

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

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10

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 CO₂ 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 CO₂ 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⁻²
39 y⁻¹ ([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 (CH₄)
44 (Moore and Roulet, 1995; Saarnio et al., 2007), and second, not all pristine peatlands appear
45 to be sinks of carbon dioxide (CO₂) (Waddington and Roulet, 2000; Riutta et al., 2007). For
46 groundwater or surface-water fed (minerotrophic) fens, CO₂ fluxes have been reported to
47 range from -208 to +190 g C m⁻² y⁻¹ (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⁻² y⁻¹ 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 (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 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 (Rocheftort 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 CH₄ and CO₂ 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 - HCO₃⁻ 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 HCO₃⁻ 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 ± 0.4 mmol kg⁻¹ fresh weight (FW) of Ca [\(determined by digestion of 200](#)
120 [mg of dry soil with 4 ml of HNO₃ and 1 ml of H₂O₂ using a microwave oven \(mls 1200](#)
121 [Mega, Milestone Inc., Sorisole, Italy\), 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 - HCO₃⁻-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 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°C [\(up to a](#)
129 [maximum of 23 °C at the end of the day\)](#) using a cryostat (NESLAB, Thermoflex 1400,
130 Breda, The Netherlands). Furthermore, a light regime of 200 μmol m⁻² 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 cm². 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 HCO_3^- and CO_2 were calculated based on the pH equilibrium.
163 Concentrations of PO_4^{3-} , NO_3^- and 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 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 *squarrosom*, *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 CO₂ using an IRGA (ABB Analytical, Frankfurt, Germany) and for CH₄ 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 CO₂ and CH₄ 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 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 NO_3^-
222 were low, since N was predominantly present as NH_4^+ in both infiltrating water and pore
223 water (Table 2). Overall, 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 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 ± 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 CO_2 ,
252 as methane (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 CH_4
254 compared with CO_2 , CH_4 contributed 10 to 30 % to the total greenhouse gas emissions

255 expressed in CO₂ equivalents. These greenhouse gas emissions were higher for plots covered
256 by *S. squarrosum* (1750±350 gCO₂-eq m⁻² y⁻¹) than for plots covered by other species
257 (580±106 gCO₂-eq m⁻² y⁻¹) or unvegetated (550±180 gCO₂-eq m⁻² y⁻¹) plots due to the higher
258 CO₂ 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 Ca²⁺ - HCO₃⁻, 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 HCO₃⁻ and Ca²⁺. When protons are released into
273 a system, they are initially buffered by the HCO₃⁻ - CO₂ buffering system (Sherlock et al.,
274 1995; Lamers et al., 2015). Once most of the HCO₃⁻ has been consumed, protons (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 H⁺ (Lamers et al., 2015). Ca²⁺ usually is the main component of
277 the CEC, since it is the dominant divalent cation in many peatlands (Bache, 1984; Rippy and
278 Nelson, 2007).

279 Several studies have indicated that *Sphagnum* can be sensitive to calcareous groundwater or
280 surface water due to Ca - HCO₃⁻ 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 HCO₃⁻-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 Ca²⁺ content
295 (35-40 μmol L⁻¹) (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^{2+} concentrations of 270-500 $\mu\text{mol L}^{-1}$ (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^{-1} , 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⁻¹ (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⁻² yr⁻¹ (Gerdol, 1995; Graf and Rochefort, 2009; Hajek, 2009; Samaritani et
326 | al., 2011), which corresponds to a CO₂ fixation rate of approximately 28 to 240 g C m⁻² yr⁻¹.
327 | If we extrapolate the daily CO₂ 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 CO₂ fixation rates of approximately 100-450 g C
330 | m⁻² y⁻¹. 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 | CO₂ 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⁻² d⁻¹ (Fig. 4), which consisted for
335 | 98% of CO₂ and 2% of CH₄. Both bare peat and vegetated plots were a small source of CH₄.

336 with average emission rates of 2 to 20 mg C m⁻² d⁻¹, which fall within the range of 4 to 500
337 mg C m⁻² d⁻¹ usually reported for saturated peatlands (e.g. Salm, Barlett & Harris, Saarnio,
338 Byrne 2004). Still, the contribution of CH₄ to the greenhouse gas emission is much higher in
339 terms of CO₂ equivalents, since the global warming potential of CH₄ is 34 times that of CO₂
340 (IPCC, 2013). The higher greenhouse gas emissions (as CO₂ equivalents) from the plots
341 vegetated by *S. squarrosum* were, however not due to differences in CH₄ emissions, but
342 resulted from the much higher emissions of CO₂ from these plots.

343 When plots were vegetated by growing *Sphagnum* spp., CO₂ emissions increased, despite the
344 accumulation of biomass by all three species (Fig. 4), which indicates that the source of this
345 CO₂ could not solely be the decomposition of *Sphagnum* litter. The only likely explanation
346 for this remaining net CO₂ efflux is therefore the chemical reaction R. (1) that occurs when
347 HCO₃⁻ -rich water comes into contact with the acidifying mosses (Fig. 4). The transition of
348 HCO₃⁻ to CO₂ 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 Ca²⁺ (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 CO₂ sources responsible for the net CO₂ emission from
354 plots vegetated with different species, we used a mass approach (Eq. 1; Table 4). Net CO₂
355 fixation was estimated based on the difference between light and dark CO₂ fluxes, whereas

356 CO₂ emission was estimated based on dark fluxes. This CO₂ emission can be further divided
357 into separate contributors, as is shown in Eq. 1.

358 Net C flux to atmosphere = $B + R + C - F$ **Eq. 1**

359 Here, B represents the CO₂ flux from bare peat to the atmosphere, R is the dark plant
360 respiration, C represents the flux of chemically produced CO₂ according to R. 1 and F is the
361 gross CO₂ fixation, calculated as the light CO₂ flux minus the dark CO₂ 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 CO₂ emissions during the light period in growing *Sphagnum* patches clearly points out
370 that there is a considerable chemical CO₂ 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 HCO₃⁻ 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_3^- -derived CO_2 will occur in any situation where HCO_3^- -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_3^- -rich water (Lamers et al., 1999; Smolders et al.,
379 2003). Therefore, CO_2 effluxes measured from the slightly acidic bare peat in our experiment,
380 are likely at least partially derived from acid-driven CO_2 production from HCO_3^- , 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_3^- -derived CO_2 .

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_2 ranging from 0.2-1.1 g C m⁻² d⁻¹ (Table 4, Figure
387 4). While we show this phenomenon here in a controlled laboratory setting, net CO_2 effluxes
388 have indeed been reported for transitional mires, with rates ranging from -0.34 to +0.16 g C
389 m⁻² d⁻¹ (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 CO₂ 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 CO₂ production is an underestimated factor that plays a significant role in C fluxes in
402 transitional mires and other systems where calcareous (Ca and HCO₃⁻-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

429

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592 methane and carbon dioxide in three *Sphagnum*-dominated peatland ecosystems as revealed
593 by a reciprocal field peat transplant experiment, *Geomicrobiology Journal*, 17, 61-88, 2000.

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 mg l^{-1} .

	Infiltrating water	Artificial rainwater
HCO₃⁻	3000	-
SO₄²⁻	100	-
Cl⁻	8000	54
Ca²⁺	2000	17
Mg²⁺	2000	-
Na⁺	3000	-
K⁺	200	20
NH₄⁺	-	36
NO₃⁻	-	36
Sea salt (mg l⁻¹)¹	-	5

600 ¹ 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
pH	6.3±0.1	6.6±0.0	5.5±0.2	5.2±0.2
TIC	2014±268	1965±294	2496±160	2317±170
HCO₃⁻	859±318	1158±251	376±105	279±140
CO₂	1154±182	810±278	1953±300	1820±310
NO₃⁻	1.0±0.3	0.5±0.2	0.3±0.2	0.5±0.2
NH₄⁺	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
SO₄	92±13	117±26	69±6	92±21

606

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>
pH	4.5 ±0.2 ^A	5.4±0.3 ^{A,B}	5.2±0.4 ^B	6.1±0.4 ^B	0.027
Final length (mm)	85.9±4.0 ^B	66.1±7.4 ^{A,B}	70.4±7.9 ^B	42.1±1.7 ^A	0.002
Final cover (cm²)	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

613

614

615 **Table 4:** Origin and rates of C fluxes (in g C m⁻² d⁻¹) 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)).

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

620

621

622 **Captions to Figures**

623 **Fig. 1:** The Biomass dry weight (DW, \pm SEM) produced by a patch of 50 cm² 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 cm² 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 (CO₂ + CH₄) 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 HCO₃⁻ -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 HCO₃⁻ -
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.

656