Quantifying charcoal degradation and negative priming of soil organic matter with a radiocarbon-dead tracer

Emma L. Tilston[a,1], Philippa L. Ascough[a]*, Mark H. Garnett[b], Michael I. Bird[c]

[a]Scottish Universities Environmental Research Centre, Rankine Avenue, Scottish Enterprise Technology Park, East Kilbride, G75 0QF, Scotland, UK.

[b]NERC Radiocarbon Facility, Rankine Avenue, Scottish Enterprise Technology Park, East Kilbride, G75 0QF, Scotland, UK.

[c] School of Earth and Environmental Sciences and Centre for Tropical Environmental and Sustainability Science, James Cook University, Cairns, Queensland, 4870, Australia

[1]Present address: NIAB EMR, New Road, East Malling, Kent, ME19 6BJ, UK.

*Corresponding author: philippa.ascough@gla.ac.uk

ABSTRACT

Converting biomass to charcoal produces physical and chemical changes greatly increasing environmental recalcitrance, leading to great interest in the potential of this carbon form as a long-term sequestration strategy for climate change mitigation. Uncertainty remains however, over the timescale of charcoal's environmental stability, with estimates varying from decadal to millennial scales. Uncertainty also remains over charcoal's effect on other aspects of carbon biogeochemical cycling and allied nutrient cycles such as nitrogen. Radiocarbon is a powerful tool to investigate charcoal mineralization due to its sensitivity; here we report the results of a study using radiocarbon-dead charcoal (pMC = 0.137 ± 0.002) in organic rich soil (pMC = 99.76 ± 0.46), assessing charcoal degradation over 55 days of incubation. Using this method we discriminated between decomposition of indigenous soil organic matter (SOM) and

charcoal by microorganisms. SOM was the major source of carbon respired from the soil, but there was also a contribution from charcoal carbon mineralization. This contribution was 2.1 and 1.1 % on days 27 and 55 respectively. We also observed a negative priming effect due to charcoal additions to soil, where SOM mineralization was repressed by up to 14.1%, presumably arising from physico-chemical interactions between soil and charcoal.

KEYWORDS

Biochar, Black carbon, Charcoal, Molecular sieve, Soil respiration

1. Introduction

Charcoal is produced when biomass is heated in an oxygen-poor environment (pyrolysis), and is the result of natural or human-mediated burning events. Intentional production of charcoal by humans has enabled its use as a fuel, a reductant, an adsorbent and a soil amendment for millennia (Antal and Grønli 2003). These uses exploit the fact that conversion of biomass to charcoal results in a highly aromatic, carbon-rich, O/H/N/S poor material that has high porosity and reactivity, with a large surface area. The high aromaticity of charcoal confers chemical recalcitrance, leading to the persistence of at least some charcoal in the environment for thousands, or even millions of years (Preston and Schmidt 2006; Schmidt et al. 2002; Collinson et al. 2000). However, some charcoals have been observed to degrade over much shorter timescales, with loss at the decadal scale being reported for charcoal deposited in soils (Bird et al. 1999; Ascough et al. 2011; Zimmermann et al. 2012). Currently there is considerable interest in the production of charcoal as a soil amendment (in this context known as ‘biochar’) as a means of mitigating climate change through long-term terrestrial sequestration of carbon (C) in refractory forms (Lehmann et al. 2006; Ciais et al. 2013; Gurwick et al., 2013).

In this study we apply both stable isotope ratio measurements and measurement of radiocarbon by accelerator mass spectrometry (AMS) to quantify the decomposition rates of labile (i.e. indigenous) soil organic C and recalcitrant (i.e. charcoal) C in an acidic soil rich in organic matter. The use of radiocarbon content as a tracer to detect microbial utilization of charcoal C has significant advantages. Stable carbon isotope ratio measurements of natural abundance $^{13}C/^{12}C$ (expressed as ´ ^{13}C) have been used to quantify the turnover of charcoal C in soils (e.g. Major et al. 2010). These studies predominantly exploit the isotopic difference between organic matter derived from C_3 and C_4 photosynthetic pathways, for example, the incubation of C_3 charcoal (´ ^{13}C = c.-25‰) in soil containing organic matter from C_4 plants (´ ^{13}C = c.-12‰). However, the approach has insufficient measurement sensitivity for reliable deployment in situations where one source of carbon (e.g. charcoal) makes a much smaller contribution to the respired carbon in comparison with another source (e.g. SOM). In contrast, measurement of charcoal with a ^{14}C content distinct from that of SOM provides an ultra-sensitive means of quantifying contributions from charcoal mineralization within the short timescales of most experiments. In previous work, charcoal that is labelled, or artificially enriched, with ^{14}C has been used to good effect (Kuzyakov et al. 2009; Kuzyakov et al. 2014). Analyses of recent material containing bomb ^{14}C have also provided much information on the turnover rates and mean residence times (MRTs) of individual SOM pools in undisturbed systems (Pataki et al. 2003). In this study we employ an alternative approach, using charcoal prepared from material with a highly depleted ^{14}C content relative to modern biomass (i.e. '^{14}C-dead'), which avoids the logistical issues associated with the use of enriched material in most ^{14}C laboratories (e.g. use of dedicated lines to avoid cross-contamination). This approach has been used to study C turnover where ^{14}C-dead CO_2 from fossil fuel sources has been used to introduce a distinctive ^{14}C-depleted signal to living plants e.g. field-scale free-air CO_2 enrichment (FACE) experiments (Leavitt et al. 1994; Staddon et al. 2003), but has not

to our knowledge been used to study charcoal mineralization rates. The present study therefore employs this approach to distinguish between the decomposition of indigenous SOM and charcoal, thereby resolving some of the uncertainties surrounding the rate of charcoal decomposition in soil.

2. Material and methods

2.1. Soil and amendments

'Radiocarbon dead' *Nothofagus* spp. wood (Table 1) was obtained from the Yallourn seam of brown coal (13.6 – 16.3 Ma), south eastern Australia (38.195 S, 146.360 E). Despite the great age of this wood, it retains much of its original ligno-cellulosic structure and despite some post-depositional alteration it is representative of typical woody biomass (see section 3.1 below). Large (up to 5 m) fragments of tree trunks, often retaining their original bark, are among the common readily identifiable macroscopic plant remains present in the Yallourn seam (Holdgate et al. 2009). One hundred gram sub-samples of dried (105°C, 24 h), chipped (2-4 mm) wood were pyrolyzed to charcoal for 1 hour at 300°C under a stream of nitrogen (3.5 L min^{-1}). Soil (0-10 cm depth) from the A-horizon of a Typic Humaquept (Soil Survey Staff 2006) was collected from under a mixed stand of mature trees in west central Scotland (55.757 N, 04.163 W). The soil was sieved to 2 mm in the field moist state (Table 1) and stored in a sealed plastic bag at 5˚C for 48 hrs before use.

2.2. Soil microcosms and CO_2 measurement

Triplicate microcosms comprising aliquots of field moist soil equivalent to 30 g dry weight were established for each treatment in gas-tight glass preserving jars (1011 mL capacity), which had been modified to accommodate two gas sampling ports (Fig. 1). The moisture content of the soil was adjusted to 70% water-holding capacity and based on the

104 different carbon contents and substrate availability, the microcosms were amended with
105 either 3.0 g wood chips or 1.7 g charcoal, with unamended soil as a control sample. The
106 mass of wood or charcoal mixed with the soil was calculated on the basis that the added
107 carbon comprised 20% of C in the wood/soil or charcoal/soil mixture, based on a wood %C
108 of 40% and charcoal %C of 70% (see Ascough et al., 2008). These values are
109 commensurate with typical standard applications of organic amendments (including
110 biochar) to arable soil (Soffe, 1995), and should allow for a sensitive test for charcoal CO_2
111 emissions under representative conditions, without danger of major alteration of the soil
112 physico-chemical properties and allied impacts on microbial activity.
113 The microcosms were incubated with one sampling port open for ventilation, in the
114 dark at 20-21°C for 55 days. Soil respiration rates were determined on a weekly basis by
115 measuring headspace CO_2 accumulation during a 4 hour period with an infra-red gas
116 analyser (EGM-4, PP Systems, Hitchin, UK); after which the soil moisture content was
117 checked and adjusted with additions of distilled water as required.
118
119 *2.3. Elemental and isotope ratio analyses*
120 Four days prior to gas sampling, the headspace gases of each jar were pumped
121 through a soda lime cartridge to exclude CO_2 and the jars were sealed. Four days later,
122 on days 27 and 55 of the incubation, the CO_2 accumulated in headspace gases was
123 collected from the unamended and charcoal-amended microcosms using a pump-based
124 molecular sieve technique (Hardie et al. 2005). To provide sufficient sample for analysis,
125 CO_2 concentrations reached between 1.0-1.7 %; while high CO_2 concentrations can affect
126 microbial activity, these concentrations were well within the usual range experienced by
127 soils (1-5 %; ` antro• ková and ` imek 1997). One aliquot of CO_2 was analysed for ´ ^{13}C by
128 isotope ratio mass spectrometry (VG Optima, Micromass, Manchester, UK) for
129 normalization of sample $^{14}C/^{13}C$ and a second aliquot was cryogenically purified and

converted to graphite for analysis using Fe/Zn reduction (Slota et al. 1987). Sample $^{14}C/^{13}C$ ratios were measured by AMS at the Scottish Universities Environmental Research Centre. Measured $^{14}C/^{13}C$ ratios were normalized to ´ ^{13}C of -25‰ and expressed as %modern carbon (pMC) according to Stuiver and Polach (1977). The background ^{14}C measurement for the molecular sieve process was 0.37% modern, based upon analysis of ^{14}C-dead radiocarbon CO_2 standards collected using the same molecular sieve sampling system (Garnett and Murray, 2013). Although a specific incubation process background was not obtained, this was deemed unnecessary due to the fact that the respired CO_2 had a modern $^{14}C/^{13}C$ ratio.

Total C and nitrogen (N) contents and ´ ^{13}C values of the soil, wood and charcoal samples described above were determined on ball-milled sub-samples using a Deltaplus XL isotope ratio mass spectrometer (IRMS, Thermo Finnigan GmbH, Bremen, Germany), linked to a Costech elemental analyzer (Milan, Italy) via a ConFlo III (Werner et al. 1999). Each sample sequence run included a mix of samples, laboratory standards and blanks, with precision better than ± 0.20 (1Ã) for ´ ^{13}C. The stable C isotope values (´ ^{13}C) are reported as per mil (‰) deviations from the VPDB international standard. Total O and H contents were also determined on ball-milled sub-samples using a high temperature conversion elemental analyzer (TC/EA) connected via a Conflo III to a DeltaPlus XP IRMS (all Thermo Finnigan, Bremen, Germany). Precisions for the quality control standard (benzoic acid) were: total H = 5.26 ± 0.07% and total O = 26.74 ± 0.27% (mean ± sd, n = 5).

2.4. ^{13}C CP-SS NMR

Solid-state ^{13}C Nuclear Magnetic Resonance Spectroscopy, using cross-polarization magic angle spinning (^{13}C-CP-SS NMR spectroscopy) was used to characterize the uncharred wood and the charcoal produced at 300°C. The ^{13}C CP-SS

NMR spectra were recorded using a 4-mm MAS probe at a ^{13}C frequency of 100.56 MHz on a Varian VNMRS instrument. Samples were spun at 10 or 12 kHz with a CP contact time of 1 or 2 ms and a 1-s recycle time. Spectra were referenced to neat tetramethylsilane and interpreted according to the following chemical shift limits (after Wilson, 1987): 0 to 45 ppm = methyl- and alkyl-C (indicative of aliphatic compounds including amino acids, lipids and waxes); 45 to 60 ppm = methoxyl-C and N-alkyl-C (indicative of lignin substituents, amino acids and amino sugars); 60 to 110 ppm = O-alkyl-C, acetal- and ketal-C (indicative of monomeric and polymeric sugars); 110 and 145 ppm = aromatic-C (indicative of phenyl compounds including lignin and tannins); 145 and 160 ppm = O-aromatic-C (indicative of phenyl compounds); 160 and 190 ppm = carbonyl-C (indicative of organic acids and peptides).

2.5. Wet chemical analyses

Soil pH was determined on 1:2.5 w/v aqueous suspensions of soil. Colorimetric methods were used to determine the phenolic content of hot water-extracts (1:500 w/v, 100°C, 1 h) of wood and charcoal (Box 1983), the concentrations of extractable (1:4 w/v 0.5 M K_2SO_4) nitrate (Cataldo et al. 1975) and ammonium (Anderson and Ingram 1993) and to estimate the microbial biomass content after chloroform-fumigation (Amato and Ladd 1988). Estimates of ninhydrin-reactive microbial biomass N were converted to microbial biomass carbon using a conversion factor of 35.3, as proposed by Joergensen (1996) for soils with a pH of 5 or less. The total metal contents of the wood and charcoal were determined by inductively coupled plasma – optical emission spectroscopy (Optima 5300 DV, Perkin Elmer, Waltham, USA) after aqua-regia digestion (0.1 g sample + 1.5 mL ARISTAR grade concentrated hydrochloric acid and 1.5 mL ARISTAR grade concentrated nitric acid, VWR, Lutterworth, UK).

182 *2.6. Soil microbiology*

183 The size of culturable microbial populations in the presence of unextracted and hot
184 water-extracted wood and charcoal were determined using spread agar plates and the
185 wood and charcoal samples used to determine the concentration of hot water-extractable
186 phenolics. Fifty milligram aliquots of surface sterilized (5 min. 10% commercial sodium
187 hypochlorite, 1.5% free chlorine; rinsed 2× sterile distilled water) wood or charcoal were
188 suspended in 100 µL sterile distilled water and applied to the surface of 10% tryptic soy
189 agar (Sigma-Aldrich, Gillingham, UK). The plates were then inverted and allowed to dry
190 before 100 µL of a 10^{-3} dilution of a 1:20 w/v soil suspension was spread over each plate.
191 The diluent throughout was 10% tryptic soy broth. Plates were incubated at 20°C and
192 enumerated after 7 and 14 days.
193
194 *2.7. Data handling and statistics*

195 Decomposition rates were modelled using the first order negative exponential decay
196 equation (Jenny et al. 1949) (Eq. 1):
197
198 $$C_t / C_0 = C_0 \bullet e^{-kt} \quad (1)$$
199
200 where C_t is the amount of original carbon (C_0) remaining at time *t*, and *k* is the
201 decomposition rate constant, or the slope term in the logarithmic transformation of Eg. 1
202 (Eq. 2):
203
204 $$\log_n (C_t / C_0) = \log_n C_0 - k \bullet t \quad (2)$$
205
206 If the mean residence time of a given substrate is defined as being the time taken for 90%
207 decomposition to occur, i.e. $C_t / C_0 = 10 / 100 = 0.1$, or -1 after $\log_{10}$ transformation; then

by rearranging Eq. 3, the mean residence time can be estimated as the reciprocal of *k* (Cresser et al 1993).

$$k \bullet t = -1 \quad (3)$$

The % contribution of ^{14}C-depleted charcoal to respired CO_2-C ($C_{Charcoal}$%) was calculated by isotope mass balance using a two-component mixing model (Eq. 4):

$$C_{Charcoal}\% = ((\%mod_{AResp} - \%mod_{UnResp}) / (\%mod_{Charcoal} - \%mod_{UnResp})) \bullet 100 \quad (4)$$

where the ^{14}C content of CO_2 emitted from unamended soil ($\%mod_{UnResp}$), represents a 100% contribution from the SOM fraction being actively turned over by the microorganisms (cf. ^{14}C total soil, Table 1) and the ^{14}C content of the pure charcoal ($\%mod_{Charcoal}$ = 0.137% modern (Bird and Ascough 2012)) represents 100% charcoal-derived CO_2; $\%mod_{AResp}$ is the ^{14}C content of CO_2 respired by charcoal-amended soil. The propagated standard deviation ($SDC_{Charcoal}$%) of values obtained were calculated using the standard deviations of %modern values for CO_2 respired by charcoal-amended soil (SD_A) and unamended soil (SD_U) and the dynamic range associated with each treatment based on the %modern values for respired CO_2, the added charcoal and for total soil ($\%mod_{Soil}$ = 99.76%modern) according to Eq. (5):

$$SDC_{Charcoal}\% = \bullet (SD_A / (\%mod_{AResp} - \%mod_{Charcoal}))^2 + (SD_U /(\%mod_{UnResp} - \%mod_{Soil}))^2 \quad (5)$$

The SOM contribution to respired CO_2 ($SOM_{Resp}C_A$) in charcoal-amended soil was calculated with Eq. (6) using the respired C flux from charcoal-amended soil ($RespC_A$) on days 29 and 50 (the nearest days for which we have flux measurements) and the % contribution of ^{14}C-depleted charcoal to respired CO_2-C ($C_{Charcoal}$%, from Eq. 4):

$$SOM_{Resp}C_A = RespC_A - (RespC_A \bullet (C_{Charcoal}\% / 100)) \quad (6)$$

In Eq. (4) the reduction in SOM mineralization in charcoal-amended soil was estimated as the relative mineralization intensity (RMI) using the SOM contribution to respired CO_2 ($SOM_{Resp}C_A$) in charcoal-amended soil as calculated in Eq. (6) and the amount of C respired by unamended soil ($RespC_U$):

$$RMI = ((RespC_U - SOM_{Resp}C_A) / RespC_U) \bullet 100 \quad (7)$$

All statistical analyses (two-way analysis of variance, repeated measures analysis of variance, residual maximum likelihood analysis and the Bartlett-Box F-test) were performed using Genstat 13 (VSN International, Hemel Hempstead, UK). Significant differences between means were identified with Fisher's least significant difference test ($P < 0.050$).

3. Results and discussion

3.1. Charcoal chemistry

The C content of the wood (~56%, Table 1) used in this study was slightly greater than that of contemporary *Nothofagus* trees (~49% C) (Clinton et al. 2009), and the ^{13}C CP-SS NMR spectrum shows some depletion of the most labile fractions (cellulose and hemicellulose) (Fig. 2). The overall spectrum remains however, similar to that of typical

woody biomass (Hopkins and Chudek 1997), with distinct signals in shift ranges 20-45 ppm and 60-90 ppm, with a number of smaller peaks between 90-160 ppm, indicating the presence of waxes and fatty acids, hemicellulose and cellulose and carboxylic acids, plus various phenolic components (lignin). After pyrolysis the relative abundance of peaks between 60 and 90 ppm (cellulosic polymers) is reduced, but increased for peaks between 110 and 145 ppm (ligninaceous phenolics) (Fig. 2). Therefore, the charcoal produced contains predominantly aromatic forms of C, but some untransformed lipids, waxes and cellulosic polymers remain. It is also notable that as a consequence of the partially transformed chemistry of the woody starting material the C content of the charcoal produced at 300°C for use in this study is similar to that of charcoals produced at greater temperatures e.g. 400 or 550°C for the studies of Cross and Sohi (2011) and Kasozi et al. (2010) respectively.

3.2. Soil respiration dynamics and ^{14}C measurements

Consistent with the contrasting abundances of labile components such as hemi-cellulose and cellulose, as indicated by the NMR spectra, soil respiratory activity was significantly greater in the three days after amendment with wood than after amendment with charcoal (Fig. 3). Thereafter the respiration rates of all three treatments converged and continued to decline for the remainder of the incubation period. After day-15 the respiration rates of the treatments diverged resulting in small (~6.5%), but statistically significant, reductions in soil respiration from day-29 onwards for wood amendment and on day-50 for charcoal amendment. Soil respiration rate typically shows a bi-phasic response to the addition of organic matter, whereby mineralization of the labile fraction results in the short-lived increase in respiratory activity and the longer-lived decline is attributed to decomposition of more recalcitrant components (Marstorp 1997). The results of ^{14}C measurements on samples of head-space gases accumulating above charcoal-amended

285 and unamended control soils are given in Table 2. Over the course of the experiment the
286 total amount of C respired, as calculated on the basis of ^{14}C measurements, was
287 equivalent to 1.22% of total C present in unamended soil, with significantly less C being
288 mineralized after amendment with either wood or charcoal (Tables 2 and 3). These
289 differences between treatments are also reflected in similarly reduced decomposition rate
290 constants (Table 3) and derived mean C residence times of 11.2 years for C in
291 unamended soil and 15.4 and 13.8 years for C in wood- and charcoal-amended soils
292 respectively. The reduced amount of C mineralized from both wood- and charcoal-
293 amended soils was also associated with about a 30% reduction in microbial biomass in
294 charcoal-amended soil or a 9% reduction in wood-amended soil (Table 4), compared with
295 that measured in unamended soil.
296
297 *3.3. Assessment of amendment toxicities*
298 It is unlikely that the reductions in microbial biomass and allied responses are due
299 to the amendments being toxic, or inducing toxicity by concurrently reducing soil pH and
300 increasing the free-ion concentration of heavy metals (Lofts et al. 2004). Not only were the
301 concentrations of potentially toxic elements in both the wood and the charcoal (Table 1)
302 considerably less than the threshold values proposed for biochar (International Biochar
303 Initiative 2013), their addition had a negligible impact on soil pH (Table 3). Furthermore, in
304 agar-plate based microbial toxicity tests no statistically significant (P = 0.411) differences
305 in the number of colonies of culturable microorganisms were observed between either
306 amendment, or after removal of soluble components by hot water extraction (Table 4).
307
308 *3.4. Resource availability limits microbial respiration*
309 Instead of amendment toxicity, we propose that the size of the microbial population
310 declined because the availability of a key nutrient, such as nitrogen, became limiting

311 resulting in the death of a proportion of the microbial community (Miltner et al. 2012).
312 Extractable (available) nitrogen, as nitrate-N and ammonium-N, concentrations declined
313 after wood and charcoal addition (Table 3). Unusually for soils recently amended with
314 organic matter with a high C-to-N ratio there was no concomitant increase in microbial
315 biomass N (from which the microbial biomass C data in Table 3 are derived), attributable
316 to the immobilization of mineral N within microbial tissues. Losses of N through
317 conversion of mineral N to nitrous oxide are unlikely to have occurred because the soil
318 used had been sieved to promote aeration and the moisture content was maintained below
319 that required for the development of anaerobic conditions and denitrification (Linn and
320 Doran 1984). Furthermore, acidic soils often have lower denitrification potential than more
321 neutral soils because of the indirect action of limitations such as the availability of labile C
322 and slower rates of N mineralization (Šimek and Cooper 2002; Šimek et al. 2002). There
323 are, however, two physico-chemical explanations for the reductions in mineral N observed
324 following the addition of both amendments. Not only do charcoals have considerable
325 adsorption and cation exchange capacity for mineral N, especially NH_4^+ (Clough et al.
326 2013); but also the wood used was rich in extractable polyphenolics which can form stable
327 complexes with organic forms of nitrogen (Northup et al. 1995), such as the amino sugars
328 released during lysis of microbial necromass (Miltner et al. 2012).
329 The presence of conditions that constrained microbial activities on day 50 is further
330 indicated by consideration of metabolic quotients (qCO_2), which are defined as the
331 microbial respiration rate (measured CO_2 efflux) per unit of microbial biomass (Anderson
332 and Domsch 1993). Although larger quotients are associated with the addition of more
333 respirable substrates (Sparling, 1997), they are also associated with the greater energetic
334 requirements of cell maintenance under environmental stress conditions (Odum 1985;
335 Anderson and Domsch 1993). The chemistry (high C-to-N ratio) of both the charcoal and
336 wood suggests that neither were labile decomposition substrates, so it is more likely that

337 the smaller elevation of qCO_2 in charcoal-amended soil (Table 3) is due to the microbial
338 community experiencing less favorable conditions.
339
340 *3.5. Isotopic partitioning of respiratory C sources*
341 Although both the indigenous SOM and the added charcoal had distinct $\delta^{13}C$
342 isotopic signatures (Table 1), this did not translate into a significant difference between the
343 $\delta^{13}C$ values for the C respired by unamended and charcoal-amended soils (Tables 2 and
344 3). However, the greater analytical sensitivity and potential dynamic range (0.137 pMC to
345 ~100 pMC) associated with measuring the natural abundance of ^{14}C compared with ^{13}C
346 (where the dynamic range is -21 to -28‰), did enable attribution of the relative
347 contributions of SOM and charcoal to soil respiration. The abundance of ^{14}C in respired
348 CO_2 was diminished by only ~1.75 pMC after charcoal amendment (average of both 27
349 and 55-day incubations, Tables 2 and 3); this indicates that SOM was the predominant
350 source of respiratory C. Furthermore, the slight ^{14}C-enrichment relative to the
351 contemporary atmosphere indicates that most of the respired C had been originally fixed
352 within the last few decades. Even though the reduction in the ^{14}C content of the CO_2
353 emitted by charcoal-amended soil relative to unamended soil was small (Tables 2 and 3),
354 it was nonetheless statistically significant (P <0.001) and was unaffected by sampling day
355 (P = 0.093, two-way ANOVA interaction term). The contribution of charcoal C to respired
356 C (calculated according to equation 4) was 2.1% (1 sd = 0.05) on day 27, declining to
357 1.1% (1 sd = 0.07) on day 55 and these values are similar to those reported by Kuzyakov
358 et al. (2009) and Major et al. (2010) following the addition of charcoals with comparable C
359 contents to soil. Furthermore, if the C-respiration data are revised accordingly to reflect
360 the decomposition of charcoal only, the decomposition rate constant declines to -3.03 d^{-1} x
361 10^{-6} and the corresponding mean residence time increases to 903.7 years. These values
362 are slightly slower than the values reported by Kuzyakov et al. (2009, 2014) for charcoal

363 decomposing under laboratory conditions; but the ~85-fold difference from indigenous soil
364 C is nonetheless consistent with the expected greater recalcitrance of charcoal.
365 The reduction in SOM mineralization (Eqs 6 and 7) in charcoal-amended soil (RMI)
366 was estimated to be 11.7% on day 27 and 14.1% on day 55. Other studies have also
367 reported retardation of SOM decomposition after the addition of high temperature charcoal
368 to organic C-rich soils (e.g. Kuzyakov et al. 2009 and 2015; Cross and Sohi 2011;
369 Zimmerman et al. 2011). Various physico-chemical mechanisms by which the availability
370 of both decomposition substrates and key nutrients can be reduced have been proposed
371 to account for this response, including the sorption of mineral N (as previously discussed)
372 and the sorption of labile SOM fractions to charcoal (Kasozi et al. 2010). Reduced
373 accessibility of SOM fractions, and an allied depletion of the range of respiratory
374 substrates used by the microbial community in the presence of charcoal is also suggested
375 by the significantly greater homogeneity of variance (i.e. smaller standard deviations) in
376 the ´ ^{13}C signature of respired C (Table 3; P = 0.026).
377
378 *3.6. Positive and negative priming*
379 The addition of charcoals to soil is frequently associated with so-called ‘priming’
380 effects (Zimmerman et al. 2011), which are short-term changes in the decomposition of
381 SOM brought about by the addition of organic or mineral substances (Jenkinson et al.
382 1985; Kuzyakov et al. 2000). As documented by the expanding and often contradictory
383 literature, charcoal-SOM-microbe relationships are complicated and dynamic, with a range
384 of priming effects occurring both concurrently and sequentially. With respect to the results
385 of our experiment, it is probable that initial decomposition of the labile fraction of the
386 charcoal was facilitated by synchronous provision of additional labile C and N by microbial
387 decomposition of indigenous SOM, i.e. positive priming. In the longer term, the loss of the
388 most labile component of charcoal increased its recalcitrance to further decomposition

processes. As the incubation proceeded, the effects of kinetically slower charcoal-soil interactions such as sorption of SOM and N became more influential with the result that not only was SOM mineralization repressed (Zimmerman et al. 2011), but the amount of microbial biomass present was also reduced (Dempster et al. 2012), resulting in negative priming of SOM decomposition (Blagodatskaya and Kuzyakov 2008). As reported by this study, negative priming typically develops in the 4- to 5-weeks after the addition of 200-500% more substrate C than that present as microbial biomass C, which was also observed in the work by Blagodatskaya and Kuzyakov 2008.

The absence of other treatments such as amendment with ^{14}C-labelled glucose to introduce an isotopic signature for microbial biomass (Blagodatskaya et al. 2011) or additional analyses such as ^{14}C dating of the microbial biomass (Garnett et al. 2011) means that we are unable to partition the respired C between microbial biomass C and SOM C sources. Consequently, with particular reference to the wood amended soil, it is not possible to distinguish between 'real' and 'apparent' priming effects. During a 'real' priming effect there is additional decomposition of recalcitrant SOM as a result of co-metabolism and greater enzyme production, possibly because of activation of previously dormant microbes with substrate-specific metabolic capabilities. However, an apparent priming effect is one in which there is a change in the turnover of microbial biomass that is unrelated to change in SOM decomposition (Jenkinson et al. 1985; Kuzyakov et al. 2000). The period of maximal respiration occurring in the 1- to 3-days after the addition of the wood chips is most likely to be an apparent priming effect arising from pool substitution and related to changes in activity within the microbial community and the microbial demand for other nutrients such as N (Blagodatskaya and Kuzyakov 2008). In addition, further work is required to investigate the longer-term effects, such as the duration of the resource-limited conditions.

4. Conclusions

The approach used in this study employing ^{14}C-dead charcoal as a tracer enabled partitioning of respired CO_2-C into charcoal and indigenous SOM sources. Not only was it possible to measure the small contributions of charcoal C to respired C (<2.1%) at specific times during the incubation, but quantification of opposing concurrent effects, specifically charcoal decomposition and inhibition of indigenous SOM decomposition (by up to 14.1% on day 55) was also possible. Further work is required in order to determine the direction, persistence and extent of these effects in the long-term. The use of ^{14}C-depleted source material for quantification of charcoal-C mineralization rates represents a viable alternative to ^{14}C-enriched tracers. Potential sources of ^{14}C-depleted material include controlled environment plant growth facilities with regulated atmospheres (Romer 2001) using commercially available 'industrial' radiocarbon-dead CO_2 (Topham 1986). Overall, we recommend that our approach is most suited to fine-scale studies of functional microbial ecology at the soil-charcoal interface.

Acknowledgments

Samples of ^{14}C-depleted *Nothofagus* wood were kindly provided by Mr B. Wood, (TRUenergy, Australia). We thank David Apperley and the EPSRC NMR facility (Durham, UK) for the NMR experiments and Barry Thornton (James Hutton Institute, UK) for the elemental analyses for oxygen and hydrogen. Andrew Tait, Caroline Donnelly and Julie Dougans provided excellent technical assistance at SUERC, as did Lorna English at the University of Stirling. The cooperation of Gillian MacKinnon (SUERC) and Philip Wookey (University of Stirling) for access to laboratory facilities is also gratefully acknowledged. Support for ^{14}C measurements within this study was provided through the NERC Radiocarbon Facility NRCF010001 (alloc. 1510.1010). Overall financial support was provided by NERC (NE/F017456/1).

441

442 **References**

443 Amato M, Ladd JN. 1988. Assay for microbial biomass based on ninhydrin-reactive
444 nitrogen in extracts of fumigated soils. *Soil Biology & Biochemistry* 20:107–114.

445 Anderson JM, Ingram JSI. 1993. *Tropical Soil Biology and Fertility: A Handbook of*
446 *Methods.* Wallingford: CAB International.

447 Anderson T-H, Domsch DKH. 1993. The metabolic quotient for CO_2 (qCO_2) as a specific
448 activity parameter to assess the effects of environmental conditions, such as pH, on
449 the microbial biomass of forest soils. *Soil Biology & Biochemistry* 25:393-395.

450 Antal MJ, Grønli M. 2003. The art, science and technology of charcoal production.
451 *Industrial Engineering and Chemistry Research* 42:1619-1640.

452 Ascough PL, Bird MI, Francis SM, Thornton B, Midwood A, Scott AC, Apperley D. 2011.
453 Variability in oxidative degradation of charcoal: influence of production variables
454 and environmental exposure. *Geochimica et Cosmochimica Acta* 75:2361-2378.

455 Bird MI, Ascough PL. 2012. Isotopes in pyrogenic carbon: a review. *Organic*
456 *Geochemistry* 42:1529-1539.

457 Bird MI, Moyo E, Veenendaal E, Lloyd JJ, Frost P, 1999. Stability of elemental carbon in a
458 savanna soil. *Global Biogeochemical Cycles* 13:923–932.

459 Blagodatskaya E, Kuzyakov Y. 2008. Mechanisms of real and apparent priming effects
460 and their dependence on soil microbial biomass and community structure: critical
461 review. *Biology and Fertility of Soils* 45:115-131.

462 Blagodatskaya E, Yuyukina T, Blagodatsky S, Kuzyakov Y. 2011. Three-source-
463 partitioning of microbial biomass and of CO_2 efflux from soil to evaluate
464 mechanisms of priming effects. *Soil Biology & Biochemistry* 43:778-786.

465 Box JD. 1983. Investigation of the Folin-Ciocalteau phenol reagent for the determination
466 of polyphenolic substances in natural waters. *Water Research* 17:511-525.

Cataldo DA, Haroon M, Schrader LE, Youngs VL. 1975. Rapid colorimetric determination of nitrate in plant tissues by nitration of salicylic acid. *Communications in Soil Science and Plant Analysis* 6: 71–80.

Ciais P, Sabine C, Bala G, Bopp L, Bovkin V, Canadell J, Chhabra A, DeFreis R, Galloway J, Heimann M, Jones C, Le Quéré C, Myneni RB, Piao S, Thornton P, 2013. Carbon and other biogeochemical cycles. In: Stocker TF, Quin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM, editors. *The Physical Science Basis. Contribution of Working Group I to the fifth assessment report of the Intergovernmental Panel on Climate Change.* Cambridge: Cambridge University Press.

Clinton PW, Buchanan PK, Wilkie JP, Smail, SJ, Kimberley MO. 2009. Decomposition of *Nothofagus* wood *in vitro* and nutrient mobilization by fungi. *Canadian Journal of Forest Research* 39:2193-2202.

Clough TJ, Condron LM, Kammann C, Müller C. 2013. A review of biochar and soil nitrogen dynamics. *Agronomy* 3:275-293.

Collinson ME, Featherstone C, Cripps JA, Nichols GJ, Scott AC. 2000. Charcoal- rich plant debris accumulations in the lower cretaceous of the Isle of Wight, England. *Acta Palaeobotanica* 164:93–105.

Cresser M, Kilham K, Edwards T. 1993. *Soil Chemistry and its Applications*. Cambridge: Cambridge University Press.

Cross A, Sohi SP. 2011. The priming potential of biochar products in relation to labile carbon contents and soil organic matter status. *Soil Biology & Biochemistry* 43:2127-2134.

Dempster DN, Gleeson DB, Solaiman ZM, Jones DL, Murphy DV. 2012. Decreased soil microbial biomass and nitrogen mineralisation with Eucalyptus biochar addition to a coarse textured soil. *Plant and Soil* 354:311-324.

493 Garnett MH, Bol R, Bardgett RD, Wanek W, Bäumler R, Richter A. 2011. Natural
494 abundance radiocarbon in soil microbial biomass: results from a glacial foreland.
495 *Soil Biology & Biochemistry* 43:1356-1361.
496 Garnett MH, Murray C. 2013. Processing of CO_2 samples collected using zeolite molecular
497 sieve for ^{14}C analysis at the NERC Radiocarbon Facility (East Kilbride UK).
498 Radiocarbon 55, 410–415
499 Gurwick NP, Moore LA, Kelly C, Elias P. 2013. A systematic review of biochar research,
500 with a focus on its stability in situ and its promise as a climate change mitigation
501 strategy. *PLOS One* 8: e75932.
502 Hardie SML, Garnett MH, Fallick AE, Rowland AP, Ostle NJ. 2005. Carbon dioxide
503 capture using a zeolite molecular sieve sampling system for isotopic studies (^{13}C
504 and ^{14}C) of respiration. *Radiocarbon* 47:441–451.
505 Holdgate GR, McGowran B, Fromhold T, Wagstaff BE, Gallagher SJ, Wallace MW, Sluiter
506 IRK, Whitelaw M. 2009. Eocene-Miocene carbon isotope and floral record from
507 brown coalseams in the Gippsland Basin of southeast Australia. *Global and*
508 *Planetary Change* 65:89-103.
509 Hopkins DW, Chudek JA. 1997. Solid-state NMR investigations of organic transformations
510 during the decomposition of plant material in soil. In: Cadisch G, Giller KE, editors.
511 *Driven by Nature: Plant litter quality and decomposition*. Wallingford: CAB
512 International. p 85-94.
513 International Biochar Initiative. 2013. Standardized product definition and product testing
514 guidelines for biochar that is used in soil. http://www.biochar-
515 international.org/characterizationstandard (Accessed 16 February 2016).
516 Jenkinson DS, Fox RH, Rayner JH. 1985. Interactions between fertilizer nitrogen and soil
517 nitrogen –the so-called 'priming' effect. *Journal of Soil Science* 36:425-444.

518 Jenny H, Gessel S, Bingham F. 1949. Comparative study of decomposition rates in
519 temperate and tropical regions. *Soil Science* 68: 419-432.
520 Joergensen RG. 1996. Quantification of the microbial biomass by determining ninhydrin-
521 reactive N. *Soil Biology & Biochemistry* 28:301-306.
522 Kasozi GN, Zimmerman AR, Nkedi-Kizza P, Gao B. 2010. Catechol and humic acid
523 sorption onto a range of laboratory-produced black carbons (biochars).
524 *Environmental Science & Technology* 44:6189-6195.
525 Kuzyakov Y, Bogomolova I, Glaser B. 2014. Biochar stability in soil: Decomposition during
526 eight years and transformation as assessed by compound-specific ^{14}C analysis.
527 Soil Biology & Biochemistry 70:229-236.
528 Kuzyakov Y, Friedel JK, Stahr K. 2000. Review of mechanisms and quantification of
529 priming effects. *Soil Biology & Biochemistry* 32:1485-1498.
530 Kuzyakov Y, Subbotina I, Chen H, Bogomolova I, Xu X. 2009. Black carbon
531 decomposition and incorporation into soil microbial biomass estimated by ^{14}C
532 labelling. *Soil Biology & Biochemistry* 41:210-219.
533 Leavitt SW, Paul EA, Kimball BA, Hendrey GR, Mauney JR, Rauschkolb R, Rogers H,
534 Lewin KF, Nagy J, Pinter PJ, Johnson HB. 1994. Carbon isotope dynamics of free-
535 air CO_2-enriched cotton and soils. *Agricultural and Forest Meteorology* 70:87-101.
536 Lehmann J, Gaunt J, Rondon M. 2006. Bio-char sequestration in terrestrial ecosystems –
537 A review. *Mitigation and Adaptation Strategies for Global Change* 11:403-427.
538 Linn DM, Doran JW. 1984. Effect of water-filled pore space on carbon dioxide and nitrous
539 oxide production in tilled and non- tilled soils. *Soil Science Society of America*
540 *Journal* 48:1267-1272.
541 Lofts S, Spurgeon DJ, Svendsen C, Tipping E. 2004. Deriving soil critical limits for Cu, Zn,
542 Cd and Pb: A method based on free ion concentrations. *Environmental Science &*
543 *Technology* 38:3623-3631.

544 Major J, Lehmann J, Rondon M, Goodale C. 2010. Fate of soil-applied black carbon:
545 downward migration, leaching and soil respiration. *Global Change Biology*
546 16:1366-1379.
547 Marstorp H. 1997. Kinetically defined litter fractions based on respiration measurements.
548 In: Cadisch G, Giller KE, editors. *Driven by Nature: Plant litter quality and*
549 *decomposition*. Wallingford: CAB International. p 95–104.
550 Miltner A, Bombach P, Schmidt-Brücken B, Kästner M. 2012. SOM genesis: microbial
551 biomass as a significant source. *Biogeochemistry* 111:41–55.
552 Northup RT, Yu ZS, Dahlgren RA, Vogt KA. 1995. Polyphenol control of nitrogen release
553 from pine litter. *Nature* 377:227-229.
554 Odum E. 1985. Trends expected in stressed ecosystems. *Bioscience* 35:419-422.
555 Pataki DE, Ellsworth DS, Evans RD, Gonzalez-Meler M, King J, Leavitt SW, Lin G,
556 Matamala R, Pendall E, Siegwolf R, van Kessel, C, Ehleringer JR. 2003. Tracing
557 changes in ecosystem function under elevated carbon dioxide conditions.
558 *BioScience* 53:805-818.
559 Preston CM, Schmidt MWI. 2006. Black (pyrogenic) carbon: a synthesis of current
560 knowledge and uncertainties with special consideration of boreal regions.
561 *Biogeosciences* 3:397-420.
562 Romer MJ. 2001. Carbon dioxide within controlled environments, the commonly neglected
563 variable. Proceedings of the International Conference: Controlled Environments in
564 the New Millennium, Norwich. http://biology.mcgill.ca/Phytotron/Romer2001-
565 CO2.pdf (Accessed 16 February 2016).
566 ` antro• ková H, ` imek M. 1997. Effect of soil CO_2 concentration on microbial biomass.
567 *Biology and Fertility of Soils* 25: 269-273.
568 Schmidt MWI, Skjemstad JO, Jäger C. 2002. Carbon isotope geochemistry and
569 nanomorphology of soil black carbon: Black chernozemic soils in central Europe

originate from ancient biomass burning. *Global Biogeochemical Cycles* 16:1123-1130.

Šimek M, Cooper JE. 2002. The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years. *European Journal of Soil Science* 53:345-354.

Šimek M, Jíšová L, Hopkins DW. 2002. What is the so-called optimum pH for denitrification in soil? *Soil Biology & Biochemistry* 34:1227–1234.

Slota P, Jull AJT, Linick T, Toolin LJ. 1987. Preparation of small samples for ^{14}C accelerator targets by catalytic reduction of CO. *Radiocarbon* 29:303–306.

Soffe RJ. (ed.) 1995. The Agricultural Notebook. Blackwell Science, Oxford. Pp. 646.

Soil Survey Staff, 2006. *Keys to Soil Taxonomy,* 10th edition. Washington: United States Department of Agriculture and Natural Resources Conservation Service.

Sparling GP. 1997. Soil microbial biomass, activity and nutrient cycling as indicators of soil health, In: Pankhurst CE, Doube BM, Gupta VVSR, editors. *Biological Indicators of Soil Health*. Wallingford: CAB International. p 97–119.

Staddon PL, Ramsey BC, Ostle H, Ineson P, Fitter AH. 2003. Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of ^{14}C. *Science* 300:1138-1140.

Stuiver M, Polach HA. 1977. Reporting of ^{14}C data. *Radiocarbon* 19:355–363.

Topham S. 1986. Carbon dioxide. In: *Ullmann's Encyclopedia of Industrial Chemistry*. New York: John Wiley. p 165-183.

Werner RA, Bruch BA, Brand WA. 1999. ConFlo III – An interface for high precision ´ ^{13}C and ´ ^{15}N analysis with an extended dynamic range. *Rapid Communications in Mass Spectrometry* 13:1237-1241.

Wilson MA. 1987. *NMR Techniques and Applications in Geochemistry and Soil Chemistry*. Oxford: Pergamon Press.

596 Zimmerman AR, Gao B, Ahn M-Y. 2011. Positive and negative carbon mineralisation
597 priming effects among a variety of biochar-amended soils. *Soil Biology &*
598 *Biochemistry* 43:1169-1179.
599 Zimmermann M, Bird MI, Wurster C, Saiz G, Goodrick I, Barta J, Capek P, Santruckova H
600 Smernik R. 2012. Rapid degradation of pyrogenic carbon. *Global Change Biology*
601 18:3306–3316.
602

603 **Table 1**

604 Selected physico-chemical characteristics of soil, wood and charcoal. Values are the

605 means of three replicates (unless otherwise stated), 1Ã standard deviation given in

606 brackets. ND = Not detected.

	Soil	Wood	Charcoal
Total C (mg g^{-1})	221 (7.0)	557 (0.8)	637 (1.5)
Total N (mg g^{-1})	18 (0.4)	ND	ND
Total H (mg g^{-1})		59	53
Total O (mg g^{-1})		314	261
´ ^{13}C (V-PDB‰)	-27.9 (0.12)	-21.1(0.09)	-21.1 (0.03)
^{14}C activity (% modern ± 1σ, n = 1)	99.76 ± 0.46		0.137 ± 0.002[a]
$pH_{[H2O]}$	4.74 (0.037)		
Extractable phenolics (µg phenol equivalent g^{-1})		1011 (26.9)	229 (44.7)
Al (mg kg^{-1})		41 (19.7)	5 (5.8)
Ca (mg kg^{-1})		734 (207.7)	445 (188.2)
Cd (mg kg^{-1})[b]		ND	ND
Cr (mg kg^{-1})		11 (6.3)	1 (0.2)
Cu (mg kg^{-1})		8 (2.8)	1 (0.5)
Fe (mg kg^{-1})		573 (33.14)	655 (267.6)
Hg (mg kg^{-1})[b]		ND	ND
Mg (mg kg^{-1})		355 (65.2)	432 (32.0)
Mn (mg kg^{-1})		6 (2.2)	7 (1.4)
Ni (mg kg^{-1})		14 (5.2)	27 (29.0)
Pb (mg kg^{-1})		2 (3.6)	4 (4.7)
Zn (mg kg^{-1})		19 (4.1)	5 (0.2)

607 [a]Bird and Ascough (2012). Note that this value was obtained on charcoal prepared at

608 450°C, instead of the 300°C charcoal used in this paper. The starting material was the

609 same for both samples however, so differences in ^{14}C content are not expected.

610 [b]Limits of detection are 100 µg kg^{-1} for cadmium and 1 mg kg^{-1} for mercury.

Table 2

Publication codes and the ^{13}C and ^{14}C content of respired CO_2.

Publication code	Treatment	Sampling day	^{14}C content (%modern ± 1σ)	$\delta^{13}C_{V\text{-}PDB}$‰ (±0.1)
SUERC-33154	Unamended control	27	110.21 ± 0.48	-28.1
SUERC-33155	Unamended control	27	110.50 ± 0.48	-29.1
SUERC-33156	Unamended control	27	111.27 ± 0.51	-28.8
SUERC-33157	Charcoal amended	27	108.74 ± 0.50	-28.8
SUERC-33158	Charcoal amended	27	108.32 ± 0.50	-28.4
SUERC-33159	Charcoal amended	27	107.86 ± 0.50	-28.7
SUERC-33160	Unamended control	55	109.61 ± 0.51	-28.3
SUERC-33161	Unamended control	55	110.01 ± 0.51	-28.1
SUERC-33164	Unamended control	55	111.10 ± 0.51	-29.9
SUERC-33165	Charcoal amended	55	109.32 ± 0.51	-29.0
SUERC-33166	Charcoal amended	55	108.96 ± 0.48	-29.0
SUERC-33167	Charcoal amended	55	108.91 ± 0.50	-28.7

Table 3

Selected physico-chemical characteristics of unamended soil and soil amended with either wood or charcoal after 55 days of incubation and the ^{13}C and ^{14}C contents of respired CO_2. Values are the means of three replicates, standard deviation given in brackets. Values within a row identified with common letters are not significantly different from each other according to Fisher's least significant difference test ($P < 0.050$).

	Amendment			
	Unamended	Wood	Charcoal	*P*
C mineralized (%)	1.22 (0.081) b	0.89 (0.025) a	0.98 (0.016) a	<0.001
Decomposition rate (k $d^{-1} \times 10^{-3}$)	-0.25 (0.016) c	-0.18 (0.005) a	-0.20 (0.004) b	<0.001

Microbial biomass C (mg C_{mic} g^{-1})	2.4 (0.30) c	2.1 (0.12) b	1.7 (0.16) a	0.016
$pH_{[H2O]}$	4.8 (0.04)	4.8 (0.01)	4.8 (0.04)	0.797
Extractable NO_3-N (µg N g^{-1} soil)	59.4 (0.36) c	9.5 (3.95) a	34.4 (7.92) b	< 0.001
Extractable NH_4-N (µg N g^{-1} soil)	16.8 (3.49)	14.5 (1.99)	12.5 (2.91)	0.259
Metabolic quotient (µg CO_2-C mg^{-1} C_{mic} hr^{-1})	0.9 (0.03) a	0.9 (0.03) a	1.1 (0.07) b	0.003
´ ^{13}C respired CO_2, day 27	-28.7 (0.51)		-28.6 (0.21)	
´ ^{13}C respired CO_2, day 55	-28.8 (0.99)		-28.9 (0.17)	
^{14}C %modern respired CO_2, day 27	110.7 (0.55)		108.3 (0.44)	
^{14}C %modern respired CO_2, day 55	110.2 (0.77)		109.1 (0.22)	

Table 4

Culturable microbial population sizes (Log_{10} colony forming units (CFU) g^{-1} soil) in the presence of unextracted and hot water-extracted wood and charcoal. Values are the mean of three replicates (except soil suspension n = 6), standard errors are given in brackets.

	Wood	Charcoal
Unextracted	7.0 (0.04)	6.9 (0.06)
Extracted	6.9 (0.04)	6.9 (0.04)
Soil suspension only	7.0 (0.04)	

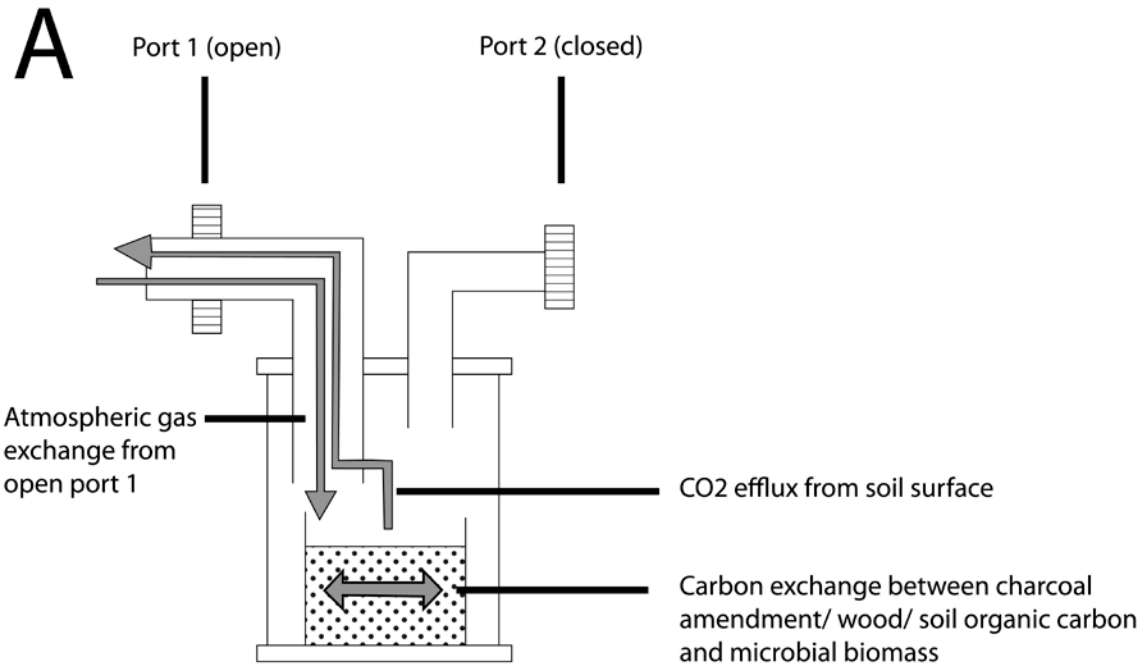
FIGURE CAPTIONS

Fig. 1. Schematic of experimental apparatus showing carbon pathways during (A) incubation and (B) measurement and collection of CO_2. Prior to measurement or collection of CO_2 port 1 was closed for 4 days to allow build up of the gas evolved from the soil surface.

Fig. 2. Solid state ^{13}C CP-MAS NMR spectra of the uncharred beech wood (A) and charcoal prepared at 300°C (B). Major peaks of interest are highlighted on Figure 2B.

Fig. 3. Soil respiration rates in unamended (○), wood-amended (▲) and charcoal-amended (■) soil. Values are the mean of three replicates, error bars where visible indicate one standard deviation. Bar indicates the least significant difference of means at the same time point (P = 0.003; d.f. = 10.51) based on a repeated measures analysis of variance.

Fig. 1. Schematic of experimental apparatus showing carbon pathways during (A) incubation and (B) measurement and collection of CO_2. Prior to measurement or collection of CO_2 port 1 was closed for 4 days to allow build up of the gas evolved from the soil surface.


A
Port 1 (open)
Port 2 (closed)
Atmospheric gas exchange from open port 1
CO2 efflux from soil surface
Carbon exchange between charcoal amendment/ wood/ soil organic carbon and microbial biomass

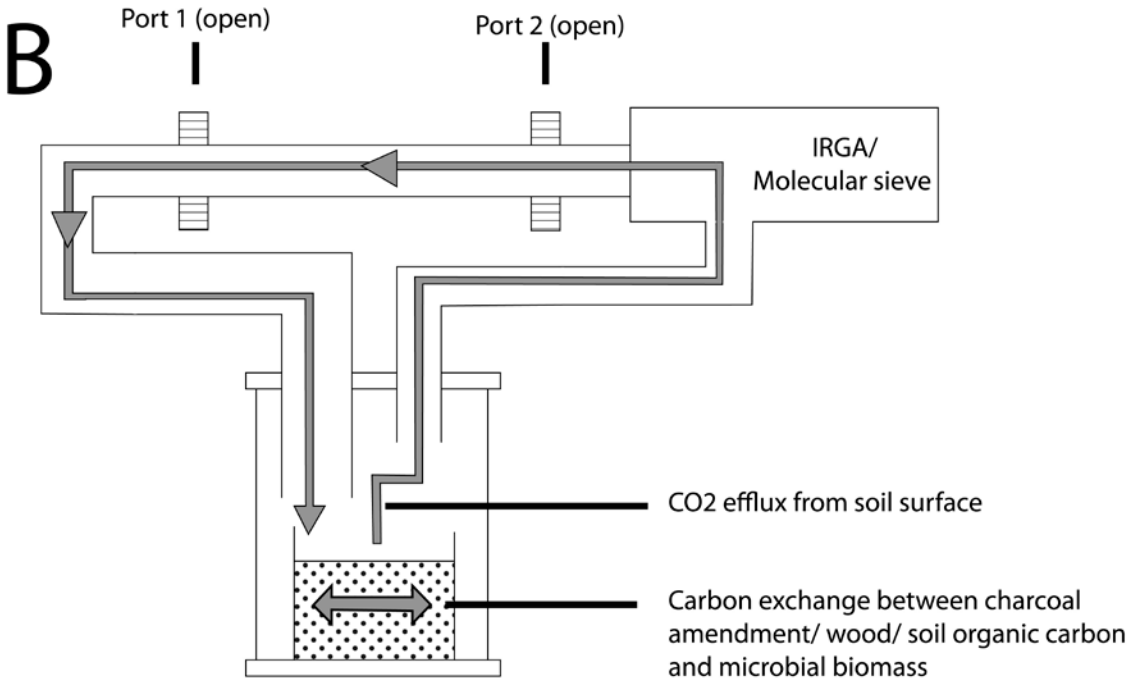

B
Port 1 (open)
Port 2 (open)
IRGA/ Molecular sieve
CO2 efflux from soil surface
Carbon exchange between charcoal amendment/ wood/ soil organic carbon and microbial biomass

Fig. 2. Solid state ^{13}C CP-MAS NMR spectra of the uncharred beech wood (A) and charcoal prepared at 300°C (B). Major peaks of interest are highlighted on Figure 2B.

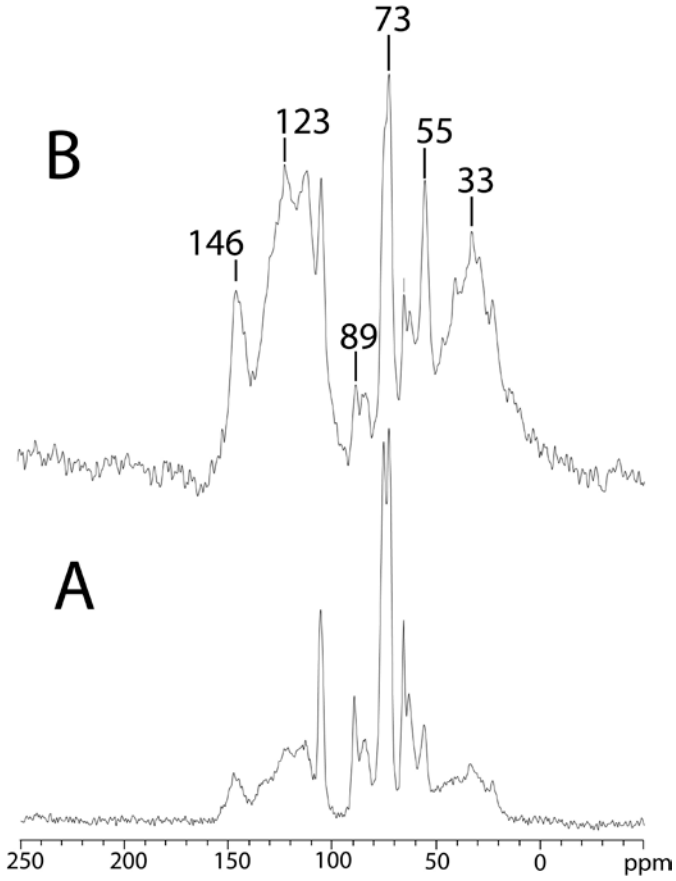
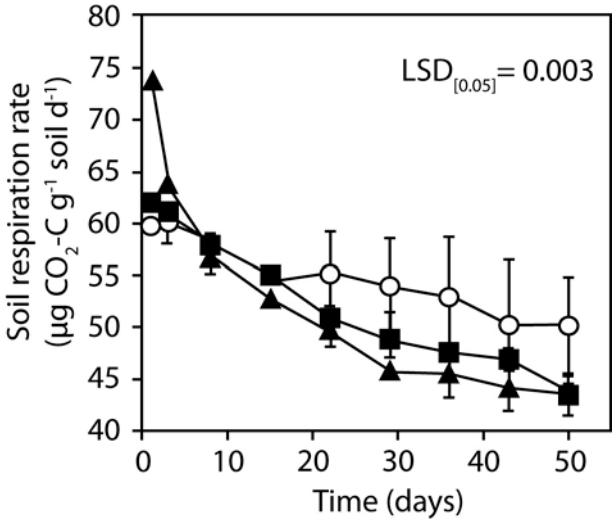


Fig. 3.


LSD[0.05]= 0.003
Soil respiration rate
(µg CO2-C g-1 soil d-1)
Time (days)
80
75
70
65
60
55
50
45
40
0
10
20
30
40
50