Appendix 14 of BD5104
Mycorrhizal and charcoal impacts on peat decomposition

The purpose of this Appendix is to further describe the methods and findings relating to the pot experiment investigating the effects of mycorrhizal fungi on the decomposition of ancient peat and modern charcoal, which are summarised in Section 4.4.4 of the main body of the report. The method summaries, results and discussions are given here with full details. Additionally, a table containing the ^{13}C and ^{14}C content of each sample are given at the end of this Appendix, along with the publication codes relating to these values (as provided by the NERC Radiocarbon Facility at the Scottish Universities Environment Research Centre (SUERC), East Kilbride, UK).

Methods

Peat preparation

Peat was collected between 1 and 2 m depth from an exposed peat bank in the Peak District (this site was chosen as no age information was available for the project sites), which was expected to have a minimum age of 3,000 years based on evidence from Tallis (1991). Using very old peat was to avoid problems associated with ^{14}C dating during (nuclear test) bomb-peak C ages (Evans *et al.*, 2007) and to provide a strong contrasting age against any recently produced C (e.g. from plant root exudates). Living roots were removed and pieces of peat with a diameter more than about 3 cm were broken up. Plastic boxes (64 L capacity; Really Useful Storage Boxes, London), lined with polythene sacks, were filled with peat to a depth of 30 cm. Filled boxes were gamma-irradiated at 34.5 kGy (Bradford Synergy Health PLC, Bradford). Although a dose of 10 kGy should have been sufficient to destroy the filamentous fungi (Parker & Vincent, 1981), 34.5 kGy was the only available dose at the facility and increased the chance that all fungal spores were rendered inviable. Both autoclaving and microwaving were considered as sterilisation methods, but were deemed impractical for the quantity of peat required (see Trevors (1996) for details of appropriate weights and layer thicknesses required for adequate sterilisation). Additionally, autoclaving releases more nitrates, ammonium and soluble organic matter than gamma-irradiation (Salonius *et al.*, 1967) and can destroy the soil structure (Trevors, 1996). In order to reduce the impact gamma-irradiation had on the peat, the soil was air-dried (in a vented greenhouse on top of a thick PVC sheet) before irradiating to reduce the release of radiation-induced soluble organic matter as much as possible (Salonius *et al.*, 1967), which involved breaking up larger peat clumps and mixing to remove moisture. This process also homogenised the peat which was necessary to obtain a consistent average age throughout.

The sterile peat was rehydrated by being submerged (by a cover pressing down on the peat) in deionised water (pH adjusted to 3.6) for 2 weeks in a laboratory environment to reduce the risk of contamination by fungal spores. The peat was drained in a 24 cm tinned mesh sieve (Kitchen Craft, Birmingham) and lumps were further broken up to smaller than 2 cm in diameter. Every sieve full of peat was also rinsed with fresh deionised water to wash out excess DOC caused by the gamma-irradiation process (Genney *et al.*, 2000) before peat was placed into plant pots. Finer peat fragments were retrieved by filtering through pieces of cotton cloth.

Pot preparation

Forty eight 25 cm diameter and 22 cm tall PVC pots (ref: 101; LBS Horticulture Ltd, Colne, UK), forty eight 32 cm diameter by 4 cm tall saucers (ref: SAU 019; LBS Horticulture Ltd, Colne, UK) ninety six 11 cm diameter 3 cm tall uPVC collars (Plumb Center, Wolseley UK Ltd, Leamington Spa, UK) were wiped with a 7.5% calcium hypochlorite solution (Technical grade $Ca(ClO)_2$, Sigma-Aldrich Company Ltd., Dorset, UK) to sterilise them and rinsed five times with deionised water. Pieces of 1 µm nylon mesh (Normesh Limited, Oldham) were glued over the pot drainage holes (2 cm by 4 cm) with Bostik All Purpose Glue (Bostik Ltd, Stafford) and sealed around the edges with non-toxic aquarium silicone sealant (Everbuild Building Products Ltd, Leeds).

Pots were filled in batches of 12 with the sterilised washed peat. Filled pots were placed in the saucers, which were filled with deionised water (pH adjusted to 3.6). This simulated a water table depth (WTD) in the pots of -18 cm, which was chosen as it was deemed to represent a typical summer WTD on a blanket bog managed by burning (see WTD values in Section 4.2.7 of the main report). Additionally, lower WTDs tend to cause increased decomposition due to more aerobic conditions (e.g. Frolking *et al.*, 2011) and it was hoped this would help to provide sufficient C release (in both water and air) for radiocarbon analysis. A pre-soaked Rhizon soil moisture sampler (pore size 0.15 µm, Rhizosphere Research Products B.V., Wageningen, Netherlands) was inserted through a hole drilled 18 cm from the top of each pot (so it was just above the WTD) and sealed in place with the aquarium silicone sealant. For the top of each pot, a pair of sterile collars was glued together with a 26 cm diameter circle of the 1 µm nylon mesh sandwiched between. All joins were sealed with aquarium silicone sealant (see **Figure A14.1** for the pot set-up).

Pre-treatment measurements

Water samples were collected twice from each pot during a five week period (see **Table A14.1** for dates) by attaching 50 ml luer-lock syringes to the Rhizon samplers. The syringes were held open with a retainer to create a vacuum. The DOC concentrations were determined using a total carbon analyser (LiquiTOC, Elementar Analysensysteme GmbH, Hanau, Germany). Prior to analysis, samples were acidified and sparged with oxygen to remove any inorganic carbon. A five-point calibration was determined with a sodium carbonate and potassium hydrogen phthalate (SC/KHP) solution and standards of 50 ppm SC/KHP were regularly analysed during a machine run to reduce machine drift and errors. All samples were analysed in duplicate. The absorbency of the water samples was measured in a 1 cm wide quartz cell at 254, 400, 465 and 665 nm (Abs_{254}, Abs_{400}, Abs_{465} and Abs_{665}) using an ultra-violet spectrophotometer (Lambda 25 UV-Vis spectrophotometer, PerkinElmer Ltd, Beaconsfield, UK), with a blank (deionised water) reading subtracted from each sample. An infrared gas analyser (IRGA; Model 8100, Li-Cor, Lincoln, NE, USA) and an Ultraportable Greenhouse Gas Analyser (UGGA; Model 915-0011, Los Gatos Research, Inc., San Jose, CA, USA) were connected in sequence to a 10 cm automated survey chamber (Model 8100-102, Li-Cor, Lincoln, NE, USA) to allow for simultaneous CO_2 and CH_4 measurements (the effective total air volume was used in flux calculations). Although the UGGA is able to measure both CO_2 and CH_4, the IRGA was connected in order to enable the chamber to be raised and lowered, thus pinpointing the exact measurement periods, which aided derivation of the fluxes. The chamber was placed over the top collar, which had been pressed onto the peat surface, and fluxes measured for a 90 s period on four occasions during a three week period (see **Table A14.1**).

On 29th January 2015, all pots were weighed to the nearest gram during the gas measurements, having been watered to excess with pH-adjusted (pH 3.6) deionised water 12 hours before. The offset from the top of the pot to the peat surface was also measured to the nearest half centimetre. On 30th January, a teaspoonful of peat was removed from the subsurface of each pot, weighed and placed in a foil tray in an oven at 80°C until a stable weight was reached. The water content was calculated from the wet and dry peat weights for each pot and the dry weight for each pot calculated.

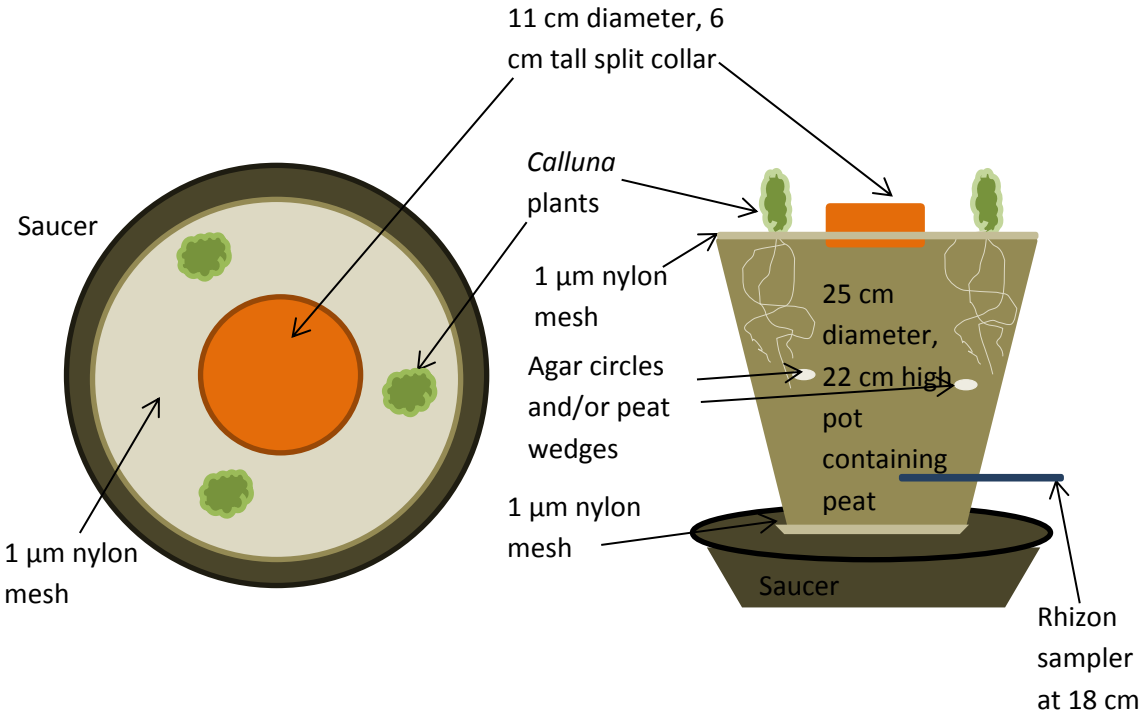

Figure A14.1 Schematic of a full pot set-up. The diagram on the left shows a pot as viewed from above. The diagram on the right shows a cross-section of a pot viewed from the side. All pieces of 1 µm nylon mesh, Rhizon samplers and collars were glued and sealed around to prevent fungal spore entry. Not all pots contained *Calluna* plants and all pots had either agar, agar inoculated with *H. ericae* culture or agar and non-sterile peat wedges buried below the peat surface (see **Table A14.2** and text for treatments).

Table A14.1 Dates (all in 2015) when plant pots were measured for CO_2 and CH_4 and when water samples were collected from the pots. Water samples were tested for DOC and absorbency at various wavelengths (see main text) with one month of collection. No CH_4 samples were collected for ^{14}C analysis.

Measurement set	CO_2 measurements	CH_4 measurements	Water samples
Pre-treatment measurements (all 2015)	22nd January	22nd January	
	29th January	29th January	19th January
	30th January	30th January	11th February
	5th February	5th February	
Post-treatment measurements (all 2015)	12th August	12th August	12th August
	13th October	13th October	13th October
	6th November	6th November	9th November
	10th November	10th November	13th November
	19th November	19th November	19th November
^{14}C sample collection (all 2015)	14th October*		
	15th–16th October	-	Continuous multiple collections between 20th and 26th October
	19th October		

*No CO_2 sample collection for radiocarbon analysis was made on 14th October; the CO_2 fluxes measured on this date were used in the mass balance equations.

Making charcoal

On 3rd July 2014, all *Calluna* plants (which were 30-40 cm tall; age estimate about 15-20 years based on visual growth increment based on branching patterns) from a previous burn area at Whitendale (but outside the project's study catchment) were cut at the base from a 5 x 1 m patch. On 31st July, the air-dried *Calluna* plants were burnt on a clean brick barbeque pit, with the burnt *Calluna* caught underneath in a metal tray lined with aluminium foil. The burnt *Calluna* residue was stored in air-tight plastic containers at ambient temperature. Although this burnt residue is referred to as charcoal throughout the rest of this Appendix and in the main report, it should be noted that the residue is not solely charcoal; it is likely a mixture of ash and charcoal which is representative of the burn impact on grouse moors.

Culturing ericoid mycorrhizas

Due to its extensive use in laboratory trials, *Hymenoscyphus ericae* (Read) Korf and Kernam (Strain He 101; supplied by Prof. JR Leake, University of Sheffield) was chosen as the single ericoid mycorrhiza fungus. The fungus was cultured on Modified Melin Norkrans agar (MMN) media acidified to pH 4.7 with 10%HCl and containing 50 mg $CaCl.2H_2O$, 25 mg NaCl, 155 mg $MgSO_4.7H_2O$, 250 mg $(NH_4)_2HPO_4$, 500 mg KH_2PO_4, 7.2 mg $FeCl_3.6H_2O$, 1 mg thiamine, 2.5 g d-glucose, 10 g malt extract and 15 g agar per litre. The fungal plates were stored at 4°C to slow growth.

Growing *Calluna* plants

Seed trays and propagator lids were sterilised with 7.5% calcium hypochlorite solution, in the same manner as the pots and saucers (see "Pot preparation" above). Each tray was filled with sterilised coarse sand. The sand was sterilised by microwaving when saturated with deionised water in a 900W microwave for 4 min kg^{-1} sand, as this was deemed quickest and most cost-effective for killing fungal spores (Ferriss, 1984).

Stem and heel cuttings were taken from young (less than one year old) *Calluna* plants (Cheviot Trees, Berwick-upon-Tweed, UK). The lower leaves were stripped off and cuttings were immediately placed in deionised water to prevent desiccation. The cuttings were sterilised in 3.25% calcium hypochlorite solution for 5 minutes then rinsed five times in autoclaved (121°C for 15 minutes) deionised water.

The cuttings were planted into the sterilised wet sand with the bottom end of each cutting dipped into rooting powder (Doff Portland Ltd, Nottingham, UK). Cuttings were watered in with half-strength ericaceous feed (containing 24% total N, 12% P_2O_5, 8% K_2O, 2% MgO, 0.02% B, 0.01% Cu, 0.2% Fe, 0.02% Mn, 0.002% Mo and 0.05% Zn; Chempak, Ipswich, UK) mixed with deionised water and covered with the sterilised propagator lids. Trays were placed inside a growth cabinet (Sanyo MLR-352, Sanyo Electric Co., Ltd., Tokyo, Japan) with a 16 hour light (at 18°C)/8 hour dark (at 14°C) cycle. The light source was provided from the sides by white fluorescent tubes with an average light level of 150 µmol m^{-2} s^{-1}. The day length was reduced by 2 hours per week in the month prior to removal from the cabinet and temperature was also lowered to adjust to outside conditions: the final settings were 10 hours light (at 12°C)/14 hours dark (at 10°C). Sand was kept moist by regular addition of deionised water. Half-strength ericaceous feed was added once every three weeks.

Treatment set-up

The pre-treatment measurements were used to partition the pots into four blocks. The pre-treatment DOC concentrations showed a pattern which corresponded to the four batches used to fill the pots. No such patterns

were observed in the CO_2 or CH_4 fluxes. Therefore, four blocks were defined as the four batches in which the pots were filled.

Treatments were administered between 6th March and 14th April 2015 and randomly allocated to one pot per block. Each pot received either *H. ericae* fungal culture (H), a wedge of non-sterile peat to introduce a mixed natural fungal and microbial community (M) or sterile agar, and either *Calluna* plants (C), or not, and charcoal (B), or not. This produced a fully crossed replicated design of 12 treatments. Treatment codes from **Table A14.2** will be used henceforth. Where a treatment component is generically referred to, the component code parts not referred to will be represented by X, e.g. XXB represents all pots with charcoal, XC- represents all pots with *Calluna* plants and no charcoal.

Burning has been used for grouse moor management since at least the 1850s (Lovat, 1911) and many moors manage on a 10-20 year cycle (Grant *et al.*, 2012). The *Calluna* from the 5 x 1 m patch produced 88 g of charcoal. As the surface area of each pot was 490 cm^2, the 3.5 g (± 0.012 g) of charcoal added to each XXB pot was the equivalent of about 4-5 burns, simulating a site managed by burning over the past 50-100 years. The charcoal was spread evenly across the peat surface and mixed into the top ~5 cm.

The *H. ericae* culture was added to the relevant pots by means of punching out approximately 1 cm diameter circles of agar from the growing edges of the culture plates and burying these about 5 cm below the peat surface. Three circles were added to each pot, evenly spaced round the edge zone (see **Figure A14.1**). All pots without *H. ericae* culture addition (including MXX pots) received similar sized circles of sterile MMN media agar in the equivalent places. Non-sterile peat wedges of approximately 3 x 1 x 1 cm were cut from the root zone of a *Calluna*-covered peat monolith from Mossdale and inserted in the peat to a similar depth as the agar circles.

Table A14.2 Codes used for the 12 pot treatments and the components of each pot treatment.

Treatment Code	Treatment Components
---	Peat only (and sterile agar)
-C-	*Calluna* plants (and sterile agar)
--B	Burnt material/charcoal (and sterile agar)
-CB	*Calluna* plants and burnt material/charcoal (and sterile agar)
H--	*H. ericae* fungal culture
HC-	*H. ericae* fungal culture and *Calluna* plants
H-B	*H. ericae* fungal culture and burnt material/charcoal
HCB	*H. ericae* fungal culture, *Calluna* plants and burnt material/charcoal
M--	Non-sterile peat wedges
MC-	Non-sterile peat wedges and *Calluna* plants
M-B	Non-sterile peat wedges and burnt material/charcoal
MCB	Non-sterile peat wedges, *Calluna* plants and burnt material/charcoal

A single tray of *Calluna* plants was initially used for all relevant pots in a block. Plants were carefully separated and visually sorted by size into three groups based on root size. Three small slits were cut in the mesh circles. The slits were between the pot edge and collar and corresponded to the buried agar locations. One plant was pushed through each slit meaning each pot received one plant from each size group. *Calluna* plants were fixed in the mesh by sealing both sides of the slit around the stem with non-toxic aquarium silicone sealant. Once dry, the central collar was pushed into the peat in the centre of each pot and the peat packed around the base to ensure a seal. The roots of the three attached plants were buried directly next to the agar/fungal circles or peat wedges,

placing the growing roots in the vicinity of the fungal mycelium. All pots, including those without plants, received 500 ml of full-strength ericaceous feed. The mesh circles were then glued to the pot edges and sealed with silicone sealant to prevent contamination from fungal spores.

Pots were kept in a normally unheated greenhouse (heaters were automatically switched on only when the greenhouse air temperature dropped below 6°C) and watered by filling the central collars with deionised water, acidified to pH 3.6 with hydrochloric acid. Once every three weeks, half strength ericaceous feed was used instead of water until plants were fully established and new shoots appeared. Some plants did not establish in the pots and 11 dead *Calluna* plants were replaced (on 30th April 2015). To reduce the disturbance to the established plants, new plants were sealed through a slit in a 2 x 2 cm piece of the 1 µm nylon mesh using a similar method to the initial planting. Dead plants were removed from the pots and new plants were immediately pushed through the holes and roots buried in the peat. The extra piece of mesh was glued to the mesh circle and sealed around. All pots were moved outside to a sheltered courtyard on 29th June 2015. Due to colder temperatures, pots were returned to the unheated greenhouse on 3rd November 2015.

Pot measurements

Five sets of water samples were collected from the Rhizon samplers in all pots post-treatment over three months (**Table A14.1**). The collection procedure and method of measuring absorbency were identical to those used before treatments were added (see "Pre-treatment measurements" above). The DOC concentrations were also measured using the same method but were made using a different total carbon analyser (vario TOC cube, Elementar Analysensysteme GmbH, Hanau, Germany).

The CO_2 and CH_4 fluxes were measured in parallel five times over three months (**Table A14.1**). The methods and equipment were identical to those described in "Pre-treatment measurements" (see above), apart from flux measurements ranging between 2 and 5 minutes in length due to lower fluxes in cooler weather. The CO_2 fluxes were also measured over 5 minute periods prior to ^{14}C sampling (see "^{14}C sampling" below).

Soil moisture content was monitored and maintained at 75% (± 5%) by regular weighing of pots and watering with pH-adjusted (pH 3.6) deionised water. Pots were watered through the mesh in the central collars and water was removed from the saucers of any overweight pots to aid drainage. This was to remove soil moisture as a factor in explaining any measured differences between treatments.

^{14}C sampling

Not all treatments could be sampled for ^{14}C analysis due to funding limitations. As MXX treatments were expected to exhibit similar effects to HXX treatments, it seemed prudent to only assess one form of fungal addition. HXX was chosen as it was the more controlled fungal introduction and therefore the fungal component fluxes from these pots were less likely to include other potentially confounding component fluxes, such as peatland bacterial contributions. The selected treatments were ---, --B, H--, HC- and HCB, as this combination enabled ages of all components to be derived. Three replicates were chosen for each treatment based on blocks. However, due to total plant death before sampling in two HCB replicates, one MCB pot was used as a substitute for one HCB replicate. All selected pots were arranged randomly outside on an electric blanket (Silentnight Comfort Control, Amazon, UK) to prevent frost impacts on sampling) for the entire collection period (warming the pot base by about 3°C).

When sampling for ^{14}C analysis, it is important to prevent contamination by atmospheric CO_2. As such, 26 cm diameter circles were cut from plastic vacuum storage bags to fit over the mesh on top of the pots, with a hole in

the centre so the central collar was not covered. These were sealed into place with the silicone sealant. For XCX pots, the plastic circles were slit to sit around the stems and sealed in place. Custom built 20 cm long and 10 cm diameter uPVC chambers (Biology Mechanical Workshop, University of York, UK) were fixed over the central collars in the pots by means of thick rubber bands to create an airtight seal. Each chamber had a male and female auto-shutoff coupling (Colder Products Company, St Paul, MN, USA) glued into holes 4 cm from the bottom and 4 cm from the top of the chamber, respectively.

An established molecular sieve sampling system (MS^3; see Hardie *et al.* (2005) and Garnett & Murray (2013) for full details) was used to collect samples from ^{14}C analysis. The only modification to the system was that the IRGA used here (Li-Cor 8100) contained an integral pump with an adjustable flow rate, negating the need for a pump in sequence. This reduced the number of connections and lengths of tubing where potential leaks could occur. The MS^3 was coupled to each chamber in succession and the chamber air was passed through the soda lime cartridge at 3 L min^{-1} for 3.5 minutes to remove atmospheric CO_2. This scrubbed the chamber air approximately five times and caused measured CO_2 concentrations to fall below 10 ppm. Scrubbed chambers were left to build up respired CO_2, with concentrations periodically checked. After 28-33 hours (exact time depended on the pot), the chamber air was forced through a zeolite molecular sieve cartridge (type 13X, 1.6 mm pellets, Sigma-Aldrich, Dorset, UK) at 500 ml min^{-1} to capture the CO_2. Collection was terminated when the CO_2 concentrations in the chamber dropped below 500 ppm to reduce the risk of atmospheric CO_2 contamination.

As there was insufficient CO_2 retrieved from the first sampling event (based on the measured chamber CO_2 concentrations during CO_2 capture), a second collection was made on the same molecular sieve cartridges. To prevent plant roots becoming oxygen starved due to the plastic covers on the mesh, there was a two day gap after CO_2 collection, during which the plastic covers were removed, before the chambers were resealed. For the second collection period, the plastic covers were resealed, chambers rescrubbed and left for 6-9 hours to allow respired CO_2 to accumulate before collection. Two pots required a third collection event.

The DOC samples for ^{14}C analysis were collected by attaching acid-washed 50 ml luer-lock syringes to the pot Rhizon samplers. Syringes were held open with a retainer to create a vacuum. Syringes were placed inside cardboard tubes during collection to prevent photo-degradation of the DOC. Once full, syringes were emptied into acid-washed bottles (Nalgene, Thermo Scientific, Rochester, NY, USA) through pre-ashed, pre-rinsed (with deionised water) 0.7 µm glass-fiber filters (Whatman glass microfiber filters, Grade GF/F, 25 mm diameter, Sigma-Aldrich, Dorset, UK) and reset on the same Rhizon sampler. The DOC concentrations from 13th October 2015 were used to calculate the minimum water volume required to obtain sufficient DOC for ^{14}C analysis and appropriately sized samples were taken. Bottled samples were stored at 4°C in the dark.

The molecular sieve cartridges and DOC samples, along with three peat samples and charcoal sample which were obtained before treatment addition, were sent to the NERC Radiocarbon Facility. The CO_2 was thermally retrieved (500°C), dried and cryogenically purified. The DOC was retrieved by rotary evaporation, freeze-drying and hydrolysis in hydrochloric acid, and combusted in an elemental analyser (Costech Instruments) for conversion to CO_2. The peat samples were heated with CuO in a sealed quartz tube and recovered as CO_2.

Each sample was split into subsamples. One of each subsample was analysed for $^{13}C/^{12}C$ ratio on a dual-inlet isotope ratio mass spectrometer (VG Optima, UK) and expressed ‰ relative to the Vienna PDB reference standard. Another subsample was converted to graphite by Fe/Zn reduction and analysed for ^{14}C by accelerator mass spectrometry at the NERC Radiocarbon Facility.

Data analysis

All data manipulation was performed in Microsoft Excel and all statistical analyses were undertaken in R version 3.3.1 (R Core Team, 2016) unless otherwise stated. Following Zuur *et al.* (2009), residuals were plotted against fitted values and visually assessed for normality and homogeneity of variance. The critical p-value chosen for significance was 0.05.

Pot measurements (pre- and post-treatment)

Raw DOC concentrations were corrected for machine drift using the standards and blank samples. All absorbency values were standardised to absorbance units per metre (m^{-1}) by multiplying the cell length by the appropriate value (here 100). Abs_{254} was divided by the weight-adjusted (see below) DOC concentration to obtain specific ultra-violet absorbency ($SUVA_{254}$) values and expressed in L mgC^{-1} m^{-1} kg dry $soil^{-1}$. $SUVA_{254}$ is often used by water companies as a proxy for the aromaticity of DOC and to determine the need for, or amounts of, enhanced coagulation and softening prior to treatment (Weishaar *et al.*, 2003). Water colour was expressed in Hazen units by multiplying Abs_{400} by 12, following Watts *et al.* (2001). The relative proportions of fulvic to humic acids were expressed as E4/E6 ratios (Thurman, 1985) by dividing Abs_{465} by Abs_{665}.

The LiCor Viewer software was used to derive the CO_2 fluxes from the most linear 50 s portion of each measurement. Similarly, CH_4 fluxes were derived by regressing the most linear 30-60 s section of each measurement over time and calculating the increase in CH_4 s^{-1}. With CH_4, the flux was discarded and recorded as zero if the linear relationship gave $R^2 < 0.4$ (there were no such problems for CO_2). Whilst this R^2value is low, this was due to very low CH_4 fluxes meaning that measurement variability was very large. All fluxes were also assessed by eye to verify the best section of a linear trend.

All weight-dependent values were adjusted to compensate for the different amounts of peat in each pot using:

$$V_{corr} = \frac{V}{P_{DW}} \qquad \textbf{Eq.A14.1}$$

where V is the pot CO_2 or CH_4 flux or DOC concentration, P_{DW} is the dry weight of peat in the pot in kg and V_{corr} is V per kg of dry peat. For a summary of all but the CO_2 fluxes (which are shown in the main report) see **Table A14.3.**

Table A14.3 Pot treatment averages in the pre- and post-treatment periods of the DOC concentrations, $SUVA_{254}$ values, Hazen units, E4/E6 ratios and CH_4 fluxes. Numbers in brackets are 95% confidence intervals. Treatment codes are explained in TableA14.2.

Treatment	DOC concentration (mgC L^{-1} kg dry $soil^{-1}$)		$SUVA_{254}$ (L mgC^{-1} m^{-1} kg dry $soil^{-1}$)		Hazen units		E4/E6 ratio		CH_4 flux (nmol m^{-2} s^{-1} kg dry $soil^{-1}$)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
HCB	96.94 (±14.23)	14.62 (±2.50)	2.13 (±0.60)	6.89 (±1.37)	231.45 (±25.40)	204.24 (±53.02)	1.97 (±0.20)	2.20 (±0.41)	0.005 (±0.010)	-0.014 (±0.027)
HC-	102.71 (±11.93)	21.78 (±4.93)	1.86 (±0.33)	5.68 (±0.57)	224.33 (±18.63)	227.52 (±53.86)	1.96 (±0.13)	2.24 (±0.36)	-0.018 (±0.034)	0.010 (±0.020)
H-B	101.28 (±12.45)	21.08 (±3.91)	1.93 (±0.43)	6.66 (±0.93)	227.48 (±22.66)	282.52 (±60.35)	1.98 (±0.16)	2.67 (±0.40)	-0.024 (±0.027)	-0.002 (±0.024)
H--	94.88 (±7.70)	20.40 (±2.46)	2.05 (±0.30)	7.12 (±0.81)	236.55 (±25.27)	281.65 (±47.80)	1.98 (±0.13)	2.66 (±0.31)	-0.022 (±0.065)	-0.029 (±0.039)
MCB	108.55 (±12.35)	23.15 (±6.65)	1.70 (±0.41)	6.18 (±0.51)	284.48 (±79.77)	285.59 (±95.03)	1.88 (±0.27)	2.50 (±0.55)	-0.006 (±0.011)	-0.033 (±0.060)
MC-	111.37 (±14.66)	20.07 (±4.68)	1.79 (±0.26)	6.89 (±0.77)	231.98 (±19.29)	268.17 (±60.37)	2.00 (±0.13)	2.59 (±0.40)	0.022 (±0.031)	0
M-B	93.73 (±26.75)	19.81 (±5.42)	2.07 (±0.43)	9.14 (±1.25)	224.40 (±43.19)	365.31 (±86.65)	1.93 (±0.24)	3.10 (±0.49)	0.009 (±0.018)	0
M--	95.20 (±16.68)	19.07 (±4.58)	2.23 (±0.63)	8.02 (±1.11)	233.63 (±19.09)	306.09 (±70.31)	2.04 (±0.15)	2.68 (±0.41)	0.024 (±0.040)	-0.014 (±0.023)
-CB	98.07 (±15.56)	24.98 (±5.84)	2.07 (±0.39)	7.95 (±1.06)	241.73 (±25.90)	343.89 (±74.31)	1.99 (±0.15)	2.99 (±0.45)	-0.041 (±0.071)	0
-C-	93.65 (±10.14)	18.52 (±3.80)	2.13 (±0.35)	7.15 (±0.70)	229.65 (±24.32)	261.90 (±70.42)	1.98 (±0.14)	2.52 (±0.42)	0.001 (±0.028)	0
--B	88.08 (±13.78)	20.04 (±4.18)	2.20 (±0.89)	7.75 (±1.16)	220.43 (±45.59)	253.09 (±38.40)	1.89 (±0.29)	2.45 (±0.24)	-0.004 (±0.034)	-0.006 (±0.011)
---	105.60 (±21.48)	24.63 (±5.88)	2.12 (±0.40)	7.35 (±1.14)	239.49 (±26.88)	312.96 (±56.53)	2.01 (±0.11)	2.87 (±0.36)	0.031 (±0.092)	-0.019 (±0.025)

^{14}C processing

Following convention (Stuiver & Polach, 1977), all ^{14}C data were normalised to -25‰ $\delta^{13}C$ to correct for mass-dependent isotopic fractionation and were expressed as %Modern relative to the activity of the NBS Oxalic Acid international radiocarbon standard. One --B replicate was removed from both the $^{14}CO_2$ and $DO^{14}C$ analyses due to producing unrealistically high values (i.e. values of 110.99 and 111.81 %Modern, which were substantially higher than both the peat and charcoal components, suggesting potential contamination from a bomb-peak C source).

A multi-component isotope mass balance approach, based on the two- and three-component mass balance approaches used by Hardie *et al.* (2009), was employed to derive the fluxes and isotopic concentrations of each pot component. The full equation was:

$$D_E F_E = D_P F_P + D_H F_H + D_C F_C + D_B F_B \qquad \textbf{Eq.A14.2}$$

where *D* represents the average isotopic concentration (^{14}C %modern) and *F* the average flux (either DOC or CO_2) kg dry $soil^{-1}$ (see **Eq.A14.1**) apportioned to each component. *E* represents the concentration or flux from the whole 'pot ecosystem', *P* represents that from the peat component, *H* that from the *H. ericae* fungus component (or natural mixed fungal community in one case), *C* that from the *Calluna* root component and *B* that from the burnt material/charcoal. The CO_2 fluxes used were measured less than 24 hours prior to CO_2 collection for radiocarbon analysis (**Table A14.1**) and the DOC concentrations used were those measured in the water samples taken for ^{14}C analysis. For pots only containing some components, appropriate simpler mass balance equations were used. It was assumed that F_E from the --- pots represented the F_P in all other pots meaning that:

$$F_H = F_E - F_P \qquad \textbf{Eq.A14.3}$$

where F_E represented the average flux of the H-- pots. The isotopic concentration of the *H. ericae* fungal component was calculated using:

$$D_H = \frac{D_E F_E - D_P F_P}{F_H} \quad \textbf{Eq.A14.4}$$

where D_E represented the average isotopic concentration of the H-- pots. Similarly, D_B and F_B were calculated using **Eq. A14.3** and **Eq. A14.4** but substituting D_B for D_H and F_B for F_H and using D_E and F_E from the --B pots. Due to the combination of treatments chosen for radiocarbon analysis, the charcoal component flux contributions were calculated in two different ways, using either the --B and --- pots or the HCB and HC- pots. As the -C- pots were not sampled for ^{14}C, the *Calluna* component flux and isotopic concentration were calculated using:

$$F_C = F_E(HC-) - F_E(H--) \quad \textbf{Eq.A14.5}$$

and

$$D_C = \frac{D_E(HC-)F_E(HC-) - D_E(H--)F_E(H--)}{F_C} \quad \textbf{Eq.A14.6}$$

where D_E(HC-) and D_E(H--) represent the average isotopic concentrations from HC- and H-- pots, respectively, and F_E(HC-) and F_E(H--) represent the average fluxes from HC- and H-- pots, respectively. Similarly, D_B and F_B were calculated using **Eq. A14.5** and **A14.6** but substituting D_B for D_C, F_B for F_C, D_E(HCB) and F_E(HCB) for D_E(HC-) and F_E(HC-), and D_E(HC-) and F_E(HC-) for D_E(H--) and F_E(H--).

The component ages were determined using **Eq. A14.7**:

$$Years\ BP = -8033 \ln \frac{D}{100} \quad \textbf{Eq.A14.7}$$

where 'Years BP' is the radiocarbon age in years before present, where 0 years BP = AD 1950, and 8033 represents the mean lifetime of ^{14}C (Stuiver & Polach, 1977). Any samples or components for which the ^{14}C content was >100 %Modern, radiocarbon ages were derived using data from Levin *et al.* (2008) (see Hardie *et al.* (2009) for details).

Statistical analysis

Linear mixed effects models employing the "lmer" function from the "lmerTest" package (Kuznetsova *et al.*, 2016) were used to test for treatment effects on the CO_2 and CH_4 fluxes, DOC concentrations, $SUVA_{254}$ values, Hazen units, E4/E6 ratios and ecosystem $^{14}CO_2$ and ecosystem $DO^{14}C$ content. The treatment, the pre-treatment/post-treatment period and interaction between them were the fixed effects (except for the $^{14}CO_2$ and $DO^{14}C$ content which were only measured post-treatment). For all tests apart from $^{14}CO_2$ and $DO^{14}C$ content, random terms were included for pot (as a repeated measure) and block. For $^{14}CO_2$ and $DO^{14}C$ content, the block was the random term.

Following the 10-step protocol in section 5.10 of Zuur *et al.* (2009), models were checked to determine whether a variable should be kept by removing variables stepwise from each linear mixed effects model and assessing the log-likelihood ratio and AIC value. The "glht" function with the "Tukey" option from the "multcomp" package (Hothorn *et al.*, 2008) was used to determine between which treatments significant differences occurred.

Linear regression tests (employing the function “lm” in the R “stats” package; R Core Team, 2016) were used to determine whether there was a relationship between the DOC concentrations and any of the measured water quality indicators (Abs_{254}, Abs_{400}, Abs_{465} and Abs_{665}). Separate linear regressions were used for each wavelength in each period (pre- and post-treatment).

Results

The ^{14}C content of the measured samples ranged from 61.67 to 111.81 %Modern (**Table A14.4**). As one --B replicate produced unrealistically high values of 110.99 and 111.81 %Modern for $^{14}CO_2$ and DO^{14}C respectively, which were substantially higher than both the peat and charcoal components and suggested significant pot contamination from a bomb-peak C source, these were removed from the analysis (and are highlighted in *italics* in the below **Table A14.4**).

Table A14.4 NERC Radiocarbon Facility - Scottish Universities Environmental Research Centre (SUERC) publication codes and sample types. The ^{14}C and $\delta^{13}C$ content of each sample are shown. Treatment codes refer to the pot treatments, which are laid out in full in **Table A14.2**. Two likely contaminated samples with very high %Modern ^{14}C values are highlighted in *italics*.

Publication code	Sample type	Treatment code	^{14}C content (%Modern ± 1σ)	$\delta^{13}C$ content (‰)
SUERC-64843	CO_2	H--	62.96 ± 0.34	-24.3
SUERC-64844	CO_2	---	61.67 ± 0.34	-24.7
SUERC-64848	CO_2	HCB	83.45 ± 0.39	-26.6
SUERC-64849	CO_2	--B	73.23 ± 0.36	-19.7
SUERC-64850	CO_2	HC-	89.25 ± 0.41	-27.1
SUERC-67911	CO_2	---	68.03 ± 0.35	-19.9
SUERC-67912	CO_2	HC-	83.68 ± 0.37	-27.0
SUERC-67913	*CO_2*	*--B*	*110.99 ± 0.51*	*-20.7*
SUERC-67917	CO_2	H--	62.97 ± 0.35	-23.9
SUERC-67918	CO_2	HCB	85.91 ± 0.40	-26.8
SUERC-67919	CO_2	---	67.86 ± 0.33	-21.2
SUERC-67920	CO_2	H--	67.31 ± 0.34	-22.3
SUERC-67921	CO_2	HC-	78.84 ± 0.38	-21.9
SUERC-67922	CO_2	--B	66.43 ± 0.35	-24.3
SUERC-67923	CO_2	MCB	74.79 ± 0.37	-26.6
SUERC-65920	DOC	H--	71.57 ± 0.33	-26.5
SUERC-65921	DOC	---	71.80 ± 0.32	-27.0
SUERC-65922	DOC	HCB	75.36 ± 0.33	-26.7
SUERC-65923	DOC	--B	67.99 ± 0.32	-27.7
SUERC-65924	DOC	HC-	74.30 ± 0.33	-25.9
SUERC-67898	DOC	---	74.00 ± 0.34	-26.6
SUERC-67899	DOC	HC-	74.34 ± 0.33	-26.9
SUERC-67900	*DOC*	*--B*	*111.81 ± 0.49*	*-27.6*
SUERC-67901	DOC	H--	72.53 ± 0.33	-26.8
SUERC-67902	DOC	HCB	74.48 ± 0.34	-26.8
SUERC-67903	DOC	---	70.64 ± 0.33	-27.4
SUERC-67907	DOC	H--	72.25 ± 0.33	-27.3
SUERC-67908	DOC	HC-	74.26 ± 0.34	-26.8
SUERC-67909	DOC	--B	71.38 ± 0.31	-27.0
SUERC-67910	DOC	MCB	74.94 ± 0.33	-27.0
SUERC-65527	Peat	n/a	69.54 ± 0.32	-27.7
SUERC-65528	Peat	n/a	68.06 ± 0.31	-27.8
SUERC-67897	Peat	n/a	68.38 ± 0.32	-27.5
SUERC-68198	Charcoal	n/a	104.95 ± 0.48	-30.2

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