Interactive comment on "Simultaneous high C fixation and high C emissions in Sphagnum mires" by S. F. Harpenslager et al.

Anonymous Referee #1

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The manuscript investigates the relation between soil pH and Sphagnum moss population in transitional mires using a mesocosm experiment. Species succession and changing soil properties are timely research subjects relevant for Biogeosciences. The manuscript is well written but I have some concerns about the data analysis, which will have to be addressed before the manuscript can be considered for publication.

We thank Referee #1 for his or her critical review of our manuscript. The highly relevant points raised by this referee have been carefully considered and we feel that this has improved our manuscript considerably.

Comments:

1. P. 6 (Page 19 in this document): explain why you grow species of Sphagnum on a substrate they do not favour in natural conditions.

Transitional mires are peatlands that are characterised by a transition from buffered to acid conditions. In these systems, *Sphagnum* species are ecosystem engineers and as such responsible for the production of acid. While some species, such as *S. magellanicum*, are more sensitive to HCO_3^- and will thus grow towards the more acid part of the system, other species, such as *S. squarrosum*, are more tolerant of buffered conditions and typically start the acidification process and succession towards more acidic vegetation types. Heterogeneity in the system will therefore result in diversity in the *Sphagnum* species pool, as is illustrated by the habitat preferences of these species (Clymo, 1973; Vitt and Slack, 1984). We chose our species based on the natural gradient that exists in transitional mires. There are many studies that present examples of such heterogeneous systems, where different *Sphagnum* species are present, including Kooijman and Bakker (1995), van Diggelen et al. (1996), Bragazza and Gerdol (2002) and Hajkova and Hajek (2004).

2. Line 199-204, P. 11 (Line 498-503; P. 24 in this document): I do not understand the correction factor applied to the C-fluxes based on the ratio of the surface areas of the mosses. You assume that all growth occurred horizontally, which is probably not the case. What you measure is the net flux per chamber volume and you must clarify what this is an underestimate of. If the aim is to quantify the increase in C-fluxes due to changes in biomass, you could consider normalising the fluxes at time 0 (t0) and time 1 (t1) with the bulk volume of the mosses at t0 and t1 respectively. Using the ratio of surface areas most probably over-estimates the fluxes you are trying to characterise.

We regret that our description of the correction factor was not clear in our Method section. By mentioning that the growth occurred horizontally, we mean that the natural vertical elongation of moss fragments causes them to fall sideways in our setup, since the moss patches were too small to provide support for long fragments. The entire moss patch should, as it were, have been erected and would then have fit underneath the chamber. During measurement, however, only part of the long fragments was present in the chamber, which means that the total flux of the patch with long Sphagnum fragments (*S. squarrosum*) was underestimated. We thus corrected for this underestimation by multiplying with the area covered by all these moss fragments. We have made this more clear by replacing "expansion occurred partly horizontally" in the Methods section with: "elongated moss fragments fell over, causing an apparent horizontal growth".

3. Line 199-204, P. 11 (Line 498-503; P. 24 in this document): these numbers must be revisited in the light of the previous comment.

We hope to have clarified the confusion concerning our correction factor in question number 2 and have, consequently, not altered our calculations.

4. Line 358, P. 19 (Line 659; P. 32 in this document): You forget the plant respiration term (the biomass increases, and hence the respiration too).

We fully agree with the referee that we should include plant respiration here. We have therefore changed this in the revised document. Although we cannot present actual respiration data for our experiment, we have estimated the contribution of respiration to the total C emission by using literature data. In literature, *Sphagnum* respiration ranges from 12 to 31 % of photosynthetic C fixation (Haraguchi et al., 2003; Laine et al., 2011; Kangas et al., 2014). From this range we have taken the maximum value (31 %) to determine a conservative estimate of the C emitted as HCO_3^--derived CO_2. and present these values in Table 4 in the revised manuscript.

5. P. 17 (P. 30 in this document): you use C and CO2 inter-changeably. Please, change to CO2, especially when defining the terms of eq. (1) which all seem to have to do with respiration, etc... Not sure where CH4 fits in.

We agree that using both C and CO_2 inter-changeably causes confusion. In the revised document, we now first discuss the CH_4 fluxes, which we compare to ranges from literature. In the rest of the discussion, we then focus on CO_2-fluxes, since these show the most relevant results of our study. To avoid confusion, we have now made clear in these paragraphs whether the C fluxes we discuss concern CO_2, CH_4 or both.

6. Line 367-370, P. 18 (Line 668; P. 32 in this document): you omitted the temperature dependence of the fluxes (e.g. respiration follows an exponential relationship of the form R = a exp(bT)). You may find that the daytime fluxes > night-time fluxes due to this temperature dependence. This might give rise to a "residual" respiration term, which would then feed into the "C" term of eq. (1) and thus overestimate it.

We thank the referee for remarking on the importance of temperature dependence in C-dynamics. Although the temperature in our set-up was fairly constant due to the use of a temperature controlled water-bath, daytime and night-time temperatures differed slightly due to the heat generated by the lamps during daytime (reaching maximum daytime temperatures of 23 °C). This indeed increases the confidence interval of our "conservative" (see point 4) estimate of HCO_3^- derived CO_2**.** Although assumptions (point 4) and the likely higher respiration rates during the day impede an exact quantification of this HCO_3^- derived CO_2, the fact that we find CO_2 emissions during the day in growing *Sphagnum* patches clearly points out that there is a considerable chemical CO_2 source. To acknowledge this uncertainty we have added "Although the likely higher respiration rates during the day and the use of estimated *Sphagnum* respiration impede an exact quantification of factor C, the fact that we find CO_2 emissions during the day in growing *Sphagnum* patches clearly points out that there is a considerable chemical CO_2 source" to the discussion.

7. The values of C-fluxes in the rest of the manuscript must be revised in light of the previous comments.

We now point out that the exact magnitude of the HCO_3^- derived CO_2 flux should be interpreted with caution, due to reasons mentioned above.

8. Overall, CH4 fluxes seem underused or amalgamated into a general C-term. It would be interesting to treat CH_4 and CO_2 independently and compare them to literature values.

We agree that the contribution of CH_4 to the total C-flux could be emphasized more. We have therefore added the range of CH_4 emissions that were measured in our experiment in our Results and Discussion section, and have compared this range to data reported in literature in the revised manuscript. For CO_2, this comparison to literature data was already included on P20, L388. To include the effect of these emissions on the climate change, we now also include the global warming potential (GWP) of CO_2 and CH_4 to look at the effect of the greenhouse gas emissions from these floating soils covered by *Sphagnum* spp. in a climate perspective.

Specific comments:

9. Line 64, p. 4 (Line 362-364; P. 17 in this document): in which way is Sphagnum "strongly influenced" by water?

We agree that we should specify this influence of water on *Sphagnum*. We have therefore changed the sentence "As *Sphagnum* spp. lack stomata, water conducting tissue and roots, they are strongly influenced by the surrounding water (Robroek et al., 2009)" into "As *Sphagnum* spp. lack stomata, water conducting tissue and roots, their growth, nutrition and vitality depend on the chemical composition of the surrounding water (Robroek et al., 2009)"

10. Line 68, p. 4 (Line 366-367; P. 17 in this document): again, qualify the "direct influence".

We agree that we should specify this "direct influence of buffered surface water". We have therefore changed the latter part of the sentence "… and the **direct influence** of buffered surface water in the moss layer is therefore relatively low" into "…and are fed by rain water, which accumulates in the top (moss) layer and dilutes the buffered surface water."

11. Line 112, p. 6 (Line 410; P. 19 in this document): move (n = 8) to the end of the parentheses block, which provides details of the monolith dimensions.

We thank the referee for this comment and have changed this according to his/her advice.

12. Line 118, p. 7 (Line 416; P. 20 in this document): how was the organic matter content determined?

We thank the reviewer for spotting the missing method explanation on determining OM content of our soils and the Ca content. We have added the following paragraph to this section of our Methods: "The peat had an organic matter content of 92.7±0.4 % (determined by loss on ignition; 3h at 550°C) and contained 3.6±0.4 mmol kg^{-1} fresh weight (FW) of Ca (determined by digestion of 200 mg of dry soil with 4 ml of HNO_3 and 1 ml of H_2O_2 using a microwave oven (mls 1200 Mega, Milestone Inc., Sorisole, Italy), after which digestates were analysed by inductively-coupled plasma spectrometry (ICP-OES iCap 6000; Thermo Fischer scientific))".

13. Lines 123-125. P. 7 (Line 421-423; P. 20 in this document): define "rich" in Ca-HCO3-. Clarify whether the Ca-... rich water was replenished over the course of the experiment.

We agree that "rich" is not a clear definition, although we do also refer to the exact composition of the infiltrating water used in our experiment, which is presented in Table 1. Here, we show that the infiltrating water, which is being pumped into the aquaria at a rate of 5 L week^{-1}. To clarify that this flushing of the water layer is being carried out with the treatment water, we have changed this sentence to: "The underlying water layer was subsequently refreshed with treatment water at a rate of 5 L week^{-1} using peristaltic pumps."

14. Line 132, p. 7 (Line 430; P. 20 in this document): 16 hours of daylight; this doesn't seem realistic for the end of March in the NL!

While we carried out our experiment in March, it was conducted entirely indoors with artificial lighting. We simulated summer conditions for a period of three months, with temperatures and daylight roughly corresponding to the period of May until July. We have clarified this in the revised manuscript by adding the sentence: "This regime of temperature and light was chosen to mimic summer conditions"

15. Line 134, P. 7 (Line 432; P. 20 in this document): it is unclear whether each vegetated monolith had all four species of Sphagnum growing on it or only 1.

We agree that this can be presented clearer. We have therefore added the word "together" to the sentence: On four floating peat monoliths, four different species of *Sphagnum* (*Sphagnum squarrosum, S. fallax, S. palustre* and *S. magellanicum*) were planted together.

16. Overall, the linkage between the body of the manuscript and the figures and tables is insufficient. Much of the tables and figures data is described only in their captions and insufficiently referenced in the text. This makes the latter seem data-poor.

We feel that we may indeed have underused the table and figure referred to in the Discussion of our text. We therefore elaborated more on the references to Table 4 and Figure 4 in the Discussion section. Furthermore, we included the CH_4 emission rates and elaborated on these measurements in the Results section of our revised manuscript, as suggested by the referee in point 8.

References

Bragazza, L., and Gerdol, R.: Are nutrient availability and acidity-alkalinity gradients related in Sphagnum-dominated peatlands?, J Veg Sci, 13, 473-482, 2002.

Clymo, R. S.: Growth of *Sphagnum* - Some Effects of Environment, Journal of Ecology, 61, 849-869, 1973.

Hajkova, P., and Hajek, M.: Bryophyte and vascular plant responses to base-richness and water level gradients in Western Carpathian Sphagnum-rich mires, Folia Geobot, 39, 335-351, 2004.

Haraguchi, A., Hasegawa, T., Iyobe, T., and Nishijima, H.: The pH dependence of photosynthesis and elongation of *Sphagnum squarrosum* and *S. girgensohnii* in the Picea glehnii mire forest in Cape Ochiishi, north-eastern Japan, Aquat Ecol, 37, 101-104, 2003.

Kangas, L., Maanavilja, L., Hajek, T., Juurola, E., Chimner, R. A., Mehtatalo, L., and Tuittila, E. S.: Photosynthetic traits of Sphagnum and feather moss species in undrained, drained and rewetted boreal spruce swamp forests, Ecol Evol, 4, 381-396, 2014.

Kooijman, A. M., and Bakker, C.: Species replacement in the bryophyte layer in mires - the role of water type, nutrient supply and interspecific interactions, Journal of Ecology, 83, 1-8, 1995.

Laine, A. M., Juurola, E., Hajek, T., and Tuittila, E. S.: Sphagnum growth and ecophysiology during mire succession, Oecologia, 167, 1115-1125, 2011.

van Diggelen, R., Molenaar, W. J., and Kooijman, A. M.: Vegetation succession in a floating mire in relation to management and hydrology, J Veg Sci, 7, 809-820, 1996.

Vitt, D. H., and Slack, N. G.: Niche Diversification of Sphagnum Relative to Environmental-Factors in Northern Minnesota Peatlands, Can. J. Bot.-Rev. Can. Bot., 62, 1409-1430, 1984.

Interactive comment on “Simultaneous high C fixation and high C emissions in Sphagnum mires” by S. F. Harpenslager et al.

Anonymous Referee #2

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These authors grew different kinds of Sphagnum in the lab under conditions of rather high alkalinity. They tested 4 different types of Sphagnum. Only one type grew well, one did quite poorly, and two grew a bit. The one that grew well produced significant acidity resulting in the titration of the bicarbonate in the media water and subsequent release of CO2. The authors make the point that even with growth and organic matter formation, such a system may serves as a CO2 emitter. They suggest that the conditions under which they grew the moss represent a pioneering stage for Sphagnum and that when this acid producing moss moves into an environment with high alkalinity similar results may occur. They cite examples from the literature when CO2 emission from mires has been detected. In general, I think that these results are important, as many researchers probably have not considered such a mechanism. I do have a couple of suggestions for the authors.

We thank Referee #2 for pointing out the relevance of our work. Furthermore, we thank this referee for his/her interesting points raised about our manuscript. We feel that the processing of these points has considerably improved the clarity of our manuscript.

1. Please right away in your introduction where you cite the CO2 fluxes, say what convention you are using. Do negative fluxes indicate CO2 uptake by the surface? Or emission.

We agree that mentioning the convention used in our study early on improves the clarity of our paper. We have changed this in the revised version of our manuscript by inserting "With all presented values of C fluxes, positive values represent net C losses to the atmosphere, whereas negative values represent net storage of C in growing peat throughout the manuscript" in line 39 on page 3 (line 337 on page 16 in this document).

2. Could you explore the relationship between biomass production and acid production a bit more? Is this a linear relationship or what? How does this work? I don't mean to suggest doing more experiments, but just see if you can find more on this in the Literature.

We thank the reviewer for raising this issue. In literature, we have found that the acidification rate of Sphagnum species is dependent on the availability of nutrients (Kooijman and Paulissen, 2006). From this, we hypothesised that there should also be a relationship with biomass production. We therefore checked our own data and found a clear linear relationship between pH within the vegetation and biomass increase, which is a valuable addition to the manuscript. We have included the existence of this linear relationship in our Results section and added this graph as a Supplementary Figure.

3. All the acid produced by mosses, where does this end up? If mosses grow and grow, they continue to produce H+ right? Eventually does this acid migrate some place, to some more alkaline surface water and result in more CO2 emission, thus cancelling out the carbon sequestration of mosses?

This is a very interesting point. In *Sphagnum* dominated fens and bogs, the acid produced by the mosses lower pH, which can be as low as pH 3.5. Furthermore, these systems will show an outflow of acid water, which, when running into more buffered surroundings, may indeed result in the reaction we presented in our manuscript, leading to higher CO_2 emissions in these areas. Including this flux could have implications for the C sink function of peatlands, which we speculated on in the final part of our conclusion in the revised manuscript.

4. So is the carbon that is fixed into moss tissue from the water? The Total inorganic carbon? Or is it from the air? If the former, and the groundwater has ancient CO2, say from limestone dissolution, could this result in ancient appearing moss? 14C –wise?

To determine the exact carbon source that *Sphagnum* uses for photosynthesis, we would have needed to introduce isotope labelled C into the medium. From previous research, however, it has become clear that although part of the C used by *Sphagnum* will be derived from the air, ambient CO_2 concentrations are not high enough to meet the C requirements of *Sphagnum* (Smolders et al., 2001), which means that they need an additional C source. It is therefore not surprising to learn that *Sphagnum* utilises both previously respired CO_2 (Rydin and Clymo, 1989) and soil-derived CO_2 from decomposition processes (Turetsky and Wieder, 1999) for C fixation. Apart from the CO_2 in the air, C was present in our system as TIC (HCO_3^- and CO_2) in the soils and as TIC in the treatment water. In our case, however, we cannot distinguish between the different sources of C that *Sphagnum* may have used, and we therefore just cannot calculate how much of the fixed C is derived from the atmosphere based on our results.

5. I note that the tables contain CO_2 concentration and HCO_3 concentration. Please give total inorganic carbon too, just for ease of comparison, in the table.

We have added TIC concentrations to Table 2 in the revised manuscript, according to the referee's suggestion.

6. Interesting in the moss pore water, why was the pH higher in the pore water without the moss>? How are the moss values in Table 2 obtained, when the 4 mosses used behaved so differently?

Since the soils were floating on top of the surface water, we cannot distinguish between the water layer that stood in (indirect) contact with the different moss species. Furthermore, our sediments contained two soil moisture cups to take pore water samples, which were pooled to minimise the effect of variation within the soil. All data from Table 2 is therefore derived from pooled samples of water layer or pore water of moss-covered or bare peat soils. All pore water was sampled from the middle of the aquarium. The differences that we found in pH between soils with moss and without moss might be explained by the higher intrusion of O_2 into sediments without mosses, leading to oxidation of reduced sulphur and concomitant acid production.

References

Kooijman, A. M., and Paulissen, M. P. C. P.: Higher acidification rates in fens with phosphorus enrichment, Appl Veg Sci, 9, 205-212, 2006.

Rydin, H., and Clymo, R. S.: Transport of Carbon and Phosphorus-Compounds About Sphagnum, Proc R Soc Ser B-Bio, 237, 63-84, 1989.

Smolders, A. J. P., Tomassen, H. B. M., Pijnappel, H. W., Lamers, L. P. M., and Roelofs, J. G. M.: Substrate-derived CO2 is important in the development of Sphagnum spp., New Phytologist, 152, 325-332, 2001.

Turetsky, M. R., and Wieder, R. K.: Boreal bog Sphagnum refixes soil-produced and respired (CO2)-C-14, Ecoscience, 6, 587-591, 1999.

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

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Abstract

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

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

magellanicum) in aquaria, with floating peat influenced by the infiltration of buffered water. Surprisingly, even though the first three species increased their biomass, the moss-covered peat still showed a net efflux of CO_2 that was up to three times higher than that of bare peat. This species-dependent C release could be explained by *Sphagnum*'s active lowering of the pH, which triggers the chemical release of CO_2 from bicarbonate.

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

1. Introduction

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

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

Sphagnum spp. strongly influence their environment and are thus important ecosystem engineers in peatlands (Van Breemen, 1995). They are capable of actively acidifying their habitat by exchanging cations for protons (Clymo, 1963; Hajek and Adamec,

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

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

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

2. Material and Methods

2.1 Experimental set-up

Intact floating peat monoliths (25 x 25 cm; height 21.85 ± 2.08 cm; n=8) were cut from a floating mire in the southern part of the Netherlands (N 51^{0}24’6.1” E 6^{0}11’10.5”) in late March 2012. This floating mire was dominated by helophytes species *Typha latifolia* and *Calla palustris*, whereas the moss layer consisted mainly of *Sphagnum fallax*. After cutting, all vegetation was removed and the bare peat was transferred to glass aquaria (25 x 25 x 30 cm; length x width x height) in the field to minimize damage to the peat structure. The peat had an organic matter content of 92.7±0.4 % (determined by loss on ignition; 3h at 550°C) and contained 3.6±0.4 mmol kg^{-1} fresh weight (FW) of Ca (determined by digestion of 200 mg of dry soil with 4 ml of HNO_3 and 1 ml of H_2O_2 using a microwave oven (mls 1200 Mega, Milestone Inc., Sorisole, Italy), after which diluted digestates were analysed by inductively-coupled plasma spectrometry (ICP-OES icap 6000; Thermo Fischer scientific)).

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 refreshed with treatment water 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 (up to a maximum of 23 °C at the end of the day) using a cryostat (NESLAB, Thermoflex 1400, Breda, The Netherlands). Furthermore, a light regime of 200 µmol m^{-2} s^{-1} (PAR; 16h light/8h dark) was maintained (Master Son-T Pia Plus, Philips, Eindhoven, The Netherlands). This regime of temperature and light was chosen to mimic summer conditions.

On four floating peat monoliths, four different species of *Sphagnum* (*Sphagnum. squarrosum*, *S. fallax*, *S. palustre* and *S. magellanicum*) were planted together. *S. squarrosum* is a species of moderately rich fens and occurs in environments with pH values up to pH 7 (Clymo, 1973). *S. fallax*, on the other hand, can be quite sensitive to high pH or drought, but is also known for its high potential growth rate under minerotrophic conditions (Buttler et al., 1998). *S. palustre* is a widespread species found in habitats that are neither highly calcareous nor highly acidic (Daniels and Eddy, 1990). *S. magellanicum* is a species associated with poor fens and bogs, and it is restricted to a more acidic habitat (Vitt and Chee, 1990; Hajek et al., 2006). The first three species were collected in a peatland area in the north-western part of the Netherlands (Ilperveld; N52°26’42.5,” E4°55’45.1”), while the latter species was collected in an area in the south of the Netherlands (Maasduinen; N51°34’56.3”, E6°6’13.5”). Of all species, a patch of 50±10 g fresh material (1.6±0.8 g DW; moss length 3 cm) was applied randomly to one of the corners of the aquarium. Mosses were put upright in a patch of approximately 50 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&Lubbe, Norderstedt, Germany) using ammonium molybdate (Henriksen, 1965), hydrazine sulphate (Kamphake et al., 1967) and salicylate (Grasshof and Johannse, 1972) respectively. Concentrations of Ca, Fe, K, Mg, total-P and SO_4 were analysed by inductively coupled plasma spectrometry (ICP-OES icap 6000; Thermo Fischer scientific).

2.3 Plant data

To preserve bare control soils and monocultures of the *Sphagnum* species, all aboveground biomass of non-*Sphagnum* species was carefully removed every two weeks. This vegetation consisted mainly of *Typha latifolia* and *Juncus effusus* seedlings. Every two weeks, growth and expansion of the mosses were recorded. Mosses were allowed to grow outside of their designated quarters to include the effects of competition between species.

After 8 weeks of moss growth, pH was measured within the *Sphagnum* vegetation at 0.5-1 cm above soil level, using a pH meter (HQ 40d, Hach, Loveland, CO, USA) and Ag/AgCl pH electrode (Orion 9156BNPW, Thermo Fisher Scientific, Waltham, MA, USA). After 12 weeks, all moss biomass was harvested and the number of capitula (top 8-10 mm of the photosynthetically active tissue of the mosses) was counted for each plot. Length of the moss fragments was measured before living plant parts and dead parts were separated and weighed. Biomass was dried for 48 hours at 70 °C to determine dry weight (DW). C and N contents (%) of dried moss material were determined using an elemental analyser (Carlo Erba NA1500, Thermo Fisher Scientific, Waltham, MA, U.S.A.).

2.4 Carbon fluxes

C fluxes were determined after 6 weeks of experimental treatments. Since the cover of *S. magellanicum* had declined severely by this time, the remaining patch was too small to cover with a closed chamber and the species was excluded from these measurements. C-fluxes under both light and dark conditions were therefore only measured from soils covered with *S. squarrosum, S. palustre* or *S. fallax* and from bare control soils, using transparent and dark closed chambers (10 cm, 10 cm and 12 cm for length, width and height) respectively. Dark measurements started at the end of the 8h dark period and lights remained off during measurements, so that mosses remained dark-adapted. Samples were taken from the headspace immediately after placing the chambers on the aquaria, and subsequently after 2 and 4 hours using 1 ml syringes, which were first flushed with headspace. They were analysed for 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 grow vertically, whereas in our experiment – due to absence of supporting neighbouring mosses – elongated moss fragments fell over, causing an apparent horizontal growth. Our areal C fluxes measured with the closed chambers covering only part of the elongated fragments are therefore underestimates. We corrected for this by multiplying the areal C fluxes with the ratio of the area covered by elongated *Sphagnum* fragments and the area of the chamber.

2.5 Statistical analyses

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

3. Results

3.1 Chemical composition of infiltrating water and pore water

Growth of *Sphagnum* significantly lowered the pH of the infiltrating water ($P<0.001$), whereas pore water pH increased ($P=0.007$) when mosses were present (Table 2). Concentrations of NO_3^- ($P=0.002$) and Fe ($P=0.018$) were significantly higher in the infiltrating water of moss-covered soils, whereas concentrations of Ca ($P=0.014$) and P ($P=0.012$) were higher in pore water of bare soils (Table 2). Still, concentrations of NO_3^- were low, since N was predominantly present as NH_4^+ in both infiltrating water and pore water (Table 2). Overall, NH_4^+ concentrations in the infiltrating water were high compared to similar systems (e.g. Kooijman and Bakker (1994)), with values around 40 µmol l^{-1} (Table 2).

3.2 *Sphagnum* growth and acidification

S. squarrosum, S. fallax and *S. palustre* increased their biomass during the experiment (Fig. 1). Initial biomass of these species was increased by 340%, 250% and 600% respectively ($P<0.001$). While length of the green part of the mosses (living moss) remained approximately the same, the total length of the moss fragments increased by 5-6 cm (Table 3). In *S. fallax* and *S. palustre*, elongation appeared to be the main factor responsible for the increased biomass, since the number of capitula decreased in these species by 35% and 19% (Fig. 2). *S. squarrosum* was the only species that increased both in biomass ($P<0.001$) and in number of capitula (*P*=0.004). This species also increased its coverage in the aquaria (*P*=0.001; Table 3), thereby invading the quarters of other species and proving to be a strong competitor in this experimental set-up. In contrast, *S. magellanicum* was strongly affected by the HCO_3^--rich water and showed a strong decline in both biomass (Fig. 1) and number of capitula (Fig. 2) during the experiment. *S. squarrosum* acidified its environment most, with pH values down to 4.5 (*P*=0.027; Table 3). *S. fallax* and *S. palustre* both had pH values of around 5.2-5.4, whereas *S. magellanicum* had the highest pH with values around pH 6 (Table 3). The acidification rate was linearly correlated (*P*=0.005; R^2=0.43) to the net increase in biomass shown by the three species, with *S. magellanicum* showing both the lowest biomass increase and the highest pH and *S. squarrosum* showing the lowest pH and highest growth (Supplementary Figure 1).

3.3 Carbon exchange

Bare floating peat had a C emission of 0.3±0.1 g C m^{-2} d^{-1} (Fig. 3). Surprisingly, when *Sphagnum* species were grown on these soils, net C emission appeared to be similar to or even higher than that of bare peat (Fig. 3). Soils covered with *S. squarrosum* showed the highest net emissions of 1.1±0.2 g C m^{-2} d^{-1} (*P*<0.001), despite being the species with the highest biomass increase. *S. fallax* and *S. palustre* had net C effluxes similar to those of bare peat, with 0.5±0.1 and 0.2±0.1 g C m^{-2} d^{-1} respectively, even though significant increases in biomass were observed for these two species. C fluxes consisted almost exclusively of CO_2, as methane (CH_4) contributed less than 2.5% to net C emission and ranged from -0.8 to 43 mg C m^{-2} d^{-1} (data not shown). Still, due to the higher global warming potential of CH_4 compared with CO_2, CH_4 contributed 10 to 30 % to the total greenhouse gas emissions expressed in CO_2 equivalents. These greenhouse gas emissions were higher for plots covered by *S. squarrosum* (1750±350 gCO_2-eq m^{-2} y^{-1}) than for plots covered by other species (580±106 gCO_2-eq m^{-2} y^{-1}) or unvegetated (550±180 gCO_2-eq m^{-2} y^{-1}) plots due to the higher CO_2 emission from these plots (P=0.002; data not shown).

4. Discussion

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

4.1 Tolerance of *Sphagnum* species to buffered conditions

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

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

S. squarrosum is one of the few *Sphagnum* species that is still vital in systems with a higher influence of calcareous, and therefore HCO_3^--rich, water and is even able to tolerate (temporary) immersion in these systems (Clymo, 1973; Vitt and Chee, 1990). Other species, including *S. magellanicum,* are known to be highly sensitive to increased pH and buffered conditions in their habitat (Clymo, 1973; Granath et al., 2010), which explains why *S.*

magellanicum showed a strong decrease in our study. The typical habitats of *S. fallax*, *S. palustre* and *S. magellanicum* are all characterised by low pH (4.5-4.8) and low Ca^{2+} content (35-40 µmol L^{-1}) (Vitt and Chee, 1990; Hajek et al., 2006), although *S. fallax* and *S. palustre* can tolerate a wider range of environmental conditions in terms of acidity and trophic level than *S. magellanicum* (Daniels and Eddy, 1990). *S. squarrosum*, on the other hand often occurs in rich to moderately rich fens (Vitt and Chee, 1990; Hajek et al., 2006), which are characterised by pH values of 5.1 to 6.7 and Ca^{2+} concentrations of 270-500 µmol L^{-1} (Vitt and Chee, 1990; Kooijman and Bakker, 1994).

4.2 Succession of *Sphagnum* species

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

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

4.3 Carbon dynamics

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

Still, even with these high CO_2 fixation rates, we found net C emissions from both bare peat and from peat covered with growing *Sphagnum* mosses.

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

When plots were vegetated by growing *Sphagnum* spp., CO_2 emissions increased, despite the accumulation of biomass by all three species (Fig. 4), which indicates that the source of this CO_2 could not solely be the decomposition of *Sphagnum* litter. The only likely explanation for this remaining net CO_2 efflux is therefore the chemical reaction R. (1) that occurs when HCO_3^- -rich water comes into contact with the acidifying mosses (Fig. 4). The transition of HCO_3^- to CO_2 is the first step in the ANC of aquatic systems and will occur much faster than other buffering mechanisms, such as cation-exchange of Ca^{2+} (Lamers et al., 2015). Active acidification was mainly observed in *S. squarrosum*, while *S. fallax* and *S. palustre* did not significantly lower pH more than the dying *S. magellanicum*.

$HCO_3^- + H^+ \rightarrow H_2O + CO_2$ **R. 1**

To further disentangle the different CO_2 sources responsible for the net CO_2 emission from plots vegetated with different species, we used a mass approach (Eq. 1; Table 4). Net CO_2 fixation was estimated based on the difference between light and dark CO_2 fluxes, whereas CO_2 emission was estimated based on dark fluxes. This CO_2 emission can be further divided into separate contributors, as is shown in Eq. 1.

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

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

Table 4 shows the different sources of the C fluxes as presented in Eq.1. Furthermore, the implications that these values have on a landscape scale are depicted in a schematic overview of a floating transitional mire that is being fed by HCO_3^- rich water (Figure 4).

Here, we show simultaneous C fixation and C emission of the three growing *Sphagnum* species from our experiment, with their different growth and acidification rates.

The production of HCO_3^- -derived CO_2 will occur in any situation where HCO_3^- -rich water comes into contact with an acid environment, such as in the highly acidic lower layers of floating bog systems influenced by HCO_3^- -rich water (Lamers et al., 1999; Smolders et al., 2003). Therefore, CO_2 effluxes measured from the slightly acidic bare peat in our experiment, are likely at least partially derived from acid-driven CO_2 production from HCO_3^-, as is illustrated in Figure 4. Our finding that the most strongly acidifying and fastest growing mosses such as *Sphagnum squarrosum* show the highest C effluxes strongly suggests that active acidification enhances the production of HCO_3^- -derived CO_2.

This leads to the apparent contradiction that while growth of *Sphagnum* will lead to accumulation of organic matter and thus contributes to the build-up of a peat layer, it is accompanied by a large net efflux of CO_2 ranging from 0.2-1.1 g C m^{-2} d^{-1} (Table 4, Figure 4). While we show this phenomenon here in a controlled laboratory setting, net CO_2 effluxes have indeed been reported for transitional mires, with rates ranging from -0.34 to +0.16 g C m^{-2} d^{-1} (Moore and Knowles, 1987; Koch et al., 2008; Salm et al., 2009). As mentioned before, however, this phenomenon may not be limited to transitional mires. For example, bogs typically show an outflow of acid water (H^+ and organic acids) and therefore *Sphagnum* produced acids may also cause chemical CO_2 production outside the peatland system, thereby counteracting at least a part of the C sequestration realised by peat growth.

5. Conclusion

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

Author Contributions

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

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References

Alm, J., Schulman, L., Walden, J., Nykänen, H., Martikainen, P. J., and Silvola, J.: Carbon balance of a boreal bog during a year with an exceptionally dry summer, Ecology, 80, 161-174, 1999.

Andrus, R. E.: Some aspects of *Sphagnum* ecology, Canadian Journal of Botany, 64, 416-426, 1986.

Bache, B. W.: The role of calcium in buffering soils, Plant Cell Environ, 7, 391-395, 1984.

Belyea, L. R., and Malmer, N.: Carbon sequestration in peatland: patterns and mechanisms of response to climate change, Global Change Biology, 10, 1043-1052, 2004.

Bortoluzzi, E., Epron, D., Siegenthaler, A., Gilbert, D., and Buttler, A.: Carbon balance of a European mountain bog at contrasting stages of regeneration, New Phytologist, 172, 708-718, 2006.

Bubier, J. L., Bhatia, G., Moore, T. R., Roulet, N. T., and Lafleur, P. M.: Spatial and temporal variability in growing-season net ecosystem carbon dioxide exchange at a large peatland in Ontario, Canada, Ecosystems, 6, 353-367, 2003.

Buttler, A., Grosvernier, P., and Matthey, Y.: Development of *Sphagnum fallax* diaspores on bare peat with implications for the restoration of cut-over bogs, Journal of Applied Ecology, 35, 800-810, 1998.

Carroll, P., and Crill, P.: Carbon balance of a temperate poor fen, Global Biogeochemical Cycles, 11, 349-356, 1997.

Clymo, R. S.: Ion exchange in *Sphagnum* and Its relation to bog ecology, Ann. Bot., 27, 309-&, 1963.

Clymo, R. S.: Experiments on Breakdown of *Sphagnum* in Two Bogs, Journal of Ecology, 53, 747-758, 1965.

Clymo, R. S.: Growth of *Sphagnum* - Some Effects of Environment, Journal of Ecology, 61, 849-869, 1973.

Coulson, J. C., and Butterfield, J.: An investigation of the biotic factors determining the rates of plant decomposition on blanket bog, Journal of Ecology, 66, 631-650, 1978.

Daniels, R. E., and Eddy, A.: Handbook of European Sphagna, 2nd ed., edited by: Ecology, I. o. T., HMSO, London, UK, 1990.

Du Rietz, G. E.: Die Mineralbodenwasser- zeigergrenze als Grundlage einer natürlichen Zweigliederung der nord- und mitteleuropaischen Moore, Vegetatio, 5-6, 571-585, 1954.

Freeman, C., Ostle, N., and Kang, H.: An enzymic 'latch' on a global carbon store - A shortage of oxygen locks up carbon in peatlands by restraining a single enzyme, Nature, 409, 149-149, 2001.

Gerdol, R.: The growth dynamics of *Sphagnum* based on field measurements in a temperate bog and on laboratory cultures, Journal of Ecology, 83, 431-437, 1995.

Giller, K. E., and Wheeler, B. D.: Acidification and succession in a flood-plain mire in the Norfolk Broadland, Uk, Journal of Ecology, 76, 849-866, 1988.

Golovatskaya, E. A., and Dyukarev, E. A.: Carbon budget of oligotrophic mire sites in the Southern Taiga of Western Siberia, Plant Soil, 315, 19-34, 2009.

Gorham, E.: Northern peatlands - Role in the carbon cycle and probable responses to climatic warming, Ecological Applications, 1, 182-195, 1991.

Gorham, E., Janssens, J. A., and Glaser, P. H.: Rates of peat accumulation during the postglacial period in 32 sites from Alaska to Newfoundland, with special emphasis on northern Minnesota, Canadian Journal of Botany, 81, 429-438, 2003.

Graf, M., and Rochefort, L.: Examining the peat-accumulating potential of fen vegetation in the context of fen restoration of harvested peatlands, Ecoscience, 16, 158-166, 2009.

Granath, G., Strengbom, J., and Rydin, H.: Rapid ecosystem shifts in peatlands: linking plant physiology and succession, Ecology, 91, 3047-3056, 2010.

Grasshof, K., and Johannse, H.: New sensitive and direct method for automatic determination of ammonia in seawater, Journal Du Conseil, 34, 516-521, 1972.

Hajek, M., Horsak, M., Hajkova, P., and Dite, D.: Habitat diversity of central European fens in relation to environmental gradients and an effort to standardise fen terminology in ecological studies, Perspect Plant Ecol, 8, 97-114, 2006.

Hajek, T.: Habitat and species controls on *Sphagnum* production and decomposition in a mountain raised bog, Boreal Environment Research, 14, 947-958, 2009.

Hajek, T., and Adamec, L.: Mineral nutrient economy in competing species of *Sphagnum* mosses, Ecol Res, 24, 291-302, 2009.

Haraguchi, A., Hasegawa, T., Iyobe, T., and Nishijima, H.: The pH dependence of photosynthesis and elongation of *Sphagnum squarrosum* and *S. girgensohnii* in the Picea glehnii mire forest in Cape Ochiishi, north-eastern Japan, Aquat Ecol, 37, 101-104, 2003.

Henriksen, A.: An automated method for determining low-level concentrations of phosphate in fresh and saline waters, Analyst, 90, 29-34, 1965.

IPCC: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergoernmental Pane on Climate Change, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535, 2013.

Kamphake, L. J., Hannah, S. A., and Cohen, J. M.: Automated analysis for nitrate by hydrazine reduction, Water Res., 1, 205-&, 1967.

Kangas, L., Maanavilja, L., Hajek, T., Juurola, E., Chimner, R. A., Mehtatalo, L., and Tuittila, E. S.: Photosynthetic traits of Sphagnum and feather moss species in undrained, drained and rewetted boreal spruce swamp forests, Ecol Evol, 4, 381-396, 2014.

Koch, O., Tscherko, D., Kuppers, M., and Kandeler, E.: Interannual ecosystem CO2 dynamics in the alpine zone of the Eastern Alps, Austria, Arct Antarct Alp Res, 40, 487-496, 2008.

Kooijman, A. M., and Bakker, C.: The acidification capacity of wetland bryophytes as influenced by simulated clean and polluted rain, Aquatic Botany, 48, 133-144, 1994.

Kooijman, A. M., and Bakker, C.: Species replacement in the bryophyte layer in mires - the role of water type, nutrient supply and interspecific interactions, Journal of Ecology, 83, 1-8, 1995.

Laine, A. M., Juurola, E., Hajek, T., and Tuittila, E. S.: Sphagnum growth and ecophysiology during mire succession, Oecologia, 167, 1115-1125, 2011.

Lamers, L. P. M., Farhoush, C., Van Groenendael, J. M., and Roelofs, J. G. M.: Calcareous groundwater raises bogs; the concept of ombrotrophy revisited, Journal of Ecology, 87, 639-648, 1999.

Lamers, L. P. M., Vile, M. A., Grootjans, A. P., Acreman, M. C., Van Diggelen, R., Evans, M. G., Richardson, C. J., Rochefort, L., Kooijman, A. M., Roelofs, J. G. M., and Smolders, A. J. P.: Ecological restoration of rich fens in Europe and North America: from trial and error to an evidence-based approach, Biological Reviews, 2015.

Limpens, J., and Berendse, F.: How litter quality affects mass loss and N loss from decomposing *Sphagnum*, Oikos, 103, 537-547, 2003.

Martikainen, P. J., Nykänen, H., Alm, J., and Silvola, J.: Change in fluxes of carbon-dioxide, methane and nitrous-oxide due to forest drainage of mire sites of different trophy, Plant Soil, 168, 571-577, 1995.

Moore, P. D.: The future of cool temperate bogs, Environ Conserv, 29, 3-20, 2002.

Moore, T. R., and Knowles, R.: Methane and carbon dioxide evolution from subarctic fens, Canadian Journal of Soil Science, 67, 77-81, 1987.

Moore, T. R., and Roulet, N. T.: Methane emissions from Canadian peatlands, in: Soils and Global Change, Advances in Soil Science, edited by: Lal, R., Kimble, J., Levine, R., and Stewart, B. A., Lewis Publishers, Baco Raton, Fl, 153-164, 1995.

Nijp, J. J., Limpens, J., Metselaar, K., van der Zee, S. E. A. T. M., Berendse, F., and Robroek, B. J. M.: Can frequent precipitation moderate the impact of drought on peatmoss carbon uptake in northern peatlands?, New Phytologist, 203, 70-80, 2014.

Rippy, J. F. M., and Nelson, P. V.: Cation exchange capacity and base saturation variation among Alberta, Canada, moss peats, Hortscience, 42, 349-352, 2007.

Riutta, T., Laine, J., and Tuittila, E. S.: Sensitivity of CO2 exchange of fen ecosystem components to water level variation, Ecosystems, 10, 718-733, 2007.

Robroek, B. J. M., van Ruijven, J., Schouten, M. G. C., Breeuwer, A., Crushell, P. H., Berendse, F., and Limpens, J.: *Sphagnum* re-introduction in degraded peatlands: The effects of aggregation, species identity and water table, Basic and Applied Ecology, 10, 697-706, 2009.

Rochefort, L., Vitt, D. H., and Bayley, S. E.: Growth, production, and decomposition dynamics of *Sphagnum* under natural and experimentally acidified conditions, Ecology, 71, 1986-2000, 1990.

Rowson, J. G., Gibson, H. S., Worrall, F., Ostle, N., Burt, T. P., and Adamson, J. K.: The complete carbon budget of a drained peat catchment, Soil Use and Management, 26, 261-273, 2010.

Rydin, H., and Jeglum, J. K.: Biology of peatlands, Oxford University Press, Oxford, 2006.

Saarnio, S., Morero, M., Shurpali, N. J., Tuittila, E. S., Makila, M., and Alm, J.: Annual CO2 and CH4 fluxes of pristine boreal mires as a background for the lifecycle analyses of peat energy, Boreal Environment Research, 12, 101-113, 2007.

Salm, J. O., Kimmel, K., Uri, V., and Mander, U.: Global warming potential of drained and undrained peatlands in Estonia: a synthesis, Wetlands, 29, 1081-1092, 2009.

Samaritani, E., Siegenthaler, A., Yli-Petays, M., Buttler, A., Christin, P. A., and Mitchell, E. A. D.: Seasonal net ecosystem carbon exchange of a regenerating cutaway bog: how long does it take to restore the C-sequestration function?, Restoration Ecology, 19, 480-489, 2011.

Sherlock, E. J., Lawrence, R. W., and Poulin, R.: On the neutralization of acid rock drainage by carbonate and silicate minerals, Environ Geol, 25, 43-54, 1995.

Sjörs, H., and Gunnarsson, U.: Calcium and pH in north and central Swedish mire waters, Journal of Ecology, 90, 650-657, 2002.

Smolders, A. J. P., Tomassen, H. B. M., Lamers, L. P. M., Lomans, B. P., and Roelofs, J. G. M.: Peat bog restoration by floating raft formation: the effects of groundwater and peat quality, Journal of Applied Ecology, 39, 391-401, 2002.

Smolders, A. J. P., Tomassen, H. B. M., Van Mullekom, M., Lamers, L. P. M., and Roelofs, J. G. M.: Mechanisms involved in the re-establishment of *Sphagnum*-dominated vegetation in rewetted bog remnants, Wetlands Ecology and Management 11, 403-418, 2003.

Van Breemen, N.: How *Sphagnum* bogs down other plants, Trends in Ecology & Evolution, 10, 270-275, 1995.

Verberk, W. C. E. P., Leuven, R. S. E. W., van Duinen, G. A., and Esselink, H.: Loss of environmental heterogeneity and aquatic macroinvertebrate diversity following large-scale restoration management, Basic and Applied Ecology, 11, 440-449, 2010.

Verhoeven, J. T. A., and Toth, E.: Decomposition of *Carex* and *Sphagnum* litter in fens - Effect of litter quality and inhibition by living tissue-homogenates, Soil Biology & Biochemistry, 27, 271-275, 1995.

Vitt, D. H., and Chee, W. L.: The relationships of vegetation to surface-water chemistry and peat chemistry in fens of Alberta, Canada, Vegetatio, 89, 87-106, 1990.

Waddington, J. M., and Roulet, N. T.: Carbon balance of a boreal patterned peatland, Global Change Biology, 6, 87-97, 2000.

Waddington, J. M., Rotenberg, P. A., and Warren, F. J.: Peat CO2 production in a natural and cutover peatland: implications for restoration, Biogeochemistry, 54, 115-130, 2001.

Wheeler, B. D., and Proctor, M. C. F.: Ecological gradients, subdivisions and terminology of north-west European mires, Journal of Ecology, 88, 187-203, 2000.

Wilcox, D. A., and Andrus, R. E.: The role of *Sphagnum fimbriatum* in secondary succession in a road salt impacted Bog, Canadian Journal of Botany, 65, 2270-2275, 1987.

Yavitt, J. B., Williams, C. J., and Wieder, R. K.: Controls on microbial production of methane and carbon dioxide in three *Sphagnum*-dominated peatland ecosystems as revealed by a reciprocal field peat transplant experiment, Geomicrobiology Journal, 17, 61-88, 2000.

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}) [1]	-	5

[1] Pro Reef, Tropic Marine, aQua united LTD, Telgte, Germany.

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

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

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.

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

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

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

Captions to Figures

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

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

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

Fig. 4: Schematic overview of a transitional floating mire influenced by HCO_3^- -rich groundwater or surface water, illustrated by dashed arrows in the figure above. Due to differences in the thickness of the floating peat or the origin and composition of the HCO_3^- -rich water, there is a high heterogeneity within these systems. Part of the floating raft is shown in more detail below. Here, peat soils are covered with different *Sphagnum* species.

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

Supplementary Fig. 1: A linear relationship (R^2=0.43; P=0.005) exists between biomass production of *Sphagnum* mosses and acidification of the environment. Here, ♦ = *S. magellanicum*, ■ = *S. fallax*, ▲ = *S. palustre* and ● = *S. squarrosum*. *Sphagnum* species that show the highest biomass increases also have a higher acidification rate, leading to lower pH in their environment than slower growing species.