Global Change Biology
celebrating 20 years

Global Change Biology (2014) **20**, 1629–1642, doi: 10.1111/gcb.12459

Transformation and stabilization of pyrogenic organic matter in a temperate forest field experiment

NIMISHA SINGH*, SAMUEL ABIVEN*, BERNARDO MAESTRINI*, JEFFREY A. BIRD†, MARGARET S. TORN‡ and MICHAEL W. I. SCHMIDT*

*Department of Geography, University of Zurich, Winterthurerstrasse 190, Zürich 8057, Switzerland, †School of Earth and Environmental Sciences, Queens College, City University of New York, Flushing, 11367 NY, USA, ‡Lawrence Berkeley National Laboratory, Berkeley, 94720 CA, USA

Abstract

Pyrogenic organic matter (PyOM) decomposes on centennial timescale in soils, but the processes regulating its decay are poorly understood. We conducted one of the first studies of PyOM and wood decomposition in a temperate forest using isotopically labeled organic substrate, and quantified microbial incorporation and physico-chemical transformations of PyOM *in situ*. Stable-isotope (^{13}C and ^{15}N) enriched PyOM and its precursor wood were added to the soil at 2 cm depth at ambient (N0) and increased (N+) levels of nitrogen fertilization. The carbon (C) and nitrogen (N) of added PyOM or wood were tracked through soil to 15 cm depth, in physically separated soil density fractions and in benzene polycarboxylic acids (BPCA) molecular markers. After 10 months *in situ*, more PyOM-derived C (>99% of initial ^{13}C-PyOM) and N (90% of initial ^{15}N-PyOM) was recovered than wood derived C (48% of ^{13}C-wood) and N (89% under N0 and 48% under N+). PyOM-C and wood-C migrated at the rate of 126 mm yr^{-1} with 3–4% of PyOM-C and 4–8% of wood-C recovered below the application depth. Most PyOM C was recovered in the free light fraction (fLF) (74%), with 20% in aggregate-occluded and 6% in mineral associated fractions – fractions that typically have much slower turnover times. In contrast, wood C was recovered mainly in occluded (33%) or dense fraction (27%). PyOM addition induced loss of native C from soil (priming effect), particularly in fLF (13%). The total BPCA-C content did not change but after 10 months the degree of aromatic condensation of PyOM decreased, as determined by relative contribution of benzene hexa-carboxylic acid (B6CA) to the total BPCA C. Soil microbial biomass assimilated 6–10% of C from the wood, while PyOM contributions was negligible (0.14–0.18%). The addition of N had no effect on the dynamics of PyOM while limited effect on wood.

Abbreviations

PyOM = pyrogenic organic matter
BPCA = benzene polycarboxylic acids
fLF = free light fraction
oLF = occluded light fraction
DF = dense fraction
SOM = soil organic matter
PE = priming effect
SMB = soil microbial biomass

Keywords: ^{13}C, ^{15}N, benzene polycarboxylic acids, density fractionation, microbial biomass, nitrogen deposition, priming effect, pyrogenic organic matter, soil organic matter

Received 27 June 2013; revised version received 11 October 2013 and accepted 17 October 2013

Introduction

Pyrogenic organic matter (PyOM), a product of incomplete combustion of biomass (Goldberg, 1985), is ubiquitous in soils (Schmidt & Noack, 2000) and can account for up to 45% of total soil organic carbon (SOC) (Bird *et al.*, 1999; Schmidt *et al.*, 1999b; Skjemstad *et al.*, 2002; Lehmann *et al.*, 2008; Rovira *et al.*, 2009). In recent years, PyOM has received considerable interest by researchers, in part, because of its potential relevance to the carbon (C) cycle of terrestrial ecosystem (Schmidt *et al.*, 2011). Climate change projections predict an increase in the wildfire frequency and intensity in temperate and boreal regions (Westerling *et al.*, 2006; Moritz *et al.*, 2012), which would increase the inputs of PyOM to soils. The addition of PyOM to soils could also constitute a method for sequestering C (Lehmann *et al.*, 2006; Deluca & Aplet, 2008), if its residence time

Correspondence: Samuel Abiven, tel. +41 44 6355183, fax +41 44 6356841, e-mail: samuel.abiven@geo.uzh.ch

in soil were sufficiently longer than its precursor biomass. However, many uncertainties remain about the dynamics of PyOM C and nitrogen (N) in soils including *in situ* PyOM turnover rates, degradation pathways and stabilization mechanisms (Knicker, 2011; Singh *et al.*, 2012).

Most wood decomposition studies estimate decay rates as either mass loss or density change per unit time (Chambers *et al.*, 2001). These studies have estimated yearly to decadal turnover time for fine woody debris and litter (Abbott & Crossley, 1982; Busse, 1994; Clark *et al.*, 2002; Guo *et al.*, 2006; Zell *et al.*, 2009; Fasth *et al.*, 2011). Degradation rates of pyrolyzed wood are slower than its initial precursor biomass and becomes even slower with increasing pyrolysis temperatures (Baldock & Smernik, 2002). The slow decomposition of pyrolyzed wood, and pyrogenic matter in general, has been attributed to changes in physical and chemical structure, including an increase in the degree of aromatic condensation as the pyrolysis temperature increase from 200° to 600 °C (Baldock & Smernik, 2002; Keiluweit *et al.*, 2010; Schneider *et al.*, 2010; Zimmerman, 2010; Chatterjee *et al.*, 2012).

In the last decade, studies on PyOM degradation in soils based on field (Bird *et al.*, 1999; Hammes *et al.*, 2008; Nguyen *et al.*, 2008) and incubation studies (Baldock & Smernik, 2002; Hamer *et al.*, 2004; Kolb *et al.*, 2009; Kuzyakov *et al.*, 2009; Hilscher & Knicker, 2011; Santos *et al.*, 2012) challenges the view that PyOM persists in soils for millennia (Schmidt & Noack, 2000). A recent synthesis of current knowledge of PyOM degradation in soil estimated turnover times on centennial scales (Singh *et al.*, 2012). In addition to losses via mineralization, PyOM is also vertically mobile in mineral soil (Skjemstad *et al.*, 1999; Dai *et al.*, 2005; Rumpel *et al.*, 2006; Brodowski *et al.*, 2007) and can eventually be lost from soil by leaching (Shinogi *et al.*, 2003; Hockaday *et al.*, 2006; Cheng & Lehmann, 2009; Major *et al.*, 2009b; Abiven *et al.*, 2011). PyOM dissolution from soils could therefore be an important translocation mechanism in the terrestrial system.

Recent research on soil organic matter (SOM) concludes that long-term persistence of SOM, that is, on centennial scales, depends on its inaccessibility to microorganisms, such as through organo-mineral interactions (Von Lützow *et al.*, 2006; Schmidt *et al.*, 2011). PyOM interacts with the soil mineral phase (Glaser *et al.*, 2000; Brodowski *et al.*, 2006; Cheng *et al.*, 2006; Cheng & Lehmann, 2009) and has been posited to act as a binding agent for soil aggregates (Brodowski *et al.*, 2005a). Vasilyeva *et al.* (2011) observed that 70% of PyOM was associated with the dense fraction (>2 g cm^{-3}) under 55 years of fallow management (fire suppression) in the Streletzkaya steppe (Russia), and suggested its stabilization by clay micro-aggregation. These studies indicate that aggregation and mineral interactions may be important mechanisms for stabilizing PyOM in soil. However, we do not know when and to what extent such stabilizing mechanisms occur during the PyOM decomposition pathway.

Atmospheric N deposition has increased in recent decades and is predicted to further rise (Galloway *et al.*, 2008). This is important because C and nitrogen (N) cycles are closely linked at all scales and interact in many ways and therefore cannot be considered separately (Norby, 1998). N addition has decreased (Fog, 1988; Turunen *et al.*, 2004; Waldrop *et al.*, 2004; Janssens *et al.*, 2010), increased (Mack *et al.*, 2004; Waldrop *et al.*, 2004; Bragazza *et al.*, 2006), or had no effect on (Knorr *et al.*, 2005) SOM decomposition rates. There are fewer studies on the effects of increased N on wood and PyOM decomposition. These studies observed increased (Micks *et al.*, 2004; Wal *et al.*, 2007; Allison *et al.*, 2009; Bebber *et al.*, 2011) or level (Mccoll & Powers, 1998) decomposition rates for wood, with no effect on PyOM (Santos *et al.*, 2012).

Recent observations showed that C mineralization rates for native SOM could be influenced from PyOM addition to mineral soils (i.e. priming effect). However, the direction of this priming effect is under debate. PyOM has been shown to inhibit (negative priming) (Jones *et al.*, 2011), have no effect (Hilscher *et al.*, 2009; Kolb *et al.*, 2009; Kuzyakov *et al.*, 2009; Abiven & Andreoli, 2010; Santos *et al.*, 2012), or increase native SOM mineralization rates (positive priming) (Steinbeiss *et al.*, 2009; Zimmerman *et al.*, 2011). Moreover, in an incubation study using different types of PyOM, Zimmerman *et al.* (2011) observed a change in priming effects from positive to negative with time. Several mechanisms have been proposed to explain these contradictory priming effects associated with added PyOM, including (i) differences in the physicochemical state of PyOM in different soils (Santos *et al.*, 2012), (ii) encapsulation and/or sorptive protection of SOM by PyOM (Zimmerman *et al.*, 2011), (iii) nutritional competition and balance between r and K strategist microbial population (Fontaine *et al.*, 2003), or (iv) changes in microbial community structure (Blagodatskaya & Kuzyakov, 2008). For wood-amended soil, only a few studies reported positive priming effects on SOM mineralization (Sulzman *et al.*, 2005; Crow *et al.*, 2009) or no priming effect (Santos *et al.*, 2012).

No reported field studies have compared the C and N dynamics of PyOM and its precursor biomass during its decomposition in soil. To evaluate the potential of PyOM as a long-term C sink in soil as compared to its precursor biomass, we conducted a field study to quantify the fate of added $^{13}C/^{15}N$ labeled PyOM and its

precursor biomass (*Pinus ponderosa* wood) to a forest soil during 10 months, *in situ*. To determine the effect of added inorganic N on PyOM and wood dynamics in soil, we experimentally manipulated N deposition to half of the plots by adding ammonium nitrate (NH_4NO_3) at (+60 kg N ha^{-1} yr^{-1}). The objectives of our study were to (i) determine recovery of C and N from PyOM and its precursor wood in soil (0–15 cm) after 10 months; (ii) quantify the vertical movement of PyOM and wood C and N in the soil profile; (iii) investigate the main stabilization mechanism for PyOM and/or wood by determining the partitioning of PyOM and wood C and N within operational-defined SOM fractions; (iv) determine chemical changes in PyOM, both in quality and quantity in comparison to initial input, and (v) assess the effect of N treatment on the decomposition dynamics of PyOM and wood in soil.

Materials and methods

Field site

The experimental site is a mixed, beech-dominated temperate forest, located 20 km northwest of Zurich, CH (47°28′40.8′N, 8°21′55.2′E) on the south-facing slope of Lägeren Mountain (eastern-most part of the Jura Mountain range) at 680 m above sea level. The mean annual temperature is 8.4 °C, and mean annual precipitation is 930 mm (Ruehr & Buchmann, 2010). The soil at the site is classified as a Cambisol (F.A.O.-U.N.E.S.C.O., 1998). Chemical and physical properties of the soil (0–10 cm) are presented in Table 1. Soil volumetric moisture content and soil temperature were monitored every 30 min at two depths (5 cm and 10 cm below the surface) within each field replicates, using soil moisture temperature sensors (ECH2O-TE/EC-TM, Decagon Devices, Pullman, WA, USA) connected to a data-logger. Soil moisture in the field site ranged from 20 to 50%, while temperatures ranged from 0 to 25 °C (at 5 cm depth) during the first year of the study.

Experimental design

The experiment is located within a forest gap created by a natural windthrow (in 1999), which has been subsequently mowed to maintain open conditions. The site was chosen to provide similar micro-climatic conditions to a post-fire gap. The experimental setup was a randomized block design, having the factorial combination of three types of organic inputs (i.e., pinewood, PyOM, and no input as control) and two treatment levels of N (ambient = N0 and added N = N+) with three field replicates (n = 3) per treatment combination. The ambient, natural N deposition at the field site was estimated to be 20 kg N yr^{-1} ha^{-1} (Kloeti *et al.*, 1989). The N+ treatment corresponds to a level of ca. 80 kg N yr^{-1} ha^{-1} (60 kg N yr^{-1} ha^{-1} added to the ambient N deposition). The wood used for the study was primary stem biomass from two-year-old *Pinus ponderosa* saplings grown under controlled

Table 1 Physical and chemical characteristics of the soil in the 0–15 cm depth. Values correspond to the mean (n = 3) and values between brackets correspond to SE

Texture %			Bulk density g cm^{-3}				CEC	Elemental analysis g kg^{-1} soil										
Sand	Silt	Clay	0–5 cm	5–10 cm	10–15 cm	pH	mmol kg^{-1}	C	H	N	Na	Mg	Al	Si	P	K	Ca	Mg	Fe
45.5	24.2	31.5	1.20	1.21	1.60	5.9	74.3	33.7	8.9	2.4	7.8	11.2	71.6	317	0.3	19.5	4.6	1.4	32.8
(2.0)	(2.5)	(1.4)	(0.1)	(0.2)	(0.2)	(0.3)	(8.6)	(2.8)	(0.4)	(0.1)									

greenhouse conditions and labeled with $^{13}CO_2$ and $^{15}NO_3^-$ (Bird & Torn, 2006). PyOM was obtained by charring the labeled wood at 450 °C for 5 h under N_2 flux according to Hammes *et al.* (2006). The chemical characteristics of both labeled wood (^{13}C = 2.05 atom% and ^{15}N = 4.3 atom%) and PyOM (^{13}C = 2.03 atom% and ^{15}N = 4.2 atom%) are described in Santos *et al.* (2012). The C and N elemental composition of wood was 499 g kg^{-1} and 4.3 g kg^{-1}, respectively. For PyOM, C concentration was 799 g kg^{-1} and N was 7.1 g kg^{-1}. The structure of the PyOM and wood is detailed by magnetic resonance, mid-infrared spectroscopy and mass spectrometry in Chatterjee *et al.* (2012). The wood and PyOM were uniformly labeled (Yarnes *et al.*, 2011; Santos *et al.*, 2012). Both wood and PyOM were ground (<2 mm) prior to soil addition.

In each plot, we inserted 20 cm long and 10 cm diameter mesocosms (polyethylene tubes, smoothed at the top and sharpened at an angle at the bottom) into the soil up to a depth of 15 cm from the surface. Each mesocosm had two 4 cm diameter windows (at 7.5 cm and 12 cm distance from the bottom and aligned at 120º to one another), fit with 0.7 mm stainless steel mesh to allow fungal hyphae and some fine roots to penetrate the core and limit lateral movement of the added substrate (Bird & Torn, 2006). Mesocosms were placed >1 m from large trees and >0.5 m from the adjacent mesocosms. In April 2009, the mesocosms were installed at the field site and allowed to equilibrate for 180 days before the addition of the organic inputs.

In October 2009, ^{13}C and ^{15}N enriched-wood and PyOM were applied to the mesocosms at a rate of 189 g C m^{-2} for wood or 397 g C m^{-2} for PyOM, at 2 cm soil depth and mixed gently with 1–2 mm of mineral soil. The PyOM application rate was based on a previous estimate of PyOM inputs to soil after a fire in a similar forest type (Eckmeier *et al.*, 2007b). The amount of wood was based on estimation of twig and other wood contribution to the litter (Kammer & Hagedorn, 2011; Jones *et al.*, 2011). Unamended-control mesocosms were similarly disturbed to those that received wood or PyOM. Beginning in March 2010, 11.4 mg of $NH_4^+NO_3^-$ dissolved in 10 ml of water was added monthly for 10 months (equivalent to 60 kg N ha^{-1} yr^{-1}) to increased N (N+) treatment mesocosms, while an equivalent amount of distilled water was added to ambient N (N0) treatment mesocosms.

Soil sampling and analysis

We sampled the intact mesocosms (n = 18) 10 months after the PyOM or wood additions to the soil mesocosms. The soil within the mesocosms was separated immediately into 0–5 cm, 5–10 cm, and 10–15 cm depth. Soil fauna, stones (>2 mm), and roots (>2 mm) were manually removed and stored separately. Soil subsamples were air-dried and ball-milled for physico-chemical analysis. Soil water content was determined by drying 1 g of soil (n = 3) at 105 °C for 24 h. Microbial biomass analysis (chloroform fumigation extraction method) was performed immediately after sampling on fresh soil (Vance *et al.*, 1987). Total C and N contents in soil samples were determined with a CHN elemental analyzer (EA 1108; Carlo Erba, Cornaredo, Italy). The soil pH values were measured on air-dried soil at mass-to-volume ratio of 1 : 2.5 (soil : water ratio) (Jackson, 1958).

Soil organic matter fractionation

We used a density fractionation approach to partition SOM into three main pools that differ in their main stabilization mechanisms and turnover times. For each fraction, we quantified the ^{13}C and ^{15}N excess from added PyOM or wood to assess the distribution of PyOM and wood into discrete physical fractions as a means for identifying mechanisms such as organo-mineral interactions. In our study, the free light fraction (fLF) was separated using a density of 1.6 g cm^{-3} (Glaser *et al.*, 2000; Cerli *et al.*, 2012) and the occluded light fraction (oLF) was separated after gentle ultrasonic dispersion using a sonifier (Bandelin Sonoplus HD 3400; Berlin, Germany; calibrated according to Schmidt *et al.* (1999a). We applied 250 J ml^{-1} of disruptive energy per sample. This rate was based on analysis of oLF yield and C content across a dispersive energy range (0–300 J ml^{-1}, data not shown). For the density fractionation, a subsample (10 g) of air-dried sample (0–5 cm) was suspended in 50 ml of 1.6 g cm^{-3} sodium polytungstate (SPT) solution (TC-Tungsten compounds), the suspension was allowed to settle for 1 h and centrifuged (3237 *g*, 30 min; Heraeus Megafuge 1.0, Newport Pagnell, UK). The floating material (≤1.6 g cm^{-3}) was collected as fLF on a glass microfiber filters with 1.5 μm particle retention (934-AH, Whatman, Maidstone, UK), washed thoroughly with deionized water to remove any SPT (conductivity of supernatant water <50 μS cm^{-1}) and freeze-dried. The remaining pellet for each sample was resuspended in SPT and treated with ultra sonification (250 J ml^{-1}) to destroy aggregates. The suspension was allowed to settle for 4 h, followed by centrifugation (3237 *g*, 30 min). The oLF (<1.6 g cm^{-3}) was collected similarly as above on glass microfiber filters and washed thoroughly with deionized water (conductivity of supernatant water <50 μS cm^{-1}). The remaining dense fraction (DF) was washed until the SPT was removed completely (conductivity of supernatant water <50 μS cm^{-1}). The DF was not further separated via physical fractionation into sand or clay/silt fractions. It is considered a heterogeneous mixture of organic matter in different types of association with minerals (i.e., denser than 1.6 g cm^{-3}). Some part of organic matter present in DF could be uncomplexed organic matter in sand sized separates. The density fractions (fLF, oLF, DF) were ball-milled to homogenize the samples. C and N concentration was measured with an elemental analyzer (EA 1108; Carlo Erba) and ^{13}C and ^{15}N was measured using an isotope ratio mass spectrometer (IRMS) (Delta S, Thermo Finnigan, MAT, Bremen, Germany). The recovery of C and N was calculated based on the amount of C and N present in 0–5 cm depth soil after 10 months.

Benzenepolycarboxylic acid (BPCA) analysis

The BPCA molecular marker method was employed to quantify and characterize the PyOM before and after its addition to the soil (Glaser *et al.*, 1998; Brodowski *et al.*, 2005b; Schneider *et al.*, 2010). Subsamples (400–500 mg) of PyOM-amended

air-dried soil (0–5 cm, $n = 3$) and PyOM (30–40 mg) were pre-treated with 4 M trifluroacetic acid (4 h, 105 °C) to remove Fe and Al, followed by conversion of PyOM into BPCAs by nitric acid oxidation (8 h, 170 °C). The digested extract was further purified using cation-exchange resin to remove any polyvalent cations. The extracts were freeze-dried and subsequently derivatized into trimethylsilyl derivatives to be analyzed on a gas chromatograph (Agilent 6890, Palo Alto, CA, USA) equipped with a flame ionization detector and a DB-5MS capillary column (50 m × 0.2 mm i.d., 0.33 μm film thickness). Each analysis was performed in triplicate. The acids with 3, 4, 5, and 6 carboxyl functions (B3CA, B4CA, B5CA, and B6CA, respectively) were identified and summed up to represent the total amount of pyrogenic molecular markers in the PyOM.

Microbial biomass by chloroform fumigation direct extraction

Moist soil, equivalent of 20 g of oven-dried soil (105 °C, 24 h), was fumigated with alcohol free chloroform in desiccators for 24 h in the dark (Vance *et al.*, 1987). The fumigated soil and an equivalent amount of non-fumigated soil for each sample was then extracted using 1 M KCl (1 : 5 soil solution ratio) for 1 h, filtered (Whatman GF 934-AH; Whatman), and extracts stored at –20 °C until analysis. The total organic C (TOC) in fumigated and non-fumigated extracts were analyzed using a TOC analyzer (TOC-V; Shimadzu Corporation, Kyoto, Japan). A conversion factor of 0.45 (K_c) (Wu *et al.*, 1990) and 0.68 (K_n) (Shen *et al.*, 1984) was applied for incomplete extraction for microbial C and N, respectively. PyOM could adsorb lysed cells and may influence microbial biomass recovery (Durenkamp *et al.*, 2010; Liang *et al.*, 2010). Nevertheless, the amount of PyOM C contributing to total SOC is inversely correlated to the extraction efficiency (Liang *et al.*, 2010). In this study, the applied PyOM C contributed to 11% of total SOC and therefore, adsorption of lysed microbial biomass on PyOM is assumed to be negligible. The $\delta^{13}C$ and $\delta^{15}N$ of extracts were analyzed on freeze-dried extracts using IRMS (Delta S, Thermo Finnigan) (Murage & Voroney, 2007).

The $\delta^{13}C$ (‰) of soil microbial biomass C (SMB-C) was estimated as the $\delta^{13}C$ of the C extracted from the fumigated sample in excess of that extracted from the non-fumigated sample, using Eqn (1) (Murage & Voroney, 2007),

$$\delta^{13}C = \frac{(\delta^{13}C_f \times C_f) - (\delta^{13}C_{nf} \times C_{nf})}{(C_f - C_{nf})} \quad (1)$$

where C_f and C_{nf} were the amounts of C extracted from the fumigated and non-fumigated samples (μg C g^{-1} dry soil) and $\delta^{13}C_f$ and $\delta^{13}C_{nf}$ were the ^{13}C natural abundance of the fumigated and non-fumigated extracts (%), respectively.

The soil under no treatment (control plots) was taken as the reference, and the proportion of labeled substrate-derived-C in SMB was calculated using Eqn (2),

$$f_{C-\text{substrate}}, \% = \left(\frac{\delta_{\text{treated}-\text{microbes}} - \delta_{\text{control microbes}}}{\delta_{\text{substrate}} - \delta_{\text{control microbes}}}\right) \quad (2)$$

where $\delta_{\text{treated-microbes}} = \delta^{13}C$ value of SMB extracted from the substrate-treated mesocosms, $\delta_{\text{substrate}} = 842‰$ for wood and 800‰ for PyOM, and $\delta_{\text{control microbes}} = \delta^{13}C$ the value of SMB extracted from the control mesocosms.

Calculations

The amount of labeled substrate-C (or N) (PyOM and wood) recovered in the bulk soil and density fractions was calculated for each mesocosms using a two end-member linear mixing model Eqns (3) and (4) (Bernoux *et al.*, 1998),

$$f_{\text{substrate}} = \frac{\delta_{\text{soil sample}} - \delta_{\text{control soil}}}{\delta_{\text{substrate}} - \delta_{\text{control soil}}} \quad (3)$$

$$\text{Amount of substrate}_{C\,or\,N}, \% = C \text{ or } N_{\text{sample}}(\%) \times f_{\text{substrate}}, \quad (4)$$

where $f_{\text{substrate}}$ is the fraction of substrate in the soil, $\delta_{\text{soil sample}}$, $\delta_{\text{control soil}}$, $\delta_{\text{substrate}}$ is isotopic value (either $\delta^{13}C$ or $\delta^{14}N$, ‰) of soil sample, corresponding control soil within field replicates, and substrate (PyOM or wood), respectively. *C (or N)* is the amount of C (or N) in% of the soil sample.

To calculate excess ^{13}C or ^{15}N values in soil and microbial biomass, we used Eqn (5) (Dawson *et al.*, 2002),

$$\text{Excess } ^{13}C \text{ or } ^{15}N = \frac{\text{atom}\%_{\text{sample}} - \text{atom}\%_{\text{background}}}{100} \times C \text{ or } N \quad (5)$$

where Excess ^{13}C or ^{15}N (μg g^{-1} dry soil for microbes) is the total amount of ^{13}C or ^{15}N added by labeled PyOM or wood to soil or microbial biomass, $\text{atom}\%_{\text{sample}}$ is the atom % of soil or microbial biomass in substrate-treated sample, and $\text{atom}\%_{\text{background}}$ is the atom% of soil or microbial biomass in the control treatments averaged over three plots. C or N is the total organic C or N content of soil (g kg^{-1} soil) or microbial biomass (μg g^{-1} dry soil).

To estimate the potential migration rate of substrate-C in the soil profile, we use Eqn (6)

$$\text{Migration rate}_{\text{input}-C} = \frac{d_{max}}{t} \quad (6)$$

where d_{max} (mm) is the maximum depth where PyOM was recovered below its application depth after time t (in years). We estimated d_{max} as 10.5 cm based on the recoveries of PyOM and wood at depth 10–15 cm (average of 12.5 cm as soil was homogenized) and application depth at 2 cm below the surface.

Statistical analysis

We performed an analysis of variance (ANOVA) using SPSS (IBM-SPSS statistics 20.0 package for Mac, Armonk, NY, USA) to determine the effect of the different input treatments and the two levels of N application, and the interactions between the two factors at different depths (0–5 cm, 5–10 cm, and 10–15 cm). Differences in the relative contribution of individual molecular markers and BPCA-C (g kg^{-1} OC) at $t = 0$ and

t = 10 months were tested with independent t-test, when the data were normally distributed (Shapiro–Wilk test), and non-parametric Mann–Whitney U-test when the normality test failed. Levene's test for equality of variance was used to determine homogeneity in the data, and significance test was used accordingly. We considered differences between treatments with $P \leq 0.05$ as significant.

Results

Total C and N and recovery of substrate-C or N in the topsoil

We observed no change in soil total C in mesocosms (0–15 cm) due to different organic inputs or N ($P > 0.05$, $n = 3$), except in wood-amended soil under the N+ treatment which had a significantly higher total C than N0 at 0–5 cm depth ($P = 0.007$). Including all depth and across all N treatments, we recovered 99.6 ± 0.2% of the initial ^{13}C-PyOM and 48.3 ± 4.4% of the initial ^{13}C-wood after 10 months (Table 2). Most of the ^{13}C-PyOM (95 ± 1.7% for N0 and 96.5% ±1.4% for N+) and ^{13}C-wood (40.0 ± 13.2% under N0 and 44.5 ± 1.4% under N+) were recovered between 0 and 5 cm. Both PyOM-C and wood-C were recovered at the depth 10–15 cm, indicating vertical movement with a migration rate of 126 mm yr^{-1} and 3–4% of PyOM-C and 4-8% of wood-C migrated below the incorporation depth of 2 cm (Table 2). Given the three depth intervals of sampling, the migration rate of PyOM and wood C could range between 100 mm yr^{-1} to 150 mm yr^{-1}. We observed no effect of the N treatment on either on the vertical movement (amount or rates) or C recovery of wood or PyOM.

Similarly, total soil N in mesocosms was not affected by added PyOM, wood or from added N. We recovered >90% of applied ^{15}N-PyOM, mainly at the application depth (0–5 cm; Table 2). The recovery of ^{15}N-PyOM across all N treatment (93.6 ± 2.2%, $n = 6$) in mesocosms was significantly lower than ^{13}C-PyOM ($P \leq 0.05$). More wood-^{15}N was recovered in mesocosms under ambient N levels (88.5 ± 6.4%) than with elevated (N+) levels (48.0 ± 6.0%, Table 2).

Soil organic matter fractions

The average recovery of C and N of bulk soil across all treatments ($n = 18$) after density fractionation was 88.8 ± 2.9% of total C and 84.8 ± 2.6% of the total N, respectively (Table 3). The loss of C and N is due to mobilization into SPT solution or as dissolved organic C and N during washing of density fractions with water to remove SPT, which were discarded during the process. After density fractionation, we recovered on average all of ^{13}C-PyOM (100 ± 3.7%, $n = 6$) and ^{15}N-PyOM (100 ± 6%, $n = 6$), but recovery of ^{13}C-wood (72.0 ± 9.7%; $n = 6$) and ^{15}N-wood (70 ± 14%; $n = 6$) was highly variable. The recovery of ^{13}C-wood was significantly lower than ^{13}C-PyOM ($P \leq 0.05$), while ^{15}N-wood recovery was highly variable among replicates, leading to no significant difference in ^{15}N-PyOM recoveries. The recoveries of PyOM and wood C and N are expressed as% of the amount recovered in the bulk soil between 0 and 5 cm depth after 10 months.

For all treatments, most of the SOC was in the DF. The C concentration and C : N ratios of density fractions increased in the order DF < fLF < oLF (Table 4). Relative to the unamended control, the C : N ratio of both PyOM and wood-amended soil was significantly higher ($P \leq 0.05$) in fLF and oLF density fractions, while DF had similar values to the control soils across

Table 2 Recovery of applied ^{13}C/^{15}N-labeled wood or PyOM and excess ^{13}C and ^{15}N in the soil at depths of 0–5 cm, 5–10 cm, and 10–15 cm and bulk soil, 10 months after application. The initial C : N ratio of added PyOM was 110 and wood was 115. Different letters in the same column (within the same depth) are significantly different ($P \leq 0.05$)

	Soil depth				Excess ^{13}C, mg kg^{-1} soil		
Substrate	0–5 cm	5–10 cm	10–15 cm	Bulk soil	0–5 cm	5–10 cm	10–15 cm
^{13}C-substrate recovered (% of the applied after 10 months)							
Wood, N0	40.0 (13.2)^{a}	6.5 (3.9)^{a}	1.6 (0.9)^{a}	48.2 (4.6)^{a}	14.7 (5.3)	2.0 (1.1)	0.5 (0.3)
Wood, N+	44.5 (5.1)^{a}	2.1 (0.3)^{a}	1.8 (0.9)^{a}	48.4 (8.8)^{a}	14.5 (3.0)	0.7 (0.1)	0.5 (0.2)
PyOM, N0	95.1 (1.7)^{b}	3.1 (0.9)^{a}	1.1 (0.7)^{a}	99.4 (0.3)^{b}	64.0 (8.5)	1.5 (0.3)	0.5 (0.2)
PyOM, N+	96.5 (1.4)^{b}	2.3 (0.7)^{a}	1.1 (0.9)^{a}	99.9 (0.4)^{b}	71.2 (5.8)	1.5 (0.5)	0.5 (0.4)
^{15}N-substrate recovered (% of the applied after 10 months)					Excess ^{15}N mg kg^{-1} soil		
Wood, N0	78.2 (3.8)^{a}	7.1 (2.8)^{a}	3.1 (0.8)^{a}	88.5 (6.4)^{a}	0.9 (0.05)	0.1 (0.02)	0.03 (0.01)
Wood, N+	35.1 (7.5)^{b}	7.8 (0.8)^{a}	5.1 (2.0)^{a}	48.0 (6.0)^{b}	0.4 (0.12)	0.1 (0.00)	0.05 (0.03)
PyOM, N0	81.5 (2.6)^{a}	6.1 (2.0)^{a}	2.0 (1.0)^{a}	89.2 (0.4)^{a}	2.0 (0.32)	0.1 (0.04)	0.03 (0.02)
PyOM, N+	89.2 (2.3)^{a}	5.8 (0.9)^{a}	2.7 (0.2)^{a}	97.7 (3.0)^{a}	2.4 (0.21)	0.6 (0.46)	0.05 (0.01)

Table 3 Total C and N recovery of bulk soil and added substrate after density fractionation. Values correspond to the mean (n = 3) and values between brackets correspond to SE

	Total C recovery, %		Total N recovery, %	
Treatments	Bulk soil	Added substrate	Bulk soil	Added substrate
Wood, N0	88.6 (18)	78.8 (1)	93.0 (17)	45.2 (14)
Wood, N+	91.4 (4)	67.4 (17)	85.0 (5)	94.7 (14)
PyOM, N0	88.4 (1)	102.5 (10)	83.0 (2)	103.9 (10)
PyOM, N+	90.4 (3)	106.3 (2)	85.2 (2)	97.8 (9)
Control, N0	86.1 (7)		85.5 (2)	
Control, N+	88.2 (3)		77.6 (5)	

all treatments. The important contribution of PyOM (22% of total C in fLF and 7% of total C in oLF was PyOM C) and wood (5% of total C in fLF and 3% of total C in oLF-C was wood C) in these fractions explains their higher C : N ratio. We did not observe any significant effects of N treatment on the C : N ratios in density fractions across treatment.

The distribution of PyOM-C and wood-C among the density fractions after 10 months of their application was not similar (Fig. 1). Under ambient N, PyOM-C was recovered on average (n = 3) mostly in the fLF (70.2 ± 4.8%) followed by oLF (22.7 ± 3.9%), while wood C was recovered mostly in oLF (42.6 ± 21.5%) and fLF (39.4 ± 20.8%). The DF showed least recovery for both organic-input C under ambient N but wood C (14.6 ± 9.4%) recovery in DF was double as compared to PyOM C (7.1 ± 1.2%). A similar trend was observed in the increased N treatment for PyOM C with 77.4 ± 2.2% in fLF, 17.3 ± 1.9% in oLF and 5.4 ± 0.9%. Wood C under increased N treatment, however, showed maximum recovery in the DF (38.6 ± 5.4%) followed by fLF (38.0 ± 11.5%) and oLF (23.3 ± 9.3%). Added N resulted in significantly higher wood C recovery in DF as compared to wood C recovery under ambient N (F = 9.6, P = 0.02) and PyOM C recovery under increased N treatment (F = 18.3, P = 0.003).

In PyOM-amended soils, across all N treatments (n = 6) we observed a decrease of 13 ± 3% in fLF, 4 ± 3% in oLF and 0.2 ± 0.3% in DF in native soil C concentration (priming effect – Fig. 2). Native soil C concentration in wood-amended soils did not show a significant change in fLF (4.5 ± 4.6%, P = 0.061) or oLF (3.3 ± 3.5%, P = 0.068). Under the added N treatment, wood-amended soil showed a significant increase in the DF-C (0.7 ± 0.3%, P = 0.04) and bulk soil (1.3 ± 0.3%, P = 0.01) with respect to zero. Added N did not affect native SOC content or interact with PyOM effects on SOC content (Fig. 2). Native soil organic N showed little modification in either wood or PyOM-amended soil.

PyOM quality and quantity using BPCA marker molecules

The BPCA-C content of the labeled PyOM used in this study was 145.9 ± 6.8 g BPCA-C kg^{-1} OC (without using any conversion factor) and is similar to standard PyOM produced at 450 °C (Schneider *et al*., 2010). The BPCA-C content of the labeled PyOM mixed with soil (1:137 PyOM: dry soil mass ratio that corresponds to the application rate at 0–5 cm depth) at time t = 0 was 28.7 ± 2.6 g BPCA-C kg^{-1} OC. Ten months after PyOM addition to soils, total BPCA-C content in the 0–5 cm depth was not significantly different than at t = 0 (28.5 ± 2.6 g BPCA-C kg^{-1} OC for N0 and 23.7 ± 14 g BPCA-C kg^{-1} OC for N+, P > 0.05).

We observed a significant increase in benzene tetra-carboxylic acids (B4CA, P = 0.004) and a significant decrease in benzene hexa-carboxylic acids (B6CA, P = 0.023), and therefore a shift in the relative contribution of individual molecular markers after 10 months (Fig. 3). We did not observe any significant change in

Table 4 Total yield (% of soil), total C and N in SOM fractions (g kg^{-1} fraction) and bulk soil (g kg^{-1} soil) from 0 to 5 cm depth. Values correspond to the mean (n = 3) and values between brackets correspond to SE

	Density Fractions (g cm^{-3})										
	fLF			oLF			DF			Bulk soil	
Treatments	Yield	C	N	Yield	C	N	Yield	C	N	C	N
Wood, N0	1 (0.1)	299 (21)	9 (0.8)	1 (0.3)	393 (17)	12 (0.5)	98 (0.3)	20 (2)	2 (0.1)	30 (5)	2 (0.3)
Wood, N+	3 (0.2)	296 (14)	10 (0.5)	2 (0.3)	404 (8)	14 (0.5)	99 (0.5)	32 (2)	2 (0.2)	48 (2)	3 (0.2)
PyOM, N0	2 (0.5)	394 (19)	8 (1.6)	2 (0.3)	421 (27)	13 (2.2)	96 (0.9)	21 (2)	2 (0.2)	41 (6)	2 (0.3)
PyOM, N+	3 (0.2)	387 (32)	8 (0.1)	3 (0.3)	428 (5)	14 (0.5)	95 (0.5)	23 (3)	2 (0.2)	45 (3)	3 (0.2)
Control, N0	1 (0.2)	288 (28)	11 (0.3)	2 (0.1)	393 (9)	15 (0.3)	97 (0.2)	24 (2)	2 (0.2)	36 (4)	2 (0.2)
Control, N+	1 (0.2)	297 (13)	11 (0.3)	2 (0.3)	405 (1)	16 (0.5)	97 (0.5)	24 (3)	2 (0.1)	36 (2)	3 (0.1)

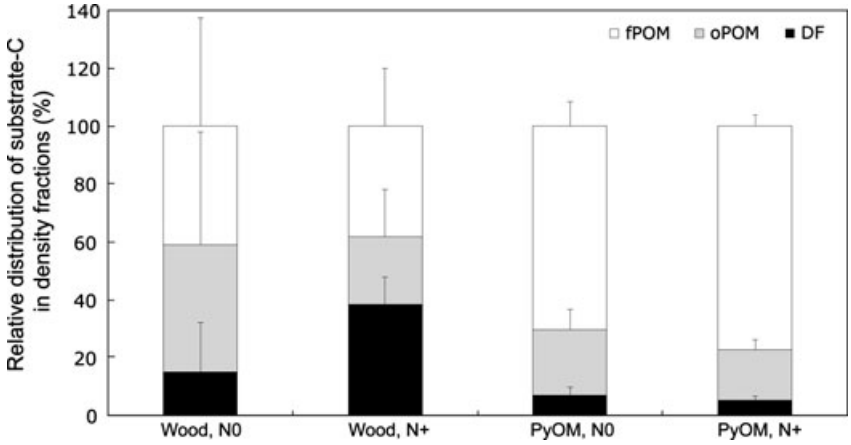

Fig. 1 Distribution of ^{13}C excess derived from wood or PyOM among soil organic matter (SOM) fractions (fLF: free light fraction; oLF: occluded light fraction and DF: dense fraction) 10 months after organic substrates application to the soil. SOM fractions shown are from 0 to 5 cm soil depth. The values correspond to the mean (n = 3) and the bars to the SE.

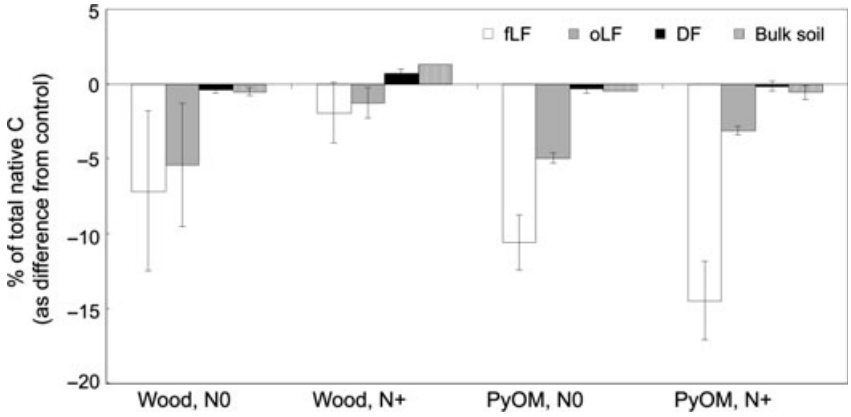

Fig. 2 Relative changes of native SOC among SOM fractions (fLF, oLF and DF) and bulk soils 10 months after addition of $^{13}C/^{15}N$-labeled wood or PyOM, expressed as differences from control plots. The error bars represent SE, n = 3.

the relative proportion of either benzene tricarboxylic acids (B3CA) or benzene penta-carboxylic acid (B5CA) and no significant effect of added N on BPCA distribution patterns.

Microbial C and N

Microbial biomass C (μg per g dry soil) was unaffected by addition of wood or PyOM after 10 months in the mesocosms (Table 4). Microbial biomass C declined significantly with increasing depths (0–5, 5–10 and 10–15 cm) averaged across all treatments ($P < 0.05$). ^{13}C-wood contributed 6–10% of microbial biomass, two levels of magnitude higher than from ^{13}C-PyOM (between 0.14 and 0.18% PyOM-^{13}C).

Microbial biomass N (μg per g dry soil) decreased with increasing depth from 0 to 5 cm to 10 cm to 15 cm (Table 4), except for wood-amended soil under N0 treatment. In the 0–5 cm depth, microbial biomass N increased ($P = 0.09$) with added N in wood-amended soil (51 ± 17 μg N g^{-1} dry soil under N0 and 89 ± 5 μg N g^{-1} dry soil under N+), while PyOM showed an opposite trend (94 ± 51 μg N g^{-1} dry soil under N0 and 64 ± 30 μg N g^{-1} dry soil under N+) but it was not significant (Table 5). Despite the highly labeled material used in this study, the ^{15}N signal was not detectable from added PyOM.

Discussion

Loss of PyOM and wood by decomposition and downward migration in soil

We recovered >99% PyOM-C but only half of wood-C in the soil mesocosms after 10 months *in situ* averaged across all treatments, which is consistent with the

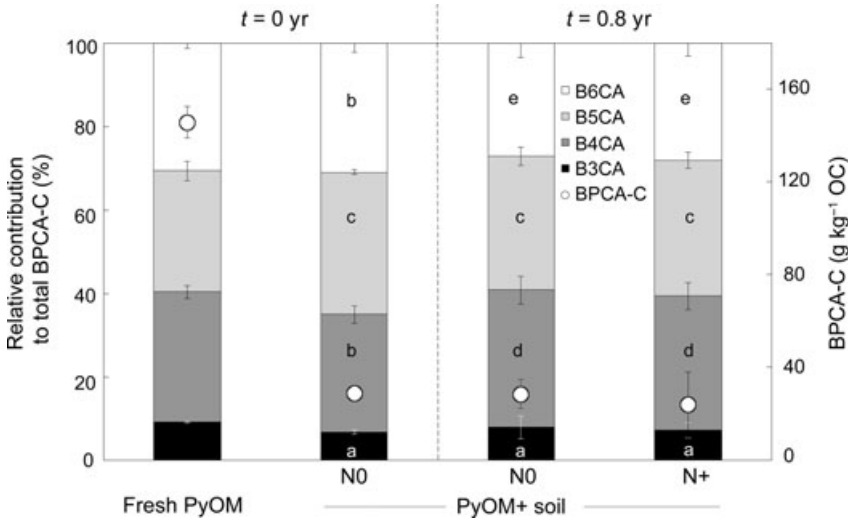

Fig. 3 Relative contributions of individual benzene polycarboxylic acids (BPCA) marker molecules (left, *y*-axis as bars) and total BPCA-C contents (right, *y*-axis as circles) in fresh PyOM, PyOM mixed with soil before (t = 0) and 10 months after the experiment started under ambient N (N0) and increased N (N+) treated soils. Different letters indicate significance difference ($P < 0.05$). The values correspond to the mean (n = 3) and the bars to the SE; B3CA, benzene tri-carboxylic acid; B4CA, benzene tetra-carboxylic acid; B5CA, benzene penta-carboxylic acid; B6CA, benzene hexa-carboxylic acid.

results from the same site for wood (Kammer & Hagedorn, 2011; Jones *et al.*, 2011). The loss of pine wood C corresponds to annual to decadal turnover times, which is similar to that estimated by a 180 days laboratory incubation of the same wood in two Alfisols of different parents material (Santos *et al.*, 2012) and by a 1 year litter experiment using ^{13}C labeled twigs (Beech tree, 1–8 mm) in the same field study area (Kammer & Hagedorn, 2011; Jones *et al.*, 2011).

In the present field study, the mean difference of PyOM stocks over 10 months was approximately 1% of the amount added, but this difference was not significant so we did not estimate a turnover time of PyOM. Nevertheless, we can conclude that PyOM decomposed much slower than the plant biomass from which it was derived after pyrolysis. Several field and laboratory studies have found PyOM turnover times on centennial scale (Singh *et al.*, 2012). Our results provide *in situ* confirmation of the relative rates reported by previous laboratory studies. (Baldock & Smernik, 2002; Hilscher & Knicker, 2011; Santos *et al.*, 2012).

We recovered 3–4% of applied PyOM below the application depth (0–5 cm) suggesting downward vertical movement of PyOM. Leifeld *et al.* (2007) estimated migration rate of 630–1160 mm yr^{-1} in a peat soil with very low bulk density where 21–69% migrated below the incorporation depth in 95 years. Compared to the previously cited study, the soil here was denser, more clayey and so probably less favorable to organic matter transfer through the profile. Nevertheless, the amount of translocated PyOM to lower depth in the present case was higher than Major *et al.* (2009a) who observed 100 mm yr^{-1} migration rate in a sandy Oxisol under native savanna vegetation with only 0.45% of applied amount. Therefore, translocation of PyOM to lower depth in the present case could either be due to soil faunal mixing (Carcaillet, 2001; Topoliantz & Ponge, 2003; Eckmeier *et al.*, 2007a) or leaching. The downward vertical movement could either result in its accumulation at lower depths (Skjemstad *et al.*, 1999; Dai *et al.*, 2005; Rumpel *et al.*, 2006; Brodowski *et al.*, 2007) or loss from soil as dissolved organic carbon (DOC) (Hockaday *et al.*, 2007; Dittmar *et al.*, 2012; Ding *et al.*, 2013). On the other hand, we observed that wood C translocated to lower depths is double (4–8%) in comparison to PyOM C. Transport of wood-C into deeper soil horizons usually occurs as DOC (Yano *et al.*, 2005; Zalamea *et al.*, 2007). This could partly explain the larger amount of wood being translocated to deeper soil profile.

Quality of PyOM changes within one year in soil

After 10 months in soil, the total BPCA-C content of PyOM was unchanged, but the proportion of various BPCAs had changed. Aromatic condensation, measured as the relative contribution of B6CA to the total BPCA-C (Brodowski, 2005; Schneider *et al.*, 2010), decreased after 10 months, suggesting that PyOM partially degraded into smaller aromatic moieties. In contrast, Hammes *et al.* (2008), in a field study, observed a relative increase

Table 5 Microbial biomass 10 months after $^{13}C/^{15}N$-labeled wood or PyOM addition to soil mesocosms across all N treatments. Values correspond to the mean ($n = 6$) and values between brackets correspond to SE

	Soil microbial biomass C			Soil microbial biomass N			Excess ^{13}C in microbial biomass			C : N ratio of soil microbial biomass		
	$= (C_{fum}-C_{nfum})/0.45$, μg g^{-1} dry soil			$= (N_{fum}-N_{nfum})/0.68$, μg g^{-1} dry soil			μg g^{-1} dry soil					
Treatment	0–5 cm	5–10 cm	10–15 cm	0–5 cm	5–10 cm	10–15 cm	0–5 cm	5–10 cm	10–15 cm	0–5 cm	5–10 cm	10–15 cm
Wood	699 (86)	581 (86)	427 (86)	70 (11)	87 (6)	51 (8)	0.22 (0.05)	0.23 (0.15)	0.02 (0.01)	9.8 (2.3)	6.9 (1.9)	8.8 (2.1)
PyOM	671 (77)	454 (77)	360 (86)	79 (27)	42 (8)	32 (7)	0.01 (0.00)	0.00 (0.00)	0.00 (0.00)	8.4 (4.2)	10.9 (3.3)	11.4 (3.7)
Control	661 (77)	496 (77)	417 (94)	78 (10)	63 (7)	12 (4)	–	–	–	8.5 (1.5)	7.9 (1.9)	31.8 (13.2)

in the B6CA molecular marker after 100 years *in situ* (no absolute change) suggesting relative preservation of condensed aromatic structures. Moreover, Schneider *et al.* (2011) found no change in the BPCAs pattern in a 100-year chronosequence. It is not clear yet if the changes in the relative contribution of various BPCAs could be linked directly to decomposition mechanism. Abiven *et al.* (2011) observed an increase in the B3CA at the expense of B5CA between a fresh and a 10 year aged PyOM while Brodowski (2005) also observed similar results in an incubation study on the decomposition of PyOM. The dominance of B3CA and B4CA indicates small aromatic cluster size (Schneider *et al.*, 2011) and indicates depolymerization of the highly condensed aromatic backbone of PyOM. These previous findings, together with our data, suggest that PyOM in soil degrades by the breaking of condensed aromatic structures into smaller clusters, at least within the first stages of degradation after the input of PyOM to the soil.

PyOM was physically associated with the soil mineral fraction after 10 months

About one third of the applied PyOM C was recovered in aggregates (i.e., oLF) plus dense fraction of soil within 1 year. Studies of the ambient distribution of PyOM in soil fractions, in other words, in soils collected from sites without intentional addition of PyOM also find significant portion of PyOM in these fractions (Glaser *et al.*, 2000; Brodowski *et al.*, 2006; Laird *et al.*, 2008; Liang *et al.*, 2008; Vasilyeva *et al.*, 2011). However, these studies do not report the temporal scale over which aggregation and/or organo-mineral interaction of PyOM with soil occurs, as they have no data for PyOM inputs to these soils. This study highlights, for the first time, that significant interaction between PyOM and the mineral phase of soil can occur *in situ* within a year. Glaser *et al.* (2000) posit that interaction of PyOM and soil mineral phases might stabilize PyOM by aggregation and organo-mineral associations. As one mechanism, oxidation of PyOM surface has been hypothesized to favor its interaction with the soil mineral phase (Brodowski *et al.*, 2006). It was, however, not possible to directly link oxidized forms of PyOM to a specific interaction with soil minerals that led to recovery of PyOM in the dense fraction. Moreover, the presence of PyOM in DF does not mean that all of it was stable organo-mineral associates; PyOM in the DF could also occur as an uncomplexed sand fraction.

PyOM accelerated the loss of native C from fLF

We observed positive priming in the free particulate native C pool in soil (i.e., in the fLF) by both PyOM and

wood, with significantly larger priming by PyOM than wood of native SOC in the fLF. However, there is no consistent effect of PyOM on native SOC across the literature. For example, Santos *et al.* (2012) did not observe priming in SOM using the same substrates but different soils in an incubation study, which indicated that the type of soil has an influence on priming effects rather than the organic substrate itself. In a recent study under controlled conditions, Stewart *et al.* (2013) observed an exponential relationship between initial SOC and cumulative soil C primed by PyOM addition with high negative priming at low soil C% and positive priming at high soil C%. Our study, for the first time, indicated which pool of SOM is affected due to priming by organic input. The native SOM associated to the minerals was not affected by the input while the SOM that was free or occluded in aggregates decreased significantly within few months.

One property supporting priming is PyOM's porous structure which is known to sorb organic substrates (Raveendran & Ganesh, 1998; Sudhakar & Dikshit, 1999; Accardi-Dey & Gschwend, 2002; Kwon & Pignatello, 2005; Chen *et al.*, 2008) and may offer favorable microsites for microorganisms and shelter them against soil faunal predators (Pietikäinen *et al.*, 2000). If so, the physical effects of PyOM amendments could increase microbial activity and lead to increased mineralization of readily decomposable substrates such as the fLF. However, we were not able to detect a change in microbial biomass (see No change in microbial biomass), and further research is needed to develop a predictive understanding of the temporal course of priming-type effects.

No change in microbial biomass

Pyrogenic organic matter addition had no effect on SMB-C 10 months after addition to the soil mesocosm. Bruun *et al.* (2008) observed a similar lack of effect in soil treated with PyOM (^{14}C-labeled roots of barley). On the contrary, Steinbeiss *et al.* (2009) observed a reduction in microbial biomass in soil to which charred glucose had been added to a forest soil in an incubation study after 4 months. Several studies observed increased microbial biomass and activity in soil (Steiner *et al.*, 2008; Kolb *et al.*, 2009; Bruun *et al.*, 2011) within days to few months after PyOM addition or higher SMB-C in PyOM rich soils compared to control or adjacent soils with no PyOM (Liang *et al.*, 2010). Our study was comparatively longer than the studies cited above (10 months vs. a couple of weeks) and therefore we cannot exclude the possibility that the microbial biomass could have increased in the first few weeks after PyOM addition and reverted to its initial value over time. Moreover, this study is the case of a single input of PyOM to the soil without a real wildfire and therefore its effect on the soil properties and consequently on microbial biomass is not similar to PyOM rich soils. In addition, the inconsistency in the response of microbial biomass to PyOM treatment in soil may be attributed to type of PyOM used that differed in the degree of condensation, intensity of pyrolysis and amount of precombustion material present in various studies (Zavalloni *et al.*, 2011). PyOM could have influenced the extraction efficiency but in our study it is not likely that it would have resulted in a major change, as the amount of PyOM used was small as compared to total SOC. Our results suggest that, under these conditions, the total microbial biomass is not durably affected by the substrate addition.

We observed a small amount of PyOM-C within the microbial biomass 10 months after organic inputs to soil, as did Bruun *et al.* (2008) and Zavalloni *et al.* (2011). Kuzyakov *et al.* (2009) and Kolb *et al.* (2009) observed a higher assimilation of PyOM (1.5–2.6% of initial PyOM input) by microbial biomass after 624 days of incubation compared to our 10 months field study. In all these studies, the amount of PyOM incorporated into the SMB was large enough to be detected clearly, and hence indicate that microbes can utilize PyOM as a C source.

Effect of N fertilization

The addition of N had little impact on the parameters considered in this study. Under the added N treatment, we observed a significant increase in the total C content at 0–5 cm depth in wood-amended soil, higher loss of wood-N, a higher transfer of wood derived C in the DF and a higher N content in the microbial biomass. However, these changes are limited and affect the C and N cycles only marginally. These results are in line with Santos *et al.* (2012). However, the effect of N addition is often seen in the longer term and these results need to be validated for longer periods.

Summary and environmental implications

The novel use of a dual isotope label for PyOM and wood in this field study provided insight into the dynamics of PyOM and wood C and N during the first year after its application to soil. The PyOM C was almost completely retained in the soil, although there was a small but significant change in the overall chemical structure of PyOM. In contrast, 48% of the initial amount of wood C was lost. Our results showed that PyOM persistence in soil need not solely be due to its chemical structure, as one third of the PyOM C was quickly incorporated into physically protected fractions

(i.e., oLF and DF). PyOM primed the loss of native soil C in the free light fraction, suggesting a significant loss of decadal C pool of SOM.

This study shows that PyOM seems to be a promising tool to stabilize C in soils. After 10 months, PyOM-C losses from soil were negligible; PyOM-C was hardly assimilated by microbes and seemed to interact substantially with the mineral phase of the soil, leading to a potential long-term stabilization of the C. However, this study also shows that the application of PyOM increased the priming of the easily decomposable organic matter of the soil and may offset its stabilization. While a lot of attention has been brought to the chemistry of the PyOM as main driver of its stability in the soil, our study rather highlights that its interactions with the mineral and organic phases of the soil may be quantitatively more relevant and more dynamic than its chemical structure, at least on the short term. This new information would advocate a better understanding of PyOM relationships to soil characteristics in future mechanistic investigations.

Acknowledgements

The Swiss National Science Foundation (SNSF) financially supported this study. This work was also supported by the Director, Office of Science, Office of Biological and Environmental Research, Climate and Environmental Science Division, of the US Department of Energy under Contract No. DE-AC02-05CH11231 to Berkeley Laboratory. This study was supported by the University of Zurich Research Priority Program (URPP) 'Global Change and Biodiversity'. We thank Sarah Bösch and Ryan Christinger for their help in setting the experimental field plots, and Ivan Woodhatch for the technical help and support in the setup. We also thank Michael Hilf, Bruno Kägi, and Claudia Schreiner for their assistance in various laboratory analyses carried at the University of Zurich. We further extend our thanks to Alois Zürcher for support in TOC analysis at the Institute for Forest, Snow and Landscape research (WSL). We would also like to acknowledge Dr. Rolf Siegwolf, Dr. Matthias Saurer, and Catharina Lötscher for support in the isotope analysis at Paul Scherrer Institute (PSI), Switzerland. We also extend our thanks to Dan Hawkes, Berkeley Laboratories for editing and proofreading this manuscript. We thank subject editor M. Francesca Cotrufo and two anonymous reviewers for their constructive comments on our manuscript.

References

Abbott DT, Crossley DA (1982) Woody litter decomposition following clear-cutting. *Ecology*, **63**, 35–42.

Abiven S, Andreoli R (2010) Charcoal does not change the decomposition rate of mixed litters in a mineral cambisol: a controlled conditions study. *Biology and Fertility of Soils*, **47**, 111–114.

Abiven S, Hengartner P, Schneider MPW, Singh N, Schmidt MWI (2011) Pyrogenic carbon soluble fraction is larger and more aromatic in aged charcoal than in fresh charcoal. *Soil Biology & Biochemistry*, **43**, 1615–1617.

Accardi-Dey A, Gschwend PM (2002) Assessing the combined roles of natural organic matter and black carbon as sorbents in sediments. *Environmental Science & Technology*, **36**, 21–29.

Allison SD, Lebauer DS, Ofrecio MR, Reyes R, Ta AM, Tran TM (2009) Low levels of nitrogen addition stimulate decomposition by boreal forest fungi. *Soil Biology & Biochemistry*, **41**, 293–302.

Baldock JA, Smernik RJ (2002) Chemical composition and bioavailability of thermally, altered Pinus resinosa (Red Pine) wood. *Organic Geochemistry*, **33**, 1093–1109.

Bebber DP, Watkinson SC, Boddy L, Darrah PR (2011) Simulated nitrogen deposition affects wood decomposition by cord-forming fungi. *Oecologia*, **167**, 1177–1184.

Bernoux M, Cerri CC, Neill C, De Moraes JFL (1998) The use of stable carbon isotopes for estimating soil organic matter turnover rates. *Geoderma*, **82**, 43–58.

Bird JA, Torn MS (2006) Fine roots vs. Needles: a comparison of C-13 and N-15 dynamics in a ponderosa pine forest soil. *Biogeochemistry*, **79**, 361–382.

Bird MI, Moyo C, Veenendaal EM, Lloyd J, Frost P (1999) Stability of elemental carbon in a savanna soil. *Global Biogeochemical Cycles*, **13**, 923–932.

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

Bragazza L, Freeman C, Jones T *et al.* (2006) Atmospheric nitrogen deposition promotes carbon loss from peat bogs. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 19386–19389.

Brodowski S (2005) Origin, function, and reactivity of black carbon in the arable soil environment. PhD Thesis, Institut für Bodenkunde, Bonn.

Brodowski S, Amelung W, Haumaier L, Abetz C, Zech W (2005a) 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, Rodionov A, Haumaier L, Glaser B, Amelung W (2005b) Revised black carbon assessment using benzene polycarboxylic acids. *Organic Geochemistry*, **36**, 1299–1310.

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

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

Bruun S, Jensen ES, Jensen LS (2008) Microbial mineralization and assimilation of black carbon: dependency on degree of thermal alteration. *Organic Geochemistry*, **39**, 839–845.

Bruun EW, Muller-Stover D, Ambus P, Hauggaard-Nielsen H (2011) Application of biochar to soil and N2O emissions: potential effects of blending fast-pyrolysis biochar with anaerobically digested slurry. *European Journal of Soil Science*, **62**, 581–589.

Busse MD (1994) Downed bole-wood decomposition in lodgepole pine forests of central Oregon. *Soil Science Society of America Journal*, **58**, 221–227.

Carcaillet C (2001) Are Holocene wood-charcoal fragments stratified in alpine and subalpine soils? Evidence from the Alps based on AMS C-14 dates. *Holocene*, **11**, 231–242.

Cerli C, Celi L, Kalbitz K, Guggenberger G, Kaiser K (2012) Separation of light and heavy organic matter fractions in soil - Testing for proper density cut-off and dispersion level. *Geoderma*, **170**, 403–416.

Chambers JQ, Schimel JP, Nobre AD (2001) Respiration from coarse wood litter in central Amazon forests. *Biogeochemistry*, **52**, 115–131.

Chatterjee S, Santos F, Abiven S, Itin B, Stark RE, Bird JA (2012) Elucidating the chemical structure of pyrogenic organic matter by combining magnetic resonance, mid-infrared spectroscopy and mass spectrometry. *Organic Geochemistry*, **51**, 34–44.

Chen BL, Zhou DD, Zhu LZ (2008) Transitional adsorption and partition of nonpolar and polar aromatic contaminants by biochars of pine needles with different pyrolytic temperatures. *Environmental Science & Technology*, **42**, 5137–5143.

Cheng CH, Lehmann J (2009) Ageing of black carbon along a temperature gradient. *Chemosphere*, **75**, 1021–1027.

Cheng CH, Lehmann J, Thies JE, Burton SD, Engelhard MH (2006) Oxidation of black carbon by biotic and abiotic processes. *Organic Geochemistry*, **37**, 1477–1488.

Clark DB, Clark DA, Brown S, Oberbauer SF, Veldkamp E (2002) Stocks and flows of coarse woody debris across a tropical rain forest nutrient and topography gradient. *Forest Ecology and Management*, **164**, 237–248.

Crow SE, Lajtha K, Filley TR, Swanston CW, Bowden RD, Caldwell BA (2009) Sources of plant-derived carbon and stability of organic matter in soil: implications for global change. *Global Change Biology*, **15**, 2003–2019.

Dai X, Boutton TW, Glaser B, Ansley RJ, Zech W (2005) Black carbon in a temperate mixed-grass savanna. *Soil Biology & Biochemistry*, **37**, 1879–1881.

Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics*, **33**, 507–559.

Deluca TH, Aplet GH (2008) Charcoal and carbon storage in forest soils of the Rocky Mountain West. *Frontiers in Ecology and the Environment*, **6**, 18–24.

Ding Y, Yamashita Y, Dodds WK, Jaffe R (2013) Dissolved black carbon in grassland streams: is there an effect of recent fire history? *Chemosphere*, **90**, 2557–2562.

Dittmar T, Paeng J, Gihring TM, Suryaputra IGNA, Huettel M (2012) Discharge of dissolved black carbon from a fire-affected intertidal system. *Limnology and Oceanography*, **57**, 1171–1181.

Durenkamp M, Luo Y, Brookes PC (2010) Impact of black carbon addition to soil on the determination of soil microbial biomass by fumigation extraction. *Soil Biology & Biochemistry*, **42**, 2026–2029.

Eckmeier E, Gerlach R, Skjemstad JO, Ehrmann O, Schmidt MWI (2007a) Minor changes in soil organic carbon and charcoal concentrations detected in a temperate deciduous forest a year after an experimental slash-and-burn. *Biogeosciences*, **4**, 377–383.

Eckmeier E, Rosch M, Ehrmann O, Schmidt MWI, Schier W, Gerlach R (2007b) Conversion of biomass to charcoal and the carbon mass balance from a slash-and-burn experiment in a temperate deciduous forest. *Holocene*, **17**, 539–542.

F.A.O.-U.N.E.S.C.O. (1998) *ISSS, ISRIC, FAO. World Reference Base for Soil Resources*. World Soil Resources Reports 84. FAO, Rome.

Fasth BG, Harmon ME, Sexton J, White P (2011) Decomposition of fine woody debris in a deciduous forest in North Carolina. *Journal of the Torrey Botanical Society*, **138**, 192–206.

Fog K (1988) The effect of added nitrogen on the rate of decomposition of organic-Matter. *Biological Reviews of the Cambridge Philosophical Society*, **63**, 433–462.

Fontaine S, Mariotti A, Abbadie L (2003) The priming effect of organic matter: a question of microbial competition? *Soil Biology & Biochemistry*, **35**, 837–843.

Galloway JN, Townsend AR, Erisman JW *et al.* (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science*, **320**, 889–892.

Glaser B, Haumaier L, Guggenberger G, Zech W (1998) Black carbon in soils: the use of benzenecarboxylic acids as specific markers. *Organic Geochemistry*, **29**, 811–819.

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

Goldberg ED (1985) *Black Carbon in the Environment: Properties and Distribution*. John Wiley and Sons, New York.

Guo LB, Bek E, Gifford RM (2006) Woody debris in a 16-year old Pinus radiata plantation in Australia: mass, carbon and nitrogen stocks, and turnover. *Forest Ecology and Management*, **228**, 145–151.

Hamer U, Marschner B, Brodowski S, Amelung W (2004) Interactive priming of black carbon and glucose mineralisation. *Organic Geochemistry*, **35**, 823–830.

Hammes K, Smernik RJ, Skjemstad JO, Herzog A, Vogt UF, Schmidt MWI (2006) Synthesis and characterisation of laboratory-charred grass straw (Oryza saliva) and chestnut wood (Castanea sativa) as reference materials for black carbon quantification. *Organic Geochemistry*, **37**, 1629–1633.

Hammes K, Torn MS, Lapenas AG, Schmidt MWI (2008) Centennial black carbon turnover observed in a Russian steppe soil. *Biogeosciences*, **5**, 1339–1350.

Hilscher A, Knicker H (2011) Degradation of grass-derived pyrogenic organic material, transport of the residues within a soil column and distribution in soil organic matter fractions during a 28 month microcosm experiment. *Organic Geochemistry*, **42**, 42–54.

Hilscher A, Heister K, Siewert C, Knicker H (2009) Mineralisation and structural changes during the initial phase of microbial degradation of pyrogenic plant residues in soil. *Organic Geochemistry*, **40**, 332–342.

Hockaday WC, Grannas AM, Kim S, Hatcher PG (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.

Hockaday WC, Grannas AM, Kim S, Hatcher PG (2007) The transformation and mobility of charcoal in a fire-impacted watershed. *Geochimica Et Cosmochimica Acta*, **71**, 3432–3445.

Jackson ML (1958) *Soil Chemical Analysis*. Prentice-Hall, Englewood Cliffs, NJ, USA.

Janssens IA, Dieleman W, Luyssaert S *et al.* (2010) Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience*, **3**, 315–322.

Jones DL, Murphy DV, Khalid M, Ahmad W, Edwards-Jones G, Deluca TH (2011) Short-term biochar-induced increase in soil CO_2 release is both biotically and abiotically mediated. *Soil Biology & Biochemistry*, **43**, 1723–1731.

Kammer A, Hagedorn F (2011) Mineralisation, leaching and stabilisation of C-13-labelled leaf and twig litter in a beech forest soil. *Biogeosciences*, **8**, 2195–2208.

Keiluweit M, Nico PS, Johnson MG, Kleber M (2010) Dynamic Molecular Structure of Plant Biomass-Derived Black Carbon (Biochar). *Environmental Science & Technology*, **44**, 1247–1253.

Kloeti P, Keller HM, Guecheva M (1989) Effects of forest canopy on throughfall precipitation chemistry. *Atmospheric Deposition, Proceedings of the Baltimore Symposium*. IAHS Publ, Wallingford, UK, **179**, 203–209.

Knicker H (2011) Pyrogenic organic matter in soil: its origin and occurrence, its chemistry and survival in soil environments. *Quaternary International*, **243**, 251–263.

Knorr M, Frey SD, Curtis PS (2005) Nitrogen additions and litter decomposition: a meta-analysis. *Ecology*, **86**, 3252–3257.

Kolb SE, Fermanich KJ, Dornbush ME (2009) Effect of charcoal quantity on microbial biomass and activity in temperate soils. *Soil Science Society of America Journal*, **73**, 1173–1181.

Kuzyakov Y, Subbotina I, Chen HQ, Bogomolova I, Xu XL (2009) Black carbon decomposition and incorporation into soil microbial biomass estimated by C-14 labeling. *Soil Biology & Biochemistry*, **41**, 210–219.

Kwon S, Pignatello JJ (2005) Effect of natural organic substances on the surface and adsorptive properties of environmental black carbon (char): pseudo pore blockage by model lipid components and its implications for N-2-probed surface properties of natural sorbents. *Environmental Science & Technology*, **39**, 7932–7939.

Laird DA, Chappell MA, Martens DA, Wershaw RL, Thompson M (2008) Distinguishing black carbon from biogenic humic substances in soil clay fractions. *Geoderma*, **143**, 115–122.

Lehmann J, Gaunt J, Rondon M (2006) Bio-Char sequestration in terrestrial ecosystems- A review. *Mitigation and Adaptation Strategies for Global Change*, **11**, 403–427.

Lehmann J, Skjemstad J, Sohi S *et al.* (2008) Australian climate-carbon cycle feedback reduced by soil black carbon. *Nature Geoscience*, **1**, 832–835.

Leifeld J, Fenner S, Muller M (2007) Mobility of black carbon in drained peatland soils. *Biogeosciences*, **4**, 425–432.

Liang B, Lehmann J, Solomon D *et al.* (2008) Stability of biomass-derived black carbon in soils. *Geochimica Et Cosmochimica Acta*, **72**, 6069–6078.

Liang BQ, Lehmann J, Sohi SP *et al.* (2010) Black carbon affects the cycling of non-black carbon in soil. *Organic Geochemistry*, **41**, 206–213.

Mack MC, Schuur EAG, Bret-Harte MS, Shaver GR, Chapin FS (2004) Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. *Nature*, **431**, 440–443.

Major J, Lehmann J, Rondon M, Goodale C (2009a) Fate of soil-applied black carbon: downward migration, leaching and soil respiration. *Global Change Biology*, **16**, 1366–1379.

Major J, Steiner C, Downie A, Lehamann J (2009b) Biochar effects on nutrient leaching. In: *Biochar for Environmental Management Science and Technology*. (eds Lehman J, Joseph S), pp. 271–287. Earthscan, London.

Mccoll JG, Powers RF (1998) Decomposition of small diameter woody debris of red fir determined by nuclear magnetic resonance. *Communications in Soil Science and Plant Analysis*, **29**, 2691–2704.

Micks P, Downs MR, Magill AH, Nadelhoffer KJ, Aber JD (2004) Decomposing litter as a sink for N-15-enriched additions to an oak forest and a red pine plantation. *Forest Ecology and Management*, **196**, 71–87.

Moritz MA, Parisien M-A, Batllori E, Krawchuk MA, Dorn JV, Ganz DJ, Hayhole K (2012) Climate change and disruptions to global fire activity. *Ecosphere*, **3**, 1–22.

Murage EW, Voroney PR (2007) Modification of the original chloroform fumigation extraction technique to allow measurement of delta C-13 of soil microbial biomass carbon. *Soil Biology & Biochemistry*, **39**, 1724–1729.

Nguyen BT, Lehmann J, Kinyangi J, Smernik R, Riha SJ, Engelhard MH (2008) Long-term black carbon dynamics in cultivated soil. *Biogeochemistry*, **89**, 295–308.

Norby RJ (1998) Nitrogen deposition: a component of global change analyses. *New Phytologist*, **139**, 189–200.

Pietikäinen J, Kiikkila O, Fritze H (2000) Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. *Oikos*, **89**, 231–242.

Raveendran K, Ganesh A (1998) Adsorption characteristics and pore-development of biomass-pyrolysis char. *Fuel*, **77**, 769–781.

Rovira P, Duguy B, Vallejo VR (2009) Black carbon in wildfire-affected shrubland Mediterranean soils. *Journal of Plant Nutrition and Soil Science*, **172**, 43–52.

Ruehr NK, Buchmann N (2010) Soil respiration fluxes in a temperate mixed forest: seasonality and temperature sensitivities differ among microbial and root-rhizosphere respiration. *Tree Physiology*, **30**, 165–176.

Rumpel C, Alexis M, Chabbi A, Chaplot V, Rasse DP, Valentin C, Mariotti A (2006) Black carbon contribution to soil organic matter composition in tropical sloping land under slash and burn agriculture. *Geoderma*, **130**, 35–46.

Santos F, Torn MS, Bird JA (2012) Biological degradation of pyrogenic organic matter in temperate forest soils. *Soil Biology & Biochemistry*, **51**, 115–124.

Schmidt MWI, Noack AG (2000) Black carbon in soils and sediments: analysis, distribution, implications, and current challenges. *Global Biogeochemical Cycles*, **14**, 777–793.

Schmidt MWI, Rumpel C, Kogel-Knabner I (1999a) Evaluation of an ultrasonic dispersion procedure to isolate primary organomineral complexes from soils. *European Journal of Soil Science*, **50**, 87–94.

Schmidt MWI, Skjemstad JO, Gehrt E, Kogel-Knabner I (1999b) Charred organic carbon in German chernozemic soils. *European Journal of Soil Science*, **50**, 351–365.

Schmidt MWI, Torn MS, Abiven S *et al.* (2011) Persistence of soil organic matter as an ecosystem property. *Nature*, **478**, 49–56.

Schneider MPW, Hilf M, Vogt UF, Schmidt MWI (2010) The benzene polycarboxylic acid (BPCA) pattern of wood pyrolyzed between 200 degrees C and 1000 degrees C. *Organic Geochemistry*, **41**, 1082–1088.

Schneider MPW, Lehmann J, Schmidt MWI (2011) Charcoal quality does not change over a century in a tropical agro-ecosystem. *Soil Biology & Biochemistry*, **43**, 1992–1994.

Shen SM, Pruden G, Jenkinson DS (1984) Mineralization and Immobilization of Nitrogen in Fumigated Soil and the Measurement of Microbial Biomass Nitrogen. *Soil Biology & Biochemistry*, **16**, 437–444.

Shinogi Y, Yoshida H, Koizumi T, Yamaok M, Saito T (2003) Basic characteristics of low-temperature carbon products from waste sludge. *Advances in Environmental Research*, **7**, 661–665.

Singh N, Abiven S, Torn MS, Schmidt MWI (2012) Fire-derived organic carbon in soil turns over on a centennial scale. *Biogeosciences*, **9**, 2847–2857.

Skjemstad JO, Taylor JA, Janik LJ, Marvanek SP (1999) Soil organic carbon dynamics under long-term sugarcane monoculture. *Australian Journal of Soil Research*, **37**, 151–164.

Skjemstad JO, Reicosky DC, Wilts AR, Mcgowan JA (2002) Charcoal carbon in US agricultural soils. *Soil Science Society of America Journal*, **66**, 1249–1255.

Steinbeiss S, Gleixner G, Antonietti M (2009) Effect of biochar amendment on soil carbon balance and soil microbial activity. *Soil Biology & Biochemistry*, **41**, 1301–1310.

Steiner C, Das KC, Garcia M, Forster B, Zech W (2008) Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic Ferralsol. *Pedobiologia*, **51**, 359–366.

Stewart C, Zheng JY, Botte J, Cotrufo MF (2013) Co-generated fast pyrolysis biochar mitigates greenhouse gas emissions and increases carbon sequestration in temperate soils. *Global Change Biology Bioenergy*, **5**, 153–164.

Sudhakar Y, Dikshit AK (1999) Kinetics of endosulfan sorption on to wood charcoal. *Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes*, **34**, 587–615.

Sulzman EW, Brant JB, Bowden RD, Lajtha K (2005) Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO2 efflux in an old growth coniferous forest. *Biogeochemistry*, **73**, 231–256.

Topoliantz S, Ponge JF (2003) Burrowing activity of the geophagous earthworm Pontoscolex corethrurus (Oligochaeta: Glossoscolecidae) in the presence of charcoal. *Applied Soil Ecology*, **23**, 267–271.

Turunen J, Roulet NT, Moore TR, Richard PJH (2004) Nitrogen deposition and increased carbon accumulation in ombrotrophic peatlands in Eastern Canada. *Global Biogeochemical Cycles*, **18**, GB3002, doi: 3010.1029/2003GB002154.

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass-C. *Soil Biology & Biochemistry*, **19**, 703–707.

Vasilyeva NA, Abiven S, Milanovskiy EY, Hilf M, Rizhkov OV, Schmidt MWI (2011) Pyrogenic carbon quantity and quality unchanged after 55 years of organic matter depletion in a Chernozem. *Soil Biology & Biochemistry*, **43**, 1985–1988.

Von Lützow M, Kögel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H (2006) Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. *European Journal of Soil Science*, **57**, 426–445.

Wal A, Boer W, Smant W, Veen J (2007) Initial decay of woody fragments in soil is influenced by size, vertical position, nitrogen availability and soil origin. *Plant and Soil*, **301**, 189–201.

Waldrop MP, Zak DR, Sinsabaugh RL, Gallo M, Lauber C (2004) Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. *Ecological Applications*, **14**, 1172–1177.

Westerling AL, Hidalgo HG, Cayan DR, Swetnam TW (2006) Warming and earlier spring increase western US forest wildfire activity. *Science*, **313**, 940–943.

Wu J, Joergensen RG, Pommerening B, Chaussod R, Brookes PC (1990) Measurement of soil microbial biomass C by fumigation extraction - an automated procedure. *Soil Biology & Biochemistry*, **22**, 1167–1169.

Yano Y, Lajtha K, Sollins P, Caldwell BA (2005) Chemistry and dynamics of dissolved organic matter in a temperate coniferous forest on Andic soils: effects of litter quality. *Ecosystems*, **8**, 286–300.

Yarnes C, Santos F, Singh N, Abiven S, Schmidt MWI, Bird JA (2011) Stable isotopic analysis of pyrogenic organic matter in soils by liquid chromatography-isotope-ratio mass spectrometry of benzene polycarboxylic acids. *Rapid Communications in Mass Spectrometry*, **25**, 3723–3731.

Zalamea M, Gonzalez G, Ping CL, Michaelson G (2007) Soil organic matter dynamics under decaying wood in a subtropical wet forest: effect of tree species and decay stage. *Plant and Soil*, **296**, 173–185.

Zavalloni C, Alberti G, Biasiol S, Delle Vedove G, Fornasier F, Liu J, Peressotti A (2011) Microbial mineralization of biochar and wheat straw mixture in soil: a short-term study. *Applied Soil Ecology*, **50**, 45–51.

Zell J, Kandler G, Hanewinkel M (2009) Predicting constant decay rates of coarse woody debris-A meta-analysis approach with a mixed model. *Ecological Modelling*, **220**, 904–912.

Zimmerman AR (2010) Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). *Environmental Science & Technology*, **44**, 1295–1301.

Zimmerman AR, Gao B, Ahn MY (2011) Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. *Soil Biology & Biochemistry*, **43**, 1169–1179.