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CO_2 fertilization of *Sphagnum* peat mosses is modulated by water table level and other environmental factors

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Abstract

Sphagnum mosses account for most accumulated dead organic matter in peatlands. Therefore, understanding their responses to increasing atmospheric CO_2 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 CO_2 to O_2 ratio. We used climate chambers to investigate photorespiratory responses of *Sphagnum fuscum* hummocks to recent increases in atmospheric CO_2 (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 ($D6^S/D6^R$ ratio) of photosynthetic glucose. The effect of elevated CO_2 on photorespiration was highly dependent on water table. At low water table (−20 cm), elevated CO_2 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 CO_2 effect. The response was further tested for *Sphagnum majus* lawns at typical water table levels (~0 and −7 cm), revealing no effect of CO_2 under those conditions. Our results indicate that hummocks, which typically experience low water table levels, benefit from the 20th century's increase in atmospheric CO_2.

KEYWORDS
atmospheric CO_2, 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 CO_2 concentrations are still not well understood.

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

ABBREVIATIONS: c_a, atmospheric CO_2 concentrations; c_c, chloroplastic CO_2 concentrations; NPP, net primary production; RuBP, ribulose 1,5-bisphosphate; WT, water table.

constraints. Distinct *Sphagnum* species favor specific mire microhabitats, and form associated structures, including hummocks, lawns and hollows that reflect characteristic average water table (WT) levels (Moor et al., 2017; Nijp et al., 2017; Rydin, Gunnarsson, & Sundberg, 2006; Weston et al., 2015). Key elements of the species' adaptation to these microhabitats are based on physiological and anatomical traits that govern their capillary water retention (McCarter & Price, 2014; Rydin & Clymo, 1989; Weston et al., 2015). *Sphagnum fuscum* is an abundant globally distributed hummock species (Klinggräff, 1872) that is capable of growing up to 50 cm above the WT (Gunnarsson, 2005; Hogg, 1993; Rydin et al., 2006). In contrast, *Sphagnum majus* inhabits lawns and hollows and grows generally less than 10 cm above the WT (Nijp et al., 2014).

The ongoing increase in atmospheric CO_2 from preindustrial levels of 280 ppm to the contemporary ~400 ppm and concomitant changes in climate, such as higher temperatures and precipitation in the northern hemisphere, are expected to have major consequences for mire vegetation and C sequestration (Belyea & Malmer, 2004; Frolking et al., 2011; Gallego-Sala et al., 2018; Hilbert, Roulet, & Moore, 2000; Limpens et al., 2008). CO_2 "fertilization" on higher plants has been extensively studied, but its magnitude and underlying mechanisms remain unclear (Ainsworth & Long, 2005; DeLucia, Moore, & Norby, 2005; IPCC, 2013; Schimel, Stephens, & Fisher, 2015; Walker et al., 2020). Even less is known about *Sphagnum* mosses, which lack certain anatomical features of higher plants, such as the cuticle, stomata and roots, so they can only regulate CO_2 and water fluxes indirectly (Hayward & Clymo, 1982; Price & Whittington, 2010; Williams & Flanagan, 1998).

In previous manipulation experiments, conflicting results have been obtained concerning the photosynthetic response of *Sphagnum* to increases in atmospheric CO_2 levels. They found enhanced net photosynthesis, but not biomass, of *S. fuscum* (Jauhiainen & Silvola, 1999; Jauhiainen, Vasander, & Silvola, 1994). Most studies of other *Sphagnum* species also did not detect any increase in biomass (Berendse et al., 2001; Heijmans et al., 2001; Heijmans, Klees, de Visser, & Berendse, 2002; Mitchell et al., 2002; Toet et al., 2006; van der Heijden, Verbeek, & Kuiper, 2000), except for van der Heijden, Jauhiainen, Silvola, Vasander, and Kuiper (2000). However, in most of these studies, effects of confounding factors such as temperature, moisture, light intensity, and nutrient availability may have masked effects of changes in atmospheric CO_2 levels on C assimilation.

On the molecular level, C assimilation in C_3 plants such as *Sphagnum* is initiated by the reaction of CO_2 with ribulose 1,5-bisphosphate (RuBP, carboxylation), catalyzed by the enzyme Rubisco. Due to Rubisco's enzymatic properties, this reaction is accompanied by a massive side reaction of RuBP with O_2 (oxygenation). This process gives rise to the photorespiration pathway, which leads to loss of C as CO_2; therefore, this oxygenation reaction decreases net CO_2 fixation (W. A. Laing, Ogren, & Hageman, 1974). Despite anatomical differences between *Sphagnum* and higher C_3 plants, the kinetic properties of Rubisco are very similar, that is, for ~300 species (including mosses) the variation was fairly low (Flamholz et al., 2019) and $\delta^{13}C$ values (which reflect Rubisco kinetics, Tcherkez, Farquhar, & Andrews, 2006) are similar to those of higher C_3 plants (O'Leary, 1988).

Models predict that the anthropogenic increase in atmospheric CO_2 (from 280 to over 400 ppm) will generally result in suppressed C_3 photorespiration rates (under given conditions) and contribute to an increase in net photosynthesis of ~35% (Ehlers et al., 2015). Thus, robust understanding of the ratio of photorespiration to gross photosynthesis and its determinants is crucial for predicting overall net C balances of terrestrial biomass, and particularly for modeling responses of photosynthetic processes to changes in environmental conditions (Pugh, Muller, Arneth, Haverd, & Smith, 2016; Weston et al., 2015).

The above-mentioned conflicting responses in *Sphagnum* C assimilation were observed in manipulation experiments, with CO_2 increases above ambient (>350 ppm). Another question is how to constrain the response that has occurred over the 20th century. To date there are no methods to trace if there has been a suppression of photorespiration during the 20th century. However, accurate estimates of the ratio of photorespiration to gross photosynthesis, at the time of formation of both current and historical plant tissues, can be obtained using deuterium (D) isotopomers (Ehlers et al., 2015). In this method, the abundance ratio of the D isotopomers named $D6^S$ and $D6^R$ in the $C6H_2$ group of glucose derived from hydrolyzed cell wall carbohydrates is measured by NMR spectroscopy (Figure 1).

The $D6^S/D6^R$ ratio indicates the Rubisco oxygenation to carboxylation flux ratio, and thus the photorespiration to gross photosynthesis ratio. For simplicity, in the following text, we will refer to this ratio as

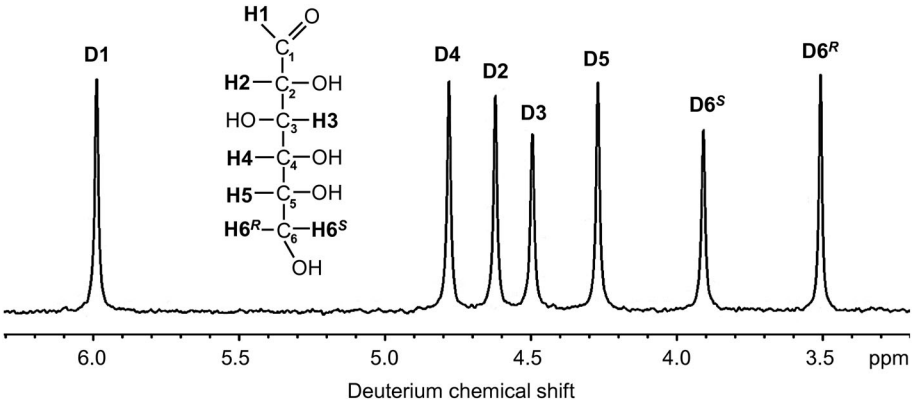

FIGURE 1 Deuterium NMR spectrum of glucose derived from structural carbohydrates of *S. fuscum*. The integral of each peak is proportional to the abundance of the deuterium (D) isotopomers at the corresponding position in the glucose molecule (H1 – H6). The ratio of the isotopomer abundance of $D6^S$ and $D6^R$ corresponds to the photorespiration/photosynthesis ratio (Ehlers et al., 2015)

photorespiration/photosynthesis ratio. Analysis with this method confirmed that the photorespiration of many C_3 plants was suppressed and their net photosynthesis rates increased by the last century's increase in atmospheric CO_2 (Ehlers et al., 2015). In the cited study, comparison of *S. fuscum* herbarium material formed at ≤300 ppm atmospheric CO_2 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 CO_2 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 isotopomer 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 CO_2 under various combinations of different atmospheric CO_2, WT, temperature and light intensity levels. To do so, we used D isotopomers as well as $\delta^{13}C$, $\delta^{15}N$ and elemental analysis (C and N). The response was further tested for the lawn species *S. majus* at different CO_2 and WT levels.

2 | MATERIALS AND METHODS

2.1 | Plant material

Peat mesocosms from hummocks dominated by *Sphagnum fuscum* (Schimp.) Klinggr. and lawns dominated by *Sphagnum majus* (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-Stormyr peatland. Degerö-Stormyr 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 cm^3 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-Stormyr: www.icos-sweden.se) under different CO_2 concentrations and WT levels. Two identical chambers were used for incubation at atmospheric CO_2 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. CO_2 levels in the growth chambers were regulated automatically, solely by removal. Thus, the CO_2 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 full 2^3 factorial design with low light intensity as a fixed factor and atmospheric CO_2 concentration, WT and temperature as variables, and the last incubation a 2^2 factorial design, with temperature and light intensity as fixed factors and CO_2 concentration and WT as variables. In the following text, the different CO_2, 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, Juurola, Hajek, & Tuittila, 2011). The response variables measured in the experiments were: photorespiration/photosynthesis ratio ($D6^S/D6^R$ ratio), moss height increment, biomass production, ^{13}C and ^{15}N 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-glucofuranose according to established protocols (Betson, Augusti, & Schleucher, 2006). To remove contamination by a mannose derivative, which has overlapping signals with the NMR spectrum of the glucose derivative, an oxidation step was introduced. Each sample was dissolved in 20 ml 0.187 M phosphate buffer (NaH_2PO_4, pH 4), 15.6 mg $NaClO_2$ was added per 100 mg sample and the pH was adjusted to 3.5 with H_3PO_4. The $NaClO_2$ addition and pH adjustment was repeated five times over a period of 3 days (every 12 hr). The complete reaction procedure was performed under the absence of light. Twelve hours after the last addition the reaction was quenched by adding a molar equivalent amount of Na_2SO_3 to the total amount of $NaClO_2$ added, then the mixture was neutralized with $CaCO_3$, vacuum-filtered and evaporated under reduced pressure. To remove remaining salts the sample was extracted three times with 20 ml ethanol. The derivative was subsequently converted to 3,6-anhydro-1,2-O-isopropylidene-α-D-glucofuranose 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 ^{1}H-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% C_6F_6 and 0.01% C_6D_6 then transferred to a 5-mm NMR tube with a PTFE valve (J. Young Scientific Glassware Ltd., Windsor, U.K.) containing ~5 mg of $NaHCO_3$. D-NMR measurements were acquired and processed as described by Betson et al. (2006) using an AVANCE III 850 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) equipped with a ^{19}F lock and a cryogenic probe optimized for D detection. D-NMR spectra were integrated by deconvolution with a Lorentzian line shape fit, using TopSpin™ 3.2 (Bruker BioSpin GmbH, Rheinstetten, Germany). The $D6^S/D6^R$ isotopomer ratio was determined as the ratio of the integrals of the $D6^S$ and $D6^R$ signals (Figure 1). For each sample, five to eight spectra were recorded and the average of the $D6^S/D6^R$ ratios was calculated.

2.6 | C and N-isotope and elemental analysis

C and N isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$, respectively) and C and N contents of dry samples of moss tissues (ca. 5 mg) were analyzed via conversion to CO_2 and N_2 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 $\delta^{13}C$; and IAEA-600, IAEA-N-2, USGS40 and USGS41 for $\delta^{15}N$.

2.7 | Calculation of chloroplastic CO_2 concentrations

Chloroplastic CO_2 concentrations (c_c) were calculated based on $\delta^{13}C$ signatures of the moss ($\delta^{13}C_{moss}$), using equations for photosynthetic fractionation ($\Delta^{13}C_{moss}$) published by Flanagan and Farquhar (2014), with modifications to account for the lack of stomatal resistance in *Sphagnum*:

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

$$\Delta^{13}C_{moss} = a_m\left(\frac{c_a - c_c}{c_a}\right) + b\left(\frac{c_c}{c_a}\right) - f\left(\frac{\Gamma^*}{c_a}\right). \quad (2)$$

Here, $\delta^{13}C_{atm}$ is the $\delta^{13}C$ signature of atmospheric CO_2 (−8.5‰, Graven et al., 2017), a_m the fractionation during CO_2 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 Γ^* the CO_2 compensation point (μmol mol^{-1}) in the absence of dark respiration (calculated from the temperature response: $\Gamma^* = 42.7 + 1.68\,(T - 25) + 0.0012\,(T - 25)^2$, 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 $\Delta^{13}C_{moss}$ values were much higher (~19‰, Figure S4) compared to other submerged mosses and aquatic plants (4–10‰, Keeley & Sandquist, 1992), suggesting that $\delta^{13}C_{moss}$ is modulated by C sources other than atmospheric CO_2. The anoxic conditions at high WT are favorable for heterotrophic production of CH_4, which is potentially oxidized to CO_2 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 CH_4 at the high WT, $\Delta^{13}C_{moss}$ was calculated according to a simple isotopic mass balance (Keeley & Sandquist, 1992; Raghoebarsing et al., 2005):

$$\Delta^{13}C_{moss} = a \cdot \delta^{13}C_{CH4} + (1-a) \cdot \delta^{13}C_{CO2} - \delta^{13}C_{moss}. \quad (3)$$

Here, $\delta^{13}C_{CH4}$ is the $\delta^{13}C$ value of respired CH_4 (−60‰, according to average from Raghoebarsing et al., 2005; Larmola et al., 2010 and Nielsen et al., 2019) and $\delta^{13}C_{CO2}$ is the $\delta^{13}C$ signature of atmospheric CO_2 (−8.5‰, Graven et al., 2017). The factor *a* represents the amount of C in *Sphagnum* derived from oxidized CH_4 and is estimated with 15% according to Raghoebarsing et al. (10%–15%, 2005) and Larmola et al. (10%–30%, 2010). Including CH_4-derived CO_2 as C source reduces $\Delta^{13}C_{moss}$ from ∼19‰ to ∼10‰ (Figure S4), which corresponds to the value of other submerged mosses (*F. antipyretica*, Keeley & Sandquist, 1992). Subsequently, c_c was calculated according to Equation (2). To account for the uncertainty of the estimated amount of C derived from CH_4 oxidation (factor *a*), we calculated the variation in c_c for values of 10% and 20%, which was ±27–38 ppm. These values were considered as the error range of c_c at high WT (Figure 3b). Correlation of c_c with the $D6^S/D6^R$ ratio, for both with and without accounting for CH_4 oxidation, revealed that considering 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 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 with 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 $D6^S/D6^R$ ratio (range: 0.85–0.94) were WT and atmospheric 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 and interactions between WT and CO_2 both explained 7% each (p = .004 and p = .005, respectively) of the variance in this ratio. Increasing atmospheric CO_2 from 280 to 400 ppm resulted in a 0.03 decrease in the $D6^S/D6^R$ ratio at low WT, but had no significant effect on it at high WT (Figure 2a). Together with the observed interaction between CO_2 and WT, this indicates WT-dependent suppression of photorespiration at the high CO_2 level. Raising the WT from −20 to ∼0 cm resulted in a significant (0.01–0.05) increase in the $D6^S/D6^R$ 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 $D6^S/D6^R$ ratio at low WT, but increasing the light intensity from 250 to 500 µmol m^{-2} s^{-1} had no significant effect on it (Figure 2a, Table 1).

Theoretically, the suppression of photorespiration at high CO_2 and low WT should have been accompanied by increases in CO_2 assimilation rates, assuming constant RuBP turnover. Therefore, biomass production was expected to increase at the high 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})

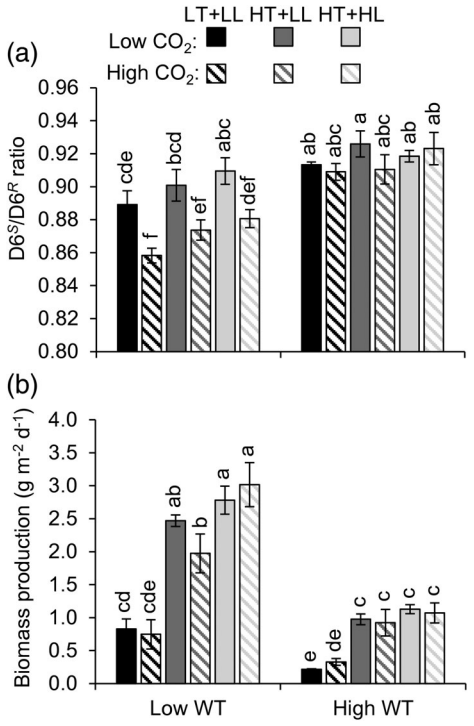

FIGURE 2 $D6^S/D6^R$ ratios and biomass production of *S. fuscum* incubated in the growth chambers at indicated atmospheric CO_2, temperature, water table (WT) and light intensity settings. Solid and striped bars indicate low and high atmospheric CO_2, respectively. LT and HT indicate low and high temperature, and LL and HL low and high light, respectively. (a) $D6^S/D6^R$ isotopomer ratios reflecting the photorespiration/photosynthesis ratio and (b) daily biomass production during the two-month incubation period. Error bars indicate ± SE, n = 3. Different letters above error bars indicate significant differences (p < .05) according to Fisher's least significant difference post-hoc test with Benjamini-Hochberg correction

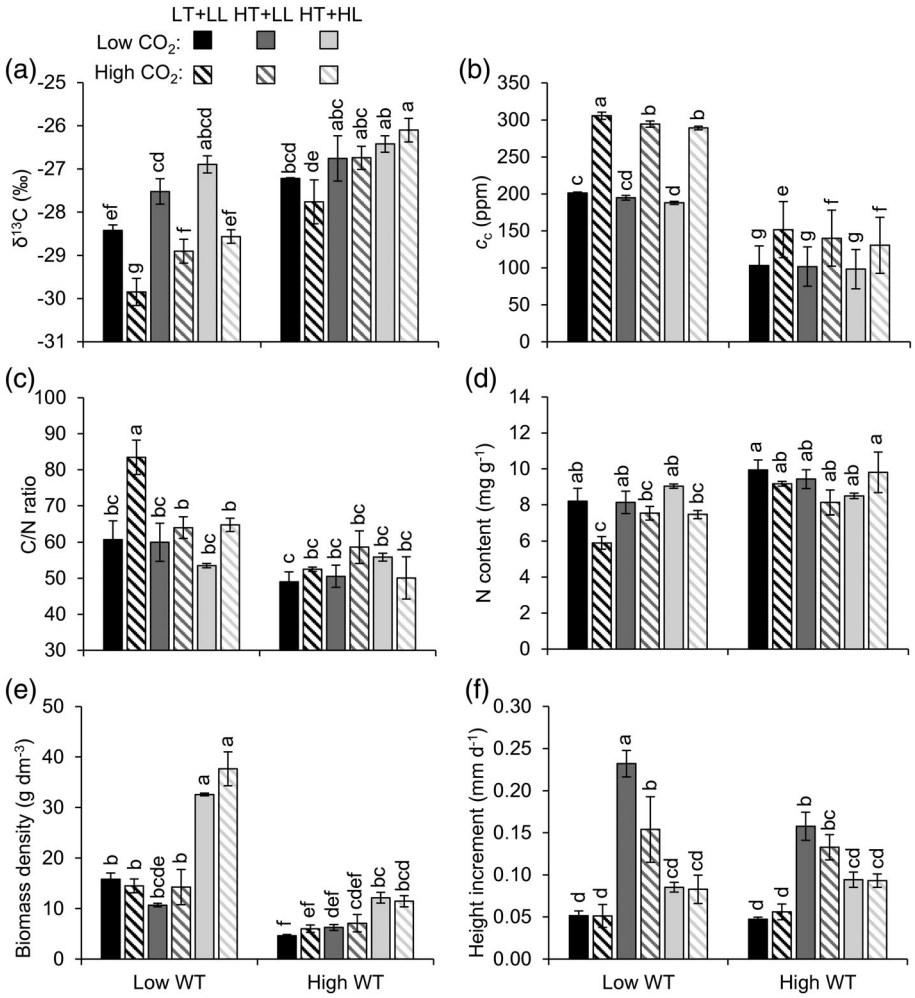

FIGURE 3 Physiological response variables of *S. fuscum* incubated in the growth chambers at indicated atmospheric CO_2, temperature, water table (WT), and light intensity settings. Solid and striped bars indicate low and high atmospheric CO_2, respectively. LT and HT indicate low and high temperature, and LL and HL low and high light, respectively. (a) whole-tissue $\delta^{13}C$ values, (b) estimated chloroplastic CO_2 concentrations (c_c), (c) whole-tissue C/N ratio and (d) N contents, (e) biomass density in total biomass dry weight per unit volume and (f) moss height increment. Error bars indicate ± SE, n = 3, except for high WT in (b), which shows the error range based on model assumptions (see materials and methods). Different letters above error bars indicate significant differences (p < .05) according to Fisher's least significant difference post-hoc test with Benjamini-Hochberg correction

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 $\delta^{13}C$ and chloroplastic to ambient CO_2 concentration

To further investigate physiological effects of increasing atmospheric CO_2 from 280 to 400 ppm, we analyzed ^{13}C discrimination by measuring whole-tissue $\delta^{13}C$ signatures (Figure 3a). $\delta^{13}C$ is commonly used as proxy for surface moisture in *Sphagnum* biomass but has also been found to be influenced by temperature, light and CO_2 concentration (Loisel, Garneau, & Hélie, 2010; Ménot & Burns, 2001). In our experiment, 48% of the variation in $\delta^{13}C$ (which ranged from −30.5 to −25.7‰) was explained by changes in WT (p < .001), 13% by atmospheric CO_2 (p < .001), 10% by temperature (p < 0.001), 3% by light intensity (p = 0.02), and 10% by the interaction between CO_2 and WT (p < .001; Table 1). Increasing atmospheric CO_2 consistently decreased $\delta^{13}C$ (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 $\delta^{13}C$. Increasing the temperature caused a significant, 0.5–1.0‰, increase in $\delta^{13}C$. Increasing the light intensity resulted in a small 0.3–0.6‰ increase in $\delta^{13}C$ at low WT. A strong positive correlation was observed between the $D6^S/D6^R$ ratio and $\delta^{13}C$ at low WT (R^2 = .88, p < .001), but there was no significant relationship between these variables at high WT (R^2 = .19, p = .069).

The $\delta^{13}C$ signature reflects the ratio of chloroplastic to atmospheric CO_2 concentration (c_c/c_a, Farquhar et al., 1989) and thus allowed estimation of c_c (see materials & methods), which is a key determinant of the photorespiration/photosynthesis flux ratio. Variation in c_c (98–306 ppm, Figure 3b) was mostly explained by WT (71%, p < .001), atmospheric CO_2 (23%, p < .001) and the interaction between CO_2 and WT (4%, p < .001; Table 1). Increasing atmospheric CO_2 at low WT significantly increased c_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

TABLE 1 Summary of four-way ANOVA models of effects of CO_2, temperature (T), water Table (WT), light intensity (LI) and their interactions on the measured physiological response variables

		Main factors				Factor interactions					Model	
Response variable		CO_2	T	WT	LI	$CO_2 \times T$	$CO_2 \times WT$	$CO_2 \times LI$	$WT \times T$	$WT \times LI$	R^2	*df*
$D6^S/D6^R$ ratio	*p*	<.001	.004	<.001	*n.s.*	*n.s.*	.005	*n.s.*	*n.s.*	*n.s.*	.77	4/31
	F	19.0	9.8	64.5			9.4					
Biomass production	*p*	*n.s.*	<.001	<.001	.003	*n.s.*	*n.s.*	*n.s.*	.005	.047	.88	5/30
	F		69.4	131.6	10.7				9.0	4.3		
$\delta^{13}C$	*p*	<.001	<.001	<.001	.020	*n.s.*	<.001	*n.s.*	*n.s.*	*n.s.*	.84	5/30
	F	23.2	17.6	88.8	6.0		19.3					
c_c conc.	*p*	<.001	.007	<.001	.024	.066	<.001	*n.s.*	*n.s.*	*n.s.*	.99	6/29
	F	1,095.4	8.6	3,392.8	5.6	3.6	210.9					
C/N ratio	*p*	.003	.083	<.001	*n.s.*	.074	.022	*n.s.*	.004	*n.s.*	.68	6/29
	F	10.9	3.2	27.5		3.4	5.9		9.5			
N content	*p*	.013	*n.s.*	<.001	*n.s.*	*n.s.*	.070	*n.s.*	.031	*n.s.*	.58	7/28
	F	7.1		19.6			3.5		5.1			
Biomass density	*p*	*n.s.*	*n.s.*	<.001	<.001	*n.s.*	*n.s.*	*n.s.*	.097	<.001	.93	6/29
	F			180.5	137.6				2.9	54.9		
Height Increment	*p*	.084	<.001	*n.s.*	<.001	.020	*n.s.*	.035	.042	.017	.88	8/27
	F	3.2	109.7		51.1	6.2		4.9	4.6	6.5		

Note: Three- and four-way interactions were excluded from the models. Non-significant factors/interactions with $p > .1$ are denoted as *n.s.*. *df*, degrees of freedom of the model and residuals.

~94 ± 27 ppm decrease in c_c at low CO_2, and ~156 ± 38 ppm decrease at high CO_2. Thus, increases in atmospheric CO_2 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 $D6^S/D6^R$ 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 CO_2 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 CO_2 (12%, $p = .003$), interaction between temperature and WT (11%, $p = .004$), and interaction between CO_2 and WT (7%, $p = .022$, Table 1). Increasing the atmospheric CO_2 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 CO_2 (11%, $p = .013$) and the interaction between WT and temperature (8%, $p = .031$, Table 1). Increasing the CO_2 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 $D6^S/D6^R$ 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 $\delta^{15}N$ 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 CO_2 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 CO_2 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 CO_2 as well as temperature and WT or CO_2 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 CO_2 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 CO_2 level, but CO_2 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}, far outside the optimal range for photosynthesis (Figure S4).

3.5 | Photorespiration response of *S. majus*

The observed CO_2-response of the $D6^S/D6^R$ 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 CO_2 levels. To test this, we analyzed $D6^S/D6^R$ ratios of *S. majus* grown at different CO_2 and WT levels (under high temperature and low light conditions). Increasing CO_2 from 280 to 400 ppm did not have any significant effect on the $D6^S/D6^R$ ratio (range: 0.90–0.98) of *S. majus* at both low (−7 cm) and high WT (~0 cm, $R^2 = .01$, $p = .153$, Table 2, Figure 4a). In contrast, raising the WT resulted in a significant increase in the $D6^S/D6^R$ ratio (0.05–0.06), indicating increased photorespiration/photosynthesis ratios at high WT levels ($R^2 = 0.94$, $p < .001$, Table 2). Biomass production did not show any significant difference in response to increasing atmospheric CO_2, but raising the WT reduced biomass production by ~40% ($R^2 = .49$, $p = .01$, Table 2, Figure 4b). Elevated atmospheric CO_2 concentrations increased biomass density by two-fold at low WT ($R^2 = .18$, $p = .048$, Table 2,

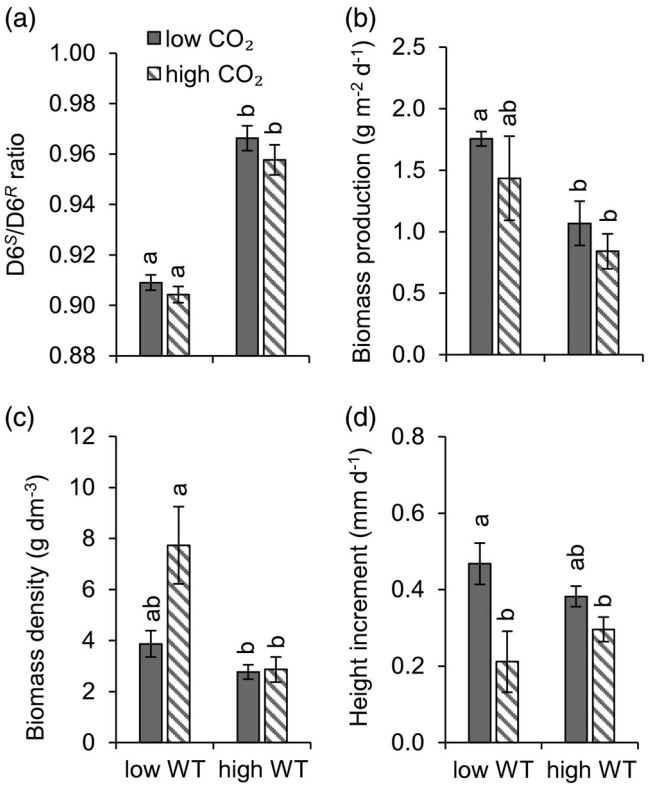

FIGURE 4 $D6^S/D6^R$ ratios, biomass production, density and height increment of *S. majus* incubated in the growth chambers at indicated atmospheric CO_2 and water table (WT) settings. Data were obtained for the high temperature and low light treatment. Solid and striped bars indicate low and high atmospheric CO_2, respectively. (a) $D6^S/D6^R$ isotopomer ratios reflecting the photorespiration/photosynthesis ratio, (b) daily biomass production during the two-month incubation period, (c) biomass density in total biomass dry weight per unit volume and (d) moss height increment. Error bars indicate ± SE, $n = 3$. Different letters above error bars indicate significant differences ($p < .05$) according to student's *t* test

TABLE 2 Summary of two-way ANOVA models of effects of CO_2 and water table (WT) and their interaction on the measured physiological response variables of *S. majus*

Response variable		CO_2	WT	$CO_2 \times WT$	R^2	*df*
$D6^S/D6^R$ 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% (R^2 = .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 photorespiration in *S. majus* is not suppressed in response to increased atmospheric CO_2, despite optimal moisture conditions.

4 | DISCUSSION

4.1 | Effects of increased atmospheric CO_2 on *S. fuscum* photosynthesis

The $D6^S/D6^R$ ratios of *S. fuscum* indicate a significant suppression of photorespiration relative to C assimilation at the higher (current) atmospheric CO_2 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 photorespiration relative to C assimilation during the 20th century. In contrast, when the *Sphagnum* plants were water-saturated, increasing atmospheric CO_2 from 280–400 ppm had no significant detected effects on the photorespiration to photosynthesis ratio (i.e. on $D6^S/D6^R$).

The estimated c_c (based on the $\delta^{13}C$ data) strongly increased under contemporary CO_2 levels at low WT (Figure 3b), suggesting that the suppression of photorespiration at low WT derives from the higher intercellular CO_2 concentration suppressing Rubisco oxygenation. At high WT, an increase in c_c 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 photorespiration significantly (Figure 2a). Considering the linear relationship between $D6^S/D6^R$ and $1/[CO_2]$ described by Ehlers et al. (2015), the increase in c_c at high WT would correspond to an increase of $D6^S/D6^R$ of 0.011 ± 0.003, which is close to the obtained average response at high WT (0.005).

The observed shift in the $D6^S/D6^R$ 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 CO_2 concentration had the largest effect, explaining 53% of the variance of $D6^S/D6^R$ under typical hydrological (low WT) conditions for *S. fuscum* (3-way ANOVA: $P_{CO2} < 0.001$, P_T = 0.01, P_{LI} = *n.s.*, F_{CO2} = 27.2, F_T = 8.8). Thus, our experimental data confirm that the detected suppression of photorespiration in herbarium samples (Ehlers et al., 2015) was due to increases in atmospheric CO_2 during the last century.

4.2 | Effect of moss water content

The increase in the $D6^S/D6^R$ ratio from low to high WT (Figures 2a and 4a) revealed an increase in the photorespiration/photosynthesis ratio for both *S. fuscum* and *S. majus*. A concomitant decrease in c_c from low to high WT was observed (Figure 3b), suggesting that the increase in the photorespiration/photosynthesis ratio was driven by a decline in c_c. In addition, the lower c_c at high WT is indicative of higher CO_2 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 CO_2 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 c_c/c_a (Rice & Giles, 1996; Williams & Flanagan, 1996, 1998).

4.3 | Relationship between the $D6^S/D6^R$ ratio and $\delta^{13}C$

At low WT the $D6^S/D6^R$ ratio and $\delta^{13}C$ were strongly correlated (R^2 = .88). $D6^S/D6^R$ 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 $\delta^{13}C$ reflects c_c/c_a and thus the Rubisco substrate concentration (Farquhar et al., 1989). However, in contrast to the $D6^S/D6^R$ ratio, the $\delta^{13}C$ value of the source C needs to be known to accurately predict c_c. This 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 $D6^S/D6^R$ ratio and $\delta^{13}C$ at high WT (R^2 = 0.19), highlighting potential difficulties in interpreting $\delta^{13}C$ under water-saturating conditions. This mismatch therefore suggests that $\delta^{13}C$ of the source C is not equal to that of atmospheric CO_2. When considering only atmospheric CO_2 as C source at the high WT, photosynthetic fractionation ($\Delta^{13}C_{moss}$) was ∼19‰ (Figure S4), much higher compared to other submerged mosses and aquatic plants (4–10‰, Keeley & Sandquist, 1992). This supports that *Sphagnum*-$\delta^{13}C$ is modulated of by another C source. The uptake of CO_2 originating from oxidation of respired CH_4 has the potential to affect $\delta^{13}C$ of *Sphagnum* significantly due to very low $\delta^{13}C$ values of CH_4 (∼ −60‰, Raghoebarsing et al., 2005; Larmola et al., 2010). When accounting for the uptake of CH_4-derived CO_2, the estimated $\Delta^{13}C_{moss}$ agrees with other submerged plants (∼10‰, Figure S4) and the derived c_c-estimates complement the $D6^S/D6^R$ ratios (R^2 = 0.74, Figures 2a and 3b and Figure S5). Altogether, this indicates potential problems in using $\delta^{13}C$ alone to estimate C fluxes in *Sphagnum*. Thus, using the $D6^S/D6^R$ ratio improves physiological interpretations of metabolic C fluxes.

4.4 | Limitations for *Sphagnum* biomass production

The decrease in the photorespiration/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 CO_2. However, there was no significant increase in biomass of *S. fuscum* under elevated CO_2 at both WT levels (Figure 2b), consistent with findings of several other studies on different *Sphagnum* species (Berendse et al., 2001; Heijmans 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 CO_2 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 CO_2-driven increase in NPP. Thus, elevated CO_2 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 CO_2 on biomass production of higher C_3 plants rely on nutrient availability and/or re-allocation 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 CO_2 (Figure 3c,d), resulting in a high correlation with the $D6^S/D6^R$ ratio (R^2 = .68 and .71 respectively). In higher C_3 plants, acclimation to increased CO_2 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 CO_2 and suggests that increasing CO_2 reduces Rubisco limitation of C assimilation (Figures 2a and 3d). However, Granath, Strengbom, and Rydin (2012) showed that net CO_2 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 CO_2 only under optimal growth conditions.

4.5 | Ecophysiological implications

Our results show that hummocks with characteristic WT levels have profited from CO_2 fertilization imposed by the 20th centuries CO_2 increase. The high WT conditions in this study resulted in abolishing the effect of CO_2 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 CO_2 (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 CO_2 during the 20th century was the major driver of *Sphagnum* photosynthetic C fluxes. During the early and mid-Holocene, atmospheric CO_2 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 CO_2 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 CO_2 suppressed *S. fuscum's* photorespiration relative to C assimilation. This response was highly dependent on WT, with water-saturating conditions abolishing the CO_2 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 CO_2 effect for those microhabitats. Our study revealed that D isotopomers are a valuable tool for understanding metabolic C fluxes in *Sphagnum*.

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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 mesocosm 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.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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