Global CO2 Fertilization of Sphagnum Peat Mosses via Suppression of Photorespiration During the 20th Century

Henrik Serk
Umeå University

Mats Nilsson (✉ mats.b.nilsson@slu.se)
Swedish University of Agricultural Sciences

Elisabet Bohlin
Swedish University of Agricultural Sciences

Ina Ehlers
Umeå University

Thomas Wieloch
Umeå University

Carolina Olid
Swedish University of Agricultural Sciences

Samantha Grover
RMIT University

Karsten Kalbitz
TU Dresden

Juul Limpens
Wageningen University & Research

Tim Moore
McGill University

Wiebke Münchberger
University of Münster

Julie Talbot
University of Montreal

Xianwei Wang
Chinese Academy of Sciences

Klaus-Holger Knorr
University of Münster

Verónica Pancotto
National Scientific and Technical Research Council

Jürgen Schleucher
Abstract

Natural peatlands contribute significantly to global carbon sequestration and storage of biomass, most of which derives from *Sphagnum* peat mosses. Atmospheric CO$_2$ levels have increased dramatically during the 20th century, from 280 to > 400 ppm, which has affected plant carbon dynamics. Net carbon assimilation is strongly reduced by photorespiration, a process that depends on the CO$_2$ to O$_2$ ratio. Here we investigate the response of the photorespiration to photosynthesis ratio in *Sphagnum* mosses to recent CO$_2$ increases by comparing deuterium isotopomers of historical and contemporary *Sphagnum* tissues collected from 36 peat cores from five continents. Rising CO$_2$ levels generally suppressed photorespiration relative to photosynthesis but the magnitude of suppression depended on the current water table depth. By estimating the changes in water table depth, temperature, and precipitation during the 20th century, we excluded potential effects of these climate parameters on the observed isotopomer responses. Further, we showed that the photorespiration to photosynthesis ratio varied between *Sphagnum* subgenera, indicating differences in their photosynthetic capacity. The global suppression of photorespiration in *Sphagnum* suggests an increased net primary production potential in response to the ongoing rise in atmospheric CO$_2$, in particular for mire structures with intermediate water table depths.

Introduction

Over one third of global soil carbon (C) is stored in boreal mires $^{1,2}$, making peat C accumulation an essential part of the global C budget. Changes in climate are expected to have strong effects on peatland C sequestration $^{1,3,4}$. During the early and mid-Holocene, the accumulation of peat C was largely determined by the retreat of the northern ice sheet and the rise in temperature because atmospheric CO$_2$ concentrations were relatively stable at 275 ± 8 ppm (SD) $^{5,6,7}$. Since the beginning of the industrial revolution in the early 19th century, CO$_2$ concentrations have risen from ca. 280 ppm to over 400 ppm today $^8$. Multiple observations indicate that recent increases in atmospheric CO$_2$ have affected peat C accumulation rates: i) the variation in acrotelm peat accumulation was mainly driven by photosynthesis $^9$, ii) peat C accumulation in Alaskan mires increased about 3-fold during the 20th century $^{10}$, and iii) the variation in net ecosystem exchange between mires was mainly controlled by differences in leaf area index $^{11}$. In addition to rising atmospheric CO$_2$ levels, ongoing climatic changes such as increases in temperature and changes in precipitation are hypothesized to influence peatland C fluxes $^{12,13,14}$.

*Sphagnum* peat mosses are primarily responsible for the accumulation of peat C because they often constitute 80–100% of the ground cover in northern peatlands $^{15}$. Compared to vascular plants, *Sphagnum* remnants are highly resistant to microbial decay, which is vital for peat C accumulation $^{16,17}$. Therefore, C accumulation and storage in the form of *Sphagnum* remains generally exceeds C losses from microbial decay. However, it is not currently clear whether increases in *Sphagnum* C accumulation driven by ongoing and projected global warming will outweigh increases in the rate of microbial peat
Understanding how *Sphagnum* C fluxes respond to recent and projected increases in atmospheric CO$_2$ is therefore crucial for predicting future peat C fluxes.

To our knowledge, responses of *Sphagnum* photosynthetic C fluxes to the recent increase in atmospheric CO$_2$ have never been explored on the global scale. Previous attempts to estimate responses of *Sphagnum* to increased atmospheric CO$_2$ were either based on free-air CO$_2$ enrichment (FACE) or greenhouse experiments$^{20-25}$. A recently developed isotopomer method$^{26,27}$ enables reconstruction of metabolic C fluxes by analyzing cell wall carbohydrates from *Sphagnum* remnants. This approach involves using NMR spectroscopy to measure the abundance ratio of the deuterium (D) isotopomers D$^6$S and D$^6$R in the C$_6$H$_2$ groups of glucose derived from hydrolyzed cell wall carbohydrates. The abundance of these D isotopomers is linked to the ratio of Rubisco oxygenation to carboxylation, which essentially depends on the substrate ratio of CO$_2$ and O$_2$ $^{26}$. Therefore, the D$^6$S/D$^6$R ratio reflects the relative rates of photorespiration and (gross) photosynthesis. Photorespiration reduces net C uptake in C$_3$ plants, and thus is an important factor for the global terrestrial C sink $^{28}$. However, most studies focus on photorespiration in higher plants and only little is known about *Sphagnum* mosses.

Using the isotopomer method, we recently experimentally investigated the response of the photorespiration to photosynthesis ratio (i.e. the D$^6$S/D$^6$R ratio) to the recent increase in atmospheric CO$_2$ levels from 280 to 400 ppm and the dependence of this response on selected climate variables including temperature, water table (WT) depth, and light intensity $^{27}$. We found that under well-aerated conditions, photorespiration was suppressed relative to photosynthesis in the hummock species *Sphagnum fuscum*. Under water-saturating conditions, however, there was no effect of atmospheric CO$_2$, indicating that WT depth strongly influences the CO$_2$ fertilization effect in *S. fuscum*. In addition, the lawn species *S. majus* did not respond to the CO$_2$ increase, suggesting a species-specific response. Therefore, *Sphagnum* photosynthetic C fluxes are expected to vary with species and microhabitat $^{29-32}$.

The aim of this study is to investigate changes in *Sphagnum* photosynthetic C fluxes during the 20th century at the global scale. To do this, we estimated the global response of the photorespiration to photosynthesis ratio by comparing the D$^6$S/D$^6$R ratios of modern *Sphagnum* tissues formed at the current atmospheric CO$_2$ concentration (ca. 400 ppm) to the D$^6$S/D$^6$R ratios of *Sphagnum* remnants from peat core sections formed at least 100 years ago under pre-industrial CO$_2$ concentrations ($\leq$ 300 ppm). Our analysis is based on 36 peat cores from 10 different sites on five continents (Fig. 1A). Effects of microhabitat were tested by including both hummock (n = 25) and lawn (n = 11) samples with WT depths ranging from ≈ 5 to 70 cm below the moss surface. The effect of species was tested by including eight different *Sphagnum* species belonging to three different taxonomic sections (subgenera). To determine whether the CO$_2$ response of the photorespiration to photosynthesis (D$^6$S/D$^6$R) ratio in *Sphagnum* was influenced by changes in temperature and precipitation during the 20th century, we estimated these changes using established climate models $^{33,34,35}$. The effects of changes in WT depth during the 20th
Global suppression of photorespiration during the 20th century

Global changes in the photorespiration to photosynthesis ratio during the 20th century were assessed by comparing the D6^S/D6^R ratios of modern and ≥100 years old Sphagnum tissues. Modern Sphagnum samples were retrieved from surface peat (top 0-2 cm) formed at contemporary atmospheric CO_2 levels (ca. 400 ppm). Conversely, historical Sphagnum tissues were retrieved from peat layers ≥ 30 cm below the surface, with an approximate age of 100 years or more (Table S1). Thus, the historical Sphagnum tissues were formed when atmospheric CO_2 concentrations were ≤ 300 ppm. The D6^S/D6^R ratio of modern Sphagnum was 0.860 ± 0.004 (average ± SE, range: 0.810-0.927), while that of historical Sphagnum was 0.901 ± 0.005 (average ± SE, range: 0.858-0.971, Figure S1). Thus, the D6^S/D6^R ratio of modern Sphagnum was significantly lower (t-test, p < 0.001) compared to ≥100 years old Sphagnum, indicating that photorespiration is suppressed relative to photosynthesis.

The peat samples differed with respect to their depth below the surface (which is related to the atmospheric CO_2 concentration when the Sphagnum biomass was formed), the Sphagnum subgenus, the present WT depth, and the geographical location (site). Effects of differences in the site are related to differences in climate, and are investigated later on. Therefore, we performed a three-way analysis of variance (ANOVA) to test the effects of atmospheric CO_2, WT and subgenus on the D6^S/D6^R ratio (Table 1). Atmospheric CO_2 and subgenus had a significant effect and explained 39% and 8% respectively (p<0.001 and p=0.002, respectively). A significant interaction was found between CO_2 and WT (p < 0.001), indicating that the effect of CO_2 on the D6^S/D6^R ratio is dependent on the WT depth.

Table 1

Three-way ANOVA models of effects of atmospheric CO_2, water table (WT) and Sphagnum subgenus (Subg.) on the D6^S/D6^R ratio.
The effect of atmospheric CO$_2$ on the D$_6^S$/D$_6^R$ ratio clearly depended on the present WT depth. For samples where the WT was < 10 cm below the moss surface, the D$_6^S$/D$_6^R$ ratios were not significantly different (t-test, $p = 0.199$) between modern and historical Sphagnum, with means of 0.893 ± 0.01 and 0.905 ± 0.009 (SE), respectively. Conversely, for samples with WT depth of 10-40 cm, the D$_6^S$/D$_6^R$ ratios of modern and historical Sphagnum clearly differed ($p < 0.001$), having means of 0.850 ± 0.004 and 0.910 ± 0.007 (SE), respectively (Figure 1 and S1). For the WT >40 cm group, the difference between modern and historical samples was again not significant ($p = 0.073$), with means of 0.860 ± 0.007 and 0.873 ± 0.004 (average ± SE), respectively.

The Sphagnum subgenus had a clear effect: the mean D$_6^S$/D$_6^R$ ratios for modern and historical samples of ACUTIFOLIA species were 0.858 ± 0.004 and 0.906 ± 0.007 (SE), respectively ($p < 0.001$), while those for species of the subgenus SPHAGNUM were 0.846 ± 0.006 and 0.885 ± 0.006 (SE), respectively ($p < 0.001$). The D$_6^S$/D$_6^R$ ratio was thus generally lower for species of the subgenus SPHAGNUM than for ACUTIFOLIA species. Modern and historical samples of CUSPIDATA species had higher mean D$_6^S$/D$_6^R$ ratios of 0.911 ± 0.008 and 0.921 ± 0.009 (SE), respectively, with no significant difference ($p = 0.211$) between modern and historical samples (Figure 1 and S1).

The differences in the D$_6^S$/D$_6^R$ ratio between modern and historical Sphagnum were normalized based on the linear relationship between the D$_6^S$/D$_6^R$ ratio and 1000/[CO$_2$] previously reported by Ehlers et al. (2015), to account for variations in atmospheric CO$_2$ concentrations due to differences in peat depth and/or age. To this end, the regression slope of this linear function was calculated as the change in the D$_6^S$/D$_6^R$ ratio per unit change in 1000/[CO$_2$] between modern and historical Sphagnum samples (denoted $\Delta$D$_6^S$/D$_6^R_N$). $\Delta$D$_6^S$/D$_6^R_N$ thus represents the degree of suppression of photorespiration; its mean was 0.044 ± 0.008 (SE) and it varied between 0.000 and 0.094 (Figure 1). $\Delta$D$_6^S$/D$_6^R_N$ varied with the WT depth: it was 0.010 ± 0.005 (average ± SE) for WT depths < 10 cm below the moss surface, 0.066 ± 0.006
(average ± SE) for WT depths between 10 and 40 cm, and 0.016 ± 0.010 (average ± SE) for WT depths >40 cm (Figure 1). These results indicate that a WT between 10 and 40 cm below the moss surface is optimal for suppressing photorespiration in response to increased atmospheric CO$_2$.

**Effect of changes in water table, temperature and precipitation during the 20$^{th}$ century**

The relationship between ΔD$_6^S$/D$_6^RN$ and the present WT depth assumes that hydrological conditions were relatively stable over the 20$^{th}$ century. To support this assumption, we estimated changes in WT depth based on available testate amoebae reconstruction data (Table 2). Data were obtained for peat cores with WT depth >10 cm only (and site 1, WT = 8 cm). In cases where data were not available for the sampled mires, data were acquired for mires from the same region (Table 2 and S2), assuming that the similarities in regional climate would result in similar changes in WT depth $^{37}$. Additionally, variations in other climate parameters such as temperature and precipitation may have affected the suppression of photorespiration (ΔD$_6^S$/D$_6^RN$). Therefore, we also estimated changes in temperature and precipitation during the 20$^{th}$ century using available climate models $^{33,34,35}$ (Table 2).

| Site | Δ WT (cm) | Δ MAT (°C) | Δ TAP (%) | Reference (WT) |
|------|-----------|------------|-----------|----------------|
| 1    | ≈ 0       | + 3.1      | + 9.9     | 38             |
| 2, 3 | + 4       | + 0.2      | - 6.7     | 39, 40         |
| 4    | - 40*     | - 1.7      | - 32.6    | 41             |
| 5    | ≈ 0*      | + 2.8      | + 19.1    | 37             |
| 6    | - 5*      | + 1.3      | + 11.1    | 37, 42         |
| 7, 8 | - 11*     | + 0.4      | + 12.5    | 37             |
| 9    | - 6       | + 1.6      | + 25.0    | 43             |
| 10   | - 30*     | + 0.1      | + 15.1    | 44             |

Changes in TAP are specified in percent change. (-) indicates a decrease and (+) indicates an increase. WT data were obtained only for peat cores with WT >10 cm, and for site 1 (WT = 8 cm), n = 30. (*) indicates WT data for another mire in the same region as the relevant site (Table S2).
Changes in WT during the 20th century were generally small (between +4 and -11 cm), except in southern Argentina and Australia, where the WT depth decreased by 40 and 30 cm, respectively (Table 2). To determine whether changes in historical climate data contribute to the $\Delta D_6^S/D_6^R_N$-response, we performed a three-way ANOVA, with WT, mean annual air temperature (MAT), and total annual precipitation (TAP) as factors. No significant effect of WT, MAT and TAP could be detected ($p > 0.2$). However, a significant interaction was found between WT and MAT ($R^2=0.16$, $F=53.8$, $p=0.028$). Further, we tested if specifically the mean summer air temperature (MSAT) or the total summer precipitation (TSP) effect the $\Delta D_6^S/D_6^R_N$-response by performing a three-way ANOVA with WT, MSAT and TSP as factors. Again no significant effect could be detected ($p > 0.2$), except for an interaction between WT and MSAT ($R^2=0.17$, $F=53.3$, $p=0.029$). Thus, a small part of the variation in $\Delta D_6^S/D_6^R_N$ (17%) of *Sphagnum* may be explained by combined changes in WT and temperature during the 20th century.

**Sphagnum $\delta^{13}C$ as proxy for changes in water table depth**

The carbon isotopic signature ($\delta^{13}C$) of *Sphagnum* peat has been proposed as proxy for surface moisture \cite{27,36,45,46}. Therefore, we tested the use of $\delta^{13}C$ as a potential indicator of changes in WT depth by measuring the $\delta^{13}C$ of both modern and $\geq 100$ years-old whole-*Sphagnum* tissues. Regression analysis revealed a highly significant correlation between $\delta^{13}C$ in modern *Sphagnum* and the present WT depth ($R^2=0.67$, $p<0.001$, Figure 2) confirming that $\delta^{13}C$ reflects changes in WT depth. The $\delta^{13}C$ values became more negative (i.e. more depleted) with increasing WT depth, from $-25.8 \pm 0.2\%o$ (average ± SE) at WT depths < 10 cm to $-30.3 \pm 0.5\%o$ (average ± SE) at WT depths > 60 cm below surface. For $\geq 100$ years-old *Sphagnum*, $\delta^{13}C$ also correlated significantly with the present WT depth ($R^2 = 0.40$, $p < 0.001$). Both modern and historical *Sphagnum* showed the same trend (with slopes of 0.07 and 0.06, respectively, Figure 2), indicating that the WT depth $\geq 100$ years ago was similar to that today. The $\delta^{13}C$ of modern *Sphagnum* was more negative than that of historical *Sphagnum* (average difference 1.9 ± 0.9\%o SD) in all peat cores bar one that showed an increase (2.9\%o, site 9, Figure S2), and was excluded from the regression in Figure 2.

**Discussion**

Our results show that the increase in atmospheric CO$_2$ during the 20th century suppressed photorespiration relative to C assimilation in *Sphagnum* mosses, thus increasing the potential net photosynthesis. However, this suppression is strongly dependent on the moisture status of the moss. High moisture contents, typical for *Sphagnum* grown at WT depths < 10 cm, resulted in no significant suppression of photorespiration, i.e. the mean $\Delta D_6^S/D_6^R_N$ value of the < 10 cm WT group (0.010; Fig. 1) was not significantly different from zero (t-test, $p = 0.066$). In contrast, the mean $\Delta D_6^S/D_6^R_N$ for WT depths between 10 and 40 cm was 0.066 ($p < 0.001$), clearly reflecting a strong suppression of photorespiration. This latter response is identical to that obtained from CO$_2$ manipulation experiments.
with higher C_3 plants and corresponds to an increase in net photosynthesis of 35%, assuming constant ribulose 1,5-bisphosphate (RuBP) turnover rates^{26,47}. The similarity of the kinetic properties of Rubisco between different C_3 plants (including mosses)\(^{48}\), suggests that the observed response in \textit{Sphagnum} also corresponds to an increase in net photosynthesis of 35%. Conversely, the low \(\Delta D_6^S/D_6^R_N\) value for \textit{Sphagnum} grown at WT < 10 cm indicates no significant increase in net photosynthesis. This suggests that hollow and lawn \textit{Sphagnum} communities that experience high WT do not profit from CO\(_2\) fertilization. However, CO\(_2\) fertilization is beneficial in mire structures that experience intermediate WT depths, mostly hummocks, and may therefore stimulate hummock formation and topographic development as atmospheric CO\(_2\) concentrations rise.

Some factors may limit the CO\(_2\) fertilization effect in hummocks. In particular, our data show that a very low WT (> 40 cm) has a negative effect on CO\(_2\) fertilization, \textit{i.e.} on the suppression of photorespiration: the mean \(\Delta D_6^S/D_6^R_N\) value for samples in the > 40 cm WT group (0.016; Fig. 1) was not significantly different from zero (\(p = 0.122\); Fig. 1). This indicates that \textit{Sphagnum} mosses do not respond to increased atmospheric CO\(_2\) under water limiting conditions, \textit{i.e.} drought. Climate modelling of peatland C fluxes indicates that \textit{Sphagnum} gross primary production decreases significantly at WT > 40 cm\(^{49}\).

Concomitantly, increased releases of CO\(_2\) from sub-surface peat decomposition during drought\(^{50,51}\), may decouple the mosses’ response to changes in atmospheric CO\(_2\).

The \(D_6^S/D_6^R\) ratio of \textit{S. fuscum} responded strongly to experimental manipulation of the CO\(_2\) concentration, rising by 0.03 on average (\(\Delta D_6^S/D_6^R_N\)) when CO\(_2\) levels increased by 120 ppm at a WT of 20 cm. This trend was largely unaffected by varying the temperature\(^{27}\). In \textit{S. fuscum} samples from peat cores with WT depths > 10 cm, the suppression of photorespiration (\(\Delta D_6^S/D_6^R_N\)) was 0.06 on average in response to a CO\(_2\) concentration increase of ca. 100 ppm (Fig. 1). This suggests that the response to changes in atmospheric CO\(_2\) levels under field conditions has been stronger than in growth chambers. In the field, environmental conditions such as light and nutrient levels may differ from the growth chamber conditions\(^{52,53}\). Whether these factors affect the CO\(_2\)-driven suppression of photorespiration requires further investigation.

The peat core data in this study showed that higher CO\(_2\) concentrations did not cause any suppression of photorespiration in \textit{S. majus} grown at typical WT depths close to the mire surface (\(\Delta D_6^S/D_6^R_N\): 0.00, Fig. 1). This result is consistent with the response observed in CO\(_2\) manipulation experiments with \textit{S. majus} for WT levels of 0 and 7 cm (\(\Delta D_6^S/D_6^R_N\): 0.00)\(^{27}\). Overall, the growth chamber experiments showed that water-saturating conditions prevent the suppression of photorespiration in both hummock and lawn \textit{Sphagnum} species. The responses of the \(D_6^S/D_6^R\) ratio and \(\delta^{13}C\) in our global dataset (Fig. 1, 2, S1, S2) are consistent with these results and demonstrate that a WT depth deeper than 10 cm is needed to maximize photosynthetic C fluxes in \textit{Sphagnum}. Under these conditions, the mosses have the strongest potential to respond to the CO\(_2\) fertilization effect.
The ANOVA (Table 1) showed a significant effect of the subgenus on the \( D_6^S/D_6^R \) ratio. Species of the section SPHAGNUM had generally lower \( D_6^S/D_6^R \) ratios than ACUTIFOLIA species, suggesting that the relative rate of photorespiration is lower in SPHAGNUM species. The ratio of photorespiration to photosynthesis is directly related to the intracellular \( \text{CO}_2 \) concentration \( (c_i) \) at the site of Rubisco carboxylation \(^{54}\), suggesting differences in \( c_i \) between these two subgenera. Distinct leaf anatomical traits are responsible for different water holding capacities of these two subgenera \(^{55}\), and potentially influence \( c_i \). Altogether, this indicates that species of the subgenus SPHAGNUM have higher photosynthetic capacities than ACUTIFOLIA species, assuming that the RuBP turnover rates are similar.

Concerning the \( \text{CO}_2 \)-driven suppression of photorespiration, both ACUTIFOLIA and SPHAGNUM species showed a high suppression of photorespiration, and no difference in this response between these two subgenera (average \( \Delta D_6^S/D_6^R_{\text{N}} = 0.05 \) for both). Thus, no significant interaction was found between \( \text{CO}_2 \) and subgenus (Table 1). In contrast, species of the subgenus CUSPIDATA showed no suppression of photorespiration (Fig. 1). Unlike the ACUTIFOLIA and SPHAGNUM samples (WT range 8 to 67 cm, both), the CUSPIDATA samples came from high WT cores (WT ca. 5 cm), making it impossible to determine whether this response is species-specific. However, CUSPIDATA species require high WT levels to maintain growth \(^{56}\), suggesting that they will not respond to changes in atmospheric \( \text{CO}_2 \) in any case.

Our dataset revealed that the extent of \( \text{CO}_2 \)-driven suppression of photorespiration in \textit{Sphagnum} mosses depends on the WT depth. Among the sites included in this study, the shifts in the WT appear to have been relatively modest over the last 100 years (Table 2). This is consistent with the observed dependency of \( \delta^{13}\text{C} \) on WT depth, which is conserved for historical \textit{Sphagnum} samples. These results support our assumption that changes in hydrological conditions during the 20th century did not attenuate the suppression of photorespiration caused by rising atmospheric \( \text{CO}_2 \) levels.

Our data did not indicate any relationship between the temperature-increase during the 20th century and the suppression of photorespiration. This is consistent with the absence of an effect of temperature on the suppression of photorespiration observed in climate chamber experiments \(^{27}\), where a temperature increase of 5°C did not affect the magnitude of suppressed photorespiration (\( \Delta D_6^S/D_6^R_{\text{N}} \)). However, the photorespiration to photosynthesis (\( D_6^S/D_6^R \)) ratio increased slightly with elevated temperature (0.002°C\(^{-1}\)) \(^{27}\). Thus, the concomitant increase in temperature with atmospheric \( \text{CO}_2 \) during the 20th century may have reduced the \( \text{CO}_2 \)-driven suppression of photorespiration by approximately 0.002 units (for a temperature increase of 1°C) \(^{8}\).

On the molecular scale, the \( D_6^S/D_6^R \) ratio reflects changes in the Rubisco oxygenation to carboxylation flux ratio. Here we extended this molecular model to study global responses in these metabolic C fluxes to environmental drivers in \textit{Sphagnum}. Our results indicate that ongoing increases in atmospheric \( \text{CO}_2 \) suppress photorespiration relative to C assimilation in \textit{Sphagnum}. According to photosynthesis models, this suppression may increase C uptake by up to 35% (depending on the WT level), which points towards
an increase in net primary production (NPP) during the 20th century. However, NPP is influenced by many other factors such as temperature, precipitation and sink limitations $^9, ^{57}$. Modelling of peatland C dynamics $^{49, 58}$ suggests that temperature and precipitation have opposing influences on the C uptake response to the recent increase in atmospheric CO$_2$. An increase in temperature combined with a decrease in precipitation reduces C uptake, whereas a small increase in temperature combined with a large increase in precipitation results in enhanced C uptake. We found a significant interaction between the mean annual/summer temperature and WT during the 20th century, indicating a possible link between the observed changes in metabolic fluxes on the molecular level and global peat C assimilation. Thus, upscaling of the isotopomer data to global responses in peat C fluxes provide valuable information for the mechanistic understanding of photosynthetic responses of _Sphagnum_ mosses to ongoing and future climate changes.

**Conclusion**

Here we used deuterium isotopomers to study global responses in photosynthetic C fluxes in _Sphagnum_ mosses to the 20th century’s increase in atmospheric CO$_2$. This method allowed us to upscale changes in metabolic C flux ratios from the molecular level to the global scale. We were able to track historical changes of metabolic C fluxes over long time scales using peat archives, and link these results to observations from short-term manipulation experiments. Thus, our results will help to: i) develop mechanistic models of global metabolic C fluxes, ii) assess the role of peatlands for the global C budget during the 20th century, and iii) improve the prediction of future responses of peatlands to increases in atmospheric CO$_2$ and climate change. Furthermore, our results point out that mire structures with intermediate WT depths, such as hummocks, will benefit strongly from CO$_2$ fertilization, unlike lawn and hollow _Sphagnum_ communities that often experience high WT depths.

**Materials And Methods**

**Plant material - peat cores.**

Hummock and lawn peat cores were retrieved from 10 sites located in Sweden (2), Italy (2), Canada (3), China (1), Argentina (1), and Australia (1) between 2014 and 2018. The sites’ latitudes ranged from 55°S to 64°N, their mean annual air temperatures from -3.9°C to 6.3°C, mean annual precipitation levels from 369 mm to 1270 mm, and elevations from 35 m to 1700 m asl. Detailed descriptions of each site are provided in the Supplement (Table S2). Historical _Sphagnum_ samples were retrieved from peat depths between 30 and 40 cm, except in the cores from southern Argentina (60 cm). All historical samples were found to be approximately 100 years or older, based on $^{210}$Pb radiometric dating or estimates obtained using published age-depth profiles for each site (Table S1). The atmospheric CO$_2$ concentration when the historical _Sphagnum_ samples formed was estimated for the respective year, and ranged between 280 and 310 ppm (Table S1) $^{59}$. Since no major changes in atmospheric CO$_2$ levels occurred between year 0 and
1900, uncertainties in the age-estimates are not expected to have major effects on the estimated CO$_2$ concentration.

Peat cores were extracted using a sharp knife or a peat corer, yielding cores with dimensions of 8 x 8 cm or diameters of 10 cm, respectively. Peat cores were either wrapped in plastic film and stored at -20°C or sliced into 2 cm sections, oven dried (at 60°C), and transferred to Ziploc-bags before further transport to Sweden. In the case of frozen cores, the topmost and bottom-most 2 cm were sliced off and thawed, and vascular plant material and mosses other than _Sphagnum_ were removed. In cases where no intact species could be retrieved from the bottom of the core (6 cores), the material was washed through a series of sieves (mesh sizes: 3 mm, 1.6 mm, 0.8 mm and 0.5 mm). The residue of the 0.5 mm sieve consisted almost exclusively of _Sphagnum_ leaves. _Sphagnum_ species from the top and bottom of the core were identified using a stereomicroscope. Peat cores in which the dominant _Sphagnum_ species differed between top and bottom were excluded because such differences may indicate a change in microtopography. After processing as described above, samples were dried at 60°C for 3 days.

**Sample preparation for Deuterium isotopomer measurements**

The dried _Sphagnum_ samples were ground to a fine powder at 30 Hz for 2 min using a MM 400 ball mill (Retsch®, Haan, Germany), and 200-700 mg portions were used to prepare samples for Deuterium isotopomer measurements. Glucose-containing structural polymers were hydrolyzed to glucose and converted to 1,2-O-isopropylidene-α-D-glucofuranose according to established protocols$^{60}$. To remove contamination by a mannose derivative whose NMR signals overlap with those of the glucose derivative, an oxidation step was applied as previously described$^{27}$. The derivative was subsequently converted into 3,6-anhydro-1,2-O-isopropylidene-α-D-glucofuranose following published procedures$^{61}$. The latter compound was purified by flash chromatography using silica gel and diethyl ether. Pure fractions were identified by thin-layer chromatography and pooled. Diethyl ether was evaporated, the sample was washed with amylene-stabilized chloroform, and its purity was checked by $^1$H-NMR.

**Deuterium isotopomer quantification**

For NMR measurements of intramolecular deuterium abundances, each sample of the glucose derivative prepared as described above was dissolved in a mixture of 83% v/v acetonitrile, 17% C$_6$F$_6$, and 0.01% C$_6$D$_6$, then transferred to a 5-mm NMR tube with a PTFE valve (J. Young Scientific Glassware Ltd., Windsor, U.K.) containing ca. 5 mg of NaHCO$_3$. Deuterium NMR spectra were acquired and processed as previously described$^{60}$, using an AVANCE III 850 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) equipped with a $^{19}$F lock and a cryogenic probe optimized for deuterium detection. Deuterium NMR spectra were integrated by deconvolution with a Lorentzian line shape fit, using TopSpin™ 3.2
(Bruker BioSpin GmbH, Rheinstetten, Germany). The D6\textsuperscript{S}/D6\textsuperscript{R} isotopomer ratio was determined as the ratio of the integrals of the D6\textsuperscript{S} and D6\textsuperscript{R} signals\textsuperscript{26}. For each sample, five to eight spectra were recorded and the average D6\textsuperscript{S}/D6\textsuperscript{R} ratio was calculated.

**C-isotope analysis**

C-isotopic signatures (δ\textsuperscript{13}C) of dry moss tissue samples (ca. 5 mg) were analyzed by conversion into CO\textsubscript{2} by combustion and quantification by mass spectrometry\textsuperscript{62}, using an elemental analyser (Flash EA 2000, Thermo Fisher Scientific, Bremen, Germany) coupled to an isotope ratio mass spectrometer (DeltaV, Thermo Fisher Scientific, Bremen, Germany). The data were corrected for drift and non-linear sample size effects. For quantification, we used laboratory standards consisting of wheat and maize flours calibrated against two certified δ\textsuperscript{13}C reference standards: IAEA-CH-6, and USGS40.

**Statistical analysis**

Effects of the different variables (Table 1) on the D6\textsuperscript{S}/D6\textsuperscript{R} ratio were assessed by ANOVA, which was done in R (version 3.6.1, RStudio, Inc.) by computing linear regression models using the \texttt{lm()} function. To perform ANOVA with type II sum of squares, we used the \texttt{Anova()} function of the \texttt{car} package. The models were optimized for a better tradeoff between fit and complexity by applying automated stepwise model selection based on Akaike’s information criterion using the \texttt{step()} function of the \texttt{stats} package with default settings\textsuperscript{63}. Post-hoc Fisher’s LSD tests with Benjamini-Hochberg correction were applied (in Figure S1), using the \texttt{LSD.test()} function, to account for false discovery rates\textsuperscript{64}. To test effects of atmospheric CO\textsubscript{2}, samples from the top and bottom of each core were assigned as high and low CO\textsubscript{2} respectively. Based on their present WT depths, samples were divided into five different groups (see Table S1): <10, 10-20, 21-30, 31-40 and >40 cm below surface. Individual Sphagnum species were grouped into their subgenera: ACUTIFOLIA (S. fuscum, S. warnstorfi & S. capillifolium), SPHAGNUM (S. magellanicum, S. papillosum & S. cristatum), andCUSPIDATA (S. cuspidatum & S. majus; Table S1). Geographical locations were assigned according to Table S2. All other statistical analyses were performed in Excel. Student’s t-tests were one-tailed assuming unequal variance.

**Climate data analysis**

Climate data such as annual temperature and precipitation as well as summer temperature and precipitation (June-August & December-February for northern and southern hemisphere, respectively) were obtained from existing climate reconstruction data\textsuperscript{33,34,35}. Changes during the 20\textsuperscript{th} century were estimated by calculating the 3-year averages around the approximate ages (±1 year) estimated for the modern and historical samples (Table S3). Luterbacher et al.\textsuperscript{33} and Pauling et al.\textsuperscript{34} provide a dataset of seasonal (3-month) mean air temperatures and total precipitation levels respectively, for Europe (defined...
as the region between 35.25°N and 69.75°N, and 24.75°W and 39.75°E), covering the period from 1500 to 2002 with a 0.5° gridded resolution ([https://crudata.uea.ac.uk/cru/projects/soap/data/](https://crudata.uea.ac.uk/cru/projects/soap/data/)). Willmott & Matsuura provide a global dataset of mean monthly air temperatures and total precipitation from 1900 to 2017 with a 0.5° gridded resolution ([http://climate.geog.udel.edu/~climate](http://climate.geog.udel.edu/~climate)). Consequently, the Willmott & Matsuura dataset was used to estimate the climate after 1900 and the Luterbacher et al. and Pauling et al. dataset to estimate the climate before 1900. The two databases showed differences specifically for the Italian sites; therefore, data for 1860 and 1880 were normalized against the more recent data (1990-2000) of Willmott & Matsuura. Australian precipitation data from before 1900 were obtained from the Bureau of Meteorology, Australian Government, 2020 ([http://www.bom.gov.au/climate/data](http://www.bom.gov.au/climate/data)). Temperature data were not available for the Australian site and were therefore estimated for 1900 using the Willmott & Matsuura dataset. The southern Argentinian samples were estimated to be ≈860 years old, so the temperature and precipitation data for the Argentinian site were estimated according to other climate models.

Declarations

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHORS’ CONTRIBUTIONS:

H.S., M.N., and J.S. planned and designed the research; M.N., S.G., K.K., J.L., T.M., J.T., W.M., V.P., X.W. and K.-H.K. sampled/providerd the peat cores; C.O. and J.T. dated the peat cores; H.S. and I.E. prepared the peat samples for isotopomer analysis; E.B. identified the species composition of the peat samples; J.S. and H.S. acquired the NMR spectra; H.S. and T.W. analyzed the data; and H.S., M.N., and J.S. wrote the paper and all other co-authors contributed to revision of the paper.

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**Figures**

**Figure 1**
Global changes in the deuterium isotopomer ratio (ΔD6S/D6RN) of Sphagnum during the 20th century representing changes in the photorespiration to photosynthesis ratio. (A) Global distribution of investigated sites. (B) Response of the D6S/D6R ratio per unit change in 1000/[CO2] between modern and historical Sphagnum samples (ΔD6S/D6RN). Five water table depths (WT) categories (in cm) are indicated by vertical dashed lines. Sphagnum subgenera are indicated on the x-axis by grey/white shading: AC, ACUTIFOLIA (dark grey); CU, CUSPIDATA (light grey); SP, SPHAGNUM (white). Error bars indicate standard error, n = 1-4 (see Table S1 for more information). Numbers above error bars correspond to sample sites as numbered in (A).

![Graph showing correlation between Sphagnum whole-tissue δ13C values and the present water table depth for modern (black dots) and ≥ 100 years-old (white dots) Sphagnum samples. For ≥ 100 years-old Sphagnum an outlier was removed from the regression (site 9, Figure S2). Lines represent linear regressions of modern (black) and ≥ 100 years-old (grey) Sphagnum with the functions: y=0.07x-26.1 and y=0.06x-24.4 respectively.]

Figure 2

Correlation between Sphagnum whole-tissue δ13C values and the present water table depth for modern (black dots) and ≥ 100 years-old (white dots) Sphagnum samples. For ≥ 100 years-old Sphagnum an outlier was removed from the regression (site 9, Figure S2). Lines represent linear regressions of modern (black) and ≥ 100 years-old (grey) Sphagnum with the functions: y=0.07x-26.1 and y=0.06x-24.4 respectively.

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