



Effect of air-drying pre-treatment on the characterization of forest soil carbon pools



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ABSTRACT

Archived soils could represent a valuable resource for the spatio-temporal inventory of soil carbon stability. However, archived soils are usually air-dried before storage and the impact of a drying pretreatment on physically and chemically-defined C fractions has not yet been fully assessed. Through the comparison of field-moist and corresponding air-dried (at 25 °C for 2 weeks) forest soil samples, we examined the effect of air-drying on: a) the quantity and the quality of cold- (CWEC) and hot-water (HWEC) extractable C and b) the concentration of C in physically isolated fractions (free- and intra-aggregate light and organo-mineral). Soil samples were collected from the organic (O) and mineral (A and B) horizons of three different forest soils from southeastern England: (i) cambisol under pine (*Pinus nigra*); (ii) cambisol under beech (*Fagus sylvatica*) and (iii) gleysol under oak (*Quercus robur*). CWEC concentrations for dry samples were up to 2 times greater than for corresponding field moist samples and had significantly ($p < 0.001$) higher phenolic content. However, the effect of drying pretreatment on HWEC and its phenolic content was not significant ($p > 0.05$) for most samples. Dried soils had significantly ($p < 0.001$) higher concentrations of free light-C while having lower concentrations of intra-aggregate-C when compared to moist samples ($p < 0.001$). However, fine silt and clay fractions were not significantly affected by the drying pretreatment ($p = 0.789$). Therefore, based on the results obtained from gleysol and cambisol forest soils studied here, C contents in hot-water extractions and fine particle size physical fractions ($< 25 \mu\text{m}$) seem to be robust measurements for evaluating C fractions in dried stored forest soils. Further soil types should be tested to evaluate the wider generality of these findings.

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1. Introduction

Globally, soils contain more than 2500 Pg carbon (C) and therefore store 3–4 times as much C as the land biota (560–654 Pg) and the atmosphere (845 Pg) (Batjes, 1996; Lal, 2004; Prentice et al., 2001; Tarnocai et al., 2009). However, the future of the amount of C stored in soils worldwide and the potential of soil to act as net CO₂ sink under global warming scenarios is still highly uncertain (Cox et al., 2000; Smith and Fang, 2010). Measuring and verifying changes in soil C stocks and unraveling the factors controlling long-term soil carbon stability are still major challenges that compromise our understanding of the global carbon cycle (Jandl et al., 2014; Rumpel et al., 2004; Saby et al., 2008).

Archived soils could represent a valuable resource for the spatio-temporal inventory of soil carbon stability. For instance, long-term field experiments and soil collections (e.g. the Rothamsted Archive; <http://www.rothamsted.ac.uk/sample-archive>) have been the main source of data in the parameterization of soil C models (Coleman and Jenkinson, 1995) that ultimately have been able to predict potential C sequestration rates under different land uses, management and climatic

conditions (Peltre et al., 2012; Smith et al., 1997). Worldwide, there are many other research centers and organizations holding valuable archives (Chapman et al., 2013; De Nobili et al., 2006; Jandl et al., 2014; Richter et al., 1999; Torn et al., 2002), including not only temporal but also extensive spatial soils collections spanning, in some cases, a continental scale (Baritz et al., 2010; Karssies et al., 2011). However, the use of soil samples from archives can present some complications in relation to the type of pretreatment undertaken and the soil parameters of interest. Drying is one of the most common pretreatments to ensure that samples are stored in a relatively stable state (De Nobili et al., 2006; Karssies et al., 2011). Although drying pretreatment is thought not to significantly change Total Carbon (TC) and Total Organic Carbon (TOC) measurements (Blake et al., 2000), it may alter soil properties such as pH, total S, extractable nutrients, microbial biomass and respiration (De Nobili et al., 2006; Koopmans and Groenberg, 2011), and also the quantity and quality of labile C pools obtained through cold-water extractions (Jones and Willett, 2006; Kaiser et al., 2001; Merckx et al., 2001; Qualls and Haines, 1991).

It is well-known that soil C should not only be assessed in terms of total concentration but also with respect to its quality and expected stability (Lal, 2004; Rumpel et al., 2004). Thus, several types of chemical extraction procedures and physical fractionation schemes have been

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developed with the aim of isolating C fractions differing in their chemical nature (Balara et al., 2009; Ghani et al., 2003) and degree of physical protection (Six et al., 2004; Sohi et al., 2001), while also exhibiting differences in stability and turnover rates (Gregorich et al., 2006; von Lützwow et al., 2007). Cold and hot-water extractions have been mainly used to determine readily decomposable fractions of soil organic matter (monomeric carbohydrates, aliphatic acids, low molecular weight phenols, free amino acids and peptides) and thus are used to define labile soil organic carbon (SOC) pools (Balara et al., 2009; Gregorich et al., 2006; Landgraf et al., 2006). In contrast, chemical extractions (e.g. NaOCl, H₂O₂, Na₂S₂O₈, HCl) have not yet been successful in the exclusive isolation of stable C fractions due to the preferential isolation of significant proportions of young C (Helfrich et al., 2007). While chemical extraction approaches are based on inherent chemical recalcitrance, physically based fractionation schemes examine physical stabilization of SOC in aggregates and/or by mineral complexation (Six et al., 2002; Sohi et al., 2001; von Lützwow et al., 2007). Physical and chemical methods, given that they represent different stabilization mechanisms, can be partly complementary. Some of the above methods (Ghani et al., 2003; Sohi et al., 2001) were developed for use on fresh field-moist samples and the effect of a drying pretreatment on soil C quality is not commonly assessed. Therefore, it is uncertain if C quality as estimated by fractionation of dried samples provides a good representation of that in a freshly-sampled equivalent soil sample and this undermines the application of such methods to dried soils, and consequently most archived soils.

Thus, the aim of this study was to evaluate the effect of an air-drying pretreatment on: i) the amount and the composition of two water soluble C fractions (cold- and hot-water extractable C) and ii) the concentration of C in physical fractions isolated by density flotation and particle size fractionation from forest soils under different tree species and with distinct soil properties. We focus on forest soils because of their importance as a store of C (Baritz et al., 2010; Prentice et al., 2001) and their suggested relative sensitivity to drying treatment due to their protection under field conditions from rapid changes in soil moisture content by the moderating influence of the forest canopy and litter layer (Černohlávková et al., 2009; Fierer and Schimel, 2002).

2. Materials and methods

2.1. Study site, soil sampling and pre-treatment

The sites were located within the Alice Holt Forest in Farnham (Surrey, south-east England). Alice Holt Forest has been a Forestry Commission-managed forest since 1924 and covers an area of 769 ha (National Grid reference SU 813427). The climate is characteristic of south-east England, with a mean annual (1994–2006) rainfall of 803 mm and average annual temperature of 10.1 °C (Benham, 2008). The experimental area includes a wide range of temperate forest ecosystems, with two main distinctive forest types: second-growth forests of conifers (377 ha) mainly dominated by stands of Corsican Pine (*Pinus nigra* var. *maritima*) and old-growth woodlands (392 ha) of oak (*Quercus robur* and *Quercus petraea*), beech (*Fagus sylvatica*) and mixed broadleaves with stands of up to 200 years old. Three different types of forest were selected that were representative of major forest soil types and tree species in the UK (Vanguelova et al., 2013): a gleysol under oak (*Q. robur*; S1), a cambisol under beech (*F. sylvatica*; S2) and a cambisol under pine (*P. nigra*; S3). Triplicate soil samples from each forest type were collected by horizon (O, A and B) in December 2009 and basic physico-chemical properties are given in Table 1. Field moisture contents were 60–70% for organic horizons, 20–30% for A horizons and 19–21% for B horizons. Soil samples were sieved field moist to either 2 mm (mineral horizons; isolating small macroaggregates (0.25–2 mm) and microaggregates (53–250 µm)) or 4 mm (organic horizons) and sub-samples of soils from each forest type and horizon were air dried at room temperature (25 °C) for two

Table 1

Physicochemical properties of the organic and mineral horizons of the three forest sites (S1- gleysol under *Quercus robur*, S2- cambisol under *Fagus sylvatica*, S3- cambisol under *Pinus nigra*). Data are the mean and standard error in parenthesis (n = 3).

Site	H. †	SOM ‡ (%)	TC (%) §	TN (%) §	C/N	pH ¶	Particle size analysis (%) #		
							Sand	Silt	Clay
S1	O	59.7 (0.5)	30.7 (0.2)	1.6 (0.1)	19.3	3.9 (0.1)			
	A	10.8 (0.2)	4.9 (0.1)	0.4 (0.1)	13.5	3.2 (0.1)	20.8	69.2	9.9
	B	5.5 (0.1)	2.1 (0.1)	0.2 (0.1)	13.4	3.8 (0.1)	14.2	72.2	13.6
S2	O	41.8 (1.1)	22.9 (0.8)	1.2 (0.1)	18.9	3.4 (0.1)			
	A	6.6 (0.1)	3.4 (0.8)	0.2 (0.1)	14.5	3.0 (0.1)	38.1	56.0	6.0
	B	5.2 (0.1)	2.4 (0.1)	0.2 (0.1)	15.1	3.6 (0.1)	43.7	50.0	6.3
S3	O	81.1 (0.2)	44.8 (0.4)	1.9 (0.1)	23.4	2.9 (0.1)			
	A	8.4 (0.1)	4.1 (0.1)	0.3 (0.1)	15.5	3.0 (0.1)	42.3	52.3	5.4
	B	4.9 (0.7)	2.1 (0.1)	0.2 (0.1)	12.6	3.3 (0.1)	36.0	55.8	8.2

†: Horizon. ‡: Soil Organic Matter (SOM) was determined gravimetrically by loss on ignition in a muffle furnace at 500 °C for 12 h. §: Total Carbon (TC) and Total Nitrogen (TN) contents were analysed by combustion with a LECO SC-444 autoanalyser and Europe Roboprep-VG 662, respectively. ¶ pH was determined in aqueous extracts (soil-to-solution ratio 1:10 (w/v) for organic and 1:5 (w/v) for mineral horizons). #: Particle size analysis of the mineral soil samples was carried out by using a Coulter LS Particle Size Analyzer.

weeks ('dry samples'), while the remaining soils, designated 'field moist samples', were stored in the field moist state at 4 °C. These air-dried and field moist samples were subject to cold- and hot-water extraction and physical fractionation as described below.

2.2. Cold-water extractable carbon (CWEC) and hot-water extractable carbon (HWEC)

A sequential cold-water and hot-water extraction using a method modified from (Ghani et al., 2003) was carried out. Briefly, soil samples (3–4 g, oven dry equivalent) in 50 mL polypropylene centrifuge tubes were extracted with 30–40 mL (1:10 solid-to-liquid ratio) of ultrapure water (>15 Ω Purelab Maxima) by shaking end-over-end for 3 h at 20 °C. The tubes were then centrifuged (20 min, 15,000 rpm) and the supernatant filtered through 0.45 µm cellulose nitrate membrane filters to obtain the cold-water extractable C (CWEC). A further 30–40 mL of ultrapure water (1:10 solid:liquid) was then added to the soil pellet and tubes were vortexed in order to bring all solids into suspension and placed in a water bath at 80 °C for 16 h. The resulting extracts were centrifuged and filtered as described previously for CWEC, in order to obtain the hot-water extractable C (HWEC). Total, inorganic and organic C were measured in both cold-water and hot-water extracts with a Shimadzu TOC-5000 analyser using high temperature and catalytic oxidation.

In order to assess changes in the quality of the water extractions the phenolic content of both CWEC and HWEC extractions was measured by colorimetric spectrometry using the Folin-Ciocalteu method (Folin and Ciocalteu, 1927), modified by Kalbitz et al. (2008). Changes in the phenolic content after drying might indicate that the samples had undergone strong biochemical (polymerization/depolymerization of phenolic molecules; (Sinsabaugh, 2010)) and physicochemical (altered organo-mineral sorptive interactions; (Kaiser et al., 2001)) changes during pre-treatment. Briefly, 100 µL of Folin-Ciocalteu's reagent (Sigma Aldrich, N° F9252), 200 µL of saturated Na₂CO₃ solution, 300 µL ultrapure water and 1.4 mL aliquots of sample were added to 2 mL Eppendorf tubes and vortexed to mix. Samples were incubated for 20 min at room temperature and the extent of blue-coloured complex development in the presence of phenolic compounds was determined by measurement of absorbance at 725 nm against salicylic acid standard solutions (0–40 mg L⁻¹). The concentration of phenols in CWEC and HWEC extractions was expressed as milligrams of salicylic acid equivalents per litre (mg eq. L⁻¹) and as a percentage of the total soluble C for each extraction (60.9% C per molecule of salicylic acid).

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