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 only hummocks, which typically experience low water table levels, benefit from the 20^{th} century's increase in atmospheric CO_2.

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; Wu *et al* ., 2012; Laing *et al* ., 2014; Loisel *et al* ., 2014). Thus, reliable prediction of future C sequestration and storage in peatlands requires profound understanding of *Sphagnum* C acquisition and accumulation (Frolking *et al* ., 2011; Charman *et al* ., 2013; 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 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 (Rydin *et al* ., 2006; Weston *et al* ., 2015; Moor *et al* ., 2017; Nijp *et al* ., 2017). Key elements of the species' adaptation to these microhabitats are based on physiological and anatomical traits that govern their capillary water retention (Rydin & Clymo, 1989; McCarter & Price, 2014; 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 (Hogg, 1993; Gunnarsson, 2005; 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 (Hilbert *et al* ., 2000; Belyea & Malmer, 2004; Limpens *et al* ., 2008; Frolking *et al* ., 2011; Gallego-Sala *et al* ., 2018). CO_2 'fertilization' on higher plants has been extensively studied, but its magnitude and underlying mechanisms remain unclear (IPCC, 2013; Ainsworth & Long, 2005; DeLucia *et al* ., 2005; Schimel *et al* ., 2015). 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; Williams & Flanagan, 1998; Price & Whittington, 2010).

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*et al* ., 1994; Jauhiainen & Solviola, 1999). Most studies of other *Sphagnum* species also did not detect any increase in biomass (Van der Heijden *et al* ., 2000b; Berendse *et al* ., 2001; Heijmans *et al* ., 2001, 2002; Mitchell *et al* ., 2002; Toet *et al* ., 2006), except for Van der Heijden *et al* . (2000a). 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 (Laing*et al* ., 1974). 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 (Weston *et al* ., 2015; Pugh *et al* ., 2016).

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 20^{th} century. To date there are no methods to trace if there has been a suppression of photorespiration during the 20^{th} 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 (Fig. 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 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 net photosynthesis rates of peat-forming*Sphagnum* also increased 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.

MATERIALS AND METHODS

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 x 20 x 25 cm 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.

Manipulation experiments

Triplicate mesocosms were grown in climate-controlled growth chambers (PGC-7L2/DE, Percival Scientific, USA) at Umeå Plant Science Centre for 8-10 weeks with 18/6-h 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_2concentrations 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 (Fig. S1). The WT was re-adjusted every 2 days, resulting in variations of $\pm$ 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 bathes 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 2^3factorial 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 *et al.* , 1989; Jauhiainen & Solviola, 1999; Laine *et al.* , 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.

Biomass and height increment measurements

Height increments of the moss in the mesocosms were measured weekly during the incubation period (Fig. S2), 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 h 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.

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 *et al* ., 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 h). The complete reaction procedure was performed under the absence of light. 12 h 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*et al* . (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.

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^{TM} 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 (Fig. 1). For each sample, five to eight spectra were recorded and the average of the $D6^S$ /$D6^R$ratios was calculated.

C and N-isotope and elemental analysis

C and N isotopic signatures (δ^{13}C and δ^{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 *et al* ., 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 δ^{13}C; and IAEA-600, IAEA-N-2, USGS40, and USGS41 for δ^{15}N.

Calculation of chloroplastic CO_2 concentrations

Chloroplastic CO_2 concentrations (c_c) were calculated based on δ^{13}C signatures of the moss ($\delta^{13}C_{moss}$), using equations published by Flanagan & Farquhar (2014), with modifications to account for the lack of

stomatal resistance in *Sphagnum* :

$$^{13}\mathrm{C}_{\mathrm{moss}} = 1000\ \frac{\delta^{13}\mathrm{C}_{\mathrm{atm}} - \delta^{13}\mathrm{C}_{\mathrm{moss}}}{1000 + \delta^{13}\mathrm{C}_{\mathrm{moss}}} \backslash \mathrm{n}^{13}\mathrm{C}_{\mathrm{moss}} = \mathrm{a}_{\mathrm{m}}\left(\frac{\mathrm{c}_{\mathrm{a}} - \mathrm{c}_{\mathrm{c}}}{\mathrm{c}_{\mathrm{a}}}\right) + \mathrm{b}\left(\frac{\mathrm{c}_{\mathrm{c}}}{\mathrm{c}_{\mathrm{a}}}\right) - \mathrm{f}\left(\frac{\Gamma^{*}}{\mathrm{c}_{\mathrm{a}}}\right) \backslash n$$

Here, $\delta^{13}\mathrm{C}_{\mathrm{atm}}$ is the δ^{13}C signature of atmospheric CO_2(-8.5$a_m$the fractionation during CO_2 diffusion through water to the chloroplast (1.8., 1989), b the discrimination during carboxylation by Rubisco (29photorespiration (16.2* the CO_2 compensation point (μ mol mol^{-1}) in the absence of dark respiration (calculated from the temperature response: $\Gamma^{*} = 42.7 + 1.68\ (T\text{ -}25) + 0.0012\ (T\text{ -}25)^2$, where$T$ is temperature in °C; Brooks & Farquhar, 1985). When applying this equation we assumed, following Evans & von Caemmerer (2013), that no fractionation occurred during day respiration.

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. 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 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 (Benjamni & Hochberg, 1995; Steel*et al* ., 1997) were applied, using the *LSD.test* () function, to account for false discovery rates. Microsoft Excel was used for all other data analysis.

RESULTS

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% ($P< 0.001$) and 14% ($P< 0.001$) of the total variance (Fig. 2A, Table 1). In addition, temperature and interactions between WT and CO_2 both explained 7% each ($P= 0.004$ and$P= 0.005$, 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 (Fig. 2A, Table S1). 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 (Fig. 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 (Fig. 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, and thus biomass production, but no significant increase in biomass accumulation associated with the high CO_2 level was observed under any test conditions (Fig. 2B). The variation in biomass production (0.2-3.6 g m^{-2} d^{-1}) was mostly explained by temperature (27%, $P< 0.001$), WT (52%, $P< 0.001$), and to a smaller degree light intensity (4%, $P= 0.003$) and the interaction between temperature and WT (4%, $P= 0.005$; 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% (Fig. 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 (Fig. S3). Thus, the observed changes in biomass production reflect proportional variations in C accumulation.

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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 (Fig. 3A). Variation in $\delta^{13}C$ (which ranged from -30.5 to -25.7by changes in WT (48%, $P< 0.001$), atmospheric CO_2 (13%, $P< 0.001$), temperature (10%,$P< 0.001$), light intensity (3%, $P= 0.02$), and the interaction between CO_2 and WT (10%,$P< 0.001$; Table 1). Increasing atmospheric CO_2 consistently decreased $\delta^{13}C$ (by 1.4 to 1.7 3A, Table S1). Concomitantly, raising the WT resulted in a 0.5-2.5increase in $\delta^{13}C$. Increasing the temperature caused a significant, 0.5-1.0 the light intensity resulted in a small 0.3-0.6$\delta^{13}C$ at low WT.

The $\delta^{13}C$ data allowed estimation of the chloroplastic CO_2 concentration (c_c) and subsequently the chloroplastic to ambient CO_2 ratio (c_c/c_a, Flanagan & Farquhar, 2014), which is a key determinant of metabolic C fluxes. Variation in c_c/c_a(0.63-0.80, Fig. 3B) was explained by WT (57%,$P< 0.001$), light intensity (4%, $P= 0.022$), temperature (3%, $P= 0.035$), atmospheric CO_2 (3%,$P= 0.046$) and the interaction between CO_2 and WT (13%, $P< 0.001$; Table 1). Increasing atmospheric CO_2 significantly increasedc_c/c_a, by 0.03-0.05, at low WT but had no significant effect at high WT (Fig. 3B, Table S1). Raising the WT caused a 0.02-0.09 decrease inc_c/c_a, and increasing the temperature decreasedc_c/c_a by 0.02 at low WT. Altogether, this indicates that 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 betweenc_c/c_a and the $D6^S/D6^R$ ratio at low WT (R^2=0.85, $P< 0.001$), suggesting that an increase in c_c caused the decrease in photorespiration/photosynthesis ratio, *i.e.* suppression of photorespiration at low WT. No significant relationship in these variables was observed at high WT (R^2=0.10,P =0.208).

Variation in the CO_2 diffusion gradient (72-150 ppm) from the atmosphere to the chloroplasts (c_a-c_c) was mostly explained by CO_2 (53%, $P< 0.001$), WT (26%, $P< 0.001$) and the interaction of CO_2 and WT (9%, $P< 0.001$). Increasing atmospheric CO_2 resulted in increases inc_a-c_c of 15-52 ppm (Fig. S3, Table S2). This indicates that increases in atmospheric CO_2 increased CO_2 assimilation.

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 (Fig. 3C, D). Variation in the C/N ratio (40-89 units) was mostly explained by WT (31%, $P< 0.001$), atmospheric CO_2(12%, $P= 0.003$), interaction between temperature and WT (11%,$P= 0.004$), and interaction between CO_2 and WT (7%, $P= 0.022$, Table 1). Increasing the atmospheric CO_2 concentration increased the tissue C/N ratio by 4 to 23 units at low WT, but had no significant effect at high WT (Fig. 3C, Table S1). Increasing the WT generally decreased the C/N ratio by 5 to 31 units. The changes in C/N ratio were primarily due to changes in tissue N content, the C content only showed minor variation (Fig. S3).

Accordingly, the total variation in N content (5.5-11.8 mg g^{-1}) was explained by WT (31%,$P< 0.001$), atmospheric CO_2 (11%,$P= 0.013$) and the interaction between WT and temperature (8%,$P= 0.031$, 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 (Fig. 3D, Table S1). 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=0.68, P <0.001 and R^2=0.71, P <0.001, respectively). At high WT the ratio was still correlated, but less strongly, with both the C/N ratio (R^2=0.44, P <0.003) and N content (R^2=0.40, P <0.005).

No major between-treatment differences were detected in whole-tissue $\delta^{15}N$ signatures (average: -3.63 ± 0.08 According to the 4-way ANOVA, the only significant effect was a small (˜0.45intensity (R^2=0.23, P =0.003, Table S2).

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 (Fig. 3E, F). No effect of atmospheric CO_2 on biomass density was observed,

instead the variation in density (4.1 to 42.4 g dm^{-3}) was mostly explained by WT (45%,$P< 0.001$), light intensity (34%,$P< 0.001$) and the interaction between WT and light (14%, $P< 0.001$, 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 (Fig. 3E).

Most of the variation in height increment (0.04-0.26 mm d^{-1}) was explained by temperature (51%,$P< 0.001$) and light intensity (24%,$P< 0.001$), 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%,$P <0.05$, Table 1). Increasing the temperature increased the height increment 2.4- to 4.5-fold (Fig. 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 and low light treatment, at low WT (Fig. 3F, Fig. S2).

S. 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%, $P =0.006$; Table S2, Fig. S3). Most of the variation was explained by WT (83%, $P <0.001$), temperature (5%,$P <0.001$), and the interactions between WT and temperature (6%, $P <0.001$) and between WT and light (3%, $P <0.001$, Table S2). 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 (Fig. S3).

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=0.01, P =0.153, Table 2, Fig. 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 <0.001, 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=0.49, P =0.01, Table 2, Fig. 4B). Elevated atmospheric CO_2 concentrations increased biomass density by 2-fold at low WT (R^2=0.18, P =0.048, Table 2, Fig. 4C). Concomitantly, height increment significantly decreased at low WT by 55% (R^2=0.5, P =0.012, Table 2, Fig. 4D, Fig. S2). 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 despite optimal moisture conditions for photosynthesis, increasing atmospheric CO_2 does not affect C assimilation of *S. majus* .

DISCUSSION

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

The net photosynthesis rate of*S. fuscum* , as characterized by the $D6^S$ /$D6^R$ ratio, was significantly higher at the higher (current) atmospheric CO_2 level than at the lower (pre-industrial) level at low WT (Fig. 2A). $D6^S$ /$D6^R$ ratios were lower at the high CO_2 level when the WT was low (representing typical growth conditions for *S. fuscum*), thus indicating 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 to 400 ppm had no significant detected effects on their photosynthesis, *i.e.* on the $D6^S$ /$D6^R$ ratio. The observed shift in the $D6^S$ /$D6^R$ ratio of 0.03 units at low WT (Fig. 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_2concentration had the largest effect, explaining 53% of the

variance of $D6^S$ /$D6^R$ under typical hydrological (low WT) conditions for *S. fuscum* (Table S1). 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. Estimates of c_c/c_a revealed an increase under contemporary CO_2 levels at low WT, and a high negative correlation with the $D6^S$ /$D6^R$ ratio. This implies that the suppression of photorespiration derives from the higher intercellular CO_2 concentration suppressing Rubisco oxygenation. In line with the increase in c_c, the CO_2 diffusion gradient (c_a-c_c) was higher at the high CO_2 level than at the low CO_2 level when the WT was low, supporting the increase in CO_2assimilation at the 400 ppm CO_2 level (Fig. S3).

Effect of moss water content

The increase in the $D6^S$ /$D6^R$ ratio from low to high WT (Fig. 2A, 4A) revealed an increase in the photorespiration/photosynthesis ratio, and thus reduction in net photosynthesis at the high WT for both *S. fuscum* and *S. majus* . A concomitant decrease in c_c/c_a from low to high WT (Fig. 3B) suggests that the increase in the photorespiration/photosynthesis ratio was driven by a decline in c_c. In addition, the lower c_c/c_a at high WT is indicative of higher CO_2 diffusion resistance, resulting from the higher moss water content (Fig. S3). In this respect, our data supports 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 (Titus *et al* ., 1983; Titus & Wagner, 1984; Schipperges & Rydin, 1998) and a negative relationship between water content and c_c/c_a (Rice & Giles, 1996; Williams & Flanagan, 1996, 1998).

Limitations for *Sphagnum* biomass production

The lack of observed response of biomass production to increased atmospheric CO_2 (Fig. 2B) is consistent with findings of several other studies on different *Sphagnum* species (Jauhiainen *et al* ., 1994, 1998; Van der Heijden *et al* ., 2000b; Berendse *et al* ., 2001; Heijmans *et al* ., 2001, 2002; Mitchell *et al* ., 2002; Toet *et al* ., 2006). In contrast, the decrease in photorespiration/photosynthesis ratio we detected clearly indicates increased C assimilation (assuming constant RuBP turnover rates) and thus increased NPP potential in response to increased atmospheric CO_2. At low WT, increasing the CO_2 level caused a slight but not significant increase in biomass production at high light, and a slight decrease at low light (Fig. 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 *et al* ., 2009).

Furthermore, increases in atmospheric CO_2 may only enhance biomass production of higher C_3 plants if sufficient nutrients are available (Arp *et al* ., 1998; Poorter, 1998; Kirschbaum, 2011). Accordingly, we observed a decrease in *Sphagnum* tissue N content and an increase in the C/N ratio at elevated CO_2 (Fig. 3C, D), resulting in a high correlation with the $D6^S$ /$D6^R$ ratio. 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 (Drake *et al* ., 1997; Cotrufo *et al* ., 1998). This indicates reduced N investment in Rubisco under elevated CO_2 and suggests that increasing CO_2 reduces Rubisco limitation of C assimilation (Fig. 2A, 3D). Reductions in N contents associated with increases in CO_2, which suppress photorespiration, have been observed in several *Sphagnum* species including *S. fuscum, S. recurvum, S. palustre,* and *S. magellanicum* (Jauhiainen *et al* ., 1998; Van der Heijden *et al* ., 2000b; Toet *et al* ., 2006; Heijmans *et al* ., 2001). This suggests similar effects of elevated CO_2 on N content in most *Sphagnum* species.

Ecophysiological implications

Our results show that hummocks with characteristic WT levels have profited from CO_2 fertilization imposed by the 20^{th} 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 (Fig. 4A). Together with the observed response of *S. fuscum* , this suggests a species-specific suppression of photorespiration, which may be attributed to differences in leaf-anatomy

between hummock and lawn species (Rice & Giles, 1996). Thus, in contrast to hummock-species, certain lawn-species may not be capable of responding to changes in atmospheric CO_2.

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 suggests 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.

Our results further indicate that for hummocks with typical WT conditions, the increase in atmospheric CO_2 during the 20^{th} 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 (Yu *et al.,* 2009; Loisel *et al.,* 2014). 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.

CONCLUSION

Here we show that *S. fuscum's* photorespiration is suppressed,*i.e.* net photosynthesis increased, in response to the last century's increase in atmospheric CO_2. 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. Variations in temperature and light intensity did not affect the extent of the CO_2-driven suppression of photorespiration. Our study revealed that D isotopomers are a valuable tool for understand metabolic C fluxes in *Sphagnum* . The results presented in this study will allow validation of D isotopomer analysis of peat archives and provide the basis for modelling global peatland C fluxes.

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TABLES

Table 1. Summary of 4-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.

Response variable	**Main factors**	**Main factors**	**Main factors**	**Main factors**	**Factor interactions**	**Factor interactions**	**Factor interactions**	**Factor interactions**	**Factor interactions**	**Model p.**	**M p**
	CO_2	T	WT	LI	CO_2 x T	CO_2 x WT	CO_2 x LI	WT x T	WT x LI	R^2	d
$D6^S/D6^RP$ ratio	<0.001	0.004	<0.001	*n.s.*	*n.s.*	0.005	*n.s.*	*n.s.*	*n.s.*	0.77	4
F	19.0	9.8	64.5			9.4					

Response variable		Main factors	Main factors	Main factors	Main factors	Factor interactions	Factor interactions	Factor interactions	Factor interactions	Factor interactions	Model p.	M p
Biomass production	*P*	*n.s.*	<0.001	<0.001	0.003	*n.s.*	*n.s.*	*n.s.*	0.005	0.047	0.88	5
	F		69.4	131.6	10.7				9.0	4.3		
δ^{13}C	*P*	<0.001	<0.001	<0.001	0.020	*n.s.*	<0.001	*n.s.*	*n.s.*	*n.s.*	0.84	5
	F	23.2	17.6	88.8	6.0		19.3					
c_c / c_a	*P*	0.046	0.035	<0.001	0.022	*n.s.*	<0.001	*n.s.*	*n.s.*	*n.s.*	0.80	5
	F	4.3	4.9	86.3	5.8		18.8					
C/N ratio	*P*	0.003	0.083	<0.001	*n.s.*	0.074	0.022	*n.s.*	0.004	*n.s.*	0.68	6
	F	10.9	3.2	27.5		3.4	5.9		9.5			
N content	*P*	0.013	*n.s.*	<0.001	*n.s.*	*n.s.*	0.070	*n.s.*	0.031	*n.s.*	0.58	7
	F	7.1		19.6			3.5		5.1			
Biomass density	*P*	*n.s.*	*n.s.*	<0.001	<0.001	*n.s.*	*n.s.*	*n.s.*	0.097	<0.001	0.93	6
	F			180.5	137.6				2.9	54.9		
Height increment	*P*	0.084	<0.001	*n.s.*	<0.001	0.020	*n.s.*	0.035	0.042	0.017	0.88	8
	F	3.2	109.7		51.1	6.2		4.9	4.6	6.5		

Note that 3- and 4-way interactions were excluded from the models. Non-significant factors/interactions with $P > 0.1$ are denoted as *n.s.*. *df* , degrees of freedom of the model and residuals.

Table 2 . Summary of 2-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	Response variable	CO_2	WT	CO_2 x WT	R^2 *df*
$D6^S/D6^R$ ratio	*P*	0.071	<0.001	*n.s.*	0.88 2/9
	F	4.19	62.16		
Biomass production	*P*	*n.s.*	0.01	*n.s.*	0.54 1/10
	F		10.63		
Biomass density	*P*	0.048	0.008	0.057	0.74 3/8
	F	5.46	12.4	4.93	
Height increment	*P*	0.012	*n.s.*	*n.s.*	0.57 1/10

Response variable	Response variable	CO_2	WT	CO_2 x WT	R^2	*df*
	F	10.58				

Non-significant factors/interactions with $P > 0.1$ are denoted as *n.s.*. *df* , degrees of freedom of the model and residuals.

FIGURE LEGENDS

Fig. 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).

Fig. 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 ($P < 0.05$) according to Fisher's least significant difference post-hoc test with Benjamini-Hochberg correction.

Fig. 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 and (B) chloroplastic to ambient $CO_2(c_c/c_a)$ ratios, (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. Different letters above error bars indicate significant differences ($P < 0.05$) according to Fisher's least significant difference post-hoc test with Benjamini-Hochberg correction.

Fig. 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 ($P < 0.05$) according to student's t-test.

SUPPLEMENTAL DATA

Supplemental Fig. S1. Experimental setup of the incubation of*S. fuscum* and *S. majus* mesocoms in climate growth chambers.

Supplemental Fig. S2. Time course of *S. fuscum* and*S. majus* height increment during the incubation period.

Supplemental Fig. S3. Tissue water content, C content, CO_2 diffusion gradient (c_a-c_c) and^{15}N discrimination of *S. fuscum* incubated in growth chambers.

Supplemental Table S1 . Summary of results of separate 3-way ANOVA models of effects of CO_2, temperature (T), light intensity (LI) and their interactions on the measured response variables listed in Table 1 obtained using the low and high water table (WT) datasets.

Supplemental Table S2 . Summary of the 4-way ANOVA models of effects of CO_2, temperature (T), water table (WT), light intensity (LI) and their interactions on response variables shown in Fig. S3.

Fig. 1

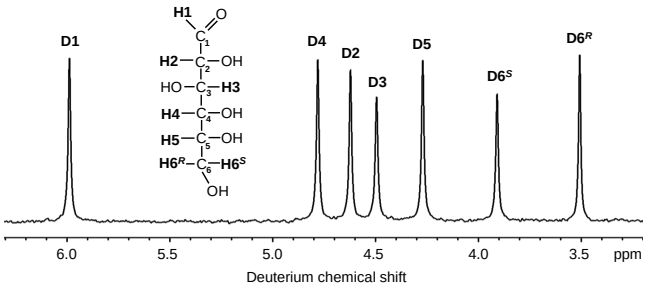

Fig. 2

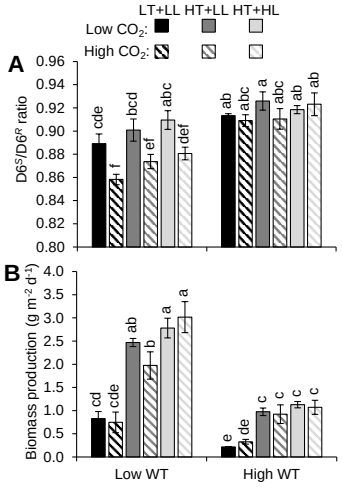

Fig. 3

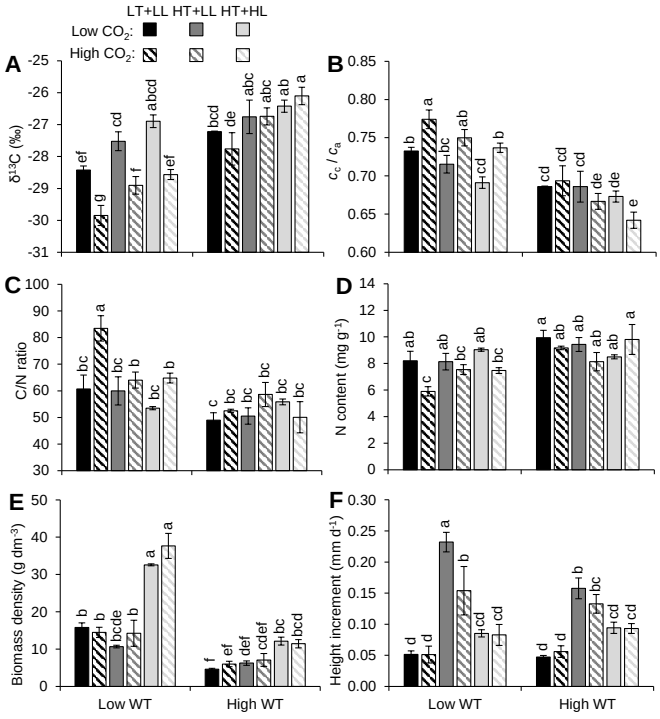

Fig. 4