Soil CO_2 efflux in a degraded raised bog is regulated by water table depth rather than recent plant assimilate

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SUMMARY

Understanding the climatic and biological factors that regulate soil carbon dioxide (CO_2) efflux is crucial in peatlands because they contain a large proportion of terrestrial carbon (C). We predicted that rainfall reduction would increase soil CO_2 efflux, and that cessation of below-ground allocation of recent plant assimilate would reduce soil CO_2 efflux. These predictions were tested in the field using rainfall shelters that allowed a maximum of 40 % of rainfall onto 2 × 2 m plots by diverting rainwater from the shelter roofs with guttering, and by girdling stems of the dominant plant, *Calluna vulgaris*, for two years. We also used $^{13}CO_2$-pulse labelling of intact monoliths at ambient CO_2 concentrations to trace recent assimilate from plant shoots to roots, bulk soil, leachate, dissolved organic carbon (DOC) and soil CO_2 efflux . Soil CO_2 efflux in the sheltered plots increased in Year 1 but not in Year 2, and we found a positive relationship between soil CO_2 efflux and water table depth. Our data indicate that lowering the water table below a critical threshold (15–20 cm) affects soil CO_2 efflux. Girdling of *C. vulgaris* shoots resulted in no measurable reduction in soil CO_2 efflux, while only ~3 % of ^{13}C fixed by shoots was recovered in soil CO_2 efflux and DOC in the 20 days after labelling. Our findings show that below-ground allocation of recent assimilate from *C. vulgaris* plants > 6 years old has little impact on soil CO_2 efflux.

KEY WORDS: *Calluna vulgaris*, carbon cycle,$^{13}CO_2$ pulse labelling, degraded peat bog, girdling

INTRODUCTION

The release of carbon dioxide (CO_2) from soil surfaces is the main pathway of carbon (C) loss from ecosystems to the atmosphere. An understanding of the factors that regulate soil CO_2 efflux is crucial if more accurate predictions are to be obtained concerning the way in which global processes such as climate change will impact biogeochemical cycles. This is particularly important in northern peatlands, which collectively hold a third of the global terrestrial C pool on 1 % of the global land surface area (Yu 2012). In the United Kingdom (UK), an estimated 18 % of the total land cover is comprised of peat soils (defined as soils with organic carbon content > 25 %), representing about 44,411 km^2 (Montanarella *et al.* 2006). In the UK, only 6 % of lowland raised bogs remain undamaged, with most of the damage occurring over the last 200 years, and in Scotland there is now estimated to be only approximately 13,000 ha of lowland raised bog remaining (JNCC 2011). Net CO_2 emissions from managed peatlands are included in the annual greenhouse gas inventory for the UK (Webb *et al*. 2014) and, to reduce uncertainty in their estimation, it is crucial that we gain a better understanding of the various processes that affect C turnover and storage in peatlands.

The characteristics of the plant communities associated with peatlands, together with their responses to climate change, are important factors governing the rate of loss of C from peatlands. In the UK, near-natural peatlands typically comprise a mosaic of *Sphagnum* moss species, sedges and ericaceous shrubs; while more damaged systems tend to be heavily dominated by ericaceous species, hypnaceous mosses or acid-tolerant grasses. Decomposition of litter is one of the main mechanisms contributing to heterotrophic pathways of C loss from soils (Mäkiranta *et al.* 2008). In contrast, the autotrophic component of CO_2 efflux often largely comprises recent plant assimilate. In many peatlands, the activity of ericoid mycorrhizal fungi, which colonise the majority of cortical cells in the fine 'hair' roots of ericaceous plants (Read & Stribley 1975), is dependent on C from host plants (Read 1996). Whilst their hyphae do not extend substantially beyond the surfaces of the roots (Read 1984), they nevertheless produce large amounts of mycelium which means they are substantial sinks for photosynthetically fixed C (Stribley & Read 1974), as are the fine roots that they colonise (Olsrud &

 DOI: 10.19189/MaP.2015.OMB.203

Christensen 2011). Experiments in plant communities that support both arbuscular and ectomycorrhizal fungi provide unequivocal evidence that recent assimilate allocated to extramatrical mycorrhizal mycelium contributes substantially to soil CO_2 efflux (Johnson *et al.* 2002a, Johnson *et al.* 2002b, Heinemeyer *et al.* 2007).

Girdling (i.e. cutting phloem tissue but maintaining water flow in the xylem) of Scots pine (*Pinus sylvestris* L.) stems has demonstrated that 55 % of soil CO_2 efflux is derived from recent photosynthate (Högberg *et al.* 2001). Other tree girdling experiments suggest that the magnitude of soil CO_2 efflux *via* autotrophic pathways is sometimes dependent on the type of ecosystem, but also on the identity of the dominant plant. For example, in eucalyptus forests, processes such as below-ground C storage offset the reduction in photosynthate flow and a clear partitioning cannot always be ascertained (Binkley *et al.* 2006). In highly disturbed ecosystems such as cut-over peatlands in early recovery phases, recent plant assimilate makes only small contributions to soil CO_2 efflux (Trinder *et al.* 2008a). By contrast, vegetation has been found to contribute 35–57 % of soil CO_2 in near-natural ombrotrophic peatlands, primarily *via* its effects on rhizodeposition and autotrophic respiration (Moore *et al.* 2002, Crow & Wieder 2005). Thus, the importance of recent assimilate in regulating soil CO_2 efflux is not consistent across ecosystems and can vary with the successional stage of the ecosystem, so there is considerable value in exploring the extent to which recent assimilate drives soil respiration in peatlands.

The accumulation of C in many peatlands is promoted by waterlogging (Laine *et al.* 2007), which regulates decomposition (Trinder *et al.* 2008c), plant growth and nutrient availability (Gorham 1991). In the UK, climate models have forecast an increase in winter rainfall with more extreme weather events and a decrease in summer precipitation (Hulme *et al.* 2002). However, the consequences of droughts on C cycling are not fully understood (Moore 2002) and the predicted changes in weather patterns as a result of climate change could have detrimental effects on the ability of peatlands to act as C sinks (Reiche *et al.* 2009). Lowered water tables in general have been widely reported to lead to increases in CO_2 losses from peatlands (Strack *et al.* 2006, Salm *et al.* 2012, Haddaway *et al.* 2014). The relationship between water content of the acrotelm (which is likely to be driven by drought events and temperature) and vegetation composition on hummocks is recognised as a key factor that determines the ability of a peatland to accumulate C (Belyea & Clymo 2001). Lowering of the water table may increase the depth of oxygenated substrates, stimulating the rate of biogeochemical cycling (Moore & Knowles 1989), and affect peat accumulation (Belyea & Clymo 2001, Swanson 2007). For example, drier soil conditions may affect the photosynthetic capacity of species either positively or negatively (Bubier *et al.* 2003) and precipitation can interact with water table depth to affect CO_2 assimilation by mosses (Robroek *et al.* 2009), which makes predicting the effects of climate change across ecosystems difficult. Recent work has shown that reduced rainfall can lead to an increase in CO_2 efflux during the drought event itself, with a larger increase in the following months and years (Fenner & Freeman 2011). Therefore, it is important to gain a better understanding of how drought may increase soil CO_2 efflux in peatlands, including those that are considered to be already degraded (Wilson *et al.* 2015).

Here we test the hypothesis that soil CO_2 efflux in a degraded raised bog is driven by both allocation of recent plant photosynthate and by water availability. We predicted that localised rainfall reduction would lead to an increase in soil CO_2 efflux by lowering the water table, and that cessation of below-ground allocation of recent plant photosynthate would lead to a rapid decline in soil CO_2 efflux. To test these predictions, we undertook a series of manipulative experiments (rainfall shelters and girdling of the dominant vegetation species *in situ*) and monitored soil CO_2 efflux and key environmental and climatic variables for a two-year period in a lowland raised bog in Scotland. In addition, we quantified the contribution of recent plant assimilate to major pools and fluxes of C using $^{13}CO_2$ pulse labelling of intact peatland monoliths *ex situ*.

METHODS

Site description

This work was undertaken at Red Moss of Candyglirach, an ombrotrophic peatland (bog) in Aberdeenshire, UK (57.103 °N, -2.411 °W). The bog has a surface area of 0.42 km^2. Perimeter drainage ditches are still in working order despite the absence of maintenance over almost two decades. Small (1–5 m^2) patches of bare peat are common throughout. The last recorded peat cutting took place > 35 years ago and burning management has not been implemented for more than 18 years. Several different ground levels (differing by 0.5–2 m) are present as a consequence of peat cutting. The bog is surrounded by mature stands of *Pinus sylvestris* L. and *Betula pubescens* Ehrh. The vegetation is a

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degraded M19 community according to the National Vegetation Classification (Rodwell 1991). The plant community is dominated by *Calluna vulgaris* (L.) Hull, mostly in the degenerate stage (Gimingham 1972). *C. vulgaris* accounts for 80–100 % of canopy cover, with the remainder comprising mainly *Eriophorum vaginatum* L. The understorey comprises mostly *Hypnum cupressiforme* with a scattered cover of *Sphagnum* species including *S. magellanicum, S. papillosum* and *S. capillifolium*. The Lowland Raised Bog Inventory undertaken by Scottish Natural Heritage (Lindsay & Immirzi 1996) listed the following additional species for Red Moss of Candyglirach: *Betula pubescens, Carex rostrata, Drosera rotundifolia, Empetrum nigrum, Erica tetralix, Eriophorum angustifolium, Juncus squarrosus, Narthecium ossifragum, Plagiothecium undulatum, Pleurozium schreberi, Sphagnum cuspidatum, S. fimbriatum, S. palustre, S. recurvum* and *S. tenellum*. There is little evidence of the typical microtopographical patterns generally found in peatlands (Lindsay *et al.* 1985), with much of the peatland consisting of a mixture of degraded T2 high ridge, damaged low T1 ridge and very occasional A1 *Sphagnum* lawns. Site restoration by ditch blocking and tree removal commenced in 2015.

Experimental treatments

Three treatments were established (n = 5 plots *per* treatment, 2 × 2 m plots) at the site in April 2009. All treatment plots were located at least 100 m from A1 *Sphagnum* lawns, in areas where *Calluna* was the most abundant higher-plant species (cover 80–100 %). The plots were located throughout the site, at least 50 m from each other. They were trenched along their edges to a depth of 45 cm and plastic barriers were inserted into the peat to prevent lateral water movement and root re-establishment between the plots and the surrounding vegetation/soil. The 2009 treatments were: 1) sheltered (rainfall reduction); 2) girdled; and 3) controls. Each sheltered plot was fitted with an open-sided frame with a Perspex slatted roof (Yahdjian & Sala 2002) that allowed a maximum of 40 % of rainfall onto the plot by diverting rainwater from the shelter roof with guttering (Figure 1). Each sheltered and control plot was equipped with five 1 m long dipwells to enable measurement of mean water table depth within the plot. The dipwells were lengths of 25 mm internal diameter plastic tubing with holes drilled in their walls at offset 5 cm intervals to allow water access. Their bases were sealed and their tops were kept closed with rubber bungs between measurements, which were made every two weeks.

In order to inhibit C transport below ground, girdled plots were established by removing ~1 cm wide rings of bark using a scalpel from all *C. vulgaris* plants with stem diameters > 5 mm, as close to the soil surface as possible (approximately 3 cm above it). Girdling took place in May 2009 and May 2010, when the main spring growth phase was expected. All plants growing on the control plots remained intact. In Year 2 of the study, a second set of girdled plots was established on account of the high mortality of plants girdled in the previous year. To utilise the previous year's girdled plots, all remaining above-ground vascular plants (*C. vulgaris*, *E. vaginatum*) were removed and these plots were incorporated into the study as 'weeded' plots for the 2010 treatment set. The weeded treatment enabled testing of the effect of complete vegetation removal, in addition to that of changing only the allocation of recent assimilate as in the girdling treatment. The bryophyte layer was left intact and did not show any signs of degradation.

Figure 1. Design of the rainfall shelters showing the arrangement of the roof drainage system

 DOI: 10.19189/MaP.2015.OMB.203

Monitoring intervals and sampling strategy

Soil CO_2 efflux (soil respiration) was measured at two-weekly intervals during the period between snowmelt (April) and first snow (November) with a Licor LI-8100 portable infra-red gas analyser (IRGA) equipped with a Licor 100 mm diameter soil survey chamber. Extra sampling events were added just before and after girdling took place. Preliminary work showed that measurements of soil CO_2 efflux obtained without using permanent collars inserted into the soil surface had a smaller coefficient of variation than when collars were present, probably because the bryophyte layer surrounding the outer rim of the measurement chamber provided a satisfactory seal. Therefore, we placed the chamber directly on the soil surface but used the same 100 mm diameter areas of peat, which were chosen as areas free of vascular plants, for each measurement. On each sampling day the following were recorded: soil CO_2 efflux (μmol m^{-2} s^{-1}), calculated using the default Licor software after checking that the fluxes were linear during the 120-second measurement period; soil temperature (°C), to 5 cm depth using the probe supplied with the IRGA; soil moisture (%), measured in control and sheltered plots only using a ML2x theta probe (Delta-T Devices, Cambridge, UK); and dipwell water table depth (cm). The data from the theta probe were converted to % water content using a custom calibration curve derived using peat from the field site.

Measurement of environmental variables

Mean annual and monthly weather data were obtained from the closest weather station, at Braemar Meteorological Office (located 71 km west of the field site). Mean monthly, mean monthly minimum and mean monthly maximum temperature data (Figure 2a) showed that the winters of 2009/2010 and 2010/2011 were colder than the previous winter. Although there was similar annual variation in rainfall during 2009 and 2010, the winter of 2008/9 was drier than that of 2009/2010 (Figure 2b).

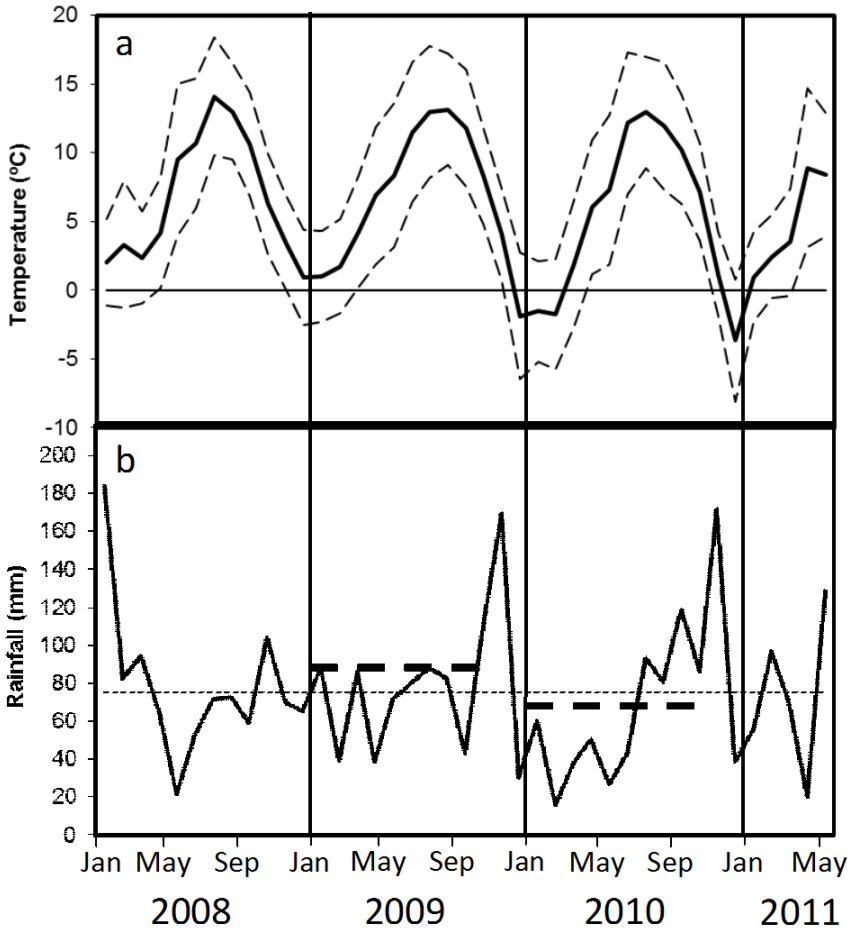

Figure 2. Braemar Meteorological Office weather data from January 2008 to May 2011. (a) Mean monthly air temperature (°C); dashed lines represent mean monthly minimum and maximum temperatures. (b) Mean monthly rainfall (mm); mean rainfall during the two study periods is indicated by bold dashed lines, and the mean monthly rainfall for 1981–2010 (75 mm) is indicated by the lighter dashed line.

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$^{13}CO_2$ pulse labelling of intact peatland monoliths

After peat thaw in April 2010, intact peat and vegetation monoliths were excavated from the site and placed in plastic boxes (250 × 200 × 170 mm deep) with holes at the bottom to allow drainage of soil leachate. The vegetation comprised *C. vulgaris* approximately six years old and 25–30 cm high in a re-growth phase with an underlying closed layer of *H. cupressiforme*. The monoliths were kept on trays and stored in a cold frame in the Aberdeen University gardens (~ 25 km east of the field site), where they received natural rainfall and experienced environmental conditions similar to those at the field site. In April 2011 the monoliths were moved into a glasshouse and placed in trays that enabled the capture of leachate. All gaps around the trays were covered with black plastic sheeting to inhibit evaporation and reaction with sunlight of the leachate. The monoliths were watered daily with 250 ml of artificial rainwater (Irwin *et al*. 1997) containing 7.5 µM $CaCl_2.2H_2O$, 3.5 µM KH_2PO_4, 17.5 µM Na_2SO_4, 20 µM $MgCl.6H_2O$, 4 µM KCl, 45 µM NaCl, 20 µM NH_4NO_3 and adjusted to pH 4.9.

On 16 July 2011, clear plastic bags (30 L) were placed over the entire monoliths and compressed air containing ~ 99 atom % $^{13}CO_2$ at 350 ppm (Spectra Gases Inc., Cambridge, UK) was blown through the bags under cylinder pressure at a rate of 0.5 L min^{-1} for four hours in natural light from 10 a.m. until 2 p.m. This rate ensured that plants did not become exposed to sub-ambient CO_2 concentrations whilst minimising wastage of the gas. Samples of *Calluna* shoot tips, leachate (10 ml) and soil cores (10 ml) were taken one day before labelling (to obtain data on natural abundance ^{13}C:^{12}C ratios), immediately after labelling and 3, 5, 8, 12 and 20 days after labelling. Soil CO_2 efflux was sampled on the same days using an IRGA (LCi-SD, ADC Bioscientific Ltd., UK) with a 100 mm soil respiration hood (V2, ADC Bioscientific Ltd., UK). The ^{13}C content of the respired CO_2 was measured by taking a soil headspace gas sample with a syringe from a 100 mm pipe, sealed with Suba-seal septa, previously inserted into the soil surface. The gas was injected into an Exetainers® vial (12 ml Soda Glass Vials, Labco, UK) for subsequent analysis by isotope ratio mass spectrometry (IRMS; Gas-bench II connected to a DeltaPlus Advantage, Thermo Finnigan, Bremen, Germany). Plant and soil samples were weighed, dried for one week at 60 ºC and milled for analysis by Isotope Ratio Mass Spectrometer (IRMS) (Flash EA 1112 Series Elemental Analyser connected *via* a Conflo III to a DeltaPlus Advantage, Thermo Finnigan, Bremen, Germany). Leachate was analysed for dissolved organic carbon (DOC) by UV-persulphate oxidation, and ^{13}C by liquid oxidation (Potthoff *et al*. 2003).

At the end of the experiment all shoots were harvested, weighed and dried for one week at 60 ºC, then green material was separated from woody stems. Only green, photosynthetically active shoots were analysed for ^{13}C. Soil cores were sieved (3 mm) and roots were separated on this basis. Thus, although we refer to these pools as bulk soil and roots, it is likely that bulk soil also contained fine roots. All solid material was analysed for ^{13}C as described above.

^{13}C calculations and statistical analysis

All ^{13}C data were converted from $\delta^{13}C$ values (‰) to ^{13}C excess values (atom %) as follows. To determine the isotopic ratio ^{13}C:^{12}C of samples (R_{sample}):

$$R_{sample} = \left(\left(\frac{\delta^{13}C}{1000} \right) \times 0.011237 \right) + 0.011237 \qquad [1]$$

where 0.011237 is the ratio of ^{13}C:^{12}C in the Pee Dee Belemnite standard. To determine the ^{13}C abundance (atom %) of samples:

$$^{13}C \text{ abundance} = \left(\frac{R_{sample}}{R_{sample} + 1} \right) \times 100 \qquad [2]$$

The atom % values were converted to ^{13}C atom % 'excess' by subtracting the % ^{13}C of unlabelled controls (i.e. samples not exposed to enriched ^{13}C) from each enriched sample.

To calculate the total amount of ^{13}C derived from the pulse of ^{13}C, the atom % excess was integrated with the total C content found in that sample (e.g. DOC in leachate, C content of roots, shoots and soil). Respired ^{13}C was calculated by multiplying ^{13}C atom % excess figures by the corresponding values of total C flux from the IRGA. The quantity of ^{13}C fixed by plants during the pulse was calculated by integrating the ^{13}C contents of shoot samples taken immediately following labelling with the final C content of total biomass of leaf tissue obtained at the end of the experiment. ^{13}C in other pools was calculated as a percentage of this initial ^{13}C uptake.

Data were analysed with SPSS Statistics for Windows v19 x86 (Version 19.0, released 2010, IBM Corporation, Armonk, NY). Linear mixed models with repeated measurements were applied to soil CO_2 efflux and temperature data. DOC data were analysed using Generalised Linear Models. Residuals were tested for normality and equality of variances. Treatment effects were tested by Tukey post-hoc multiple comparison.

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RESULTS

Effects of field-based treatments on soil surface CO_2 efflux

The flux of CO_2 from the soil in all treatments followed the same pattern during both years of the study (Figure 3) and fluctuated between 0.2 and 2.9 µmol m^{-2} s^{-1}. In the first year (2009), these high-amplitude fluctuations appeared to coincide with warm and dry spells during sampling days, but in Year 2 this was not the case and a pattern similar to the annual temperature cycle (Figure 2a) emerged. In 2009, there was a significant interaction between time and treatment ($F_{24,43}$ = 2.18, P = 0.005) and a significant overall effect of treatment alone ($F_{2,43}$ = 15.2, P = 0.002). Sheltered plots had a continually greater rate of soil respiration throughout the entire season; the mean flux (± SEM) was 1.12 (± 0.14) µmol m^{-2} s^{-1} compared to 0.62 (± 0.11) µmol m^{-2} s^{-1} in the controls. Post hoc tests showed that CO_2 efflux was greater in sheltered plots than in control and girdled plots, but there was no significant difference between control and girdled treatments (P = 0.14). In 2010, there was no significant interaction between time and treatment but the significant overall treatment effect remained ($F_{4,65}$ = 14.7, P < 0.001). In 2010, CO_2 efflux was significantly and consistently reduced in weeded plots compared to control, girdled and sheltered treatments (Figure 3). No significant interactions were found between any other treatments; the mean flux (± SEM) in the control plots was 1.30 (± 0.12) µmol m^{-2} s^{-1} compared to 1.50 (± 0.0.18) µmol m^{-2} s^{-1} in the sheltered plots.

Temperature, soil water content and water table reduction under rain shelters

None of the treatments had any significant effect on soil temperature (data not shown). The water table was consistently lower in the sheltered plots than in the control plots throughout the measurement period (mean 50 mm differential representing a 75 % reduction; Figure 4). The effect of the shelters was slightly stronger in the first year of the study ($F_{1,46}$ = 15.6, P = 0.016) compared to the second year ($F_{1,34}$ = 11.87, P = 0.026). In the first year, mean rainfall was also greater than in the second year (Figure 2b). The fluctuations in water table depth throughout the year were similar in control and sheltered plots. The reductions in water table level were mirrored by small changes in soil moisture content (Figure 5). The soil moisture content in the sheltered plots was less than in the controls on most sampling occasions. In addition, moisture content was more variable than in the control plots, particularly in 2010.

Relationship between water table depth and soil CO_2 efflux

There were clear reciprocal patterns in water table depth and soil CO_2 efflux for both years, with the highest CO_2 fluxes occurring when the water table was deepest. This was observed in control and sheltered plots in both 2009 (Figures 4a, b) and 2010 (Figures 4c, d). In 2009, the mean water table depth in the control plots was 13 cm below the peat surface. In the sheltered plots in 2009, or in the control and sheltered plots in 2010, water levels were always

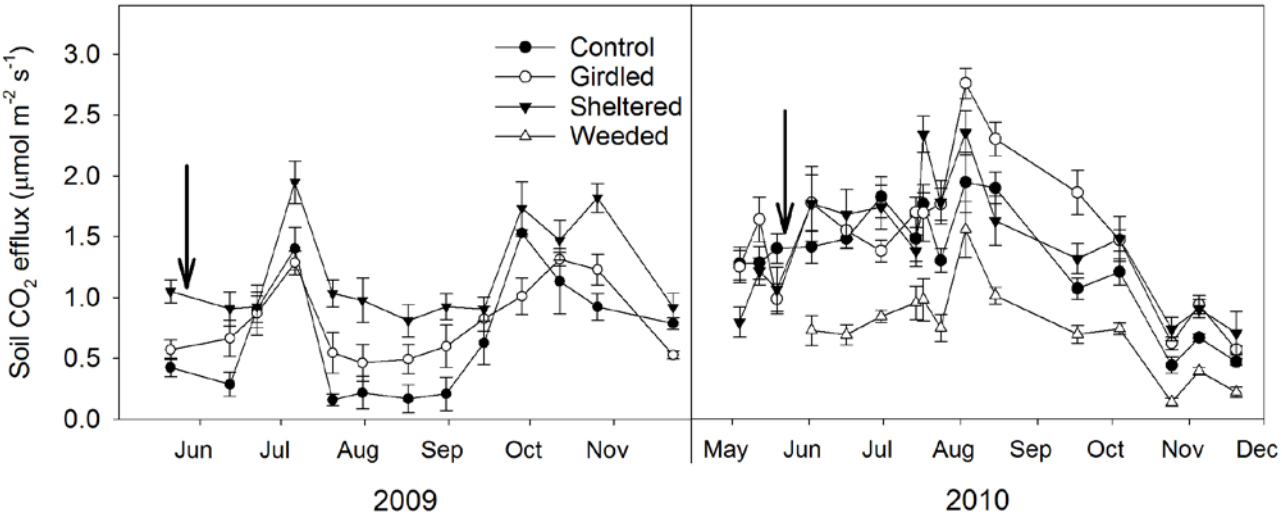

Figure 3. Soil CO_2 efflux (µmol m^{-2} s^{-1}) during the study periods in 2009 (left) and 2010 (right). Data are for the snow-free period of each year only. Arrows indicate when girdling took place.

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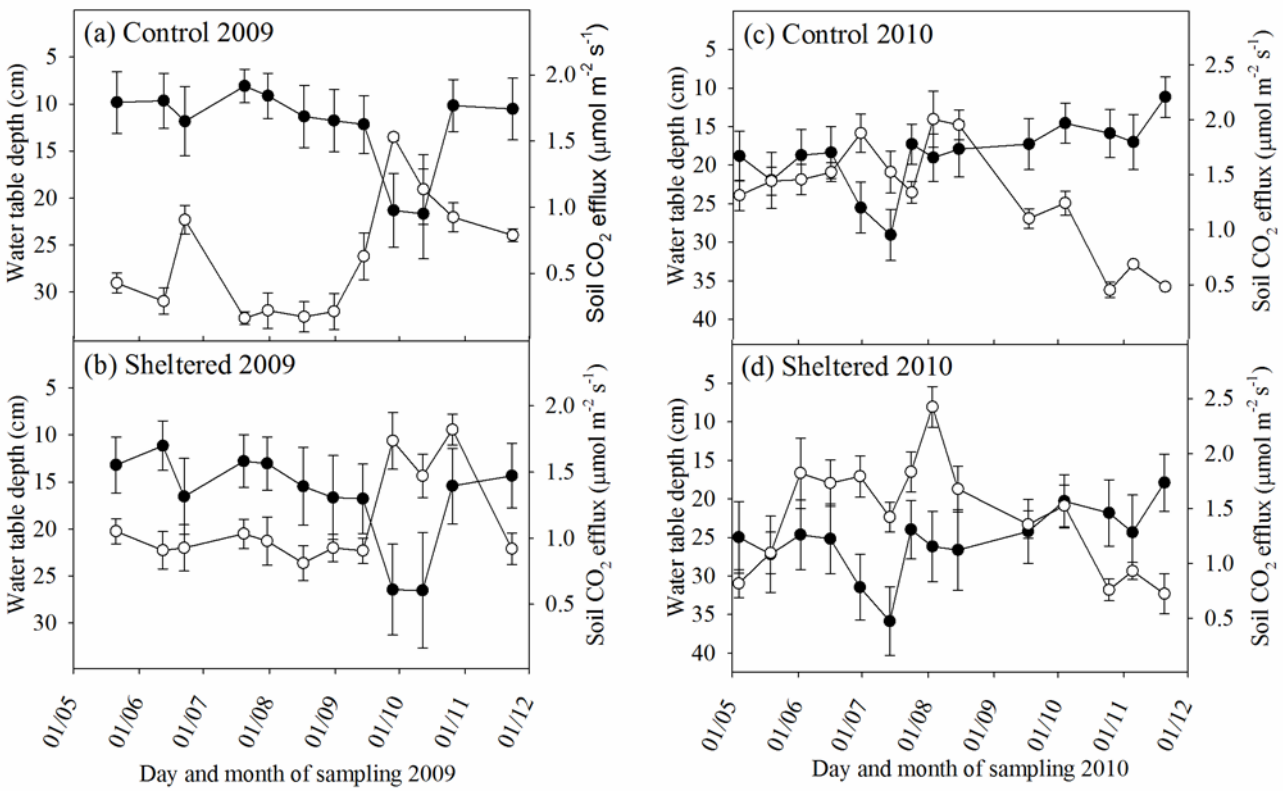

Figure 4. Water table depth (cm) (•) and soil CO_2 efflux (µmol m^{-2} s^{-1}) (○) in control and sheltered treatments (± SEM) in 2009 (a, b) and 2010 (c, d).

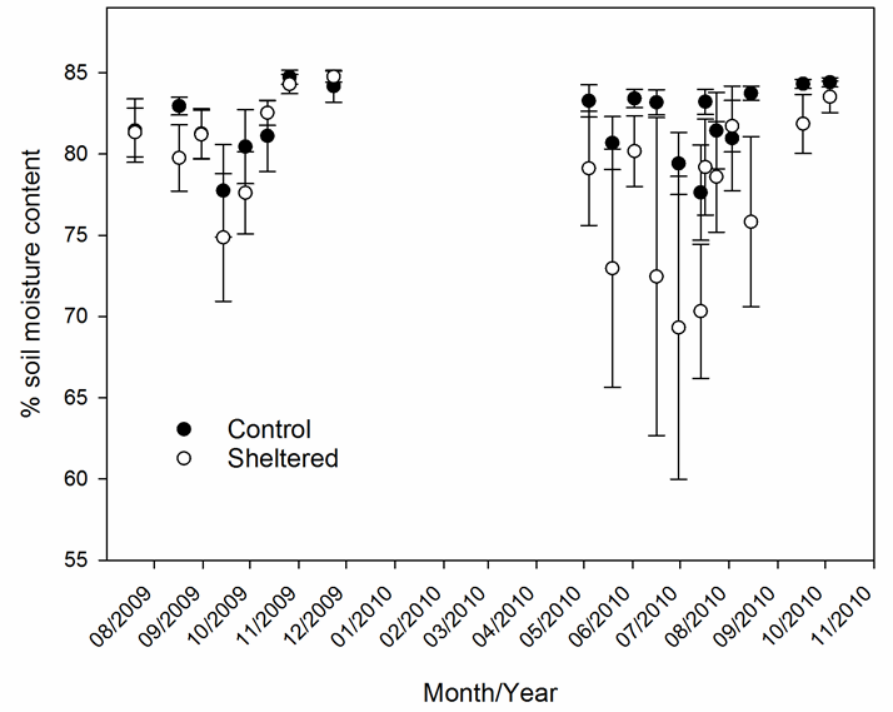

Figure 5. Mean soil moisture content (%) in control and sheltered plots (± SEM), derived from theta probe data.

below the 2009 mean value for control treatments. In 2010, CO_2 efflux did not follow water table depth as closely as in the previous year. There were significant positive linear relationships between water table depth and soil CO_2 efflux (Table 1, Figure 6); the strongest relationship was seen in 2009 in the control plots, and the weakest in 2010 in the sheltered plots. When the water table was between 15 cm and 20 cm below the peat surface, the relationship between soil CO_2 efflux and water table depth appeared to be more variable (Figure 6). There were no significant differences ($P > 0.05$) between years in either the slopes or the intercepts of regression lines fitted to the water table depth *versus* soil CO_2 efflux relationships.

Quantification of ^{13}C transfer to major pools and fluxes in monoliths

The *Calluna* plants fixed an average of 12.7 mg ^{13}C (Figure 7), and over the following 20 days, about 60 % (7.7 mg) was reallocated or respired. Most of the ^{13}C that was reallocated was recovered in the bulk soil. Only very small amounts of ^{13}C were recovered in fine roots, DOC and soil CO_2 efflux, and these collectively represented 5 % of the amount of ^{13}C initially fixed, or 8.3 % of the amount of ^{13}C reallocated or respired by shoots (Figure 7). The DOC pool was a particularly small sink of recent assimilates and contained only 1.1 µg ^{13}C.

DISCUSSION

In contrast to our predictions, we found that cessation of recent plant assimilate inputs achieved by girdling the stems of *C. vulgaris* had no significant effect on soil CO_2 efflux. While girdling of individual *C. vulgaris* plants has been undertaken in the laboratory to ascertain stem relative water content and water potential (Jackson *et al.* 1999), it has never been attempted in the field on this scale. We expected an initial decrease of CO_2 efflux in the girdled plots due to the immediate reduction in allocation of recent plant assimilate to below-ground pools. In the long term, we would expect CO_2 efflux to increase due to death and turnover of mycorrhizal fungal mycelium and the abundant fine 'hair' roots associated with *C. vulgaris*. This was not the case, but we did observe a significant reduction in CO_2 efflux in response to weeding, in which all above-ground parts of vascular plants were removed from the plots. These observations suggest that the girdling process did not kill the plants during the same growing season (otherwise soil CO_2 efflux in the girdled and weeded plots would have decreased to a similar extent), and instead suggests that recent photosynthate has little role in driving CO_2 efflux under the conditions of the experiment. Nevertheless, girdled plants flowered 2–3 weeks before those in the control plots, which may be a stress response to the treatment. The lack of an effect of girdling on soil CO_2 efflux was also supported by the $^{13}CO_2$ experiment undertaken on intact monoliths *ex situ*. In this experiment only 3 % of recently fixed ^{13}C was recovered in soil CO_2 efflux and DOC during the 20 days following labelling. These findings contrast with those of Fenner *et al*. (2004) for contributions made by *Sphagnum* recent photosynthate to CO_2 flux and DOC pools. Thus, whilst our combined approaches suggest that recent assimilate is of little importance in providing labile C that is recycled as CO_2 or dissolved in leachate, the strong reduction in soil CO_2 efflux in weeded plots (where no vascular plants were present) indicates that the collective inputs of C from plant shoots to below-ground pools are important in regulating CO_2 efflux.

Work in grassland ecosystems has shown that CO_2 efflux from external mycelium of arbuscular mycorrhizal fungi is driven both by recent assimilate (Johnson *et al.* 2002a, b) and by allocation of older pools of C (Grimoldi *et al.* 2006). Girdling of trees has shown clearly that recent photosynthate is a key regulator of soil CO_2 efflux (Högberg *et al.* 2001, Bhupinderpal-Singh *et al.* 2003), although this

Table 1: Regression analysis of soil CO_2 efflux (µmol m^{-2} s^{-1}) *versus* water table depth (cm) in 2009 and 2010 in the control and sheltered plots.

Year	Treatment	R^2	P value	Intercept	Slope
2009	control	0.62	0.002	-0.35	0.008
	sheltered	0.38	0.033	0.41	0.004
2010	control	0.33	0.031	0.09	0.007
	sheltered	0.11	0.236	0.44	0.004

 DOI: 10.19189/MaP.2015.OMB.203

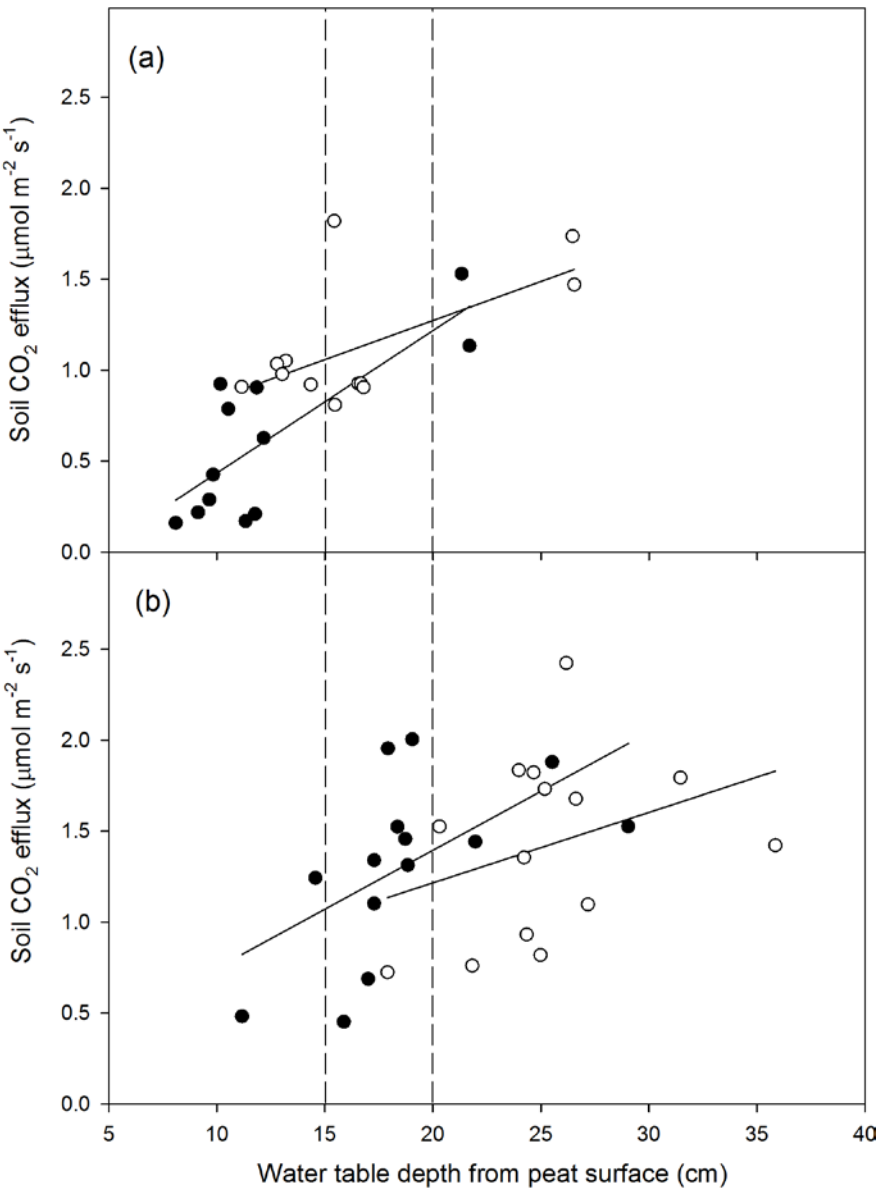

Figure 6. Regression of water table depth (cm) *versus* soil CO_2 efflux (µmol m^{-2} s^{-1}) in (a) 2009 and (b) 2010. Solid circles = control, open circles = sheltered. Vertical dashed lines indicate the range of the proposed water table tipping point.

approach can have different effects depending on the ecosystem (Binkley *et al.* 2006). In eucalyptus plantations, for example, CO_2 efflux was not affected significantly by girdling. While the reasons for different effects of girdling on CO_2 efflux amongst ecosystems are open to speculation, one potentially important factor in our study was that only *C. vulgaris* stems over 5 mm in diameter were girdled (approximately 50 *per* plot). All individual plants below this size (approximately 150–200 *per* plot, located in the understorey) were left intact, and as they were in a stronger growth phase than the older stems they potentially could have counteracted any reduction in photosynthate transport by the plants that were girdled and already in their early degenerate phase. As with all plants, age is an important factor in determining photosynthetic rates and subsequent C fixation (Grace & Woolhouse 1973). On the one hand, the comparatively small size of young plants allows for less C to be fixed or transported; whilst on the other hand, older plants in an early degenerate state have reduced rates of photosynthesis (Kulmala *et al.* 2011). However, the $^{13}CO_2$ labelling study used ~ 6-year-old *Calluna* plants in a regrowth phase, which were much younger than those in the girdled plots, and yet only a very small percentage of fixed ^{13}C was allocated into below-ground pools and CO_2 efflux. Our findings support those of other work

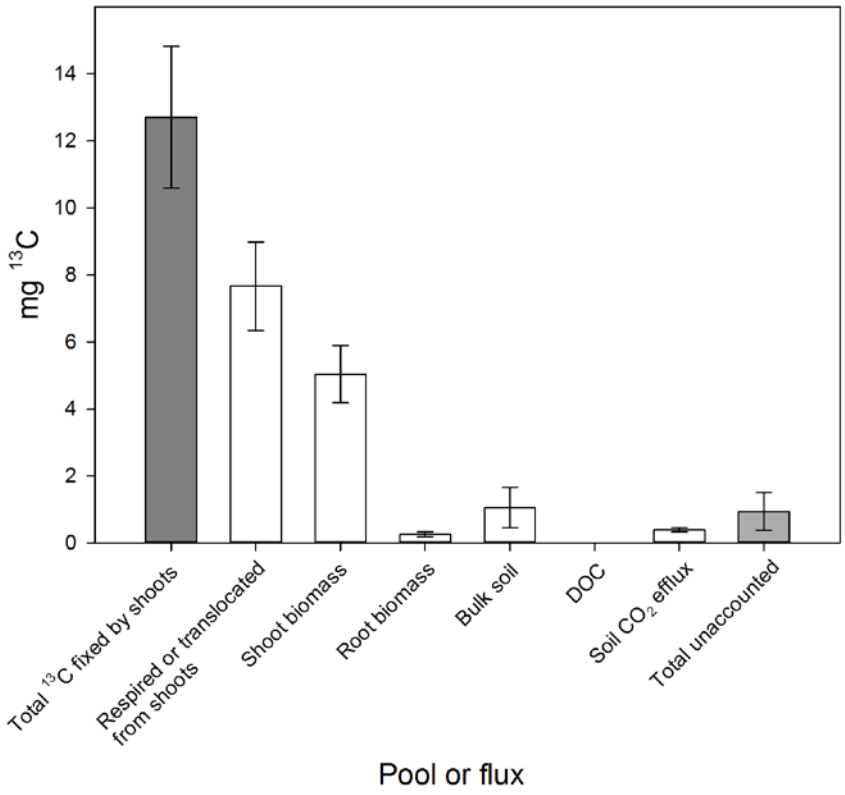

Figure 7. The amount of ^{13}C fixed by *Calluna vulgaris* shoots (dark grey) after exposure to atmospheric concentrations of ~ 99 atom % $^{13}CO_2$ for four hours, its subsequent allocation into major pools and fluxes of C, and the mean amount of ^{13}C unaccounted for (light grey) in the following 20 days (means ± SEM).

in which *C. vulgaris* stems > 8 years old were shown to translocate very small amounts of recently assimilated $^{14}CO_2$ to adventitious roots (Wallén 1983). Given that the canopy of Red Moss of Candyglirach is predominantly formed by old plants due to the absence of burning for ~18 years, it is possible that only relatively small amounts of recently fixed photosynthate are transported below ground. Despite the small size of young shoots in the girdled plots, these may be able to transport enough photosynthate below ground to maintain the biological activity that contributes to soil CO_2 efflux. If this hypothesis is correct, then we need to understand more clearly how ericaceous vegetation in the early stages of its life cycle contributes to soil CO_2 efflux, particularly in degraded peatlands such as the site investigated here, where ericaceous species dominate.

We used fixed open-sided shelters to experimentally manipulate rainfall and simulate short-term consequences of drought scenarios (as distinct from water-table drawdown experiments that reflect consequences of sustained drought events). We acknowledge that this design of shelter has the potential to affect other environmental variables such as wind speed, humidity, vapour pressure deficit and air temperature. In our study, however, it was likely that these confounding effects were minimal because the close proximity of mature trees surrounding the site provided a degree of shelter from wind, and the small surface areas of the shelters would limit their potential to significantly increase air temperatures. Indeed, we did not measure any significant change in soil temperature below the shelters. Nevertheless, the results need to be interpreted with these caveats in mind. In sheltered plots, an overall increase in CO_2 efflux was expected due to oxygenation of the peat profile leading to the release of C that is normally locked up under anaerobic conditions. In the first year of study, CO_2 efflux from sheltered plots followed our predictions. However, in the second year, no clear effect of the shelters was observed. The rain shelters lowered the water table and reduced surface moisture content, and we found significant positive linear relationships between the depth of the water table and soil CO_2 efflux. Thus, in our study, maintaining a shallow water table in a peatland restricted losses of C through soil respiration. Our observation that even a modest reduction in rainfall inputs (as occurred in 2010) can significantly affect soil CO_2 efflux supports previous findings (Funk *et al.* 1994, Aerts 1997) and emphasises the need to predict changes in

 DOI: 10.19189/MaP.2015.OMB.203

rainfall patterns accurately, especially in the context of climate change. Recent work in north Wales has also demonstrated that CO_2 loss by soil respiration in the year after a drought event is often larger than during the drought (Fenner & Freeman 2011). We also found that rates of CO_2 efflux from sheltered plots were greater in the second year than in the first, so it is possible that the control plots subjected to the natural drought event in 2010 would lose even more CO_2 through soil respiration in 2011. We do not know the exact mechanism driving the changes in CO_2 efflux from year to year or in response to the treatments. Given that our data show recent assimilate from *C. vulgaris* contributing little to soil respiration, however, we suggest that heterotrophic rather than autotrophic pathways explain most of the variation in CO_2 efflux. Nevertheless, we cannot exclude the possibility that subtle shifts in the composition of plant communities in response to the treatments, as seen in upland organic soils (Johnson *et al*. 2011) and peatlands (Breeuwer *et al*. 2009), could also have contributed to these patterns. Our data also suggest that, at a certain threshold of water table depth, the linear response between CO_2 efflux and water table depth starts to break down and other factors become more important in regulating CO_2 efflux. Lafleur *et al*. (2005) found that water table had no impact on soil CO_2 efflux in a water-stressed bog where the level of the water table fluctuated between 30 and 75 cm below the peat surface. Their findings are consistent with our suggested threshold for water-table depth and CO_2 efflux.

In conclusion, we found that CO_2 efflux from a degraded lowland raised bog is not driven by allocation of recent plant assimilate from canopy-dominant *C. vulgaris* and is sensitive to drought conditions, whether these are experimentally applied or occur naturally. The flux of CO_2 from the soil in systems where the water table is already below our proposed threshold are likely to be unaffected by subsequent drought events, and it has also been shown that the temperature of the surface 5 cm of peat is critical in regulating soil CO_2 effluxes (Alm *et al*. 2007). To minimise C losses *via* soil respiration induced by increased severity and incidence of summer droughts (as predicted by climate models), it would be advantageous to restore site hydrology so that the water table is above the threshold depth of 15–20 cm, which is also the depth at which maximum rates of peat formation are predicted to occur (Belyea & Clymo 2001). Given that soil CO_2 efflux is such an important contributor to atmospheric C inputs, the need for annual greenhouse gas inventories (Webb *et al*. 2014), and the possible implementation of C trading schemes, this work has important implications for future management strategies. We expect that improving the hydrology of drained or partially drained sites by closing existing drainage ditches, which is likely to promote species such as *Sphagnum* that are more resistant to decomposition (Trinder *et al*. 2008b), may improve the resilience of the site to climatic factors such as drought.

ACKNOWLEDGEMENTS

We thank Leys Estate for access and permission to use Red Moss of Candyglirach, particularly Thys Simpson for on-site help; and Barry Thornton, Dave Hadwen and Ken Cruickshank for technical support. This work was funded by a Natural Environment Research Council (NERC) Open CASE award (NE/F013760/1) with the James Hutton Institute (JHI).

REFERENCES

Aerts, R. (1997) Atmospheric nitrogen deposition affects potential denitrification and N_2O emission from peat soils in the Netherlands. *Soil Biology & Biochemistry*, 29, 1153–1156.

Alm, J., Shurpali, N.J., Tuittila, E-S., Laurila, T., Maljanen, M., Saarnio, S. & Minkkinen, K. (2007) Methods for determining emission factors for the use of peat and peatlands - flux measurements and modelling. *Boreal Environment Research*, 12, 85–100.

Belyea, L.R. & Clymo, R.S. (2001) Feedback control of the rate of peat formation. *Proceedings of the Royal Society B: Biological Sciences*, 268, 1325–1321.

Bhupinderpal-Singh, Nordgren, A., Ottosson Löfvenius, M., Högberg, M.N., Mellander, P-E. & Högberg, P. (2003) Tree root and soil heterotrophic respiration as revealed by girdling of boreal Scots pine forest: extending observations beyond the first year. *Plant, Cell & Environment*, 26, 1287–1296.

Binkley, D., Stape, J.L., Takahashi, E.N. & Ryan, M.G. (2006) Tree-girdling to separate root and heterotrophic respiration in two Eucalyptus stands in Brazil. *Oecologia*, 148, 447–454.

Breeuwer, A., Robroek, B.J.M., Limpens, J., Heijmans, M.M.P.D., Schouten, M.G.C. & Berendse, F. (2009) Decreased summer water table depth affects peatland vegetation. *Basic Applied Ecology*, 10, 330–339.

Bubier, J., Crill, P., Mosedale, A., Frolking, S. & Linder, E. (2003) Peatland responses to varying

 DOI: 10.19189/MaP.2015.OMB.203

interannual moisture conditions as measured by automatic CO_2 chambers. *Global Biogeochemical Cycles*, 17, 1066, doi: 10.1029/2002GB001946.

Crow, S.E. & Wieder, R.K. (2005) Sources of CO_2 emission from a northern peatland: Root respiration, exudation, and decomposition. *Ecology*, 86, 1825–1834.

Fenner, N. & Freeman, C. (2011) Drought-induced carbon loss in peatlands. *Nature Geoscience*, 4, 895–900.

Fenner, N., Ostle, N., Freeman, C., Sleep D. & Reynolds, B. (2004). Peatland carbon efflux partitioning reveals that *Sphagnum* photosynthate contributes to the DOC pool. *Plant and Soil*, 259, 345–354.

Funk, D.W., Pullman, E.R., Peterson, K.M., Crill, P.M. & Billings, W.D. (1994) Influence of water-table on carbon-dioxide, carbon-monoxide, and methane fluxes from Taiga bog microcosms. *Global Biogeochemical Cycles*, 8, 271–278.

Gimingham, C.H. (1972) *Ecology of Heathlands*. 12th edition, Chapman and Hall, London, 266 pp.

Gorham, E. (1991) Northern peatlands: role in the carbon cycle and probable responses to climatic warming. *Ecological Applications*, 1, 182–195.

Grace, J. & Woolhouse, H.W. (1973) A physiological and mathematical study of the growth and productivity of a *Calluna-Sphagnum* community. III. Distribution of photosynthate in *Calluna vulgaris* L. Hull. *Journal of Applied Ecology*, 10, 77–91.

Grimoldi, A.A., Kavanova, K.M., Lattanzi, F.A., Schaufele, R. & Schnyder, H. (2006) Arbuscular mycorrhizal colonization on carbon economy in perennial ryegrass: quantification by $^{13}CO_2/^{12}CO_2$ steady-state labelling and gas exchange. *New Phytologist*, 172, 544–553.

Haddaway, N.R., Burden, A., Evans, C.D., Healey, J.R., Jones, D.L., Dalrymple, S.E., & Pullin, A.S. (2014) Evaluating effects of land management on greenhouse gas fluxes and carbon balances in boreo-temperate lowland peatland systems. *Environmental Evidence*, 3, 30, doi: 10.1186/ 2047-2382-3-5.

Heinemeyer, A., Hartley, I.P., Evans, S.P., Carreira De La Fuente, J.A. & Ineson, P. (2007) Forest soil CO_2 flux: Uncovering the contribution and environmental responses of ectomycorrhizas. *Global Change Biology*, 13, 1786–1797.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M. & Read, D.J. (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature*, 411, 789–792.

Hulme, M., Jenkins, G.J., Lu, X., Turnpenny, J.R., Mitchell, T.D., Jones, R.G., Lowe, J., Murphy, J.M., Hassel, D., Boorman, P., McDonal, R. & Hill, S. (2002) C*limate Change Scenarios for the United Kingdom: The UKCIP02 Scientific Report*. Tyndall Centre for Climate Change Research, School of Environmental Sciences, University of East Anglia, Norwich, UK, 120 pp.

Irwin, J.G., Campbell, G., ApSimon, H., Bull, K.R., Cape, J.N., Carruthers, D.J., Derwent, R.G., Fisher, B.E.A., Fowler, D., Lee, D.S., Longhurst, J.W.S., Metcalfe, S.E., Smith, R.I., Sutton, M.A., Rea, J. & Downing, C.E.H. (1997) *Acid Deposition in the United Kingdom 1992–1994.* Fourth Report of the United Kingdom Review Group on Acid Rain, HMSO, London, 176 pp.

Jackson, G.E., Irvine, J. & Grace, J. (1999) Xylem acoustic emissions and water relations of *Calluna vulgaris* L. at two climatological regions of Britain. *Plant Ecology*, 140, 3–14.

JNCC (2011) *Towards an Assessment of the State of UK Peatlands*. Report No. 445, Joint Nature Conservation Committee (JNCC), Peterborough, 77 pp.

Johnson, D., Leake, J.R., Ostle, N., Ineson, P. & Read, D.J. (2002a) *In situ* $^{13}CO_2$ pulse-labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. *New Phytologist*, 153, 327–334.

Johnson, D., Leake, J.R. & Read, D.J. (2002b) Transfer of recent photosynthate into mycorrhizal mycelium of an upland grassland: short-term respiratory losses and accumulation of ^{14}C. *Soil Biology & Biochemistry*, 34, 1521–1524.

Johnson, D., Vachon, J., Britton, A.J. & Helliwell, R.C. (2011) Drought alters carbon fluxes in alpine snowbed ecosystems through contrasting impacts on graminoids and forbs. *New Phytologist*, 190, 740–749.

Kulmala, L., Pumpanen, J., Hari, P. & Vesala, T. (2011) Photosynthesis of ground vegetation in different aged pine forests: Effect of environmental factors predicted with a process-based model. *Journal of Vegetation Science*, 22, 96–110.

Lafleur, P.M., Moore, T.R., Roulet, N.T. & Frolking, S. (2005) Ecosystem respiration in a cool temperate bog depends on peat temperature but not water table. *Ecosystems*, 8, 619–629.

Laine, A., Byrne, K.A., Kiely, G. & Tuittila, E-S. (2007) Patterns in vegetation and CO_2 dynamics along a water level gradient in a lowland blanket bog. *Ecosystems*, 10, 890–905.

Lindsay, R.A. & Immirzi, C.P. (1996) *An Inventory of Lowland Raised Bogs in Great Britain.*

 DOI: 10.19189/MaP.2015.OMB.203

Research, Survey and Monitoring Report No. 78, Scottish Natural Heritage, Perth, 84 pp.

Lindsay, R.A., Rigall, J. & Burd, F. (1985) The use of small-scale surface patterns in the classification of British peatlands. *Aquilo: Serie Botania*, 21, 67–79.

Mäkiranta, P., Minkkinen, K., Hytönen, J. & Laine, J. (2008) Factors causing temporal and spatial variation in heterotrophic and rhizospheric components of soil respiration in afforested organic soil croplands in Finland. *Soil Biology & Biochemistry*, 40, 1592–1600.

Montanarella, L., Jones, R.J.A. & Hiederer, R. (2006) The distribution of peatland in Europe. *Mires and Peat*, 1(01), 1–10.

Moore, P.D. (2002) The future of cool temperate bogs. *Environmental Conservation*, 29, 3–20.

Moore, T.R., Bubier, J.L., Frolking, S.E., Lafleur, P.M. & Roulet, N.T. (2002) Plant biomass and production and CO_2 exchange in an ombro-trophic bog. *Journal of Ecology*, 90, 25–26.

Moore, T.R. & Knowles, R. (1989) The influence of water-table levels on methane and carbon-dioxide emissions from peatland soils. *Canadian Journal of Soil Science*, 69, 33–38.

Olsrud, M. & Christensen, T.R. (2011) Carbon partitioning in a wet and a semi-wet subarctic mire ecosystem based on in situ ^{14}C pulse-labelling. *Soil Biology & Biochemistry*, 43, 231–239.

Potthoff, M., Loftfield, N., Buegger, F., Wick, B., John, B., Joergensen, R.G. & Flessa, H. (2003) The determination of $\delta^{13}C$ in soil microbial biomass. *Soil Biology & Biochemistry*, 35, 947–954.

Read, D.J. (1984) The structure and function of the vegetative mycelium of mycorrhizal roots. In: Jennings, D.H. & Rayner, A.D.M. (eds.) *The Ecology and Physiology of the Fungal Mycelium*, Cambridge University Press, 215–240.

Read, D.J. (1996) The structure and function of the ericoid mycorrhizal root. *Annals of Botany*, 77, 365–374.

Read, D.J. & Stribley, D.P. (1975) Some mycological aspects of the biology of mycorrhiza in the Ericaceae. In: Sanders, F.E., Mosse, B. & Tinker, P.B. (eds.) *Endomycorrhizas*, Academic Press, London, 105–118.

Reiche, M., Hädrich, A., Lischeid, G. & Küsel, K. (2009) Impact of manipulated drought and heavy rainfall events on peat mineralization processes and source-sink functions of an acidic fen. *Journal of Geophysical Research*, 114, G02021, doi: 10.1029/2008JG000853.

Robroek, B.J.M., Schouten, M.G.C., Limpens, J., Berendse, F. & Poorter, H. (2009) Interactive effects of water table and precipitation on net CO_2 assimilation of three co-occurring *Sphagnum* mosses differing in distribution above the water table. *Global Change Biology*, 15, 680–691.

Rodwell, J.S. (1991) *British Plant Communities Volume 2 - Mires and Heaths*. Cambridge University Press, 640 pp.

Salm, J-O., Maddison, M., Tammik, S., Soosaar, K., Truu, J. & Mander, Ü. (2012) Emissions of CO_2, CH_4 and N_2O from undisturbed, drained and mined peatlands in Estonia. *Hydrobiologia*, 692, 41–55, doi:10.1007/s10750-011-0934-7.

Strack, M., Waddington, J.M., Rochefort, L. & Tuittila E-S. (2006) Response of vegetation and net ecosystem carbon dioxide exchange at different peatland microforms following water table drawdown, *Journal of Geophysical Research*, 111, G02006, doi: 10.1029/2005JG 000145.

Stribley, D.P. & Read, D.J. (1974) Biology of mycorrhiza in Ericaceae. IV. Effect of mycorrhizal infection on uptake of ^{15}N from labelled soil by *Vaccinium macrocarpon* AIT. *New Phytologist*, 73, 1149–1151.

Swanson, D.K. (2007) Interaction of mire microtopography, water supply, and peat accumulation in boreal mires. *Suoseura*, 58, 37–47.

Trinder, C.J., Artz, R.R.E. & Johnson, D. (2008a) Contribution of plant photosynthate to soil respiration and dissolved organic carbon in a naturally recolonising cutover peatland. *Soil Biology & Biochemistry*, 40, 1622–1628.

Trinder, C.J., Artz, R.R.E. & Johnson, D. (2008b) Temporal patterns of litter production by vascular plants and its decomposition rate in cut-over peatlands. *Wetlands*, 28, 245–250.

Trinder, C.J., Johnson, D. & Artz, R.R.E. (2008d) Interactions between fungal community structure, litter decomposition and depth of water-table in a cutover peatland. *FEMS Microbiology Ecology*, 64, 433–448.

Wallén, B. (1983) Translocation of ^{14}C in adventitiously rooting *Calluna vulgaris* on peat (*Sphagnum*). *Oikos*, 40, 241–248.

Webb, N., Broomfield, M., Brown, P., Buys, G., Cardenas, L., Murrells, T., Pang, Y., Passant, N., Thistlethwaite, G. & Watterson, J. (2014) *UK Greenhouse Gas Inventory, 1990 to 2012*. Annual Report for submission under the Framework Convention on Climate Change. Compiled on behalf of the UK Department of Energy and Climate Change (DECC) Science Division by Ricardo-AEA, Didcot, Oxfordshire, 594 pp.

Wilson, D., Dixon, S., Artz, R.R.E., Smith, T.E.L.,

 DOI: 10.19189/MaP.2015.OMB.203

Evans, C.D., Owen, H.J.F, Archer, E & Renou-Wilson, F. (2015) Derivation of greenhouse gas emission factors for peatlands managed for extraction in the Republic of Ireland and the UK. *Biogeosciences*, 12, 5291–5308.

Yahdjian, L. & Sala, O.E. (2002) A rainout shelter design for intercepting different amounts of rainfall. *Oecologia*, 133, 95–101.

Yu, Z. C. (2012) Northern peatland carbon stocks and dynamics: a review. *Biogeosciences*, 9, 4071–4085, doi: 10.5194/bg-9-4071- 2012.

Submitted 07 Sep 2015, final revision 07 Jan 2016
Editor: David Wilson

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 DOI: 10.19189/MaP.2015.OMB.203