­­Running head: Black C – Physical location in soil

General research paper

# **Physical location of black carbon in grassland soil and its implication for SOC models**

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# Abstract

The ubiquity of black carbon (BC) in soils is increasingly recognised, but distributions of BC between experimentally obtained fractions have not been widely assessed or compared, despite the potential for such fractions to inform future soil C modelling. We examined fractions obtained from a long-term grassland site using three alternative fractionation procedures and assessed for average radiocarbon (14C) age. Low density fractions isolated after aggregate disruption were found to be of an average age that was both extreme (~4000 to ~7000 years BP) and higher than mineral bound fractions. A relatively small proportion of soil C resided in these fractions (1.2–9.7%), but as these fractions are typically discussed in the context of pools with high turnover there are clear implications for their use in C modelling. Direct isolation and analysis by hydropyrolysis (HyPy) established that the low density fraction of greatest average age (particulate organic matter) contained 37% of all BC in the soil and this BC has an average age assessed at 18610 ± 89 years BP. The presence of BC in other isolated fractions was inferred from fraction 14C age and FTIR analyses. Our results lend support to the inclusion of BC in soil C models, direct BC measurement, and an effort to establish default partitioning for BC between fractions.

## 1 Introduction

Experimental division of soil organic matter (SOM) into functional pools has the potential to increase the precision of C modelling, such pools being distinguished by their extent of physical protection, which in turn is affected by land-use and management practice. The presence of black carbon (BC) is a complication in this regard. BC is the product of incomplete burning of C in both vegetation and fossil fuel and chemically characterised at a molecular scale by its aromatic, polymeric and graphitic composition (Goldberg, 1985). The ubiquity of BC is increasingly acknowledged and if BC is not specifically isolated and modelled, its high level of recalcitrance will challenge the assumption in current SOM models that each pool can be defined by a single, constant level of reactivity (Lefèvre*et al*, 2014). The assignment of BC to ostensibly more active pools will tend to result in an overestimation of C turnover (Lehmann *et al*, 2008).

The extent to which the use of isolated fractions modelling C will depend on the measurement of BC, whether BC resides predictably in one fraction, and how reactivity ranges between pools that are modelled. BC has already been observed in both low density soil fractions (Glaser *et al*, 2000; Brodowski *et al*, 2005; Brodowski *et al*, 2006) and high density fractions (Brodowski *et al*, 2005; Czimczik and Masiello*,* 2007) as well as being detected as dissolved organic C (DOC) (Hockaday *et al*, 2006).

The range of different fractionation procedures presents a challenge when making comparisons between studies and fitting to a common modelling framework. Qualitative and quantitative comparisons of fractions measured using alternative fractionation schemes are scarce and have not specifically addressed the issue of BC. Different procedures isolate different numbers of SOM fractions (e.g. three for Sohi *et al*, 2001, five for Zimmermann *et al,* 2007, six for Six *et al,* 2001). Pools are associated with aggregates or minerals, dissolved in soil solution, or located in pores within aggregates or between aggregates. Isolated fractions should ideally be homogenous and display discrete differences in their *in-situ* reactivity and mean residence time (MRTs) (Lefèvre*et al*, 2014). The quantitative distribution of carbon between such fractions differs according to the procedure used, together with their measured chemical composition and conceptual function (Crow *et al*, 2007).

Isotopic analysis has provided some insights into the dynamics of carbon in soil fractions. Discrimination against the stable 13C isotope in photosynthesis differs according to fixation pathway, so the years over which the extent of depletion changes after transition from C4- to C3-dominated communities (or *vice versa*). Recalcitrant organic matter or products of microbial decomposition are usually relatedly less 13C-depleted than fresh organic material.

Radiocarbon (14C) has been used to infer the rate at which C has cycled through different SOM fractions *in situ*, providingan important means by which SOM models can be calibrated and tested over longer timeframes (Trumbore, 2009). Due to the relatively rapid exchange of CO2 between living plants and the atmosphere, the 14C/12C ratio in OM entering soil is the same as that of the atmosphere (Marschner *et al*, 2008). The 14C/12C ratio in the OM subsequently decreases owing to radioactive 14C decay; comparing this to a ‘modern’ atmospheric ratio gives an estimate for the time since C in OM was fixed from the atmosphere (conventional 14C age; Stuiver and Polach, 1977). Thermonuclear weapons testing caused a doubling of atmospheric 14C/12C in the 1950s and 60s, causing a spike in the apparent percentage of modern C (pMC). The proportion has steadily decreased from a peak of 190 pMC to ~103 pMC by 2015. The well-defined spike and subsequent decline in atmospheric 14C can be used to ascertain the turnover of SOC on a timescale of years to decades (Harkness *et al.,* 1986; Trumbore, 2009).

In the present study three commonly used fractionation methods were applied to a grassland soil. The isolated fractions were analysed for 14C and characterised chemically using Fourier transform infra-red (FT-IR) spectroscopy. The results were used to infer the relative age and recalcitrance of each fraction and interpreted in the light of soil BC content. Our interest in this study is how the choice of fractionation method affects our inference of BC presence and allocation between physical fractions, and our understanding of its interactions and fate within soils.

## 2 Methods

### 2.1 Soil and soil preparation

The sandy-loam soil used in this research was obtained from the Crichton Royal Farm in south-west Scotland (Latitude: N55:02:31 (55.041966), Longitude: W3:35:18 (-3.588243)). It is a non-calcareous Eutric Cambisol derived from red sandstone parent material (FAO, 1990). The site has been under long-term grassland management and is moderately well drained. The field sampled on this occasion is cut grass that has not received lime for over 20 years and has no long-term history of organic manure application (Bell *et al*, 2015).

Soil was collected in the spring of 2012 for the 0–10 cm depth interval. Twenty samples were taken along a ‘W’ transect across the field using a gouge auger. The fractionations were applied to a composite sample passed through a 4 mm sieve to homogenise the soil and remove stones and plant roots. The soil was air-dried and stored in a sealed plastic bag prior to fractionation. The composite soil sample had a pH of 6 and an organic C (OC) content of ~3%.

### 2.2 Soil Fractionation

Soil was fractionated using two methods commonly employed in C cycling studies and a water extraction method. These are outlined below and subsequently referred to, for brevity, as the ‘Zimmermann’, ‘Sohi’ and ‘Ghani’ methods / fractions.

The method of Zimmermann *et al* (2007) isolates five fractions density‑separated in sodium polytungstate (SPT) after disruption by sonication: 1) a low density (light) fraction (LF) – particulate organic matter (POM) > 63 μm and density < 1.8 g cm-3, 2) a soluble fraction, dissolved organic C (DOC) which is < 0.45 μm, 3) a mineral bound fractions, silt and clay (s+c) < 0.63 but > 0.45 μm, 4) sand and stable aggregates (S+A) > 63 μm and density > 1.8 g cm-3, and 5) a chemically resistant fraction (rSOC) isolated from s+c fraction after oxidation with 5% NaOCl.

The method of Sohi *et al* (2001) isolates three fractions density-separated in NaI: two LFs – free LF isolated before the disruption of aggregates and has a density < 1.8 g cm-3, and intra-aggregate LF which is isolated after aggregate disruption (as described above) and has a density < 1.8 g cm-3, and one mineral bound fraction, the organo-mineral fraction which has a density > 1.8 g cm-3.

The method of Ghani *et al* (2003) isolates two soluble fractions (< 0.45 μm): water soluble C (WSC) extracted at 20 °C and hot-water soluble C (HWEC) extracted at 80 °C.

The aim of the study is to compare the methods rather than the sensitivity of the different methods to within-site variation. It was appropriate for this purpose to use a composite sample as the comparison benefits from the homogeneity of the sample. The outcome is generalizable information on the distribution of BC between alternate fractions, rather than an understanding of variation within this single site.

Each fractionation scheme was repeated multiple times using soil randomly sub-sampled from the composite sample. Fractionations were ceased when the quantity of the smallest fraction identified by the scheme was sufficient to allow all the planned analyses to be undertaken. Individual isolated fractions from repeated fractionations were bulked after drying at 40 °C. Random sub-samples of composite fractions within each fractionation scheme were used for analyses to ensure that results were representative. Sub-samples of the relevant fractions were ball milled and transferred into glass vials and submitted for 14C or FT-IR analysis. Soluble fractions were stored in clean glass bottles.

### 2.3 Elemental analysis

Composite samples of whole soil and isolated fractions were analysed in triplicate for OC and total nitrogen (TN). Solid samples were ball milled and analysed using a Flash 2000 elemental analyser (Thermo Scientific, Waltham, MA, USA). Triplicate subsamples of the Zimmermann DOC fraction (and rinsing water from density-separated Zimmermann fractions) and Ghani WSC and HWEC fractions were acidified with two drops of orthophosphoric acid, sparged with N2 for two minutes (to eliminate carbonate carbon), then analysed for OC using a Rosemount-Dohrmann DC80 Total Carbon Analyser (Sartec, Borough Green, Kent, UK).

### 2.4 Carbon isotope analysis

Composite samples of whole soil and isolated fractions were prepared for 14C analysis at the NERC Radiocarbon Facility (SUERC, East Kilbride, UK). Duplicate graphite targets were created through a process of combustion, cryogenic purification (of the resulting CO2) and subsequent reduction using zinc and iron. The volume of liquid samples was first reduced by rotary evaporation (to a few millilitres), then freeze-dried for 48 hours on a Christ alpha 1-4 Lo plus freeze-drier (Osterodd/Harz, Germany). Any carbonates which may have been present were removed by hydrolysis using HCl fumigation. Duplicate 14C analyses were conducted by accelerator mass spectrometry (AMS), at the AMS Laboratory of the Scottish Universities Environmental Research Centre (SUERC, East Kilbride, UK). Sub-samples of the CO2 produced during the combustion of whole soil and isolated fractions were used for δ13C determination using a dual inlet stable isotope mass spectrometer (Thermo Fisher Delta V). Following convention (Stuiver and Polach, 1977) the 14C results were corrected to δ13CVPDB‰ = -25 using the δ13C results.

Additional AMS analyses were undertaken to check for possible interference of activated carbon from the recycling procedures for the media used in soil density fractionation (SPT, sodium iodide). The NERC Radiocarbon Facility standards (comprising barley mash and anthracite) were exposed to either fresh SPT or SPT that has been recycled in the usual way using activated C, plus all of the associated fractionation equipment. AMS analyses of these samples showed that the exposed organic materials were not 14C‑depleted after exposure, suggesting no effect of the recycling protocol on the experimental results.

### 2.5 Functional characterisation

### 2.5.1 Black carbon

Whole soil was initially tested for BC by thermogravimetric analysis (TGA) (TGA/DSC 1; Mettler‑Toledo, Leicester, UK) prior to fractionation. The sample was combusted in air (using nitrogen as the protective gas) heating from 35 to 650 °C at 10 °C min-1. The mass of the sample was recorded every 1.5 s.

Direct measurement of BC was made for whole soil and selected SOM fractions by hydropyrolysis (HyPy) at SUERC, according to the procedure of Meredith *et al* (2012). The OC content was assessed before and after HyPy in order to calculate the initial BC content of the samples. Measurement of OC was made offline on a Costech Elemental Analyser, with post-combustion quantification via cryogenic CO2 capture. The BC contents of the samples were calculated as follows:

### 2.5.2 Carbon functional groups

The molecular associations of carbon in whole soil and SOM fractions were assessed by FT‑IR. Analysis was conducted at the James Hutton Institute using a Bruker Vertex 70 FT-IR spectrometer fitted with a diamond attenuated total reflectance accessory. Sample spectra were acquired by averaging 200 scans at 2 cm-1 resolution over the range 4000–370 cm-1. Spectra were baseline corrected and peaks were assigned according to Palacio *et al* (2014). FT-IR analysis was applied to HWEC after freeze drying, but could not be used to characterise the Ghani WSC and Zimmermann DOC fractions as the solid mass remaining (after freeze drying) was insufficient. The WSC and DOC fractions were analysed by absorbance in the ultra violet (UV) spectrum, specifically at 254, 465 and 665 nm. Absorbance was measured on triplicate sub-samples using a HP 8453 UV-vis spectrophotometer (Hewlett‑Packard, Waldbronn, Germany) with 10 mm quartz cuvettes and using deionised water as a blank. Specific UV absorbance at 254 nm (SUVA254) values (absorbance at 254 nm normalised for C content) and E4E6, ratios (absorbance at 465 nm divided by the absorbance at 665 nm) were calculated. SUVA254 has been shown to be well correlated with DOC aromaticity (Weishaar *et al*, 2003) and E4E6, ratios have been shown to have an inverse relationship with the aromaticity of DOC (Stevenson, 1982).

### 2.6 Data analysis

### 2.6.1 Mean residence time

Mean residence times of the whole soil and isolated fractions were estimated using the 14C enrichment results and the Meathop model (Harkness *et al*, 1986). This model uses known 14C/12C ratios through the 14C-bomb spike (from Levin *et al*, 2008) to provide 14C contents expected for a range of MRTs. The MRT of a sample can then be approximated as the modelled MRT associated with the most closely matching 14C measurement in the year of sample collection.

### 2.6.2 Mass balance calculations

Mass balance calculations were carried out to show that the weighted 14C enrichment of the isolated fractions were consistent with that of the enrichment in whole soil. The 14C enrichment of each fraction isolated using the Zimmermann or Sohi methods were calculated by multiplying the higher measured 14C content by the average proportion of whole soil C recovered in that fraction. The products for each scheme were then summed to give a value which could be compared to the measurement of whole soil 14C content. The calculation was repeated for the lower measured 14C contents.

### 2.6.3 Statistical analyses

Statistical analyses were carried out using Genstat (15th edition) software (VSN International, 2011). Significant differences (p < 0.05) between fraction and whole soil C:N ratios, 14C and δ13C enrichment, SUVA254 values, E4/E6 ratios and liquid sub-sample OC concentrations were determined by one-way ANOVA followed by a Tukey-Kramer adjusted means test.

## 3 Results

### 3.1 Organic C and N in soil mass fractions

The mass recovery amongst the fractions identified by the Zimmermann and Sohi methods was 98.6 ± 1.3% and 95.2 ± 0.4% respectively. The corresponding recoveries of OC were 110.6 ± 1.6% and 90.2 ± 0.8%, for Zimmermann and 94.0 ± 0.7% and 83.0 ± 0.5% for the Sohi method. An additional 1.1% of the initial whole soil OC was present in the deionised water used to rinse SPT from the Zimmermann density-separated fractions. It is assumed that the missing 6% of initial whole soil OC from the Sohi method was lost as dissolved OC in the NaI although this was not directly quantified. The Ghani HWEC extracted 2.7% of whole soil OC, and the WSC extracted 0.3% of whole soil OC.

The SOC stock for the whole soil was 24 ± 1.5 t C ha-1 with most OC stored in mineral bound fractions (Table 1). For the Zimmermann *et al* method, the S+A fraction made up the bulk of the whole soil mass (600 ± 8 kg t-1) and the Sohiorgano‑mineral fraction mass equated to 913 ± 3 kg t-1. The organo‑mineral fraction contributed the largest proportion of C in the Sohi method. Although the rSOC mass fraction (367 ± 10 kg t-1) was only about 60% that of S+A, the S+A and rSOC fractions of the Zimmermann method each accounted for a similar amount of total SOC stock (10.2 ± 0.2 and 10.3 ± 0.3 t C ha-1, respectively).

The C concentration of the Sohi fractions followed this pattern: organo-mineral < intra‑aggregate LF < free LF (Table 1). The concentration of C was higher in free LF and the Zimmermann POM fraction than other fractions (~31 and ~13% of fraction mass respectively). Both were small mass fractions (9.2 ± 1.3 and 14.5 ± 1.4 kg t-1) and directly accounted for small C stocks (2.3 ± 0.3 and 1.9 ± 0.2 t C ha-1, respectively).

The C:N ratio of free LF and intra-aggregate LF were significantly higher than the mineral bound fractions (one-way ANOVA, p < 0.001) (Table 1). This indicates either a high proportion of less decomposed material or potentially the presence of BC.

### 3.2 Carbon isotopes

### 3.2.1 13C enrichment

Mass spectrometry showed higher 13C values in WSC, HWEC, DOC, POM, free LF and intra‑aggregate LF compared to the mineral dominated fractions. The rSOC fraction which was isolated from the s+c fraction by oxidation with NaOCl had identical δ13C values, -28.1 (±1 σ) and ‑28.2 (±1 σ) ‰, respectively (Table 2). This indicates that the oxidation may not have been fully successful in removing the labile OM component of the s+c fraction.

There was a significant negative relationship between fraction C:N and 14C content (R2 = 0.72, p < 0.001). Lower 14C content of high C:N material suggests either BC (pre-bomb, potentially extreme age) – or recent post-bomb and non-degraded organic matter. A significant positive relationship was found also between C:N ratios and δ13C (R2 = 0.74, p < 0.001). The 13C content of organic matter should generally increase through repeated decomposition, with C:N decreasing as C is progressively eliminated. There is minor discrimination of 13C in pyrolysis of organic matter (ca.1-2‰; Ascough *et al*, 2008), while near-total elimination of N results in high C:N.

### 3.2.2 14C contents

Most isolated fractions contained pre-bomb 14C; the exceptions were the Ghani HWEC, WSC fractions and the Zimmermann s+c fraction. The Zimmermann fractions fell into two distinct groups with respect to their apparent (14C) age (Table 2). The POM and DOC fractions had the oldest apparent ages (> 4000 years BP); the s+c, rSOC and S+A fractions were somewhat younger (< 1000 years BP). The pattern in 14C content was DOC < POM < S+A < rSOC < s+c. The corresponding pattern for the Sohi fractions was intra-aggregate LF < free LF < organo-mineral. The 14C enrichment of the light and soluble Zimmermann and Sohi fractions was significantly greater than that of the mineral bound fractions.

Importantly, mass balance was achieved when compiling data on the 14C content of both the Zimmermann and Sohi fraction method, i.e. the sum data was not significantly different from the directly analysed whole soil. Mass balanced results were 91.1 or 93.7 pMC for the Zimmermann method and 95.2 or 95.6 pMC for the Sohi method. Measured 14C enrichment of duplicate whole soil sub-samples were 95.5 and 96.2 pMC.

Black C isolated from the Zimmermann POM fraction by HyPy was directly assessed for 14C. Removal of non-BC resulted in a greater 14C age of 18610 ± 89 years BP (Table 2).

### 3.3 Functional characterisation

### 3.3.1 Black carbon

The TGA trace showed no distinct peak in the range 450–590 °C suggesting that BC might not be present in the soil and its sub-fractions (Leifeld, 2007). The subsequent (non-replicated) analysis by HyPy revealed that OC included a BC component in both whole soil and POM, amounting to ~5% of the analysed whole soil sub-sample C (1.7 t BC ha-1) and ~17% of the analysed POM sub-sample C (0.4 t BC ha-1) respectively.

### 3.3.2 Carbon functional groups

Major FT-IR absorption bands revealed hydroxyl, aliphatic, aliphatic carboxylate/aromatic and polysaccharide/silicate groups in all analysed fractions (Zimmermann fractions, Sohi fractions and Ghani HWEC) as well as whole soil (Figures 1 to 3). The broad absorption band around 3400 cm-1 is attributable to H-bonded OH groups associated with OM and/or the surfaces of minerals such as kaolinite. This feature was particularly pronounced in POM (Figure 1) and free LF (Figure 2). The sharpness of the band in the intra-aggregate LF (Figure 2) suggests the kaolinite surface OH doublet rather than organic or other mineral OH. Compared to whole soil and mineral-dominated fractions, those of higher OC concentration (Zimmermann POM, Figure 1; Sohi free LF and intra-aggregate LF, Figure 2) showed more pronounced absorption in bands reflecting aliphatic C-H stretching (2920 and 2850 cm‑1) and aliphatic carboxylates, and/or aromatic group bands (around 1610 cm-1). The 1610 cm‑1 band was particularly strong in the intra-aggregate LF fraction (Figure 2). Strong absorption bands around 1000 cm-1 indicated C-O stretching of polysaccharide and polysaccharide-like substances. These organic groups may be masked by Si-O stretches of silicates in the mineral bound fractions (S+A, s+c and organo‑mineral). This absorption band was weaker in the intra-aggregate LF fraction (Figure 2) compared to the other fractions and whole soil, suggesting that this fraction was more humified.

The HWEC fraction was the only soluble C fraction for which an FT-IR spectrum could be obtained. The chemical characteristics were somewhat different to the solid samples (see Figure 3). The 3400 cm-1 band was particularly strong and masked the aliphatic groups at 2850 and 2920 cm‑1. The aliphatic carboxylate and/or aromatic group absorption bands at 1610 cm-1 were also stronger than in the other fractions and whole soil. It had been expected that oxidation of the s+c fraction would result in the loss of carboxyl, aromatic and aliphatic functional groups. In fact, there were no apparent differences in the absorption bands of residual rSOC from those of s+c (Figures 4a and 4b). However, the slightly higher C:N ratio (and lower 14C enrichment) suggested that oxidation had occurred and revealed a constituent containing charcoal.

The C concentration of HWEC was more than ten times higher than WSC (Table 3). The aromaticity of the WSC fraction was revealed in the SUVA254 result (7.81 ± 1.67 L mg C-1 m-1), indicating a significantly higher aromatic content than the HWEC fraction (0.52 ± 0.12 L mg C-1 m-1). These results are in line with the view emerging from isotope analysis, that BC was an important constituent of some C fractions. The SUVA254 result for SOC was similar to that of WSC, indicating a similar high level of aromaticity despite much lower 14C content. The E4E6 ratio was significantly higher in the DOC fraction, suggesting that the OM in this fraction has a lower molecular weight than that of the Ghani fractions. However, E4E6 ratios are sensitive to OC concentration and so the HWEC cannot be accurately compared to the WSC or DOC fractions. Removing HWEC from the analysis did not result in E4E6 ratios becoming statistically significant.

### 3.4 Mean residence times of whole soil and isolated fraction C

The mean residence times inferred from the results are shown for whole soil and all fraction sets in Table 2. Where the 14C content was below the level of the atmospheric record (below 97% pMC), the Meathop model is unable to assign an MRT. Inputs of bomb-14C enriched material to these fractions are likely to become diluted by BC due to the small pool sizes. Conventional 14C age was therefore adopted as the best approach to estimate MRT. The rSOC fraction had a higher MRT (493 and > 500 years) than s+c (262 and 303 years). This is consistent with the oxidation of a less recalcitrant component revealing BC in the s+c fraction. HWEC had the shortest MRT (97.6 ± 2.6 years) suggesting that this labile fraction was comprised of the least physically or chemically protected SOC.

## 4 Discussion

### 4.1 Correspondence of C fractions obtained by different methods

Soil carbon modelling conventionally simulates conceptual pools of SOM. C is allocated between pools according to rate constants that are relatively fixed (at equilibrium), adjusted primarily according to soil texture (the absolute size of each pool is determined by the balance between C inputs for litter or transformation fluxes from other pools, versus losses via decomposition). Turnover rate (and therefore MRT) defines each pool, with rapidly reacting pools tending to be smaller in size (Sohi *et al*, 2001). Ideally, experimental measurements to inform such modelling should isolate fractions with a similar distribution of C, similar transformation pathways and similar turnover rates. SOM clearly embraces a continuum of reactivity, but distinction and homogeneity of *in-situ* reactivity is desirable (von Lützow *et al*, 2007) if soil models are to benefit from higher resolution simulation.

Based on the properties used to obtain Zimmermann POM, this fraction should encompass both the Sohi free and intra-aggregate LF fractions (density < 1.8 g cm-3 between aggregates and within aggregates), with the constraint that POM has a size > 63 µm. Free LF and intra-aggregate LF collectively accounted for a similar proportion of whole soil C as did POM in our sandy-loam soil under permanent grass (8.5 ± 0.4 and 9.7 ± 1.2%,). Based on their weighted 14C content, their aggregate modern carbon content was quite similar (62.3 ± 9.2 versus 59.6 ± 1.1 pMC, respectively). The 14C content of free LF and intra-aggregate LF individually was, however, markedly different. These findings suggest that POM comprises a mixture of OM that could be usefully separated into distinct sub-pools such as “inter‑aggregate“ and “intra-aggregate” POM.

The distinction of inter- and intra-aggregate LFs in Sohi fractionation appears potentially useful in this regard. However, the Sohi method assigns all ‘heavy’ soil particles to a single organo-mineral fraction containing sand, silt and clay size mineral particles. Due to the low surface area of sand particles they have weak affinity for SOM, whilst clay-associated SOM tends to be chemically recalcitrant as well as substantially protected through surface binding (von Lützow *et al*, 2007). Recovery of organic matter associated with sand (but not protected) using the Sohi methods would result in an under-estimation of organo-mineral reactivity; in the Zimmerman method such material could be recovered in S+A. Despite the Zimmermann S+A, s+c and rSOC fractions displaying similar 14C content in our soil, their abundance and OC concentrations was important in terms of identifying and studying the relative importance of other SOM fractions in qualitative and quantitative terms. The relationships for soils where land management impacts the extent of chemical and physical protection remains to be explored. The presence of old C in this soil suggests recalcitrant BC is present. A fractionation method that can assist modelling of soil C will depend on its successful partitioning of chemically recalcitrant BC.

### 4.2 Relevance of BC within SOM fractions

The presence of BC in this study was inferred for all solid fractions, in higher proportions for fractions defined by fine particle size and/or lower density, according to high C:N and 14C depletion (free LF, intra-aggregate LF OM), or 14C depletion increased by oxidation (s+c). Low 14C content of Zimmermann DOC was not mirrored by the soluble Ghani fractions. This is likely explained by ultrasonic disruption as part of the Zimmermann method. The extraction of the Ghani fractions is without aggregate disruption.

Selected samples were analysed for 14C content after HyPy, confirming a substantial contribution of BC to the POM fraction (~17%). The isolated BC had very low 14C enrichment but was not found to be radiocarbon ‘dead’ (14C enrichment of 9.86 pMC). This suggests that the low 14C contents in light fractions could be attributable to a mixture of charcoal, particles of broken down coal fragments from carboniferous sediments or atmospheric fallout of soot particles from fossil fuel or biomass burning. Otherwise, it may indicate the presence of ancient charcoal in the soil BC fraction.

Since this cannot explain the measured BC content of the whole soil (5% of SOC), the inference of BC in other Zimmermann fractions (rSOC, S+A) may be accurate. This by extension suggests that the Sohi fractions are similarly affected. The post-HyPy dating established an average BC age of 18610 ± 89 years BP.

The preferential situation of BC within various fractions has been explored incidentally and directly using various methods and analytical approaches (Camberdella and Elliott, 1992; Glaser *et al*, 2000; Brodowski *et al*, 2005; Brodowski *et al*, 2006; Hockaday *et al*, 2006). Brodowski *et al*(2006) studying grassland in rural Germany quantified BC in light (< 1.6 g cm-3) fractions (free and occluded POM). In their whole soil BC comprised ~3% of total OC. The OC in POM fractions was ~7% BC. Separate study of BC in industrialised parts of the same country showed corresponding whole soil proportions of ~12 to ~13% in whole soil and ~31 to ~42% of light fractions (< 2 g cm-3) (Brodowski *et al,* 2007). Brodowski *et al* (2005) examined the association of BC with soil minerals, both large BC particles with small mineral particles and *vice versa*, through interaction with oxidised functional groups on the outer surface of the BC particles. In their review of BC-soil interactions Czimczik and Masiello(2007) note that BC distribution across depth is correlated to that of organic C and that the mechanisms governing BC storage in soils are similar to those for total soil C.

Our results remind us that although established models generally simulate soil C without explicit representation of BC, fractionation may eventually lead to more precise C modelling partly by improving our understanding of BC. Realising this potential may depend, in the calibration phase, on discriminating BC within fractions and generalising the distribution of BC between fractions as affected by variables such as soil texture. In such a process it may become clear that there is a more compatible or reliable distribution of BC between fractions identified by one fractionation procedure compared to another. The apparent 14C age of SOM fractions in our study indicated the presence of BC from both charcoal and fossil sources. This highlights a complication in the use of 14C measurement to establish the contribution of BC to pool reactivity. It may be important to deploy a method such as HyPy, so that the reactivity of the non-BC component can be established in composite fractions.

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## References

Ascough, P., Bird, M.I., Wormald, P., Snape, C.E. and Apperley, D. (2008) Influence of pyrolysis variables and starting material on charcoal stable isotopic and molecular characteristics*. Geochimica et Cosmochimica Acta* 72:6090-6102.

Bell, M.J., Rees, R.M., Cloy, J.M., Topp, C.F.E., Bagnall, A. and Chadwick, D.R. (2015) Nitrous oxide emissions from cattle excreta applied to a Scottish grassland: Effects of soil and climatic conditions and a nitrification inhibitor. *Science of the Total Environment* 508:343–353

Brodowski, S., Amelung, W., Haumaier, L., Abetz, C. and Zech, W. (2005) Morphological and chemical properties of black carbon in physical soil fractions as revealed by scanning electron microscopy and energy-dispersive X-ray spectroscopy. *Geoderma* 128:116–129

Brodowski, S., John, B., Flessa, H. and Amelung, W. (2006) Aggregate-occluded black carbon in soil. *European Journal of Soil Science* 57:539–546

Brodowski, S., Amelung, W., Haumaier, L. and Zech, W. (2007) Black carbon contribution to stable humus in German arable soils. *Geoderma* 139:220–228

Camberdella, C.A. and Elliott, E.T. (1992) Particulate soil organic-matter changes across a grassland cultivation sequence. *SSSAJ* 56:777–783

Crow, S.E., Swanston, C.W., Lajtha, K., Brooks, J.R. and Keirstead, H. (2007) Density fractionation of forest soils: methodological questions and interpretation of incubation results and turnover time in an ecosystem context. *Biogeochemistry* 85:69–90

Czimczik, C.I. and Masiello, C.A. (2007) Controls on black carbon storage in soils. *Global Geochemical Cycles* 21: GB3005, doi:10.1029/2006GB002798

FAO-ISRIC, (1990) Guidelines for Soil Description, 3rd Edition, Food and Agricultural Organisation, Rome

Ghani, A., Dexter, M. and Perrott, K.W. (2003) Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. *Soil Biology and Biochemistry* 35:1231–1243

Glaser, B., Balashov, E., Haumaier, L., Guggenberger, G. and Zech, W. (2000) Black carbon in density fractions of anthropogenic soils of the Brazilian Amazon region. *Organic Chemistry* 31:669–678

Goldberg, E.D. (1985) Black Carbon in the Environment 198 pp., John Wiley, New York

Harkness, D.D., Harrison, A.F. and Bacon, P.J. (1986) The temporal distribution of ‘bomb’ 14C in a forest soil. *Radiocarbon* 28:328–337

Hockaday, W.C., Grannas, A.M., Kim, S. and Hatcher, P.G. (2006) Direct molecular evidence for the degradation and mobility of black carbon in soils from ultrahigh-resolution mass spectral analysis of dissolved organic matter from a fire-impacted forest soil. *Organic Geochemistry* 37:501–510

Lefèvre, R., Barré, P., Moyano, F.E., Christensen, B.T., Bardoux, G., Eglin, T., Girardin, C., Houot, S., Kätterer, T., Van Oort, F. and Chenu, C. (2014) Higher temperature sensitivity for stable than for labile soil organic carbon – Evidence from incubations of long-term bare fallow soils. *Global Change Biology* 20:633–640

Lehmann, J., Skjemstad, J., Sohi, S., Carter, S., Barson, M., Falloon, P., Coleman, K., Woodbury, P. and Krull, E. (2008) Australian climate-carbon cycle feedback reduced by soil black carbon. *Nature* *Geoscience* 1:832–835

Leifeld, J. (2007) Thermal stability of black carbon characterised by oxidative differential scanning calorimetry. *Organic Geochemistry* 38:112–127

Levin, I., Hammer, S., Kromer, B. & Meinhardt, F. (2008) Radiocarbon observations in atmospheric CO2: Determining fossil fuel CO2 over Europe using Jungfraujoch observations as background. *Science of the Total Environment* 391:211-216.

Marschner, B., Brodowski, S., Dreves, A., Gleixner, G., Gudem A., Grootes, P.M., Hamer, U., Heim, A., Jandi, G., Ji, R., Kaiser, K., Kalbitz, K., Kramer, C., Leinweber, P., Rethemeyer, J., Schäffer, A., Schmidt, M.W.I., Schwark, L. and Wiesenberg, G.L.B. (2008) How relevant is recalcitrance for the stabilization of organic matter in soils. *Journal of Plant Nutrition and Soil Science* 171:91–110

Meredith, W., Ascough, P.L., Bird, M.I., Large, D.J., Snape, C.E., Sun, Y. and Tilcton, E.L. (2012) Assessment of hydropyrolysis as a method for the quantification of black carbon using standard reference materials. *Geochimica et Cosmochimica Acta* 97:131–147

Palacio, S., Aitkenhead, M., Escuardero, A., Montserrat-Marti, G., Maestro, M. and Robertson, A.H.J. (2014) Gyposophile chemistry unveiled: Fourier transform infrafred (FTIR) spectroscopy provides new insight into plant adaptations to gypsum soils. *PLoS ONE* 9(9): e107285. doi:10.1371/journal.pone.0107285

Six, J., Guggenberger, G., Paustian, K., Haumaier, L., Elliott, E.T. and Zech, W. (2001) Sources and composition of soil organic matter fractions between and within soil aggregates. *European Journal of Soil Science* 52:607–618

Sohi, S.P., Mahieu, N., Arah, J.R.M., Powlson, D.S., Madari, B. and Gaunt, J.L. (2001) A procedure for isolating soil organic matter fractions suitable for modelling. *Soil Science Society of America Journal* 65:1121–1128

Stevenson, F.J. (1982) *Humus Chemistry. Genesis, Composition, Reactions*. New York, John Wiley & Sons Inc.

Stuiver, M. and Polach, H.A. (1977) Reporting of 14C data. *Radiocarbon* 19:355-363

Trumbore, S. (2009) Radiocarbon and soil carbon dynamics. *The Annual Review of Earth and Planetary Sciences* 37:47–66

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E. and Marschner, B. (2007) SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. *Soil Biology & Biochemistry* 39:2183–2207

VSN International (2011). GenStat *for Windows* 14th Edition. VSN International, Hemel Hempstead, UK.

Weishaar, J.L., Aiken, G.R., Bergamaschi, B.A., Fram, M.S., Fuji, R. and Mopper, K. (2003) Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environmental Science Technology* 37:4702–4708

Zimmermann, M., Leifeld, J., Schmidt, M.W.I., Smith, P. And Fuhrer, J. (2007) Measured soil organic matter fractions can be related to pools in the RothC model. *European Journal of Soil Science* 58:658–667

Table 1: Mean relative proportion of whole soil mass (kg t-1) and whole soil organic C (OC) (%) for isolated fractions and calculated OC stocks (t C ha-1) and C:N for whole soil and isolated SOM fractions from the Zimmermann *et al* (2007), Sohi *et al* (2001) and Ghani *et al* (2003) fractionation methods. Standard errors are given in parenthesis. Values with no letters in common after them are significantly different (one-way ANOVA followed by a Tukey-Kramer adjusted means test, p < 0.05). OM – organic matter, SOC – soil organic C, LF – light fraction.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Fraction | Relative mass of whole soil (kg t-1) | Proportion whole soil OC (%) | OC stock  (t C ha-1) | C:N |
| Whole soil | - | 1000 | 100 | 24.1 (1.5) | 11.07 (0.16)a |
| Zimmermann et al (2007) | Silt and clay | 390 (15.1) | 54.1 (1.8) | 13.0 (0.2) | 10.66 (0.02)a |
| Residual SOC | 367 (19.7) | 37.0 (1.8) | 8.9 (0.2) | 13.37 (0.06)ab |
| Sand and aggregates | 600 (16.0) | 36.7 (1.8) | 8.8 (0.2) | 12.54 (0.17)ab |
| Particulate OM | 9.2 (2.6) | 8.4 (2.1) | 2.0 (0.3) | 21.91 (1.19)bc |
| Dissolved OC | - | 1.0 (0.2) | 0.2 (0.0) | - |
| Sohi et al (2001) | Organo-mineral | 913 (3.0) | 91.0 (0.4) | 21.9 (0.1) | 11.99 (0.12)ab |
| Free LF | 14.5 (1.4) | 7.8 (0.7) | 1.9 (0.2) | 24.51 (4.66)c |
| Intra-aggregate LF | 3.5 (1.3) | 1.2 (0.5) | 0.3 (0.1) | 26.47 (1.64)c |
| Ghani et al (2003) | Hot water extractable C | - | 2.7 (0.1) | 0.6 (0.0) | - |
| Water soluble C | - | 0.3 (0.0) | 0.1 (0.0) | - |

Table 2: Duplicate sub-sample 14C enrichment expressed as % modern C (pMC), conventional 14C age (years BP), δ13C (‰) values for whole soil and isolated SOM fractions from the Zimmermann *et al* (2007), Sohi *et al* (2001) and Ghani *et al* (2003) fractionation methods. Single sub-sample 14C enrichment expressed as % modern C (pMC), conventional 14C age (years BP), δ13C (‰) values for the black C isolated from the particulate OM fraction are also presented. Errors, shown in parenthesis, are associated with analytical confidence and represent 1 standard deviation. Values with no letters in common after them are significantly different (one-way ANOVA followed by a Tukey‑Kramer adjusted means test, p < 0.05). SOC – soil organic C, OM – organic matter, LF – light fraction.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Fraction | 14C enrichment (pMC) (± 1σ) | Conventional 14C age (years BP) (± 1σ) | δ13C (‰)  (± 1σ) |  | SUERC sample IDs |
| Mean residence time (years) |
|  |
| Whole soil | - | 96.9 (0.44)c | 255 (37) | -28.4a | 500 | SUERC-51662 |
| 95.5 (0.44) | 370 (37) | -28.1 | > 500 | SUERC-51663 |
| Zimmermann *et al* | Silt and clay | 101.5 (0.46)c | Modern | -28.2a | 262 | SUERC-51664 |
| 100.5 (0.46) | -28 | 303 | SUERC-51665 |
| Residual SOC | 97.0 (0.42)c | 248 (35) | -28.1a | 493 | SUERC-51666 |
| 96.3 (0.44) | 305 (37) | -28.1 | > 500 | SUERC 51667 |
| Sand and aggregates | 90.4 (0.39)bc | 809 (35) | -28.5a | > 500 | SUERC-51670 |
| 85.8 (0.37) | 1234 (35) | -27.9 | > 500 | SUERC-51671 |
| Particulate OM | 60.7 (0.28)a | 4016 (38) | -26.5bc | > 500 | SUERC-54820 |
| 58.4 (0.27) | 4315 (37) | -26.7 | > 500 | SUERC-54821 |
| Dissolved OC | 54.1 (0.25)a | 4937 (37) | -27.0ab | > 500 | SUERC-54829 |
| 40.4 (0.20) | 7272 (39) | -27.4 | > 500 | SUERC-54830 |
| Black C isolated from Particulate OM | 9.86 (0.11) | 18610 (89) | -23.4 | - | SUERC-67369 |
| Sohi *et al* | Organo-mineral | 97.7 (0.45)c | 190 (37) | -28.0a | 451 | SUERC-51672 |
| 97.3 (0.45) | 223 (37) | -28.3 | 475 | SUERC-51673 |
| Free LF | 78.6 (0.36)b | 1932 (37) | -27.7ab | > 500 | SUERC-54822 |
| 78.0 (0.36) | 1998 (37) | -27.1 | > 500 | SUERC-54823 |
| Intra-aggregate LF | 45.2 (0.23)a | 6376 (24) | -26.0c | > 500 | SUERC-54824 |
| 47.6 (0.22) | 5968 (38) | -26.1 | > 500 | SUERC-54825 |
| Ghani *et al* | Hot water extractable C | 108.6 (0.5)c | Modern | -27.0bc | 10 | SUERC-54831 |
| 109.0 (0.5) | -26.8 | 11 | SUERC-54832 |
| Water soluble C | 101.7 (0.47)c | Modern | -26.4bc | 254 | SUERC-54833 |
| 100.1 (0.46) | -26.6 | 324 | SUERC-54834 |

Table 3: Specific UV absorbance at 245nm (SUVA254), E4/E6 ratios and organic C (OC) concentrations for water soluble C (WSC) and hot-water extractable C (HWEC) isolated using the Ghani *et al* (2003) method and dissolved OC (DOC) isolated during the Zimmerman *et al* (2007) method. Standard errors are shown in parenthesis. Values with no common letters in each row are significantly different (One-way ANOVA, n = 3).

|  |  |  |  |
| --- | --- | --- | --- |
|  | Ghani *et al* | | Zimmermann *et al* |
|  | WSC | HWEC | DOC |
| SUVA245 | 7.81 (1.67)a | 0.52 (0.12)b | 10.36 (2.61)a |
| E4/E6 | 0.68 (0.16)a | 0.78 (0.20)a | 1.59 (0.15)b |
| OC Concentration (mg l-1) | 6.80 (0.44)a | 70.67 (5.22)b | 6.03 (0.94)a |

Figure 1: FT-IR spectra for soil organic matter fractions isolated using the Zimmermann *et al* (2007) fractionation method. The indicated bands are indicative of a) H bonded OH and/or kaolinite doublet, b) aliphatic C-H stretching, c) aliphatic carboxylates and/or aromatics, and d) polysaccharides and/or Si-O stretches. SOC – soil organic C, OM –organic matter.

Figure 2: FT-IR spectra for soil organic matter fractions isolated using the Sohi *et al* (2001) fractionation method and whole soil. The indicated bands are indicative of a) H bonded OH and/or kaolinite doublet, b) aliphatic C-H stretching, c) aliphatic carboxylates and/or aromatics, and d) polysaccharides and/or Si-O stretches. LF – light fraction.

Figure 3: FT-IR spectra for hot-water extractable C fraction isolated using the Ghani *et al* (2003) method. The indicated bands are indicative of a) H bonded OH and/or kaolinite doublet, b) aliphatic C-H stretching, c) aliphatic carboxylates and/or aromatics, and d) polysaccharides and/or Si-O stretches.

Figure 4: FT-IR spectra of s+c and rSOC fractions showing a) aliphatic carboxylates and/or aromatic group bands, b) aliphatic C-H stretching bands.