2008; Salm et al., 2009). As mentioned

390 before, however, this phenomenon may not be limited to transitional mires. For example,  
391 bogs typically show an outflow of acid water ( $H^+$  and organic acids) and therefore *Sphagnum*  
392 produced acids may also cause chemical  $CO_2$  production outside the peatland system, thereby  
393 counteracting at least a part of the C sequestration realised by peat growth.

394

## 395 **5. Conclusion**

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

411 temporal variation in C fluxes in these systems. This finding may therefore help explain part

412 of the strong variation in C balances measured in seemingly similar peatland systems.

413

414 Author Contributions

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

418

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428

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594

595

596 **Table 1:** Composition of the infiltrating water and artificial rainwater used in the  
 597 experimental set-up. The rainwater composition was based on the composition of Dutch  
 598 rainwater. Note that all concentrations are in  $\mu\text{mol l}^{-1}$ , except for the sea salt addition, which  
 599 is in  $\text{mg l}^{-1}$ .

	<b>Infiltrating water</b>	<b>Artificial rainwater</b>
<b>HCO<sub>3</sub><sup>-</sup></b>	3000	-
<b>SO<sub>4</sub><sup>2-</sup></b>	100	-
<b>Cl<sup>-</sup></b>	8000	54
<b>Ca<sup>2+</sup></b>	2000	17
<b>Mg<sup>2+</sup></b>	2000	-
<b>Na<sup>+</sup></b>	3000	-
<b>K<sup>+</sup></b>	200	20
<b>NH<sub>4</sub><sup>+</sup></b>	-	36
<b>NO<sub>3</sub><sup>-</sup></b>	-	36
<b>Sea salt (mg l<sup>-1</sup>)<sup>1</sup></b>	-	5

600 <sup>1</sup> Pro Reef, Tropic Marine, aQua united LTD, Telgte, Germany.

601



602 **Table 2:** Infiltrating water and pore water characteristics. All nutrient concentrations are  
 603 given in  $\mu\text{mol l}^{-1}$ . Overall averages are given, while statistical tests were carried out over time  
 604 using linear mixed models (LMM). When these LMM showed significant differences  
 605 between moss covered and bare soils, values are given in bold.

	Infiltrating water		Pore water	
	With moss	Without moss	With moss	Without moss
<b>pH</b>	<b>6.3±0.1</b>	<b>6.6±0.0</b>	<b>5.5±0.2</b>	<b>5.2±0.2</b>
<b>TIC</b>	<b>2014±268</b>	<b>1965±294</b>	<b>2496±160</b>	<b>2317±170</b>
<b>HCO<sub>3</sub><sup>-</sup></b>	859±318	1158±251	376±105	279±140
<b>CO<sub>2</sub></b>	1154±182	810±278	1953±300	1820±310
<b>NO<sub>3</sub><sup>-</sup></b>	<b>1.0±0.3</b>	<b>0.5±0.2</b>	0.3±0.2	0.5±0.2
<b>NH<sub>4</sub><sup>+</sup></b>	38±14	46±9	99±25	59±33
<b>Total-P</b>	6.2±1.4	6.5±2.2	<b>21±8</b>	<b>42±18</b>
<b>Ca</b>	1413±86	1366±97	<b>1187±129</b>	<b>1381±139</b>
<b>Fe</b>	<b>99±8</b>	<b>63±21</b>	257±58	295±68
<b>K</b>	284±70	228±67	310±68	298±100
<b>Mg</b>	1823±118	1738±123	1444±150	1574±80
<b>SO<sub>4</sub></b>	92±13	117±26	69±6	92±21

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607

608 **Table 3:** Characteristics of the different *Sphagnum* moss patches, including pH within the  
609 vegetation (0.5-1 cm above soil level; measured after 6 weeks of growth), and final data  
610 (after 12 weeks) on C: N ratio, length of the moss fragments and cover of the patches. For all  
611 variables, *P*-values are given and significant differences between species are represented by  
612 different capital letters.

	<i>S. squarrosum</i>	<i>S. fallax</i>	<i>S. palustre</i>	<i>S. magellanicum</i>	<i>P</i>
<b>pH</b>	4.5 ±0.2 <sup>A</sup>	5.4±0.3 <sup>A,B</sup>	5.2±0.4 <sup>B</sup>	6.1±0.4 <sup>B</sup>	0.027
<b>Final length (mm)</b>	85.9±4.0 <sup>B</sup>	66.1±7.4 <sup>A,B</sup>	70.4±7.9 <sup>B</sup>	42.1±1.7 <sup>A</sup>	0.002
<b>Final cover (cm<sup>2</sup>)</b>	209±28 <sup>B</sup>	120±11 <sup>A</sup>	111±9 <sup>A</sup>	75±10 <sup>A</sup>	0.001
<b>C: N ratio (g g<sup>-1</sup>)</b>	27.0±1.3	25.3±3.6	25.2±3.2	25.2±1.7	ns

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615 **Table 4:** Origin and rates of C fluxes (in g C m<sup>-2</sup> d<sup>-1</sup>) of peat covered with different species of  
616 *Sphagnum*. Net C fluxes, gross C fixation rates and gross C emissions are based on closed  
617 chamber measurements carried out under light and dark conditions. Other fluxes are  
618 calculated using Eq. 1, with the fraction of autotrophic respiration based on the maximum  
619 value found for *Sphagnum* respiration in literature (31%, Laine et al. (2011)).

	<b>Net C flux (B+C- F)</b>	<b>Gross C Fixation (F)</b>	<b>Gross C emission (B+R+C)</b>	<b>Bare Peat (B)</b>	<b>Autotrophic respiration (R)</b>	<b>Additional HCO<sub>3</sub><sup>-</sup> derived CO<sub>2</sub> (C)</b>
<i>S. squarrosum</i>	1.1±0.2	1.9±0.5	3.0±0.7	0.3±0.1	0.6	2.1±0.7
<i>S. fallax</i>	0.5±0.1	0.9±0.2	1.5±0.2	0.3±0.1	0.3	0.9±0.2
<i>S. palustre</i>	0.2±0.1	0.4±0.1	0.6±0.3	0.3±0.1	0.1	0.2±0.3

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622 **Captions to Figures**

623 **Fig. 1:** The Biomass dry weight (DW,  $\pm$  SEM) produced by a patch of 50 cm<sup>2</sup> of four  
624 different *Sphagnum* species after 12 weeks of experimental treatments, divided into living  
625 and dead tissue (dark and light bars respectively). The smaller black bars represent initial  
626 DW. Significant differences between total final biomass of the species are indicated by  
627 different letters ( $P=0.005$ ).

628 **Fig. 2:** Number of capitula ( $\pm$  SEM) produced by a patch of 50 cm<sup>2</sup> of four different  
629 *Sphagnum* species after 12 weeks of experimental treatments (grey bars). The smaller black  
630 markers depict the number of capitula at the beginning of the experiment. Significant  
631 differences in the final number of capitula between the species are indicated by different  
632 letters ( $P=0.002$ ).

633 **Fig. 3:** Daily net C (CO<sub>2</sub> + CH<sub>4</sub>) fluxes ( $\pm$ SEM) for bare peat and peat covered with different  
634 *Sphagnum* vegetation, measured after 6 weeks of experimental treatments. Since *S.*  
635 *magellanicum* only had a few living capitula left at this moment, we excluded it from these  
636 measurements. Note that positive values represent net C emission to the atmosphere.  
637 Different letters indicate significant differences between the four species ( $P=0.012$ ).

638 **Fig. 4:** Schematic overview of a transitional floating mire influenced by HCO<sub>3</sub><sup>-</sup> -rich  
639 groundwater or surface water, illustrated by dashed arrows in the figure above. Due to  
640 differences in the thickness of the floating peat or the origin and composition of the HCO<sub>3</sub><sup>-</sup> -  
641 rich water, there is a high heterogeneity within these systems. Part of the floating raft is  
642 shown in more detail below. Here, peat soils are covered with different *Sphagnum* species.

643 Rates of C fixation in peat (downward arrow) and C emission to the atmosphere (upward  
644 arrows) are both derived from C-flux measurements and presented in  $\text{g C m}^{-2} \text{d}^{-1}$ . As the  
645 mosses showed differences in final biomass, higher or lower amounts of biomass are depicted  
646 in the figure. Furthermore, the mosses differ in acidification rate, with significantly higher  
647 amounts of acids produced by *Sphagnum squarrosum* (left) than the other species. Since  
648 *Sphagnum magellanicum* declined severely in biomass due to its sensitivity to the calcareous  
649 water, its C-fluxes could not be measured and the species was excluded from this figure.

650

651 **Supplementary Fig. 1:** A linear relationship ( $R^2=0.43$ ;  $P=0.005$ ) exists between biomass  
652 production of *Sphagnum* mosses and acidification of the environment. Here,  $\blacklozenge$  = *S.*  
653 *magellanicum*,  $\blacksquare$  = *S. fallax*,  $\blacktriangle$  = *S. palustre* and  $\bullet$  = *S. squarrosum*. *Sphagnum* species that  
654 show the highest biomass increases also have a higher acidification rate, leading to lower pH  
655 in their environment than slower growing species.

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