CO₂ fertilization of *Sphagnum* peat mosses is modulated by water table level and other environmental factors

Henrik Serk¹,² | Mats B. Nilsson² | João Figueira³ | Thomas Wieloch¹ | Jürgen Schleucher¹

¹Department of Medical Biochemistry and Biophysics, Umeå University, Umeå, Sweden
²Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, Umeå, Sweden
³Department of Chemistry, SciLife Lab, Umeå University, Umeå, Sweden

Abstract

*Sphagnum* mosses account for most accumulated dead organic matter in peatlands. Therefore, understanding their responses to increasing atmospheric CO₂ is needed for estimating peatland C balances under climate change. A key process is photorespiration: a major determinant of net photosynthetic C assimilation that depends on the CO₂ to O₂ ratio. We used climate chambers to investigate photorespiratory responses of *Sphagnum fuscum* hummocks to recent increases in atmospheric CO₂ (from 280 to 400 ppm) under different water table, temperature, and light intensity levels. We tested the photorespiratory variability using a novel method based on deuterium isotopomers (D⁶¹⁶²S/D⁶¹⁶²R ratio) of photosynthetic glucose. The effect of elevated CO₂ on photorespiration was highly dependent on water table. At low water table (−20 cm), elevated CO₂ suppressed photorespiration relative to C assimilation, thus substantially increasing the net primary production potential. In contrast, a high water table (>0 cm) favored photorespiration and abolished this CO₂ effect. The response was further tested for *Sphagnum majus* lawns at typical water table levels (−0 and −7 cm), revealing no effect of CO₂ under those conditions. Our results indicate that hummocks, which typically experience low water table levels, benefit from the 20th century’s increase in atmospheric CO₂.

KEYWORDS

atmospheric CO₂, carbon assimilation, climate change, deuterium isotopomers, NMR, photorespiration, *sphagnum*

1 | INTRODUCTION

Only 3% of the earth’s land surface is covered by peatlands, but more than a third of global soil carbon (C) is stored in boreal mires (Frolking et al., 2011; Loisel et al., 2014). Most of those mires are dominated by *Sphagnum* peat mosses, which hence contribute substantially to global peatland C sequestration (Gunnarsson, 2005; C. G. Laing, Granath, Belyea, Allton, & Rydin, 2014; Loisel et al., 2014; Wu, Roulet, Nilsson, Lafleur, & Humphreys, 2012). Thus, reliable prediction of future C sequestration and storage in peatlands requires profound understanding of *Sphagnum* C acquisition and accumulation (Charman et al., 2013; Frolking et al., 2011; Loisel et al., 2014; Wu & Roulet, 2014), but the mosses’ responses to increases in atmospheric CO₂ concentrations are still not well understood.

*Sphagnum* physiology strongly depends on local environmental conditions, including local weather, hydrology and nutritional

ABBREVIATIONS: cₐ, atmospheric CO₂ concentrations; cₐ, chloroplastic CO₂ concentrations; NPP, net primary production; RuBP, ribulose 1,5-bisphosphate; WT, water table.
Deuterium NMR spectrum of glucose derived from structural carbohydrates of S. fuscum. The integral of each peak is proportional to the abundance of the deuterium (D) isotopomers at the corresponding position in the glucose molecule (H1 – H6). The ratio of the isotopomer abundance of D6^5 and D6^6 corresponds to the photorespiration/photosynthesis ratio (Ehlers et al., 2015)
photorespiration/photosynthesis ratio. Analysis with this method confirmed that the photorespiration of many C₃ plants was suppressed and their net photosynthesis rates increased by the last century’s increase in atmospheric CO₂ (Ehlers et al., 2015). In the cited study, comparison of S. fuscum herbarium material formed at ≤300 ppm atmospheric CO₂ with modern plants showed that photorespiration of peat-forming Sphagnum was also suppressed during the last century. However, the extent to which photorespiration of Sphagnum plants is suppressed at increased atmospheric CO₂ may be influenced by other factors, particularly water content, temperature and light intensity. Thus, the overall aim was to understand variations and potential effect of suppressed photorespiration (and associated changes in C assimilation rates) in relation to biogeophysical conditions at peatland surfaces and climatic changes. This will allow us to explain D isotope data of historical Sphagnum tissues. To address this aim, we investigated the response of S. fuscum’s photorespiration/photosynthesis ratio to the recent increase in atmospheric CO₂ under various combinations of different atmospheric CO₂, WT, temperature and light intensities. To do so, we used D isotope data as well as δ¹³C, δ¹⁵N and elemental analysis (C and N). The response was further tested for the lawn species S. majus at different CO₂ and WT levels.

2 MATERIALS AND METHODS

2.1 Plant material

Peat mesocosms from hummocks dominated by Sphagnum fuscum (Schimp.) Klinggr. & Scheuchzer (Russ.) Jens. were collected in May and August of 2016, and July 2017, from the same site located at the northern end of the Degerö-Stromyr peatland. Degerö-Stromyr is a nutrient-poor minerogenic mire in northern Sweden (64°11’N, 19°33’E, 270 m asl) near Vindeln municipality that is included in the ICOS (Integrated Carbon Observation System; Franz et al., 2018) Swedish national and European research infrastructure. Mesocosms of 20 × 20 × 25 cm³ were collected using a sharp knife and transferred to 11 L square plastic containers for subsequent incubation in climate-controlled growth chambers. Vascular plants (Andromeda polifolia, Rubus chamaemorus, Vaccinium oxycoccus, Drosera rotundifolia, Empetrum nigrum, Scheuchzeria palustris, Eriophorum vaginatum and Carex spp.) and Polytrichum spp. mosses were removed before incubation.

2.2 Manipulation experiments

Triplicate mesocosms were grown in climate-controlled growth chambers (PGC-7 L2/DE, Percival Scientific, Perry, IA) at Umeå Plant Science Centre for 8–10 weeks with 18/6-hr day/night photoperiods and 70% relative humidity (corresponding to average growing season photoperiods and humidity conditions at Degerö-Stromyr: www.icos-sweden.se) under different CO₂ concentrations and WT levels. Two identical chambers were used for incubation at atmospheric CO₂ concentrations of 280 ppm and 400 ppm, respectively with two different WT levels (hummocks: 0 and –20 cm, lawns: 0 and –7 cm below capitulum) in each chamber. CO₂ levels in the growth chambers were regulated automatically, solely by removal. Thus, the CO₂ in the chambers derived from the atmosphere. Target WT levels were obtained by placing the 11 L plastic containers in larger plastic storage containers, which were then filled with deionized water to the target WT level (Figure S1). The WT was re-adjusted every 2 days, resulting in variations of ±2 cm. Mesocosms incubated at low WT were sprayed with deionized water every 2 days to maintain the high moisture levels that are naturally provided in the mire by precipitation.

The above-mentioned incubations were conducted in three batches with different temperature and light intensity settings. The first two batches were performed at light intensities of 250 μmol m⁻² s⁻¹ (at moss surface) and day/night temperatures of either 12°C/7°C or 17°C/12°C, whereas the last batch was conducted at 500 μmol m⁻² s⁻¹ and 17°C/12°C. Thus, the first two incubations represent a full² factorial design with low light intensity as a fixed factor and atmospheric CO₂ concentration, WT and temperature as variables, and the last incubation a 2² factorial design, with temperature and light intensity as fixed factors and CO₂ concentration and WT as variables. In the following text, the different CO₂, WT, temperature and light settings are referred to as low and high levels, respectively. The temperature settings were chosen according to typical growth-period conditions in the field (Peichl et al., 2014). Light intensity settings were chosen in the range of typical Sphagnum light saturation (250–500 μmol m⁻² s⁻¹; Harley, Tenhunen, Murray, & Beyers, 1989; Jauhiainen & Silvola, 1999; Laine, Juuruola, Hajek, & Tuittila, 2011). The response variables measured in the experiments were: photorespiration/photosynthesis ratio (D⁶S/D⁶R ratio), moss height increment, biomass production, ¹³C and ¹⁵N discrimination and concentrations of C and N in the plant tissue.

To rule out possible problems due to pseudo-replication and chamber effects, we used analysis of variance (ANOVA) models to test for effects of specific mesocosm/pot positions in the respective chamber of all incubation batches. No significant effect of the pot position on all measured response variables was found (Figure S2, Table S1). To test for effects of the temporal difference between the three incubation batches, we performed ANOVA models including this factor (Table S2). No significant effect of the temporal difference on all measured variables was detected.

2.3 Biomass and height increment measurements

Height increments of the moss in the mesocosms were measured weekly during the incubation period (Figure S3), using five brush wires (Rydin & Jeglum, 2013) inserted into each mesocosm container. At the end of the incubation period, biomass formed during the incubation was harvested by cutting the moss at its initial height (essentially its capitulum), based on the brush wire data. Its fresh weight was determined and the material was dried at 60°C for 48 hr to determine the dry weight and subsequently, the water content of the moss. The biomass production of each replicate (moss in each container) was...
estimated by dividing the dry weight of each replicate obtained after harvest, by its surface area (calculated based on container dimensions) and incubation time. Biomass density was calculated by dividing the dry weight of each replicate by its surface area and total height increment.

2.4 Sample preparation for D isotopomer measurements

Dry moss biomass formed during the incubations was 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 as starting material for the following sample preparation for D isotopomer measurements. Glucose-containing structural polymers were hydrolyzed to glucose and converted to 1,2-O-isopropylidene-α-D-glucurono-4-rhamnose following Schleucher, Vanderveer, Markley, and Sharkey (1999). The derivative 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 purity was checked by 1H-NMR.

2.5 D isotopomer quantification

For NMR measurements of intramolecular D abundances, each sample of the glucose derivative, prepared as described above, was dissolved in a mixture of 83% v/v acetonitrile, 17% cyclohexanone, nicotinamide, and sucrose for C; atropine, cellulose, and NIST 1515 apple leaves for N; IAEA-600, IAEA-CH-6, and USGS40 for δ¹³C; and IAEA-600, IAEA-N-2, USGS40 and USGS41 for δ¹⁵N.

2.6 C and N-isotope and elemental analysis

C and N isotopic signatures (δ¹³C and δ¹⁵N, respectively) and C and N contents of dry samples of moss tissues (ca. 5 mg) were analyzed via conversion to CO₂ and N₂ by combustion and quantification by mass spectrometry (Werner, Bruch, & Brand, 1999), using a DeltaV isotope ratio mass spectrometer (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 certified reference standards: cyclohexanone, nicotinamide, and sucrose for C; atropine, cellulose, and NIST 1515 apple leaves for N; IAEA-600, IAEA-CH-6, and USGS40 for δ¹³C; and IAEA-600, IAEA-N-2, USGS40 and USGS41 for δ¹⁵N.

2.7 Calculation of chloroplastic CO₂ concentrations

Chloroplastic CO₂ concentrations (c) were calculated based on δ¹³C signatures of the moss (δ¹³Cmoss) using equations for photosynthetic fractionation (Δ¹³Cmoss) published by Flanagan and Farquhar (2014), with modifications to account for the lack of stomatal resistance in Sphagnum:

\[
\Delta^{13}C_{moss} = 1,000 \frac{\delta^{13}C_{atm} - \delta^{13}C_{moss}}{1,000 + \delta^{13}C_{moss}}, \quad (1)
\]

\[
\Delta^{13}C_{moss} = a_m \left( \frac{c_s - c_c}{c_s} \right) + b \left( \frac{c_s}{c_c} \right) - f \left( \frac{f}{c_c} \right), \quad (2)
\]

Here, δ¹³Catm is the δ¹³C signature of atmospheric CO₂ (~8.5‰, Graven et al., 2017), a_m the fractionation during CO₂ diffusion through water to the chloroplast (~1.8‰, Farquhar, Ehleringer, & Hubick, 1989), b the discrimination during carboxylation by Rubisco (~29‰, Farquhar et al., 1989), f the fractionation during photorespiration (~11‰, Tcherkez, 2006; Evans & von Caemmerer, 2013) and f’ the CO₂ compensation point (μmol mol⁻¹) in the absence of dark respiration (calculated from the temperature response: f’ = 42.7 + 1.68(T - 25) + 0.0012(T - 25)², where T is temperature in °C; Brooks & Farquhar, 1985). In Equation (2), we assumed that fractionation during day respiration is negligible according to Evans and von Caemmerer (2013).

At high WT, the Δ¹³Cmoss values were much higher (~19‰, Figure S4) compared to other submerged mosses and aquatic plants (~4–10‰, Keeley & Sandquist, 1992), suggesting that δ¹³Cmoss is modulated by C sources other than atmospheric CO₂. The anoxic conditions at high WT are favorable for heterotrophic production of CH₄, which is potentially oxidized to CO₂ by methanotrophic bacteria and thus serves as significant C source in Sphagnum (Larmola et al., 2010; Nielsen et al., 2019; Raghoebarsing et al., 2005). To account for the uptake of respired CH₄ at the high WT, Δ¹³Cmoss was calculated according to a simple isotopic mass balance (Keeley & Sandquist, 1992; Raghoebarsing et al., 2005):
\[ \Delta^{12}\text{C}_{\text{moss}} = a \cdot \Delta^{12}\text{C}_{\text{CH}_4} + (1-a) \cdot \Delta^{12}\text{C}_{\text{CO}_2} - \Delta^{13}\text{C}_{\text{moss}}. \]  

Here, \( \delta^{13}\text{C}_{\text{CH}_4} \) is the \( \delta^{13}\text{C} \) value of respired \( \text{CH}_4 \) (−60‰, according to average from Raghoebarsing et al., 2005; Larmola et al., 2010 and Nielsen et al., 2019) and \( \delta^{13}\text{C}_{\text{CO}_2} \) is the \( \delta^{13}\text{C} \) signature of atmospheric \( \text{CO}_2 \) (−8.5‰, Graven et al., 2017). The factor \( a \) represents the amount of C in Sphagnum derived from oxidized \( \text{CH}_4 \) and is estimated with 15% according to Raghoebarsing et al. (10%–15%, 2005) and Larmola et al. (10%–30%, 2010). Including \( \text{CH}_4 \)-derived \( \text{CO}_2 \) as C source reduces \( \Delta^{12}\text{C}_{\text{moss}} \) from \( \sim 19\% \) to \( \sim 10\% \) (Figure S4), which corresponds to the value of other submerged mosses (\( \text{F. antipyretica} \), Keeley & Sandquist, 1992). Subsequently, \( c \) was calculated according to Equation (2). To account for the uncertainty of the estimated amount of C derived from \( \text{CH}_4 \) oxidation (factor \( a \)), we calculated the variation in \( c \), for values of 10% and 20%, which was \( \pm 27\text{–}38\text{ ppm} \). These values were considered as the error range of \( c \) at high WT (Figure 3b). Correlation of \( c \) with the \( \text{D}^6/\text{D}^8 \) ratio, for both with and without accounting for \( \text{CH}_4 \) oxidation, revealed that considering \( \text{CH}_4 \) as C source improves the correlation of these parameters substantially (\( R^2 = 0.74 \) vs. 0.38; Figure S5).

### 2.8 Statistical analysis

Effects of the varied environmental factors on the measured physiological parameters were assessed by ANOVA, implemented in R (version 3.6.1, RStudio, Inc.), using linear regression models. Initially, two-way ANOVA models were used to test effects of \( \text{CO}_2 \) and WT separately for each incubation batch, exhibiting different temperature and light settings (Table S3). These data indicate a consistent response for all incubations. Consequently, four-way ANOVA models were applied to test effects of all four environmental factors. Three- and four-way interactions were not included in the models to prevent overfitting and to improve interpretability of the models. To compute the ANOVA with type II sum of squares, we used the ANOVA() function of the car package. The four-way ANOVA 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 step() function of the stats package with default settings (Venables & Ripley, 2002). Post-hoc Fisher’s LSD tests were Benjamini-Hochberg correction (Benjamini & Hochberg, 1995; Steel, Torri, & Dickey, 1997) were applied, using the LSD.test() function, to account for false discovery rates. Microsoft Excel was used for all other data analysis.

### 3 RESULTS

#### 3.1 Photorespiration response and biomass production

The two most important factors explaining the variation in the \( \text{D}^6/\text{D}^8 \) ratio (range: 0.85–0.94) were WT and atmospheric \( \text{CO}_2 \) concentration, which respectively accounted for 48% (\( p < .001 \)) and 14% (\( p < .001 \)) of the total variance (Figure 2a, Table 1). In addition, temperature interactions between WT and \( \text{CO}_2 \) both explained 7% each (\( p = .004 \) and \( p = .005 \), respectively) of the variance in this ratio. Increasing atmospheric \( \text{CO}_2 \) from 280 to 400 ppm resulted in a 0.03 decrease in the \( \text{D}^6/\text{D}^8 \) ratio at low WT, but had no significant effect on it at high WT (Figure 2a). Together with the observed interaction between \( \text{CO}_2 \) and WT, this indicates WT-dependent suppression of photorespiration at the high \( \text{CO}_2 \) level. Raising the WT from −20 to −0 cm resulted in a significant (0.01–0.05) increase in the \( \text{D}^6/\text{D}^8 \) ratio, indicating that the high WT increased the photorespiration/photosynthesis ratio (Figure 2a). Increasing the day/night temperatures from 12°C/7°C to 17°C/12°C caused a small (~0.01) increase in the \( \text{D}^6/\text{D}^8 \) ratio at low WT, but increasing the light intensity from 250 to 500 \( \mu\text{mol m}^{-2} \text{s}^{-1} \) had no significant effect on it (Figure 2a, Table 1).

Theoretically, the suppression of photorespiration at high \( \text{CO}_2 \) and low WT should have been accompanied by increases in \( \text{CO}_2 \) assimilation rates, assuming constant RuBP turnover. Therefore, biomass production was expected to increase at the high \( \text{CO}_2 \) level, but no significant increase was observed under any test conditions (Figure 2b). The variation in biomass production (0.2–3.6 g m\(^{-2}\) d\(^{-1}\))
was mostly explained by temperature (27%, *p* < .001), WT (52%, *p* < .001), and to a smaller degree light intensity (4%, *p* = .003) and the interaction between temperature and WT (4% *p* = .005; Table 1). Increasing the temperature caused a massive 2.6- to 4.5-fold increase in biomass production, whereas raising the WT strongly reduced biomass production, by 53%–74% (Figure 2b). Increasing the light intensity caused a 1.1- to 1.5-fold increase in biomass. No major between-differences in C content of the biomass (48.1 ± 0.11%, SE) were detected (Figure S4). Thus, the observed changes in biomass production reflect proportional variations in C accumulation.

### 3.2 | Whole-tissue δ13C and chloroplastic to ambient CO2 concentration

To further investigate physiological effects of increasing atmospheric CO2 from 280 to 400 ppm, we analyzed 13C discrimination by measuring whole-tissue δ13C signatures (Figure 3a). δ13C is commonly used as proxy for surface moisture in *Sphagnum* biomass but has also been found to be influenced by temperature, light and CO2 concentration (Loisel, Garneau, & Hélie, 2010; Ménot & Burns, 2001). In our experiment, 48% of the variation in δ13C (which ranged from −30.5 to −25.7‰) was explained by changes in WT (p < .001), 13% by atmospheric CO2 (p < .001), 10% by temperature (p < 0.001), 3% by light intensity (p = 0.02), and 10% by the interaction between CO2 and WT (p < .001; Table 1). Increasing atmospheric CO2 consistently decreased δ13C (by 1.4–1.7‰) at low WT, but had no significant effect at high WT (Figure 3a). Concomitantly, raising the WT resulted in a 0.5–2.5‰ increase in δ13C. Increasing the temperature caused a significant, 0.5–1.0‰, increase in δ13C. Increasing the light intensity resulted in a small 0.3–0.6‰ increase in δ13C at low WT. A strong positive correlation was observed between the D6/S ratio and δ13C at low WT (R2 = .88, *p* < .001), but there was no significant relationship between these variables at high WT (R2 = .19, *p* = .069).

The δ13C signature reflects the ratio of chloroplastic to atmospheric CO2 concentration (c/ca, Farquhar et al., 1989) and thus allowed estimation of c (see materials & methods), which is a key determinant of the photorespiration/photosynthesis flux ratio. Variation in c (98–306 ppm, Figure 3b) was mostly explained by WT (71%, *p* < .001), atmospheric CO2 (23%, *p* < .001) and the interaction between CO2 and WT (4%, *p* < .001; Table 1). Increasing atmospheric CO2 at low WT significantly increased c by ~102 ppm and at high WT by ~40 ppm (±11 ppm depending on model assumptions, see materials & methods and Figure 3b). Raising the WT caused a...
~94 ± 27 ppm decrease in c_c at low CO2, and ~156 ± 38 ppm decrease at high CO2. Thus, increases in atmospheric CO2 increase c_c particularly at low WT, whereas raising the WT reduces c_c. A strong negative correlation was detected between c_c and the D6^5/D6^8 ratio (R^2 = 0.74 ± 0.01, p < .001, Figure S5), corroborating that changes in c_c cause the observed response in the photorespiration/photosynthesis ratio (Figures 2a and 3b).

### 3.3 | C/N ratio and nitrogen content

The lack of response of *S. fuscum* biomass production to high atmospheric CO2 suggested biochemical limitations of the photosynthetic machinery. To explore the nature of possible limitations, we investigated changes in whole-tissue N contents and C/N ratios (Figure 3c,d). Variation in the C/N ratio (40–89 units) was mostly explained by WT (31%, p < .001), atmospheric CO2 (12%, p = .003), interaction between temperature and WT (11%, p = .004), and interaction between CO2 and WT (7%, p = .022, Table 1). Increasing the atmospheric CO2 concentration increased the tissue C/N ratio by 4–23 units at low WT, but had no significant effect at high WT (Figure 3c). Increasing the WT generally decreased the C/N ratio by 5–31 units. The changes in C/N ratio were primarily due to changes in tissue N content, the C content only showed minor variation (Figure S4).

Accordingly, the total variation in N content (5.5–11.8 mg g^-1) was explained by WT (31%, p < .001), atmospheric CO2 (11%, p = .013) and the interaction between WT and temperature (8%, p = .031, Table 1). Increasing the CO2 level decreased the N content by 7%–28% at low WT, but had no significant effect at high WT (Figure 3d). Increasing the WT caused a 1.1- to 1.6-fold increase in N content. Both C/N ratio and N content were strongly correlated with the D6^5/D6^8 ratio at low WT (R^2 = .68, p < .001 and R^2 = .71, p < .001, respectively). At high WT the ratio was still correlated, but less strongly, with both the C/N ratio (R^2 = .44, p < .003) and N content (R^2 = .40, p < .005).

No major between-treatment differences were detected in whole-tissue δ^{15}N signatures (average: −3.63 ± 0.08 % SE; Figure S4). According to the four-way ANOVA, the only significant effect was a small (~0.45‰) increase associated with increasing the light intensity (R^2 = .23, p = .003, Table S4).

### 3.4 | Biomass density, height increment and water content

For further assessment of possible morphological effects of observed responses to increasing atmospheric CO2 on *S. fuscum*, we measured its biomass density (total biomass dry weight per volume) and height increment under all the test conditions (Figure 3e,f). No effect of atmospheric CO2 on biomass density was observed, instead the variation in density (4.1–42.4 g dm^-3) was mostly explained by WT (45%, p < .001), light intensity (34%, p < .001) and the interaction between WT and light (14%, p < .001, Table 1). Raising the WT decreased biomass density by 41%–71%, whereas light intensity...
increased it 2.1- to 3.5-fold at low WT and 1.6- to 2.6-fold at high WT (Figure 3e).

Most of the variation in height increment (0.04–0.26 mm d⁻¹) was explained by temperature (51%, p < .001) and light intensity (24%, p < .001), while the interactions between light and WT or CO₂ as well as temperature and WT or CO₂ made minor contributions (each 2%–3%, p < .05, Table 1). Increasing the temperature increased the height increment 2.4- to 4.5-fold (Figure 3f), while increasing the light intensity resulted in ~0.6- and ~0.35-fold reductions in the height increment at low and high WT, respectively. Increasing atmospheric CO₂ significantly reduced height increment by 0.19 to 0.35-fold only at the high temperature, low light, and low WT treatment (Figure 3f, Figure S3).

Sphagnum fuscum’s water content was consistently 4%–9% lower at the high CO₂ level, but CO₂ only explained a small amount of the total variation (6.6–23.4 g g⁻¹) in its water content (1%, p = .006; Table S4, Figure S4). Most of the variation was explained by WT (83%, p < .001), temperature (5%, p < .001), and the interactions between WT and temperature (6%, p < .001) and between WT and light (3%, p < .001, Table S4). At low WT, the water content varied between 7.4 and 10.6 g g⁻¹, within the reported optimal range for photosynthesis (6–10 g g⁻¹: Silvola & Aaltonen, 1984; Schipperges & Rydin, 1998). At high WT, the water content was between 15.4 and 22.3 g g⁻¹, outside the optimal range for photosynthesis (Figure S4).

### 3.5 | Photorespiration response of S. majus

The observed CO₂-response of the D⁶⁻⁷/D⁶⁻⁸ ratio of S. fuscum at high WT suggests that lawns and hollows, which generally experience relatively high WT levels, do not suppress photorespiration at high CO₂ levels. To test this, we analyzed D⁶⁻⁷/D⁶⁻⁸ ratios of S. majus grown at different CO₂ and WT levels (under high temperature and low light conditions). Increasing CO₂ from 280 to 400 ppm did not have any significant effect on the D⁶⁻⁷/D⁶⁻⁸ ratio (range: 0.90–0.98) of S. majus at both low (~7 cm) and high WT (~0 cm, R² = .01, p = .153, Table 2, Figure 4a). In contrast, raising the WT resulted in a significant increase in the D⁶⁻⁷/D⁶⁻⁸ ratio (0.05–0.06), indicating increased photorespiration/photosynthesis ratios at high WT levels (R² = 0.94, p < .001, Table 2). Biomass production did not show any significant difference in response to increasing atmospheric CO₂, but raising the WT reduced biomass production by ~40% (R² = .49, p = .01, Table 2, Figure 4b). Elevated atmospheric CO₂ concentrations increased biomass density by two-fold at low WT (R² = .18, p = .048, Table 2, Figure 4c).

| TABLE 2 | Summary of two-way ANOVA models of effects of CO₂ and water table (WT) and their interaction on the measured physiological response variables of S. majus |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Response variable | CO₂ | WT | CO₂ × WT | R² | df |
| D⁶⁻⁷/D⁶⁻⁸ ratio | p | .071 | .001 | n.s. | .88 | 2/9 |
| F | 4.19 | 62.16 |
| Biomass production | p | n.s. | .01 | n.s. | .54 | 1/10 |
| F | 10.63 |
| Biomass density | p | .048 | .008 | .057 | .74 | 3/8 |
| F | 5.46 | 12.4 | 4.93 |
| Height increment | p | .012 | n.s. | n.s. | .57 | 1/10 |
| F | 10.58 |

Note: Non-significant factors/interactions with p > .1 are denoted as n.s., df, degrees of freedom of the model and residuals.
Figure 4c). Concomitantly, height increment significantly decreased at low WT by 55% (R² = .5, p = .012, Table 2, Figure 4d, Figure S3). The water content ranged between 9.1 and 11.9 g g⁻¹ at low WT and 10.8 and 17 g g⁻¹ at high WT, thus increasing the WT shifts the water content from optimal to suboptimal conditions for photosynthesis (Schipperges & Rydin, 1998). Altogether, these data indicate that photosynthesis in S. majus is not suppressed in response to increased atmospheric CO₂, despite optimal moisture conditions.

4 | DISCUSSION

4.1 | Effects of increased atmospheric CO₂ on S. fuscum photosynthesis

The D⁶⁵/D⁶⁸ ratios of S. fuscum indicate a significant suppression of photosynthesis relative to C assimilation at the higher (current) atmospheric CO₂ level than at the lower (pre-industrial) level at low WT (Figure 2a). Because the conditions at low WT represent typical growth conditions for S. fuscum, this result indicates suppression of photosynthesis relative to C assimilation during the 20th century. In contrast, when the Sphagnum plants were water-saturated, increasing atmospheric CO₂ from 280–400 ppm had no significant detected effects on the photosynthesis to photosynthesis ratio (i.e. on D⁶⁵/D⁶⁸).

The estimated cç (based on the δ¹³C data) strongly increased under contemporary CO₂ levels at low WT (Figure 3b), suggesting that the suppression of photosynthesis at low WT derives from the higher intercellular CO₂ concentration suppressing Rubisco oxygenation. At high WT, an increase in cç was also detected, however, the increase was reduced by 61% (±11%) depending on model assumptions, see materials & methods) compared to the increase at low WT (~102 vs. ~40 ± 11 ppm, Figure 3b), which apparently does not suffice to suppress photosynthesis significantly (Figure 2a). Considering the linear relationship between D⁶⁵/D⁶⁸ and ¹/²⁴(CO₂) described by Ehlers et al. (2015), the increase in cç at high WT would correspond to an increase of D⁶⁵/D⁶⁸ of 0.011 ± 0.003, which is close to the obtained average response at high WT (0.005).

The observed shift in the D⁶⁵/D⁶⁸ ratio of 0.03 units at low WT (Figure 2a) is consistent with results of a previous comparison of contemporary samples with >100-year-old herbarium samples of S. fuscum (Ehlers et al., 2015). Of all tested environmental variables, the CO₂ concentration had the largest effect, explaining 53% of the variance of D⁶⁵/D⁶⁸ under typical hydrological (low WT) conditions for S. fuscum (3-way ANOVA: p₂CO₂ = 0.001, p₁ = 0.01, p₁₁ = n.s., FC₀₂ = 27.2, F₁ = 8.8). Thus, our experimental data confirm that the detected suppression of photosynthesis in herbarium samples (Ehlers et al., 2015) was due to increases in atmospheric CO₂ during the last century.

4.2 | Effect of moss water content

The increase in the D⁶⁵/D⁶⁸ ratio from low to high WT (Figures 2a and 4a) revealed an increase in the photosynthesis/photosynthesis ratio for both S. fuscum and S. majus. A concomitant decrease in cç from low to high WT was observed (Figure 3b), suggesting that the increase in the photosynthesis/photosynthesis ratio was driven by a decline in cç. In addition, the lower cç at high WT is indicative of higher CO₂ diffusion resistance, resulting from the higher moss water content (Figure S4). Biomass production was strongly reduced at the high WT, for both S. fuscum and S. majus (Figures 2b and 4b), suggesting that the mosses are C limited under these conditions. In this respect, our data support the hypothesis that the higher water content at high WT limits CO₂ diffusion and therefore C assimilation. This hypothesis is also supported by earlier reports of reductions in net photosynthesis with increases in water content (Schipperges & Rydin, 1998; Titus & Wagner, 1984; Titus, Wagner, & Stephens, 1983) and a negative relationship between water content and cç/cç (Rice & Giles, 1996; Williams & Flanagan, 1996, 1998).

4.3 | Relationship between the D⁶⁵/D⁶⁸ ratio and δ¹³C

At low WT the D⁶⁵/D⁶⁸ ratio and δ¹³C were strongly correlated (R² = .88). D⁶⁵/D⁶⁸ reflects the metabolic flux ratio of Rubisco oxygenation to carboxylation and is set by the proportions of reaction products formed by oxygenation and carboxylation (Ehlers et al., 2015), whereas δ¹³C reflects cç/cç and thus the Rubisco substrate concentration (Farquhar et al., 1989). However, in contrast to the D⁶⁵/D⁶⁸ ratio, the δ¹³C value of the source C needs to be known to accurately predict cç. It creates a problem, particular for plants that are not in direct contact with the atmosphere, such as submerged plants. Indeed, there was no correlation between the D⁶⁵/D⁶⁸ ratio and δ¹³C at high WT (R² = 0.19), highlighting potential difficulties in interpreting δ¹³C under water-saturating conditions. This mismatch therefore suggests that δ¹³C of the source C is not equal to that of atmospheric CO₂. When considering only atmospheric CO₂ as C source at the high WT, photosynthetic fractionation (Δ¹³Cmoss) was ~19‰ (Figure S4), much higher compared to other submerged mosses and aquatic plants (4–10‰, Keeley & Sandquist, 1992). This supports that Sphagnum-δ¹³C is modulated by another C source. The uptake of CO₂ originating from oxidation of respired CH₄ has the potential to affect δ¹³C of Sphagnum significantly due to very low δ¹³C values of CH₄ (~–60‰, Raghoebarsing et al., 2005; Lamola et al., 2010). When accounting for the uptake of CH₄-derived CO₂, the estimated Δ¹³Cmoss agrees with other submerged plants (~10‰, Figure S4) and the derived cç estimates complement the D⁶⁵/D⁶⁸ ratios (R² = 0.74, Figures 2a and 3b and Figure S5). Altogether, this indicates potential problems in using δ¹³C alone to estimate C fluxes in Sphagnum. Thus, using the D⁶⁵/D⁶⁸ ratio improves physiological interpretations of metabolic C fluxes.

4.4 | Limitations for Sphagnum biomass production

The decrease in the photosynthesis/photosynthesis ratio observed at low WT suggests increased C assimilation (assuming constant RuBP
turnover rates) and thus an increased net primary production (NPP) potential in response to increased atmospheric CO₂. However, there was no significant increase in biomass of *S. fuscum* under elevated CO₂ at both WT levels (Figure 2b), consistent with findings of several other studies on different *Sphagnum* species (Berendse et al., 2001; Heijnmans et al., 2001, 2002; Jauhiainen et al., 1994; Jauhiainen, Vasander, & Silvola, 1998; Mitchell et al., 2002; Toet et al., 2006; van der Heijden, Verbeek, & Kuiper, 2000). Nevertheless, at low WT, increasing the CO₂ level caused a slight biomass decrease at low light, but a slight increase at high light (Figure 2b), suggesting that under field conditions, where light intensities are much higher, there might well be a CO₂-driven increase in NPP. Thus, elevated CO₂ might shift the light saturation point even above 500 μmol m⁻² s⁻¹ (Hajek, Tuittila, Ilomets, & Laiho, 2009).

Furthermore, effects of increases in atmospheric CO₂ on biomass production of higher C₃ plants rely on nutrient availability and/or reallocation of limiting nutrients (Arp, Van Mierlo, Berendse, & Snijders, 1998; Kirschbaum, 2011; Poorter, 1998). We observed a decrease in *Sphagnum* tissue N content and an increase in the C/N ratio at elevated CO₂ (Figure 3c,d), resulting in a high correlation with the D₆⁅/D₆⁸ ratio (R² = .68 and .71 respectively). In higher C₃ plants, acclimation to increased CO₂ levels has been found to reduce leaf N and Rubisco contents due to reductions in demand for Rubisco (Cotrufo, Ineson, & Scott, 1998; Drake, Gonzalez Meler, & Long, 1997). This indicates reduced N investment in Rubisco under elevated CO₂ and suggests that increasing CO₂ reduces Rubisco limitation of C assimilation (Figures 2a and 3d). However, Granath, Strengbom, and Rydin (2012) showed that net CO₂ assimilation of *S. fuscum* positively correlates with its N content (below 14 mgN g⁻¹).

In addition, Limpens, Berendse, and Klees (2004) found that phosphorous (P) stimulate *Sphagnum* NPP. In our experiment, no nutrients were added, suggesting that biomass production was limited in N and/or P. Thus, we hypothesize that *S. fuscum* growth is sensitive to light and nutrient levels and responds to elevated CO₂ only under optimal growth conditions.

### 4.5 Ecophysiological implications

Our results show that hummocks with characteristic WT levels have profited from CO₂ fertilization imposed by the 20th centuries CO₂ increase. The high WT conditions in this study resulted in abolishing the effect of CO₂ for both hummocks and lawns, demonstrating the importance of the water content for photosynthetic C fluxes. Even under optimal moisture conditions for photosynthesis (at WT of ~7 cm), lawns did not respond to increased atmospheric CO₂ (Figure 4a). This suggests a species-specific suppression of photorespiration, possibly attributed to differences in leaf-anatomy between hummock and lawn species (Rice & Giles, 1996).

Predicted increases in precipitation in the northern hemisphere are suggested to be compensated for by concomitant increases in evapotranspiration due to higher temperatures (Frolking et al., 2011). This indicates that hydrological conditions of northern peatlands will remain relatively stable in a changing climate. Therefore, our data point towards changes in peatland topography in response to climate change, with the competitive advantage of hummocks over lawns and hollows.

Further, we found that, for hummocks with typical WT conditions, the increase in atmospheric CO₂ during the 20th century was the major driver of *Sphagnum* photosynthetic C fluxes. During the early and mid-Holocene, atmospheric CO₂ was relatively stable, at ~270 ppm (Indermühle et al., 1999), thus temperature appeared to control northern peatland C fluxes; peat C accumulation followed the increase in temperature during the early Holocene and the cooler and wetter climate during the neoglacial period (Loisel et al., 2014; Yu, Beilman, & Jones, 2009). Our results suggest that the ongoing increase in atmospheric CO₂ today, in marked contrast to its stability in the Holocene, has important consequences for peatland C fluxes.

### 5 CONCLUSION

Here, we show that the last century’s increase in atmospheric CO₂ suppressed *S. fuscum*’s photorespiration relative to C assimilation. This response was highly dependent on WT, with water-saturating conditions abolishing the CO₂ effect. Although those conditions are not typical for hummocks, they frequently occur for lawns. Lawns did not show any suppression of photorespiration, neither under water-saturation nor under optimal moisture conditions, suggesting a reduced CO₂ effect for those microhabitats. Our study revealed that D isotopomers are a valuable tool for understanding metabolic C fluxes in *Sphagnum*.

### ACKNOWLEDGMENTS

The authors acknowledge the help provided by the “NMR for Life” infrastructure supported by the Wallenberg Foundations, the SITES (Swedish Infrastructure for Ecosystem Science) and ICOS research infrastructures (for maintaining the Kulbäckslien/Degerö Stormyr facility) funded by the Swedish research council (VR), the SciLifeLab infrastructure, the SLU Stable Isotope Laboratory (for C- and N-isotope and elemental analysis), Umeå Plant Science Center (for providing the growth chambers), assistance from Jan Karlsson in operating the growth chambers, assistance from Prof. Larry Flanagan in calculation of C, and financial support from the Knut och Alice Wallenberg’s Stiftelse (#2015.0047), VR and the Carl Tryggers Foundation.

### CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

### AUTHOR CONTRIBUTIONS

Henrik Serk, Mats B. Nilsson, and Jürgen Schleucher planned and designed the research; Henrik Serk and Mats B. Nilsson collected the hummock mesocosms samples; Henrik Serk performed the experiments; João Figueira and Henrik Serk optimized isotopomer analysis; Jürgen Schleucher and Henrik Serk acquired the NMR spectra; Henrik
Serk and Thomas Wieloch analyzed the data; and Henrik Serk, Mats B. Nilsson, and Jürgen Schleucher wrote the paper, with input from Thomas Wieloch and João Figueira.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

**ORCID**

Henrik Serk https://orcid.org/0000-0003-4803-3664  
Mats B. Nilsson https://orcid.org/0000-0003-3765-6399  
Thomas Wieloch https://orcid.org/0000-0001-9162-2291  
Jürgen Schleucher https://orcid.org/0000-0002-4815-3466

**REFERENCES**


