Interacting effects of elevated atmospheric CO₂ and hydrology on the growth and carbon sequestration of *Sphagnum* moss

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Abstract Peatlands are a critical carbon store comprising 30% of the Earth's terrestrial soil carbon. *Sphagnum* mosses comprise up to 90% of peat in the northern hemisphere but impacts of climate change on *Sphagnum* mosses are poorly understood, limiting development of sustainable peatland management and restoration. This study investigates the effects of elevated atmospheric CO₂ (eCO₂) (800 ppm) and hydrology on the growth of *Sphagnum fallax*, *Sphagnum capillifolium* and *Sphagnum papillosum* and greenhouse gas fluxes from moss–peat mesocosms.

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Elevated CO₂ levels increased *Sphagnum* height and dry weight but the magnitude of the response differed among species. The most responsive species, *S. fallax*, yielded the most biomass compared to *S. papillosum* and *S. capillifolium*. Water levels and the CO₂ treatment were found to interact, with the highest water level (1 cm below the surface) seeing the largest increase in dry weight under eCO₂ compared to ambient (400 ppm) concentrations. Initially, CO₂ flux rates were similar between CO₂ treatments. After week 9 there was a consistent three-fold increase of the CO₂ sink strength under eCO₂. At the end of the experiment, *S. papillosum* and *S. fallax* were greater sinks of CO₂ than *S. capillifolium* and the – 7 cm water level treatment showed the strongest CO₂ sink strength. The mesocosms were net sources of CH₄ but the source strength varied with species, specifically *S. fallax* produced more CH₄ than *S. papillosum* and *S. capillifolium*. Our findings demonstrate the importance of species selection on the outcomes of peatland restoration with regards to *Sphagnum*'s growth and GHG exchange.

Keywords C sequestration · Elevated CO₂ · Greenhouse gas fluxes · Peatland · *Sphagnum* · Climate change

Introduction

Peatlands are important in the global carbon (C) cycle due to their function as a long-term carbon store. This store has built up through millennia due to very low decomposition rates that are surpassed by productivity rates (Clymo and Hayward 1982) making peatlands net carbon sinks. It's been estimated that today's peatlands store ca 30% (~ 550 Pg C) of the Earth's terrestrial soil carbon while comprising only 3% of the Earth's land area (Gorham 1991; Yu 2012).

Within peatlands in the northern hemisphere, *Sphagnum* mosses are often the dominant species, comprising up to 90% of peat (Clymo 1987). *Sphagnum* plays an important role in peatland formation and ecological resilience, through its low decomposition rate due to its recalcitrant and antibacterial nature (Kroken et al. 1996) and by creating acidic, anoxic and nutrient-poor conditions within peatlands (Amesbury 2013; Hajek and Vicherova 2014). *Sphagnum*'s extraordinary physiology allows it to hold 16–26 times its weight in water (Bold 1973; Amesbury 2013), in both live and dead non-decomposed *Sphagnum*, depending on species. This buffers the impact of dry weather conditions on peat moisture levels, preventing the peat from drying out and decomposing. Peatlands are classified as such once an organic soil layer of 30 cm or greater has formed; for northern peatlands, the average peat depth is 1.3–2.3 m, but peat depth can extend to 15–20 m in some peatlands (Clymo et al. 1998; Turunen et al. 2002). Accumulation rates of *Sphagnum* peat are approximately 0.61–3.9 mm year⁻¹ (Royles et al. 2012; Amesbury 2013), due to *Sphagnum*'s slow growth rate within the cold, nutrient poor peatlands (Strack 2008). The slow growth rate is important in the context of peatland management as once the peat is lost, the peatland will only recover slowly over centuries/millennia. Degradation of peatlands through drainage, mining and removal of *Sphagnum* allows stored C to be released back into the atmosphere CO₂, through decomposition of the exposed peat. It is estimated that UK peatlands are a net source of 3.72 Mtn CO_{2eq} year⁻¹ (Worrall et al. 2011), with only 1% of England's deep peats considered to be in an undamaged state (Natural England 2010). This makes restoring peatlands a priority, as restored peatlands not only protect the already sequestered carbon, but can reinstate their carbon sink capacity (Strack 2008).

Atmospheric CO₂ concentration has increased since pre-industrial times to ca. 408 ppm (ESRL NOAA 2015) and is predicted to increase to 800 ppm by the year 2100 (IPCC 2013). The effects of this elevation of atmospheric CO₂ on *Sphagnum* moss are currently disputed, with studies indicating an increase in growth rate (Jauhiainen and Silvde 1999; Heijmans et al. 2001a; Saarnio et al. 2003), decreases in growth rate (Grosvernier et al. 2001; Fenner et al. 2007) and no response (Van der Heijden et al. 2000; Hoosbeek et al. 2002; Toet et al. 2006). Contrasting responses between *Sphagnum* species are thought to be the cause of some of the variation. Toet et al. (2006), found increased abundance in *S. recurve* and no difference in *S. palustre* when they were subjected to an elevation of 180 ppm above ambient, while *S. fuscum* did not respond to elevated CO₂ (350, 700, 1000 and 2000 ppm) at all (Jauhiainen et al. 1997). Furthermore, contrasting responses have been seen between studies conducted on the same species. For example, *S. magellanicum*, was found to increase in growth rate by Heijmans et al. (2001b) when subjected to 560 ppm CO₂, while Heijmans et al. (2001a) reported a greatly reduced growth rate. This suggests factors other than species are affecting *Sphagnum*'s response to elevated CO₂.

Hydrology has been identified as one of the most important factors controlling *Sphagnum* growth and peatland carbon dynamics (Strack and Waddington 2007; Turetsky et al. 2012; Lanta and Kantorova 2015 etc.). Water availability impacts both C sequestration and growth rate (Glime 2007), due to there being an optimum moisture content for *Sphagnum* photosynthesis (Rice and Giles 1996). Varying physiologies mean that different *Sphagnum* species vary in sensitivity to changes in water level, each often growing best within their own hydrological niche (Rydin and McDonald 1985; Grosvernier et al. 1997; Taylor et al. 2015). For example, mature *S. fallax* is observed to struggle at low water levels (Carroll et al. 2009), while having higher growth rates than any other species in wet conditions (Buttler et al. 1998). Recent studies have also highlighted the importance of precipitation on *Sphagnum* dominated peatlands carbon dynamics and species distribution (Robroek et al. 2009; Radu and Dual 2018). Nijp et al. (2014) found precipitation reduced the negative impacts of drought, while Robroek et al. (2007) found precipitation allowed species to survive outside of their hydrological niche.

Precipitation also affects *Sphagnum* C uptake, with species responding differently to varying precipitation frequencies (Nijp et al. 2014). Water limitation has also been found to affect the impacts of other factors, such as temperature and vegetation's impact on CO₂ and CH₄ fluxes (Silvola et al. 1996; Leppala et al. 2011).

Currently large tracts of damaged peatlands are being restored in the UK via application of *Sphagnum* containing beads and gel to encourage growth on degraded peatland sites (Hinde et al. 2010; Wittram et al. 2015). However, information is limited with regards to which species are most effective in quickly establishing and recreating peatland functions such as C sequestration, particularly in the context of global change, e.g., elevated atmospheric CO₂ concentrations. Such information is urgently needed to ensure that restoration efforts are as effective as possible under future elevated atmospheric CO₂ levels. For example, some studies indicate that elevated CO₂ levels may result in increased CH₄ fluxes peatlands dominated by *Sphagnum* species (Saarnio et al. 2000, 2003; Ellis et al. 2009) but effects differ among studies made across European peatland sites (Silvola et al. 2003). To date, a hand full of studies have considered a possible interaction between hydrology and raised atmospheric CO₂ on the growth of *Sphagnum* moss (e.g., Jauhiainen et al. 1997; Toet et al. 2006) despite that interactions with hydrology may control how different *Sphagnum* species respond to elevated CO₂. Indeed, interactions between hydrology and elevated atmospheric CO₂ concentrations may cause the contrasting responses of *Sphagnum* species to elevated CO₂ found in previous studies. Such interaction would have important implications for peatland restoration, suggesting that selecting the correct species for the environment is critical for restoration success.

The aim of this study was to determine whether there is an interaction between hydrology and elevated atmospheric CO₂ on the growth and greenhouse gas (GHG) exchange of three *Sphagnum* moss species grown on peat-monoliths. To achieve this aim two specific hypotheses were tested: (i) elevated atmospheric CO₂ will have a greater positive effect on *S. fallax* growth and GHG fluxes than other species; (ii) low water table will inhibit the effect of elevated CO₂ on *Sphagnum* growth.

Materials and methods

Experimental set up

Peat cores were collected from Cadishead and Little Woolden Mosses (North West England, UK M44 5LR of Grid ref: SJ697953). This site is typical of drained and degraded cut over peatlands in the UK. The site is currently under restoration and some areas have been re-flooded. It is characterized by predominantly bare peat with cotton grass and birch growing around the edges with *Sphagnum* growing only in areas where reintroduction has been attempted.

To account for variation within the peat substrate, all 72 peat cores (approximately 18 cm × 16 cm) were collected from random locations within the non-flooded part of the peatbog. Collection and transportation of cores took place within 8 h, after collection cores were refrigerated at + 4 °C.

Three weeks after collection, peat cores were then cut into 17.4 cm × 11 cm cylinders and transplanted into 17.4 cm × 15.6 cm clear plastic, cylindrical polypropylene containers. The treatments were applied in a randomised block design (n = 4) with species (*S. fallax*, *S. papillosum* and *S. capillifolium*) and water level (1, 4 and 7 cm below the peat surface) as the treatments. Each combination of species and water level was repeated four times within each growth room. The randomized block design was used to account for any possible variations of conditions within each growth room. The two CO₂ treatments used were 400 and 800 ppm CO₂ (ambient and elevated), 800 ppm is the predicted 2100 concentration (IPCC 2013), due to the slow growth rates of *Sphagnum* this was chose as a good analogue for future restoration attempts. To achieve the two CO₂ treatments cores were randomly allocated to one of two growth rooms in which the atmosphere was adjusted to the target CO₂ concentration. Apart from the CO₂ concentration the settings in the growth room were identical: Day length consisted of a 16 h photoperiod (300 μmol m⁻²s⁻¹), including a 1 h dawn/dusk and 8 h night (0 μmol m⁻²s⁻¹). Daytime conditions were set to a temperature of 21 °C and a relative humidity of 50%, while night-time conditions were set to 15 °C and 75% respectively.

Water levels

Once the peat cores were placed in the growth rooms, the three water level treatments were applied to the cores, which were then allowed to equilibrate for 1 week. All water applied to the cores initially and throughout the experiment was rainwater that was firstly collected as runoff from glasshouses located in East Leake, Loughborough, Leicestershire. The rainwater was then filtered through a sand gravity filter, leaving the rainwater free of particulate contaminants such as dirt washed from the glasshouse surface. Rainwater was used to simulate ombrotrophic peatland conditions. Water level treatments were monitored daily for the first month and then once every 2 days thereafter via visual inspection. If needed water was applied to the cores using a pressurised water sprayer to reduce disturbance to the peat surface. This kept variation in water level to within 1 cm throughout the course of the experiment.

Sphagnum application

After 1 week, each species of *Sphagnum* was applied to the relevant core using liquid gels, currently used for application to peatlands as part of restoration programmes, containing micro propagated material of each species. The *Sphagnum* suspended within the gel was micropropagated from source material originating from the Peak Districk, UK, by MicroPropagation Services (MicroPropagation Services (EM) Ltd 2015). The micro-propagated *Sphagnum* was suspended within a gel containing basic nutrients, at an approximate concentration of 150,000 individual plants per L, which ranged from 1 to 5 mm in length (for example gel see SI. 1). The gel was applied at a rate of 3L per m² (0.072L per core) using a large pipette before being spread evenly over the core surface. The application rate is the same as trials conducted using the same gel in the restoration of the Cadishead peatland (Wright et al. 2012).

Measurements of *Sphagnum* performance and GHG fluxes

Measurements of *Sphagnum* performance and CO₂ fluxes were made bi-weekly for each parameter apart from CH₄ gas samples and dry weight, which were determined at the end of the experiment. Conditions

within the growth rooms where consistent throughout the course of the experiment: Temperature was measured at 27.5 ± 0.1 °C in direct light with an air temperature of 21.2 ± 0.1 °C, relative humidity, $52 \pm 1\%$ and finally light was 234 ± 0.4 μmol m⁻²s⁻¹.

Sphagnum height was measured using an adaption of the cranked wire method detailed by Clymo (1970). Initial height of the moss (peat level) was marked on the side of the clear plastic container; from this the height of the tallest *Sphagnum* plant in each individual container was measured to the nearest mm.

As *Sphagnum* was grown from micro propagated plants, the establishment rates of the different *Sphagnum* species could be quantified. This was inferred through the Normalized Difference Vegetation Index (NDVI), a numerical indicator that uses the visible and near-infrared bands of the electromagnetic spectrum to assess crop healthiness (Govaerts and Verhulst 2010). This was done using a hand held device (GreenSeeker®, Trimble Navigation Ltd., Sunnyvale California, USA), which gave a reading between 0 and 1 where 0 indicates bare soil and 1, established green vegetation (FSNAU 2016). The Green Seeker was held approximately 10 cm above the *Sphagnum* surface, with a reading for each core taken over 5 s while the device was continually moved over the whole surface of the core, giving an average reading for each core.

The total *Sphagnum* biomass production from each container was quantified at the end of the experiment. For this all *Sphagnum* growth in each container was removed, weighed (wet weight), dried at 70 °C and weighed again (dry weight).

CO₂ flux and evaporation rate from each core was measured using an EGM-4 Infrared Gas Analyser (IRGA) connected to a CPY-3 using an open dynamic system (PP systems, Hitchin, UK). CO₂ flux rates were recorded every minute over a 10 min period. IRGA time wishing was every 8 s, from which a linear regression is calculated to determine CO₂ flux over each 1 min period. To ensure fluxes were not affected by disturbance following the installation of the CPY-3 chamber over the mesocosms, we used only the final three readings, these were averaged and used for subsequent data analysis. Positive fluxes represent a net release of CO₂ from the mesocosms.

For CH₄ flux determination, gas samples were collected from each container using a modified version

of the closed static chamber method (Collier et al. 2014). Each container was carefully sealed using a lid with a septum in its centre giving a headspace of 0.11 dm³. Then, using a 30 ml syringe with a 25 gauge hypodermic needle, gas samples were then taken after 1, 30 and 60 min. Samples were then transferred into 12 ml pre-evacuated exetainers before each sample was analysed via gas chromatography (GC-2014; Shimadzu, UK) using a Flame Ionization Detector (FID), with hydrogen as the carrier gas. The oven temperature was 40 °C, the injector 80 °C and the FID 250 °C. The flow rate was 30 ml min⁻¹; the column was a HayeSEp N packed column, 60/80 mesh. Measurements for each container were analysed using a linear regression model and the ideal gas law to calculate gas flux rate for each container, which was then used to calculate CH₄ flux in mg m⁻² h⁻¹ where positive gas fluxes represent net CH₄ emissions.

Statistical analysis

The effects of time, hydrology, species and CO₂ treatment and their interactions on height, evaporation, CO₂ flux and NDVI were tested using repeated measures analysis of variance (ANOVA) after box-cox transformations had been performed where necessary, to normalize the data. Time was treated as a categorical variable, with each measurement week treated as a separate category. Treatment effects on dry weight and CH₄ flux were tested using ANOVA with hydrology, species, and CO₂ treatment as fixed effects, where CO₂ flux was normalised to the CO₂ flux from the first set of measurements (week 3). This removed the impact of variation in the initial CO₂ flux rates from the cores. All calculations were performed using the data analysis program GenStat 17th Edition for windows 10.1 (VSN International 2011).

Results

Growth

Different establishment rates were found between species ($F_{(2,51)} = 7.20$, $P = 0.002$), with *S. fallax* establishing fastest with NDVI > 0.8 in week 7, which was 2 weeks earlier than *S. papillosum* and 3 weeks earlier than *S. capillifolium* (Fig. 1). Under eCO₂ *S. fallax* and *S. papillosum* established faster, while *S.*

Fig. 1 The effect of elevated CO₂ and species on NDVI (mean ± SE) over the 13 week experimental period. Black lines represent atmospheric CO₂ of 400 ppm; light grey lines represent atmospheric CO₂ of 800 ppm. Diamonds and solid lines represent *S. fallax*; squares and dashes lines represent *S. papillosum*; triangles and dotted lines represent *S. capillifolium*

capillifolium established slower than under the ambient treatment ($F_{(2,51)} = 2.96$, $P = 0.061$).

Sphagnum grew taller under eCO₂ ($F_{(1,51)} = 16.34$, $P < 0.001$) but this effect varied among species ($F_{(2,270)} = 5.31$, $P = 0.008$) with *S. fallax* responding most significantly to eCO₂ ($F_{(2,51)} = 48.78$, $P < 0.001$) (Fig. 2a). *S. papillosum* height was also found to increase growth under eCO₂ (2.9 mm taller by week 13) the effect was not as large as *S. fallax* (7.4 mm taller by week 13), while *S. capillifolium* indicated no difference in height between the treatments (Fig. 2a). The lowest water level (7 cm below peat surface) had shorter plants (near significant water level effect: $F_{(2,51)} = 2.49$, $P = 0.093$) than both the medium (4 cm below peat surface) and high (1 cm below peat surface) water levels (Fig. 2b).

The eCO₂ treatment yielded 90.3% increase in biomass (294.2 ± 47.2 g m⁻²) compared to the ambient treatment (154.6 ± 23.7 g m⁻²) ($F_{(1,51)} = 30.94$, $P < 0.001$). However, the effect size varied among species ($F_{(2,51)} = 4.17$, $P = 0.021$), with *S. fallax* showing a 129.7% increase in biomass production under eCO₂ (eCO₂ 142.4 ± 17.1 g m⁻², ambient 62.0 ± 9.8 g m⁻²), followed by a 101.0% increase for *S. capillifolium* (eCO₂ 66.8 ± 16.1 g m⁻², ambient 33.2 ± 6.4 g m⁻²) and finally a 43.2% increase for *S. papillosum* (eCO₂ 85.068 ± 14.0 g m⁻², ambient 59.4 ± 7.6 g m⁻²) (Fig. 3a).

Sphagnum grown at higher water level treatments produced more biomass ($F_{(2,51)} = 7.64$, $P = 0.001$) (Fig. 3a). The eCO₂ and water level treatments interacted ($F_{(2,51)} = 4.13$, $P = 0.022$) with the high

Fig. 2 **a** The effect of elevated CO₂ and species on plant height (mean $\pm$ SE) over the 13 week experimental period. Black lines represent atmospheric CO₂ of 400 ppm; light grey lines represent atmospheric CO₂ of 800 ppm. Diamonds and solid lines represent *S. fallax*; squares and dashes lines represent *S. papillosum*; triangles and dotted lines represent *S. capillifolium*.

b The effect of water level on plant height (mean $\pm$ SE) over the 13 week experimental period. Diamonds and black line represents a water level 1 cm below peat surface; squares and dark grey line represent a water level 4 cm below peat surface; triangles and light grey line represent a water level 7 cm below peat surface

Fig. 3 **a** The effect of species on dry weight (mean $\pm$ SE) recorded at the end of the 13 week experimental period. For each species, black bars represent growth under 400 ppm atmospheric CO₂; light grey bars represent 800 ppm atmospheric CO₂. **b** The effect of water level on dry weight

(mean $\pm$ SE) recorded at the end of the 13 week experimental period. For each water level, black bars represent growth under 400 ppm atmospheric CO₂; light grey bars represent 800 ppm atmospheric CO₂

water level resulting in the largest increase of 142.0% (79.9 ± 9.2 g m⁻²) in biomass under eCO₂ compared to a 66.0% increase (35.6 ± 8.1 g m⁻²) at the 4 cm and 19.2% (8.5 ± 5.4 g m⁻²) increase at the 7 cm water level treatments (Fig. 3b).

Gas flux

In parallel with the NDVI and height data, elevated and ambient CO₂ treatments had almost identical CO₂

flux rates over the first 7 weeks period. After 7 weeks there was a large increase in the CO₂ uptake (increasingly negative CO₂ flux) under eCO₂ ($F_{(1,51)} = 14.59$, $P < 0.001$). Specifically, CO₂ uptake was more than 5-times greater in week 7 and consistently 3-times greater than the ambient treatment from week 9 to the end of the experiment (Fig. 4a; Time $\times$ eCO₂ interaction: $F_{(5,270)} = 27.28$, $P < 0.001$). Increasingly lower water levels were found to have a significantly larger CO₂ uptake ($F_{(2,51)} = 5.00$, $P = 0.010$) (Fig. 4b).

Fig. 4 **a** The effect of elevated CO₂ on CO₂ flux (mean $\pm$ SE) over the 13 week experimental period. Diamonds and black line represent 400 ppm atmospheric CO₂; squares and grey line represent 800 ppm atmospheric CO₂. **b** The effect of water level (cm below the peat surface) on CO₂ flux (mean $\pm$ SE) over the 13 week experimental period. Diamonds and black line represent 1 cm below peat surface; squares and dark grey line

represent 4 cm below the surface; triangles and light grey line represent 7 cm below the surface. **c** The effect of species on CO₂ flux (mean $\pm$ SE) over the 13 week experimental period. Diamonds and solid line represent *S. fallax*; squares and dashed line represent *S. papillosum*; triangles and dotted line represent *S. capillifolium*. **d** The effect of species on CH₄ flux (mean $\pm$ SE)

CO₂ fluxes were found to differ between species ($F_{(2,51)} = 4.29$, $P = 0.019$), with *S. capillifolium* having the smallest uptake of CO₂ (-69.12 ± 14.74 mg m⁻² h⁻¹) compared to *S. papillosum* (-101.30 ± 15.47 mg m⁻² h⁻¹) and *S. fallax* (-91.56 ± 16.43 mg m⁻² h⁻¹) by week 13 (Fig. 4c). Over the course of the experiment, CO₂ uptake increased more for *S. fallax* and *S. papillosum* over the course of the experiment than *S. capillifolium* ($F_{(10,270)} = 6.24$, $P < 0.001$) (Fig. 4c).

Only the species treatment was found to have an effect on CH₄ flux ($F_{(2,123)} = 3.66$, $P = 0.029$), with *S. fallax* producing the most CH₄ (5.28 ± 2.22 mg m⁻² h⁻¹), twice as much as *S. papillosum* (2.09 ± 1.09 mg m⁻² h⁻¹) and 10 times as much as *S. capillifolium* (0.48 ± 0.18 mg

m⁻² h⁻¹) (Fig. 4d); the species and eCO₂ treatments did not interact with the water level treatments.

Discussion

Growth responses

Our finding that eCO₂ increased *Sphagnum* growth are in agreement with multiple studies (Jauhiainen and Silvola 1999; Heijmans et al. 2001a; Saarnio et al. 2003), however as this area of research is contested, the results are also in disagreement with several studies (Berendse et al. 2001; Grosvernier et al. 2001; Fenner et al. 2007).

The greater effect of elevated CO₂ on *S. fallax* growth and establishment than either of the other species supports the first hypothesis which predicted that fast growing *S. fallax* would respond most positively to the eCO₂ treatment. This is in agreement with previous studies (Berendse et al. 2001; Mitchell et al. 2002; Granath et al. 2012) which suggested that increased CO₂ will increase the growth of certain species more than others. Variation in species responses may be caused by differences in the effectiveness of species to utilize the increased atmospheric CO₂ concentration. Under elevated CO₂ it's possible that water became the limiting factor for growth, even under the highest water level treatments. Silvola (1991) showed that the differences in water needed for photosynthesis vary between species. Additionally, Nijp et al. (2014), found that *Sphagnum* species vary in their ability to exploit transient water resources. This could account for the differences seen in growth rates under increased CO₂ between the species, with some species better able to exploit the elevated CO₂ under the hydrological conditions.

Sphagnum also have higher water level limits due to blocking of CO₂ diffusion to the leaf via the layer of water surrounding the leaf, with species differing on the extent of water level found limiting (Glime 2007). For example, optimum water content for photosynthesis for *S. fuscum* are lower than those required for *S. angustifolium*. This could have impacted *Sphagnum* growth within our experiment due to the high frequency of precipitation and surface water this provided to the *Sphagnum*.

We found that higher water levels coupled with eCO₂ caused a greater increase in dry weight supporting our second hypothesis, which predicted that low water tables would inhibit the effect of elevated CO₂ on *Sphagnum* growth. Similar results were found for *S. fuscum* by Jauhiainen et al. (1997), who suggested that this was due to water limitation of photosynthesis of *Sphagnum* at low water levels. We did not find an interaction effect of hydrology on species, in contrary to previous studies, which have identified that each species of *Sphagnum* grows best within its own hydrological niche (Grosvernier et al. 1997). It's also been found that each species responds to variation in water tables differently (Jauhiainen et al. 1997; Carroll et al. 2009) due to differences in their physiologies. In particular, *S. fallax* has been found to be highly dependent on water level, holding 30% less water than

S. capillifolium (Carroll et al. 2009). One reason for this could be the impact of precipitation rewetting on reducing the impact of drought by precipitation increasing the water contents of the top layer (Nijp et al. 2014), negating the impacts of a lower water table. However, differences between species utilization of precipitation was seen by Nijp et al. (2014) between *S. majus*, *S. balticum* and *S. fuscum*, which was not seen within our findings. This suggests that our experiment did not differentiate water level to a high enough degree to examine differences between the species.

CO₂ and CH₄ flux responses

Greater CO₂ assimilation under eCO₂ was found in *S. fallax* and *S. papillosum* than *S. capillifolium*, this lends additional support to our first hypothesis which predicted that elevated CO₂ would increase C sequestration due to increased growth rates but that responses would differ among species. Our finding of increased CO₂ uptake under eCO₂ agrees in part with findings by Van der Heijden et al. (2000), who found that photosynthesis in mature plants of *S. fallax* was initially stimulated by elevated CO₂ (700 ppm). However, in contrast to our findings, acclimation was observed with stimulated *S. fallax* returning to the levels of the controls after 3 days. This could have been due to differences between establishing and mature *S. fallax*.

Our findings somewhat contrasted with Harpenslager et al. (2015), who grew four *Sphagnum* species on peat monoliths incubated between 18 and 23 °C over a 12 week period. Harpenslager et al. (2015), found that *S. fallax* and *S. palustre* had positive net C effluxes similar to bare peat in spite of increased biomass. Harpenslager et al. (2015) suggested that these results were due to the release of CO₂ derived from bicarbonate due to lowering of the monoliths pH. While this is dissimilar to our findings, these releases were species dependant, highlighting how different species can impact total CO₂ flux beyond sequestration. These differences in growth and C fixation between the species were in congruence with our results.

Overall our findings suggest that in the context of future elevated CO₂, all three species of *Sphagnum* will increase in CO₂ assimilation due to increased growth, however, it is suggested that not all species

will react the same, with certain species seeing a much greater increase in CO₂ assimilation (in this case *S. fallax* and *S. papillosum*) compared to others. Whilst our study did not find acclimation (such as Van der Heijden et al. (2000), this could be due to our focus on establishing *Sphagnum* and not mature plants.

Greenhouse gas budgets for peatlands are often complex, with most peatlands being sources of CH₄ (Moore and Roulet 1995; Saarnio et al. 2007; Leppala et al. 2011). As CH₄ has a Global Warming Potential (GWP) of 28 over 100 years (IPCC 2013), the increased CO₂ sink strength in response to eCO₂ shown here could be offset if CH₄ emissions increase. In our 13-week study CH₄ emissions remained low, although they increased slightly at the end of the experiment.

The GWP of the CH₄ emissions over 100 years (CO₂ equivalents (CO_{2eq})) of the three species was 147.84 ± 62.12 , 58.52 ± 23.98 and 13.44 ± 5.04 mg m⁻² h⁻¹ for *S. fallax*, *S. papillosum* and *S. capillifolium* respectively. If emissions of CO₂ and CH₄ from each species are taken into account, *S. fallax* would have a positive net emission (56.28 mg m⁻² h⁻¹ CO_{2eq}), while *S. papillosum* and *S. capillifolium* would have a negative net emissions (-42.78 and -55.68 mg m⁻² h⁻¹ CO_{2eq} respectively). This suggests that while *S. fallax* was shown to have the greatest C assimilation over the course of the experiment, *S. papillosum* and *S. capillifolium* had lower net emissions. This highlights the importance of CH₄ release within these systems which can offset carbon uptake to a substantial amount and even become a net source of emissions.

The increase in CH₄ emissions towards the end of the experiment was likely due to recovery of methanogenic populations following the increased moisture content of the peat (Bellisario et al. 1999; Leppala et al. 2011) and increased C availability due to increased photosynthesis (Meronigal and Schlesinger 1997) and C transportation to the rhizosphere (Oremland 1988; Williams et al. 2000). The lack of response CH₄ fluxes to eCO₂ in our study contrasts with findings from FACE studies on UK and Finnish ombrotrophic peatlands which found modest increases in peatland CH₄ fluxes in response to eCO₂ (Saarnio and Silvola 1999; Ellis et al. 2009). An important difference between our study and those of Saarnio and Silvola (1999) and Ellis et al. (2009) are the fact that our mesocosms contained a sole *Sphagnum* species

while the plots in the other two studies encompassed both *Sphagnum* mosses, sedges and dwarf shrubs making it difficult to isolate *Sphagnum* species responses in the two field studies.

The species-specific effects on CH₄ fluxes of the three *Sphagnum* species used in this study is important as it suggests that the *Sphagnum* species composition per se, and not just the mix of functional groups (Strom et al. 2005; Leppala et al. 2011) is an important control of CH₄. Although the mechanisms behind the species-specific effects on CH₄ fluxes are unclear. They may relate to differences in production of labile carbon and/or phenolic compounds among *Sphagnum* species (Ellis et al. 2009). Transportation of C to the rhizosphere has been shown to vary with species (Reich et al. 2001). It's possible that similar variation may account for the differences seen in emissions between the *Sphagnum* species.

Furthermore, relationships between species and specific methanogenic and methanotrophic communities could have also had an impact. This has been shown for plant functional types within peatlands by Robroek et al. (2015), which showed removal of plant functional types (gramminoids and ericoids) significantly alter microbial community structure. This fits with Opelt et al. (2007) who found that diversity of bacterial communities was higher in *S. fallax* than *S. magellanicum*.

Conclusions

Our study highlights the importance of *Sphagnum* species selection when undertaking restoration of a peatland. The clear differences in species establishment and growth under increased CO₂ and CH₄ flux between the species indicates that selecting the correct *Sphagnum* species or combination of species is critical to the success and GHG balance of recolonization attempts. While water level was not shown to interact with species, it's interaction with CO₂ suggests that environmental factors may also play an important role in establishment success.

Furthermore, differences between the species CO₂ and CH₄ fluxes within the initial establishment period, suggests that using different species will impact the overall GHG balance the peatland once restored. Within the establishment phase, the increase in CO₂ uptake found in response to eCO₂ suggests that the

capacity to sequester C by *Sphagnum* dominated peatbogs may increase under future CO₂ rich atmospheric conditions. Nevertheless, there may be trade-offs between plant performance and GHG emissions as our fastest growing species *S. fallax* also had the highest CH₄ emissions, which offset assimilation and caused net GHG emission. While the estimation of the impacts to the final GHG balance of a restored peatland are beyond the scope of this paper, this highlights that species may be an important consideration when determining which *Sphagnum* species should be used for peatland restoration.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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