Forest Ecology and Management 390 (2017) 15–26

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Vertical distribution and persistence of soil organic carbon in fire-adapted longleaf pine forests

John R. Butnor[a,*], Lisa J. Samuelson[b], Kurt H. Johnsen[c], Peter H. Anderson[d], Carlos A. González Benecke[e], Claudia M. Boot[f,g], M. Francesca Cotrufo[f], Katherine A. Heckman[h], Jason A. Jackson[i], Thomas A. Stokes[b], Stanley J. Zarnoch[j]

[a] USDA Forest Service, Southern Research Station, University of Vermont, 81 Carrigan Drive, Aiken Center, Burlington, VT 05405, USA
[b] School of Forestry and Wildlife Sciences, Auburn University, 3301 SFWS Building, Auburn, AL 36849, USA
[c] USDA Forest Service, Southern Research Station, 1577 Brevard Road, Asheville, NC 28806, USA
[d] USDA Forest Service, Southern Research Station, 3041 East Cornwallis Road, Research Triangle Park, NC 27709, USA
[e] Department of Forest Engineering, Resources and Management, College of Forestry, Oregon State University, 280 Peavy Hall, Corvallis, OR 97331, USA
[f] Natural Resource Ecology Laboratory, Dept. 1499, Colorado State University, Fort Collins, CO 80523, USA
[g] Central Instrument Facility, Department of Chemistry, Colorado State University, 1872 Campus Delivery, 200 W. Lake, Fort Collins, CO 80523, USA
[h] USDA Forest Service, Northern Research Station, Forestry Sciences Laboratory, 410 MacInnes Drive, Houghton, MI 49931, USA
[i] Vance-Granville Community College, 200 Community College Road, Henderson, NC 27537, USA
[j] USDA Forest Service, Southern Research Station, Clemson, SC, 29639, USA

ARTICLE INFO

Article history:
Received 2 November 2016
Received in revised form 12 January 2017
Accepted 13 January 2017

Keywords:
C stocks
Pinus palustris
Prescribed fire
Pyrogenic C
Radiocarbon
Soil carbon

ABSTRACT

Longleaf pine (*Pinus palustris* Miller) forests in the southern United States are being restored and actively managed for a variety of goals including: forest products, biodiversity, C sequestration and forest resilience in the face of repeated disturbances from hurricanes and climate change. Managed southern pine forests can be sinks for atmospheric CO_2 in forest biomass; however, the persistence of biomass in the environment or in forest products is limited, thus making soil C the primary long-term pool. Little is known about the size of extant soil C pools, residence time of soil C or the role that frequent burning plays in C stabilization in longleaf pine ecosystems. We sampled soil from a chronosequence of longleaf pine stands ranging in age from 5 to 87 years to quantify the vertical distribution of soil organic carbon (SOC) stocks; both oxidizable (SOC_{OX}) and oxidation resistant (SOC_R) fractions, pyrogenic carbon (PyC) and the mean residence time (MRT) of SOC and its associated fractions. SOC stocks (0–1 m) ranged from 44.1 to 98.1 ($\bar{x} = 77.0$) Mg C ha^{-1}, and no effect of stand age or biomass accumulation on SOC stocks was detected. Soil C accumulation was associated with elevated clay and extractable Fe contents. While SOC concentration declined with soil depth, the proportion of SOC_R in SOC increased with depth. PyC was a minor component of soil C, representing 5–7% of SOC and the proportion was not depth dependent. The MRT of SOC was hundreds of years near the surface and many thousands of years at depth. Though SOC_R was less abundant than SOC_{OX}, SOC_R MRT was an order of magnitude greater than SOC_{OX} MRT and had a strong influence on bulk SOC MRT. The majority of the PyC was in the less persistent SOC_{ox} and not associated with long-term C storage in soil. Despite the flow of C from biomass in the form of decay products, litter fall, root turnover and pulses of PyC, these soils preserve little of recent inputs, which may be rapidly oxidized, lost to the atmosphere from periodic fires or, in the case of PyC, may be transported out of the system via erosion. Our results indicate that these soils were not strong sinks for atmospheric CO_2, especially when compared to C accumulation in biomass.

Published by Elsevier B.V.

1. Introduction

A key component of sustainable forestry is the responsible management of soil carbon (C) to ensure that site quality is maintained (Johnsen et al., 2001). Aggrading forests can rapidly accumulate atmospheric CO_2 in biomass during the early stages of a rotation,

---

* Corresponding author.
E-mail address: jbutnor@fs.fed.us (J.R. Butnor).

http://dx.doi.org/10.1016/j.foreco.2017.01.014
0378-1127/Published by Elsevier B.V.

but the persistence of this biomass C is ephemeral, thus soil is the primary long-term C pool. Stabilization of organic matter in soil is dependent on chemical composition, climate, moisture retention, soil minerology, and pyrogenic transformation, as well as physical isolation from environmental fluctuations at depth (Oades, 1988; Batjes, 1996). These factors lead to considerable variability in soil C quantity and residence time among and within sites and regions. In the southern United States, rapid turnover of soil C inputs from forests may be facilitated by coarse sands and low-activity clay mineralogy that limit long-term retention (Richter et al., 1999).

Longleaf pine forests in the southern United States are being restored and actively managed to provide forest products and a variety of ecosystem services such as biodiversity and disturbance-resistant long term C storage (Churchill et al., 2013). Longleaf pine occurs on a variety of soil types from coastal sands to eroded upland clay soils, but little is known about the quantity and persistence of soil C as well as the role of soil properties on the rate of soil C accumulation across its range. Longleaf pine ecosystems were a dominant forest type in the southeastern United States sustained by frequent low intensity fires (Frost, 1993; Schmidtling and Hipkins, 1998). As an example, in the Kisatchie National Forest in Southwest Louisiana, mean fire return from 1650 to 1905 was determined to be 2.2 years (Stambaugh et al., 2011). In the 19th and early 20th centuries the majority (>95%) of the longleaf forest was logged and converted to agriculture (Frost, 2006), and subsequent management led to considerable loss of topsoil. Thus, most current longleaf pine sites experienced millennia of frequent, low intensity fire followed by removal of forest cover, interruption of fire cycles and soil degradation. The long history of frequent fire prior to European settlement and current use of fire in restored systems has resulted in pulses of pyrogenic C (PyC) also referred to as "black carbon" into the soil. PyC residence time is estimated to be on the centennial time scale (Singh et al., 2012; Knicker et al., 2013), thus it can be an important avenue of soil C sequestration in terrestrial systems (Jaffe et al., 2013; Bird et al., 2015).

PyC found in soil is the result of incomplete combustion of biomass under low oxygen conditions. PyC is composed of a variety of C compounds; being a class rather than a specific material (Schmidt and Noack, 2000; Masiello, 2004; Preston and Schmidt, 2006; Hammes et al., 2008). In brief, fire transforms these organic compounds into a spectrum of amorphous materials dominated by fused aromatic rings. This material ranges from partially burned woody material to char. PyC becomes more recalcitrant along the spectrum associated with aromatic condensation, persisting from hundreds to thousands of years (Hammes et al., 2008; Ascough et al., 2009). There is a misconception that PyC formed from biomass under native environmental conditions is broadly inert and with long residence times in soil. Knicker et al. (2013) found that pyrogenic organic matter (PyOM) formed naturally in a severe forest fire and incorporated into the mineral soil had residence times 5–6 times longer than SOM in soils unaffected by fire. This increases the C sequestration potential of forest soils, but residence times were still modest, ranging from 500 to 600 years. The PyC products have a range of biochemical stability, though in general PyC formed at low temperature has higher O:C ratios and is more readily degraded than PyC formed at high temperatures (Inoue and Inoue, 2009; Spokas, 2010; Ascough et al., 2011). The amount of biomass converted to PyC is dependent on feedstock chemistry, combustion temperature and oxygenation. Preston and Schmidt (2006) estimated a conversion rate, of combusted organic matter, of 1–6% in boreal systems, while others contend that the mean conversion rate is substantially higher e.g. 5–15% (Santin et al., 2016).

To better understand the composition of extant soil C pools and assess the soil C sequestration potential, quantitative data are needed on the vertical distribution of C within the soil profile and persistence of C in soil. Key questions include: (1) How are SOC stocks partioned between active and resistant fractions and how much is PyC contributing to them? (2) How long does SOC persist and does PyC contribute long-term persistence? (3) Which edaphic factors are associated with SOC stocks and persistence? To address these questions we analyzed the vertical distribution and persistence of SOC to a depth of 1 m in fire-adapted longleaf pine forests aged 5–87 years old at sites with contrasting soil edaphic properties. A total of 14 stands were studied across the range of the species in the southern United States. Bulk SOC was chemically fractionated into resistant and oxidizable pools, then radiocarbon abundance measurements were applied to calculate quantitative estimates of the relative persistence of those fractions. Benzene Polycarboxylic Acid (BPCA) markers were used to quantify and characterize PyC (Glaser et al., 1998; Brodowski et al., 2005). This allowed us to analyze the effect of forest stand development (age, stand characteristics, above and belowground biomass C) and soil properties (depth, bulk density, texture, extractable iron) on the vertical distribution and persistence of C in soil. Our overall goal was to assess SOC pools by resistance to oxidation, PyC content, and mean residence in longleaf pine stands to better understand the legacy of past land use, frequent forest fires and current forest management on soil C stocks. Such quantification of soil C stocks will contribute to regional and national efforts to model C storage in soil and guide long-term management efforts.

2. Methods

2.1. Study areas

Sites selected for this study were part of a larger effort to quantify forest C density in longleaf pine forests and were previously described in detail (Samuelson et al., 2014, 2017). A chronosequence of 14 managed longleaf pine stands were selected for intensive sampling and characterization. They were located across the east-west range of the species from the Atlantic Coast Flatwoods (ACF), to the Southern Piedmont (SP) extending to the Western Coastal Plain (WCP) Major Land Resource Areas (MLRAs) in the southern United States (NRCS, 2006). Stands varied in age and basal area within each MLRA and soil characteristics varied between MLRAs (Table 1). Study sites were selected primarily on forest stand characteristics typical for each MLRA and active management with cyclical prescribed burning. Five SP stands located on Fort Benning Military Installation, Columbus, Georgia were sampled in 2012. Five WCP stands located on the Vernon District of the Kisatchie National Forest in Vernon Parish, Louisiana were sampled in 2013. Four ACP stands located on Camp Lejeune Military Installation, Jacksonville, North Carolina were sampled in 2014. Stands in ACP were typified by deep sands while those in SP and WCP had greater clay and silt fractions.

Detailed characterization of forest carbon stocks including overstory, understory, ground cover, taproots, coarse roots, fine roots, dead snags, woody debris, litter and duff were presented by Samuelson et al. (2014, 2017). All stands were even-aged with a minor component of volunteer *Pinus taeda* or hardwood recruitment and actively managed with an approximate three year fire return cycle. Four of the older stands were naturally regenerated and the others were planted.

2.2. Soil collection

The approach for defining study plots and collecting soil samples was adapted from the Global Terrestrial Observing System (GTOS) protocol for soil and root sampling (Law et al., 2008).

Table 1
Location and characteristics of longleaf pine stands sampled for soil carbon.

MLRA	Annual rainfall[1] (mm)	Annual air temp[1] (C)	Stand age (yrs)	Coordinates (Lat., Long.)	Soil series	Soil drainage class	Basal area (m^2)	SI (m)	Origin	Last three burns (yr)
ACF	1356	17.4	15	34.589, −77.271	Leon fine sand	PD	25.0	25	Planted	2005 2008 2010
			25	34.645, −77.450	Stallings loamy fine sand	PD	17.7	16	Planted	2008 2009 2010
			65	34.705, −77.252	Onslow loamy fine sand	MWD	11.6	15	Natural	2005 2009 2011
			79	34.702, −77.303	Malbis fine loamy sand	MWD	11.2	13	Natural	2007 2009 2012
SP	1180	18.7	5	32.400, −84.870	Nankin sandy clay loam	WD	0.5	19	Planted	2003 2007 2010
			12	32.373, −84.777	Nankin sandy clay loam	WD	11.5	19	Planted	2005 2008 2010
			21	32.391, −84.789	Troup sandy loam	ED	22.4	20	Planted	2005 2009 2010
			64	32.319, −85.008	Troup-Springhill-Luverne complex	WD	10.2	16	Natural	2003 2008 2010
			87	32.366, −84.785	Troup sandy loam	ED	14.5	19	Natural	2006 2008 2010
WCP	1447	18.9	8	30.944, −93.162	Troup sandy loam	MWD	5.2	21	Planted	2006 2008 2011
			18	30.943, −93.192	Beauregard-Malbis complex	MWD	8.0	21	Planted	2006 2007 2011
			34	30.991, −93.134	Briley loamy fine sand	WD	18.2	21	Planted	2007 2009 2012
			60	30.997, −93.019	Malbis fine loamy sand	MWD	20.0	19	Planted	2008 2009 2011
			83	31.024, −92.944	Malbis fine loamy sand	MWD	13.8	21	Planted	2001 2007 2011

MLRA = Major Land Resource Area (NRCS, 2006), ACF = Atlantic Coast Flatwoods, SP = Southern Piedmont, WCP = Western Coastal Plain, WD = well drained, MWD = moderately well drained, ED = excessively drained, PD = poorly drained, SI = site index at base age 50.

[1] 30 yr mean (1982–2011) Jacksonville, NC, Columbus, GA, and Leesville, LA; NOAA, National Centers for Environmental Information (http://www.ncdc.noaa.gov/cdo-webhttp://www.ncdc.noaa.gov/cdo-web).

Within each stand, a one ha circular main plot (56.4 m radius) with four 400 m^2 circular subplots (11.3 m radius) were installed. Within a main plot, one subplot was located at the center and three subplots were positioned 35 m from the center at 0°, 120°, and 240° from north. A 1.9 cm diameter push tube was used to collect soil samples for C and texture at 2 m, 5 m, and 11 m from subplot center at 0°, 90°, 180°, and 270° from north (12 locations total per subplot) at depths of 0–10 and 10–20 cm. A 10 cm diameter bucket auger was used to collect soil samples for C and texture at 20–50 and 50–100 cm from two of the soil sampling locations 2 m from the subplot center. Within each subplot, the subsamples were combined by depth resulting in 224 samples (14 sites × 4 subplots × 4 depths), that were further combined in equal portions into a composite sample by depth for each plot (14 sites × 4 depths) for a total of 56 samples. Soil samples for bulk density were collected at depths of 0–10, 10–20, 20–50 and 50–100 cm at one location 2 m north of subplot center using a 5.7 cm diameter soil core (0200 Soil Core Sampler, Soil Moisture Equipment Corp., Goleta, California).

2.3. Chemical fractionation

Oxidation resistant soil organic C (SOC_R) was isolated from bulk soil organic C (SOC) via selective oxidation with 20% H_2O_2 and 0.33 $\underline{M}$ HNO_3 with a method originally used to isolate charcoal fractions from soil and litter, described by Kurth et al. (2006) and modified by Ball et al. (2010). In this approach, oxidizable SOC (SOC_{OX}) is digested and converted to CO_2, leaving the SOC_R fraction (e.g. Eusterhues et al. (2005)). It is important to note that this was a strong oxidization reaction intended to isolate the C fraction that was highly resistant to chemical degradation. One gram of soil was placed in a pre-weighed 240 ml canning jar and 20 mL of 30% (v/v) of reagent grade H_2O_2 in water and 10 ml of 1 $\underline{M}$ HNO_3 were added to the jar to achieve a concentration of 20% H_2O_2 and 0.33 $\underline{M}$ HNO_3. The mixture was left at room temperature for 30 min with occasional swirling, then covered with a watch glass and incubated in an oven at 80 °C for 24 h. The watch glasses were then removed and the samples were left to dry uncovered at 80 °C for another 24 h. After drying, the samples (jar plus soil) were weighed again, scraped free with a spatula and ground to a fine powder using a mortar and pestle. To avoid contamination, all glassware, mortars, pestles, and chemical spatulas were cleaned with a laboratory grade cleaner (Micro SD, International Products Corp, Burlington, NJ USA) and soaked overnight in a 1% hydrochloric acid bath (HCl), and then rinsed with deionized water. Total C concentration of soil and of the oxidant resistant fraction was determined by elemental analysis with a Costech ECS 4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA) coupled to a Thermo Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA). Soils across the region have negligible inorganic C contents (Soil Survey Staff, 2000), therefore SOC was presumed to be equal to total C concentration. SOC_{OX} was calculated as the difference between SOC and $SOC_{R.}$

2.4. Pyrogenic Carbon (PyC) content

Benzene Polycarboxylic Acid (BPCA) marker quantification yields conservative, yet consistent estimates of PyC (Glaser et al., 1998; Wiedemeier et al., 2013; Cotrufo et al., 2016a). BPCAs are formed from the cleaving of condensed aromatic structures with concentrated nitric acid into single aromatic rings distinguished by a range of carboxylic acid moieties. More highly condensed ring structures have a greater proportion of B6CAs while structures with fewer fused rings have proportionally more B3CAs, providing insight into to the degree of biochemical stability (Glaser et al., 1998; Boot et al., 2015; Wiedemeier et al., 2015). Samples of SOC and SOC_R (14 stands × 4 depths) were treated with concentrated HNO_3 as described in Boot et al. (2015) to create BPCAs and analyzed using high performance liquid chromatography equipped with a photo diode array detector (Wiedemeier et al., 2013). BPCA content of SOC_{OX} was calculated as the difference between SOC and SOC_R. Since the formation of BPCAs causes a net loss of C, there is not a 1:1 ratio between PyC and BPCA markers. Depending on the molecular structure of the PyC, BPCA contents are 2–5 times lower than actual PyC contents. A conservative correction factor of 2.27 was applied to bulk BPCA contents to estimate soil PyC stocks (Glaser et al., 1998).

2.5. Radiocarbon analysis (^{14}C)

Samples for radiocarbon measurement were prepared at the USDA Forest Service, Northern Research Station, Carbon, Water & Soils Laboratory in Houghton, MI USA. Samples of SOC and SOC_R (14 stands × 4 depths) were combusted under vacuum in the presence of CuO and Ag to produce CO_2, which was then converted to graphite through heating at 570 °C in the presence of H_2 and a Fe catalyst according to Vogel et al. (1987). Radiocarbon content was analyzed by accelerator mass spectrometry at the Center for Accelerator Mass Spectrometry (CAMS), Lawrence Livermore National Lab (LLNL), Livermore, California, USA (Davis et al., 1990). SOC_{OX} Δ ^{14}C values were calculated as the difference between SOC and SOC_R $\Delta^{14}C$ values, taking into consideration C distribution between fractions. Radiocarbon data are reported as fraction modern (Fm) which is the ratio of $^{14}C/^{12}C$ (or $^{14}C/^{13}C$) of the sample to the atmospheric $^{14}C/^{12}C$ (or $^{14}C/^{13}C$) ratio in 1950, prior to extensive aboveground testing of nuclear weapons (Stuiver and Polach, 1977). Mean residence times (MRT) of SOC, SOC_{OX} and SOC_R were calculated using $\Delta^{14}C$ values and a time-dependent steady-state model (Trumbore, 1993; Torn et al., 2009).

2.6. Soil texture, extractable Fe analysis and bulk density

Soil was air-dried for several weeks and passed through a 2 mm sieve to remove roots and rocks. Particle size (soil texture) analysis was performed by hydrometer method (Bouyoucos, 1962). Fe was extracted from soil using Mehlich-3 extractant (Mehlich, 1984) and analyzed with inductively coupled plasma spectrometry by Spectrum Analytic Inc. (Washington Court House, Ohio, USA). Bulk density samples were oven-dried at 105 °C for 96 h and then passed through a 2 mm sieve, and roots and rocks were extracted and weighed separately. Rock volume was determined by water displacement. The effect of root volume on bulk density was negligible. Soil bulk density was then calculated by soil mass (minus rocks and roots)/soil volume (minus rock volume) (Law et al., 2008).

2.7. Statistical analysis

The approach taken was to first consider if forest stand development and biomass C stocks affected accumulation of soil C among fractions, then analyze the vertical distribution, residence time and soil properties related to C retention. Stepwise multiple linear regression ($SLE = 0.25$ for entry, $SLS = 0.05$ retention in model) was used to determine whether soil C stocks to a depth of 1 m (SOC, SOC_R, SOC_{OX} and PyC) were dependent on stand parameters (biomass C stocks, stand age, basal area) with Proc Reg (SAS Institute Inc., 2013) using SAS/STAT 9.4 software (SAS Institute, Cary, NC, USA).

The effect of soil depth and MLRA on the vertical distribution of soil C fractions, physical properties, fraction modern and MRT of C was analyzed with Proc Mixed (SAS Institute Inc., 2013) using composite data from each forest stand. Soil depth was the repeated measures factor, where soil was collected from several depths at a given forest stand. The depth intervals were uneven (0–10, 10–20, 20–50, 50–100 cm), so based on theoretical grounds of unequal spacing and comparison of the AICC between several types of covariance matrices, we decided to use the spatial power covariance matrix. After testing for the main effects and interaction, mean separation analysis within MLRAs for depth was performed with the Bonferroni correction. This adjustment uses the pooled variance across MLRAs and depths from the overall analysis which may result in significances that are slightly different from those based on the individual standard errors alone. The Bonferroni method performed all six possible pairs of comparisons between the four depths and ensured an experiment-wise error rate of 0.05 for the set by using the $\alpha = 0.05/6 = 0.0083$ level for testing each pair. Differences in stocks of SOC, SOC_{OX}, SOC_R, and PyC in the upper 1 m of soil among MLRAs were analyzed with one-way analysis of variance Proc Mixed (SAS Institute Inc., 2013).

Stepwise linear multiple regression was applied to analyze the relationship between concentrations of C fractions in soil (SOC, SOC_{OX}, SOC_R, and PyC) and soil properties (texture, depth, bulk density, Fe concentration) across the 14 longleaf pine stands with Proc Reg (SAS Institute Inc., 2013). Mean residence time of SOC_{OX} and SOC_R was also analyzed with stepwise linear multiple regression using the above soil properties with the addition of variables related to BPCA speciation (%B6CA, %B5CA, %B4CA, %B3CA, %B4CA/B6CA, and %B5CA/B6CA). Mean residence time of bulk soil (SOC) was similarly analyzed using soil, BPCA variables, plus the percent of SOC comprised of SOC_R and PyC.

3. Results

3.1. Stand age, biomass and soil C stocks

To analyze potential effects of forest stand biomass accumulation on soil C, it was necessary to scale soil C concentration to same units as biomass C ($Mg\ C\ ha^{-1}$). Concentrations of SOC, SOC_R, SOC_{OX} and PyC were converted to $Mg\ C\ ha^{-1}$ using soil bulk density and summed across all sample depths to yield a C stock (0–1 m) for each (Table 2). The stepwise multiple regression analysis showed no significant relationships between SOC, SOC_R, SOC_{OX} and PyC and any biomass parameter (C stocks, stand age, basal area), despite wide ranges in biomass C stocks across the 14 stands (Table 2). Stand age and biomass were thus dropped from subsequent analyses.

3.2. Carbon concentration and soil depth

SOC, SOC_{OX}, SOC_R and PyC contents of bulk soils were highest near the surface and declined with depth (Fig. 1). While soil depth accounted for most of the variability in C concentration (SOC: $F = 54.3$, $p < 0.0001$; SOC_{OX}: $F = 55.6$, $p < 0.0001$; SOC_R: $F = 22.1$, $p < 0.0001$; PyC: $F = 20.2$, $p < 0.0001$), MLRA and the interaction between MLRA and soil depth were also significant for all variables. The stands within a MLRA shared similar soil properties, making comparison of soil C fractions by MLRA useful (Table 3). WCP and ACF soils had very similar mean C concentrations among the fractions (SOC, SOC_{OX}, SOC_R and PyC) by depth; with the exception of PyC, means were within the standard error at each depth (Table 3). All WCP and ACF soil C fraction concentrations were highest near the surface and declined with depth. The SP soils differed from WCP and ACF in several ways: SOC, SOC_{OX}, PyC concentrations were approximately half that of WCP and ACF in the upper

Table 2
Minimum, maximum, and mean soil C stocks to a depth of 1 m and stand-level biomass C stocks (from Samuelson et al., 2017) for 14 longleaf pine forests.

C Stock	Minimum ($Mg\ C\ ha^{-1}$)	Maximum ($Mg\ C\ ha^{-1}$)	Mean ($Mg\ C\ ha^{-1}$)
Soil			
SOC	41.5	98.1	77.0
SOC_{OX}	33.2	87.3	61.8
SOC_R	8.3	22.9	15.2
PyC	2.1	7.2	4.6
Biomass			
Live Aboveground C	1.4	82.5	41.6
Live Belowground C	2.7	24.8	17.1
Detritus C	0.8	7.4	4.2

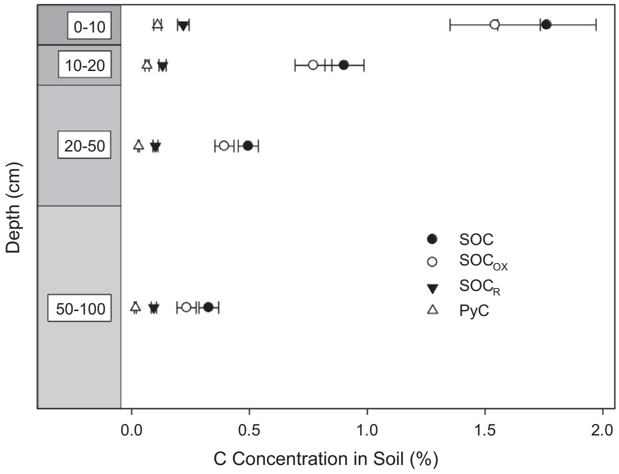

Fig. 1. Mean (SE) concentration of soil organic carbon (SOC), oxidizable SOC (SOC_{OX}), resistant SOC (SOC_R) and pyrogenic C (PyC) in soil from 14 longleaf pine stands by depth.

20 cm, and SOC_R and PyC did not significantly decline with depth. To explore these differences further, SOC_{OX}, SOC_R and PyC fractions were analyzed as a proportion of total SOC. As SOC declined with soil depth the proportion of SOC_R in SOC significantly increased at the SP and ACF stands, though there was no significant effect of depth on PyC/SOC (Table 4).

Carbon concentrations were scaled to C stocks by incorporating soil bulk density and the length of the depth interval to calculate a sum total C stock in the upper 1 m of soil. There were no differences in total SOC stocks between the MLRAs ($F = 1.55$, $p = 0.2560$), though SOC_R stocks in SP were nearly double those in WCP and ACF ($F = 22.24$, $p = 0.0001$), while WCP had the highest SOC_{OX} ($F = 4.77$, $p = 0.0322$) and PyC stock ($F = 11.57$, $p = 0.0020$) (Table 5).

3.3. PyC oxidative degradation

PyC resistant to oxidation with H_2O_2 and HNO_3 (PyC_R) was a relatively small proportion of PyC ($\bar{x}$ across depths: SP 9.2%, WCP 7.8%, ACF 13.5%) and is included in both the SOC_R and PyC fractions (Fig. 2). Using the differences between least square means (two-way ANOVA) PyC_R/PyC in the upper 10 cm of soil in ACF was significantly higher than any other MLRA/depth combination, though soil depth did not significantly affect PyC_R/PyC in SP or WCP (Fig. 2). This could indicate different formation conditions for PyC resulting in more resistant PyC_R in ACF as the coarse sands may be less conducive to C retention. The key finding is that the majority of PyC across all MLRAs is included in SOC_{OX} fraction and PyC_R accounts for only 2–5% of the C in the SOC_R fraction. This has important implications for radiocarbon as it was only possible to analyze SOC, SOC_R and calculate SOC_{OX} from the difference between SOC and SOC_R. PyC radiocarbon was not analyzed in this study, though very recent advancements have made it possible to simultaneously analyze the quantity and isotopic composition of PyC with the BPCA method (Wiedemeier et al., 2016).

3.4. PyC BPCA characterization

In addition to total BPCA contents that were scaled to estimate PyC in soil, the approach also characterized the ring structure of PyC. Benzenehexacarboxylic acid (B6CA; i.e. originating from 6 rings) relative abundance in SOC increased at deeper soil depths indicating a higher degree of aromatic condensation and biochemical stability at depth, but this was statistically significant only for SP and WCP, and not ACF (Fig. 3). The low concentration of BPCA in SOC_R precluded further analysis of that fraction (data not shown).

3.5. Radiocarbon

Soil was most ^{14}C depleted at depth; Fm declined with depth for all of the SOC pools (Table 6). Soil depth and MLRA explained most of the variation in Fm for SOC, SOC_{OX}, and SOC_R, and there were no significant MLRA by depth interactions. Both soil depth (SOC: $F = 43.1$, $p < 0.0001$; SOC_{OX}: $F = 67.1$, $p < 0.0001$; and SOC_R: $F = 35.6$, $p < 0.0001$) and MLRA (SOC: $F = 17.9$, $p = 0.0055$; SOC_{OX}: $F = 12.0$, $p < 0.0001$; and SOC_R: $F = 46.1$, $p < 0.0001$) affected Fm. Fm predicted the continuum of stability as expected where the least resistant fraction had the greatest proportion of modern C, the oxidation resistant fraction had the least proportion of modern C, indicating increased persistence in the environment, and the combination of the two was intermediate i.e.: Fm: $SOC_{OX} > SOC > SOC_R$ (Table 6). Fm values of SOC_{OX} were similar between the MLRAs, though Fm of SOC_R in SP was much lower than WCP or ACF (Table 6).

SOC is in a state of equilibrium where C is continually entering and leaving the soil, therefore a discrete "radiocarbon age" is not as appropriate as it would be with an object of a specific age such as a tree ring, bone, or char fragment. The $\Delta^{14}C$ value was used to model MRT to give greater context to the radiocarbon data. SOC MRT ranged from 123 to 6226 years depending on depth (Table 6). With our approach it was not possible to separate PyC from SOC and obtain discrete estimate of PyC MRT. As previously noted, the majority of PyC (SP 90.8%, WCP 92.2%, ACP 86.5%) was in the SOC_{OX} fraction, which is the least stable fraction with similar MRT's

Table 3
Mean (SE) SOC, SOC_{OX} SOC_R and PyC concentrations by depth in longleaf pine forests. Within each MLRA, means followed by the same letter are not significantly different at the alpha = 0.05 level using the Bonferroni adjustment.

MLRA	Depth (cm)	n	SOC (%)		SOC_{OX}		SOC_R (%)		PyC (%)	
ACF	0–10	4	2.13 (0.39)	*a*	1.85 (0.33)	*a*	0.28 (0.07)	*a*	0.117 (0.034)	*a*
	10–20	4	0.99 (0.22)	*b*	0.84 (0.19)	*b*	0.15 (0.04)	*b*	0.064 (0.010)	*ab*
	20–50	4	0.45 (0.14)	*bc*	0.37 (0.13)	*bc*	0.08 (0.02)	*b*	0.022 (0.008)	*b*
	50–100	4	0.26 (0.02)	*c*	0.18 (0.02)	*c*	0.08 (0.01)	*b*	0.014 (0.006)	*b*
SP	0–10	5	1.05 (0.05)	*a*	0.87 (0.03)	*a*	0.18 (0.02)	*a*	0.057 (0.009)	*a*
	10–20	5	0.68 (0.04)	*ab*	0.53 (0.04)	*ab*	0.15 (0.02)	*a*	0.037 (0.004)	*a*
	20–50	5	0.51 (0.05)	*ab*	0.37 (0.05)	*ab*	0.15 (0.01)	*a*	0.027 (0.005)	*a*
	50–100	5	0.35 (0.03)	*b*	0.21 (0.02)	*b*	0.14 (0.01)	*a*	0.013 (0.002)	*a*
WCP	0–10	5	2.18 (0.33)	*a*	1.97 (0.30)	*a*	0.21 (0.03)	*a*	0.158 (0.019)	*a*
	10–20	5	1.05 (0.11)	*b*	0.95 (0.10)	*b*	0.10 (0.01)	*b*	0.095 (0.017)	*b*
	20–50	5	0.51 (0.04)	*bc*	0.44 (0.04)	*c*	0.07 (0.01)	*b*	0.038 (0.005)	*c*
	50–100	5	0.36 (0.12)	*c*	0.30 (0.11)	*c*	0.06 (0.01)	*b*	0.020 (0.011)	*c*

MLRA = Major Land Resource Area (NRCS, 2006), ACF = Atlantic Coast Flatwoods, SP = Southern Piedmont, WCP = Western Coastal Plain.

Table 4
Mean (SE) ratio of C fractions in SOC by depth in longleaf pine forests. Within each MLRA, means followed by the same letter are not significantly different at the alpha = 0.05 level using the Bonferroni adjustment.

MLRA	Depth (cm)	n	SOC_{OX}/SOC * 100 (%)		SOC_R/SOC * 100 (%)		PyC/SOC * 100 (%)	
ACF	0–10	4	86.86 (1.77)	*a*	13.14 (1.77)	*a*	5.15 (1.10)	*a*
	10–20	4	84.92 (2.11)	*a*	15.08 (2.11)	*a*	7.20 (1.34)	*a*
	20–50	4	78.62 (3.68)	*a*	21.38 (3.68)	*a*	4.24 (0.77)	*a*
	50–100	4	68.40 (3.58)	*b*	31.60 (3.58)	*b*	5.34 (1.80)	*a*
SP	0–10	5	83.24 (1.38)	*a*	16.76 (1.38)	*a*	5.32 (0.68)	*a*
	10–20	5	77.94 (2.54)	*ab*	22.06 (2.54)	*ab*	5.47 (0.39)	*a*
	20–50	5	69.82 (4.50)	*b*	30.18 (4.50)	*b*	5.22 (0.48)	*a*
	50–100	5	59.12 (2.69)	*c*	40.88 (2.69)	*c*	3.65 (0.30)	*a*
WCP	0–10	5	90.24 (0.44)	*a*	9.76 (0.44)	*a*	7.46 (0.50)	*a*
	10–20	5	90.71 (0.50)	*a*	9.29 (0.50)	*a*	8.97 (1.08)	*a*
	20–50	5	85.72 (0.86)	*a*	14.28 (0.86)	*a*	7.62 (1.20)	*a*
	50–100	5	80.82 (2.99)	*a*	19.18 (2.99)	*a*	4.53 (1.21)	*a*

MLRA = Major Land Resource Area (NRCS, 2006), ACF = Atlantic Coast Flatwoods, SP = Southern Piedmont, WCP = Western Coastal Plain.

Table 5
Mean (SE) soil stocks of SOC, SOC_{OX}, SOC_R, and PyC in the upper 1 m of soil in longleaf pine forests. Across MLRAs, means followed by the same letter are not significantly different at the alpha = 0.05 level using the Bonferroni adjustment.

MLRA	n	SOC (Mg ha^{-1})		SOC_{OX} (Mg ha^{-1})		SOC_R (Mg ha^{-1})		PyC (Mg ha^{-1})	
ACF	4	71.48 (10.16)	*a*	57.93 (8.63)	*ab*	13.55 (1.92)	*b*	3.73 (0.37)	*a*
SP	5	72.12 (6.56)	*a*	51.22 (5.72)	*a*	20.90 (1.01)	*a*	3.54 (0.58)	*a*
WCP	5	86.16 (3.54)	*a*	75.42 (3.43)	*b*	10.73 (0.40)	*b*	6.39 (0.40)	*b*

MLRA = Major Land Resource Area (NRCS, 2006), ACF = Atlantic Coast Flatwoods, SP = Southern Piedmont, WCP = Western Coastal Plain.

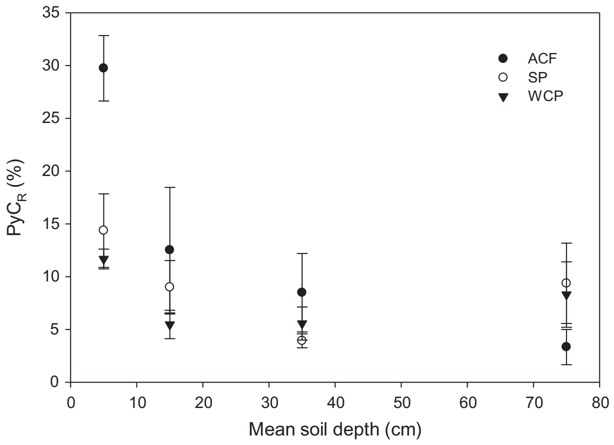

Fig. 2. Mean (SE) percent of pyrogenic C resistant to oxidation with H_2O_2 and HNO_3 (PyC_R) by depth in longleaf pine stands in the Atlantic Coast Flatwoods (ACF; n = 4), Southern Piedmont (SP; n = 5) and Western Coastal Plain (WCP; n = 5).

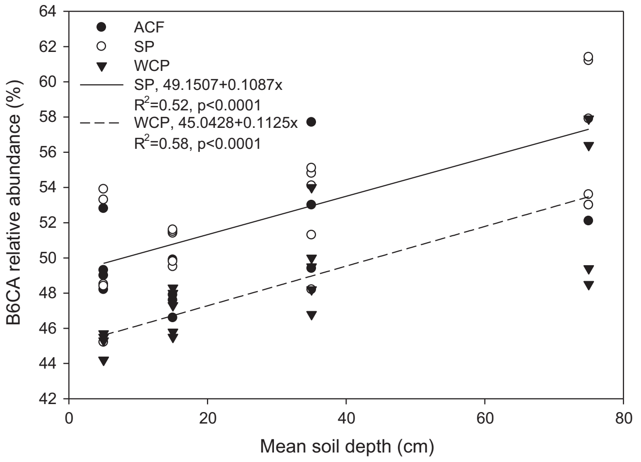

Fig. 3. Relationship between benzenehexacarboxylic acid (B6CA) relative abundance and soil depth in longleaf pine stands located in the Southern Piedmont (SP) and Western Coastal Plain (WCP). No significant relationship was observed at the Atlantic Coast Flatwoods (ACF) sites.

across MLRAs (Table 6). SOC_R is the most stable fraction, MRT values were similar for WCP and ACF; MRTs were much longer for the stands in SP averaging 22,626 years at the 50–100 cm depth interval (Table 6). These results illustrate the longevity of C that is retained in forest soil and though SOC_R is a minority component of SOC, its MRT is a magnitude higher than the oxidizable fraction. Mean residence time of C may also be considered as a measure of annual turnover (Fig. 4). The most rapid annual turnover rates (% C oxidized per year) were in the upper 10 cm of soil (SP 0.26%, WCP 0.52%, ACF, 0.82%), where rates from each MLRA were significantly different from each other.

3.6. Soil properties associated with C stabilization and retention

Soil physical properties of bulk density and texture are summarized by MLRA and depth in Table 7. A wide range of soil texture combinations were observed: ACF soils were typified by deep sandy soil, SP soils had gradual increase in clay with depth, and WCP soils had a clay profile similar to SP but with a 20–26% silt fraction throughout the profile. WCP had the highest bulk density, while ACF soils were the least dense. Fe has been shown to form

Table 6
Mean (SE) fraction modern (Fm) and modeled mean residence time of SOC, SOC_{OX} and SOC_R in soil by depth in longleaf pine forests. Within each MLRA, means followed by the same letter are not significantly different at the alpha = 0.05 level using the Bonferroni adjustment.

MLRA	Depth (cm)	n	SOC Fm		SOC_{OX} Fm		SOC_R Fm		SOC^* MRT (years)		SOC_{OX}^* MRT (years)		SOC_R^* MRT (years)	
ACF	0–10	4	1.07 (<0.01)	*a*	1.12 (0.02)	*a*	0.87 (0.03)	*a*	123 (7)	*a*	63 (13)	*a*	1345 (338)	*a*
	10–20	4	1.01 (0.01)	*ab*	1.06 (0.01)	*ab*	0.75 (0.05)	*ab*	290 (66)	*a*	142 (12)	*ab*	2870 (681)	*ab*
	20–50	4	0.88 (0.06)	*bc*	0.97 (0.03)	*bc*	0.58 (0.09)	*bc*	1473 (731)	*b*	530 (208)	*bc*	7289 (2998)	*bc*
	50–100	4	0.75 (0.04)	*c*	0.89 (0.02)	*c*	0.54 (0.07)	*c*	2891 (725)	*b*	1102(165)	*c*	7691 (2228)	*c*
SP	0–10	5	0.97 (0.03)	*a*	1.03 (0.03)	*a*	0.66 (0.03)	*a*	541 (189)	*a*	274 (144)	*a*	4344 (581)	*a*
	10–20	5	0.90 (0.03)	*a*	1.01 (0.03)	*ab*	0.48 (0.03)	*ab*	1112 (209)	*a*	322 (90)	*a*	8882 (1016)	*ab*
	20–50	5	0.76 (0.06)	*b*	0.94 (0.02)	*b*	0.41 (0.05)	*bc*	2927 (1001)	*b*	743 (149)	*b*	12917 (2790)	*bc*
	50–100	5	0.58 (0.05)	*c*	0.86 (0.04)	*b*	0.28 (0.04)	*c*	6226 (1203)	*c*	1521 (417)	*b*	22626 (3973)	*c*
WCP	0–10	5	1.03 (0.01)	*a*	1.06 (0.01)	*a*	0.88 (0.03)	*a*	200 (20)	*a*	144 (16)	*a*	1223 (243)	*a*
	10–20	5	0.98 (0.01)	*a*	0.99 (0.01)	*ab*	0.81 (0.02)	*ab*	452 (38)	*a*	360 (32)	*b*	1990 (272)	*a*
	20–50	5	0.89 (0.01)	*a*	0.94 (0.01)	*b*	0.63 (0.03)	*bc*	1090 (120)	*b*	687 (71)	*b*	4836 (610)	*b*
	50–100	5	0.67 (0.04)	*b*	0.75 (0.02)	*c*	0.51 (0.05)	*c*	4266 (702)	*c*	2802 (342)	*c*	8345 (1421)	*b*

MLRA = Major Land Resource Area (NRCS, 2006), ACF = Atlantic Coast Flatwoods, SP = Southern Piedmont, WCP = Western Coastal Plain.

[*] To meet assumption of homoscedasticity, variables were log transformed for statistical analysis. Non-transformed means and standard errors are presented.

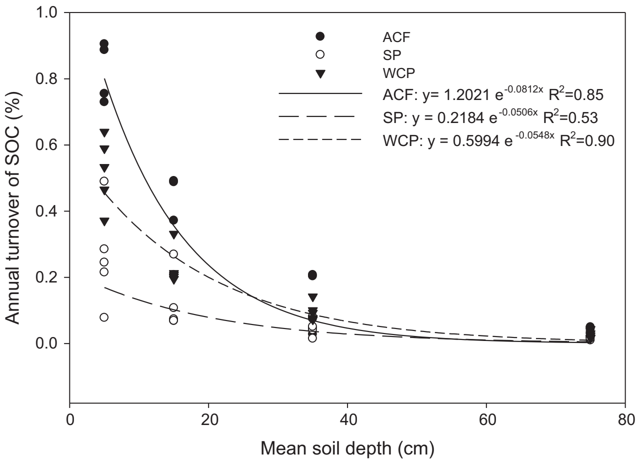

Fig. 4. Annual turnover of soil organic C (SOC) in relation to soil depth in longleaf pine stands in located in the Atlantic Coast Flatwoods (ACF), Southern Piedmont (SP) and Western Coastal Plain (WCP).

stable associations with C, therefore extractable Fe (Mehlich-3) was included in the analysis (Table 7). Extractable Fe declined significantly with depth at SP and WCP, but remained stable throughout the profile in ACF. Soils were acidic ranging from pH 4.28 to 5.04 (Table 7).

The dependence of SOC, SOC_{OX}, SOC_R and PyC concentrations on soil depth, texture, bulk density and extractable Fe was analyzed with stepwise linear multiple regression (Table 8). After examination of the residual vs predicted values, it was deemed necessary to log transform the soil C variables. Bias from log transformation was corrected using a method described by Snowdon (1991). Soil depth was the primary driver of the models as soil C across fractions was highest near the surface and declined with depth. Since soil texture, BD and Fe concentrations are all influenced by depth (Table 7), it was important to retain depth in the model to avoid causality with soil parameters that are themselves affected by depth. All of the model parameters had low variance inflation factors (<5) indicating that the parameters had minimal collinearity. Both Fe and clay can stabilize C in soil and are representative of absorptive capacity of the soil for C. Fe was positively associated with concentration of all soil C fractions, accounting for 10% of the variation in SOC_R and 7% of the variation in PyC (Table 8). Clay content was positively associated with C concentration (or conversely negative association with sand) and accounted for 15% of the variation in SOC_R. Bulk density was also negatively associated with SOC_{OX}, and SOC_R (Table 8).

3.7. Soil properties and soil C fractions associated with residence time of soil C

The dependence of SOC_{OX} MRT and SOC_R MRT concentrations on soil depth, texture, bulk density and extractable Fe was analyzed

Table 7
Mean (SE) soil physical properties plus pH and extractable Fe content by depth in longleaf pine forests. Within each MLRA, means followed by the same letter are not significantly different at the alpha = 0.05 level using the Bonferroni adjustment.

MLRA	Depth (cm)	n	Bulk Density (g cm^2)		Sand (%)		Silt (%)		Clay (%)		pH		Fe (ppm)	
ACF	0–10	4	1.09 (0.09)	*a*	91.2 (3.3)	*a*	5.8 (1.9)	*a*	3.0 (1.4)	*a*	4.28 (0.06)	*a*	96.7 (35.3)	*a*
	10–20	4	1.30 (0.04)	*b*	91.2 (3.5)	*a*	5.2 (1.9)	*a*	3.7 (1.7)	*a*	4.46 (0.08)	*b*	97.5 (37.5)	*a*
	20–50	4	1.36 (0.05)	*b*	88.8 (4.1)	*a*	5.3 (1.7)	*a*	5.8 (2.4)	*a*	4.80 (0.06)	*c*	74.9 (18.5)	*a*
	50–100	4	1.49 (0.04)	*b*	86.3 (4.2)	*a*	4.5 (1.3)	*a*	9.2 (2.9)	*a*	4.83(0.05)	*c*	93.9 (20.6)	*a*
SP	0–10	5	1.30 (0.10)	*a*	83.0 (0.6)	*a*	8.1 (0.4)	*a*	8.9 (0.4)	*a*	4.98 (0.09)	*a*	122.2 (11.5)	*a*
	10–20	5	1.55 (0.07)	*b*	79.5 (1.9)	*b*	8.0 (0.3)	*a*	12.5 (1.9)	*b*	4.97 (0.06)	*a*	94.3 (7.9)	*b*
	20–50	5	1.58 (0.05)	*b*	73.3 (1.8)	*c*	8.7 (0.6)	*a*	18.0 (2.2)	*c*	4.96 (0.09)	*a*	69.7 (7.3)	*bc*
	50–100	5	1.53 (0.03)	*b*	63.1 (4.5)	*d*	7.9 (0.4)	*a*	29.0 (4.8)	*d*	4.90 (0.08)	*a*	40.1 (2.4)	*c*
WCP	0–10	5	1.44 (0.06)	*a*	67.0 (2.5)	*a*	25.9 (2.2)	*a*	7.1 (1.1)	*a*	5.01 (0.08)	*a*	152.4 (16.2)	*a*
	10–20	5	1.69 (0.06)	*b*	64.4 (2.8)	*b*	24.8 (2.4)	*a*	10.8 (1.9)	*b*	5.02 (0.06)	*a*	114.2 (15.8)	*b*
	20–50	5	1.64 (0.03)	*ab*	58.7 (3.8)	*c*	23.4 (2.2)	*ab*	17.9 (4.0)	*c*	5.04 (0.04)	*a*	65.0 (16.9)	*c*
	50–100	5	1.77 (0.05)	*b*	54.3 (3.3)	*c*	20.5 (2.6)	*b*	25.2 (3.7)	*d*	4.94 (0.03)	*a*	34.9 (4.5)	*c*

MLRA = Major Land Resource Area (NRCS, 2006), ACF = Atlantic Coast Flatwoods, SP = Southern Piedmont, WCP = Western Coastal Plain.

Table 8

Linear regression model parameters and fit statistics for soil C fraction concentration and C mean residence time of soil samples collected at four depths from 14 longleaf pine stands (n = 56). All dependent variables were modeled with soil depth, bulk density (BD), % sand, % silt, % clay, extractable Fe (ppm). All models were highly significant ($p < 0.0001$).

Dep. variable	Significant parameters	Estimate	SE	VIF	*p*	Part. R^2	Model R^2	CF	RMSE
lnSOC	y0	−0.39876	0.22810	0	0.0865				
	Depth	−0.02126	0.00315	1.97560	<0.0001	0.6034	0.6784	1.1109	0.4391
	Fe	0.00485	0.00159	1.61999	0.0037	0.0412			
	Clay	0.01895	0.00820	1.79777	0.0249	0.0337			
lnSOC$_{OX}$	y0	2.41289	0.93447	0	0.00128				
	Depth	−0.02174	0.00320	1.80391	<0.0001	0.6280	0.7225	1.1104	0.4664
	Fe	0.00424	0.00164	1.52243	0.0126	0.0364			
	Sand	−0.01921	0.00601	1.76704	0.0024	0.0304			
	BD	−0.87857	0.39406	1.84134	0.0303	0.0276			
lnSOC$_R$	y0	−2.62703	0.89226	0	0.0049				
	Depth	−0.01067	0.00258	2.14226	0.0001	0.2641	0.6307	1.0546	0.3455
	Clay	0.05224	0.01005	4.36933	<0.0001	0.1492			
	Fe	0.00543	0.00130	1.75791	0.0001	0.1047			
	BD	−0.91864	0.29581	1.89001	0.0032	0.0771			
	Sand	0.01415	0.00651	3.77281	0.0346	0.0356			
lnPyC	y0	−2.12723	0.61669	0	0.0011				
	Depth	−0.02423	0.00423	1.71270	<0.0001	0.5456	0.6506	1.1332	0.6333
	Fe	0.00728	0.00222	1.52223	0.0019	0.0734			
	Sand	−0.01438	0.00670	1.19041	0.0366	0.0316			
lnSOC$_{OX}$ MRT	y0	2.29411	0.51102	0	<0.0001				
	Depth	0.02914	0.00289	1.22217	<0.0001	0.6981	0.7987	1.0866	0.5252
	BD	1.85970	0.36150	1.22217	<0.0001	0.1005			
lnSOC$_R$ MRT	y0	9.46139	1.47316	0	<0.0001				
	Depth	0.01101	0.00426	1.83984	0.0127	0.4178	0.6068	1.1523	0.6307
	Sand	−0.02934	0.01177	3.78014	0.0160	0.0806			
	Silt	−0.07884	0.01691	3.15899	<0.0001	0.0690			
	BD	1.22079	0.53933	1.88617	0.0279	0.0395			
LnSOC* MRT	y0	2.40683	0.48026	0	<0.0001				
	Depth	0.01962	0.00335	2.10366	<0.0001	0.7115	0.8792	0.9869	0.4526
	SOC$_R$/SOC%	0.05624	0.00762	1.72441	<0.0001	0.0960			
	BD	1.76659	0.32110	1.29820	<0.0001	0.0717			

VIF = variance inflation factor, CF = correction factor per Snowdon (1991), RSME = root square mean error.

* SOC$_R$/SOC% and PyC/SOC% included in the stepwise selection process.

with stepwise linear multiple regression similar to the approach with C concentrations (Table 8). In addition to the aforementioned parameters, $SOC_R/SOC * 100$ (%) and $PyC/SOC * 100$ (%) were included in stepwise selection process for SOC MRT to include the contribution of SOC$_R$ and PyC content to the overall model for SOC. To meet the assumption of homoscedasticity it was necessary to log transform all MRT values.

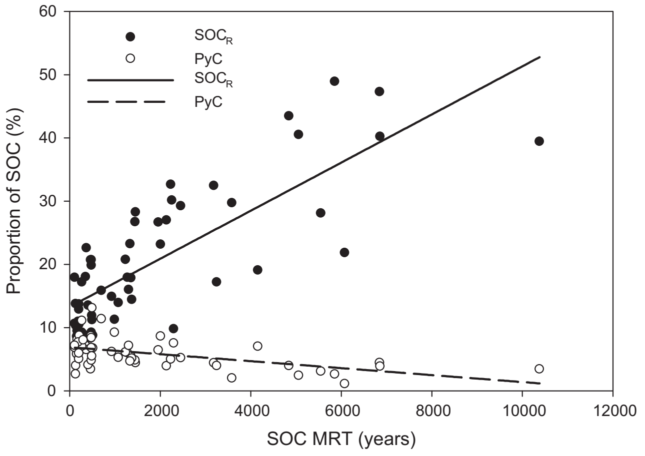

Fig. 5. Relationship between the proportion of oxidation resistant soil organic C (SOC$_R$) and pyrogenic C (PyC) in soil organic C (SOC) with mean residence time (MRT) of C in soil, SOC$_R$: $y = 13.2875 + 0.0038x$, $p < 0.0001$, $R^2 = 0.63$; PyC: $y = 6.892–0.0006x$, $p < 0.0001$, $R^2 = 0.27$.

Soil depth and bulk density were associated with increased residence time for both SOC$_{OX}$ MRT and SOC$_R$ MRT. SOC$_R$ MRT was negatively associated with sand and silt, inferring a positive relationship with clay content (Table 8). As SOC MRT is the sum total of the contributions of SOC$_{OX}$ MRT and SOC$_R$ MRT, it was positively associated with depth and BD. SOC$_R$/SOC (%) contributed an additional 10% to the SOC MRT regression model ($R^2 = 0.88$) though PyC/SOC (%) was not retained in the model at the $p < 0.05$ level. Pooling data from all depths from the 14 longleaf pine stands, SOC$_R$/SOC increased with SOC MRT, while PyC/SOC declined with SOC MRT (Fig. 5). These results indicate that SOC$_R$ and not PyC is driving long term stabilization of C in these forests.

4. Discussion

4.1. Chemical fractionation

We employed a pure chemical fractionation which had been shown to isolate charcoal in organic soils (Kurth et al., 2006; Ball et al., 2010) as well as mineral protected C in both organic and mineral soils (Eusterhues et al., 2005). This chemical-only approach simplifies the analysis and yields a highly resistant fraction, but there remains the possibility that the digest may have oxidized some C that was otherwise stable, or conversely may have retained some hydrophobic C forms or aliphatic organic matter which resist H_2O_2 degradation (Eusterhues et al., 2005). Other approaches that employ both physical and chemical fractionation with milder digests, i.e. NaOCl, are more time consuming, but further characterize fractions by C activity e.g. active, intermediate, passive (Zimmermann et al., 2007). For example, Wiesmeier et al. (2014) surveyed major soil areas in Bavaria to 1 m using this approach and was able to further characterize the nature of functional soil pools across several land uses (grass, crop, forest, etc.). Removal of dissolved and particulate organic C (active pool) lowered the intermediate activity pool in forests to 53% of SOC, which is lower than we report for our SOC_{OX} fraction.

4.2. SOC and stand age

The present study found no effect of stand age or biomass accumulation on any soil C parameter using a chronosequence approach. These results are similar to reports from other southern pine plantations where little (Richter et al., 1999) or no increase in soil C was detectable over time (Markewitz et al., 2002; Johnson et al., 2003; Johnsen et al., 2013). Though seldom available, the use of archived soils e.g. (Richter et al., 1999; Hammes et al., 2008) provides a more powerful means of detecting change over time than chronosequence, which are often point-in-time collections across diverse soil types with differing minerology (Richter et al., 2007). Across a variety of forest types, even large ecosystem disturbances from harvesting and replanting generally have little to no long-term effect on soil C (Johnson et al., 2002). In the time scale of a longleaf pine rotation (~50 to 100 yrs) sequestration of atmospheric C is driven by biomass accumulation rather than storage in soil (Samuelson et al., 2017). Concentrations of SOC and all of the representative fractions (SOC_{OX}, SOC_R and PyC) were greatest near the surface and declined with soil depth. Although subsoils have relatively low C concentrations, they account for the majority of soil C stocks when the total depth and density of soil are considered (Rumpel and Kogel-Knabner, 2011).

4.3. Residence time of soil C

C turnover was more rapid at shallow depths and C that remained at deeper depths was slower cycling and more resistant to oxidation. It is widely observed that the MRT of soil C typically increases with soil depth, though the specific mechanisms of C persistence may be complex and difficult to identify (Rumpel and Kogel-Knabner, 2011). Long-term persistence may result from the formation of highly stabilized C compounds via biochemical processes or association with clay minerals and Fe (Scharpenseel and Becker-Heidmann, 1992; Eusterhues et al., 2003, 2005; Dick et al., 2005; Rumpel et al., 2008). It is also possible that microbes in deep soils function in a relatively closed system, where old C is recycled and reincorporated until disturbance allows entry of new, younger labile C forms (Gleixner et al., 2002). Additionally, persistent SOC fractions have been shown to be highly hydrophobic (von Lutzow et al., 2006), a property which would limit enzymatic/microbial access.

In the present study, SOC, SOC_{OX}, and SOC_R MRTs ranged from hundreds of years in the near surface to several thousand years at depths of 1 m. The efficiency of oxidation declined with depth, and MRT values were similar to those reported by Eusterhues et al. (2005). Isolating the contribution of the mechanisms that control soil C persistence is confounded by interactions with soil depth, though we were able to identify associations between bulk density, clay content and SOC_R ratio with MRT. Once C is stabilized into a clay complex it is not readily exchanged with younger organic matter moving through the soil profile (Scharpenseel and Becker-Heidmann, 1992). Maximum ^{14}C MRT of the soil profile and clay content was compared to values reported for six Alfisols by Scharpenseel and Becker-Heidmann (1992) (Fig. 6). The slopes of the regression lines were similar, though the Y-intercept of the Ultisols in the present study was more than double that of Alfisols (Fig. 6). In general, Ultisols are highly acidic and have reduced cation exchange capacity compared to well-buffered Alfisols, which have greater plant available nutrients and productivity that may affect C turnover. Values of SOC_R MRT were similar to those reported by Eusterhues et al. (2005), with the exception of the SP sites which were 3–4 times longer than either study. Based on clay content and extractable Fe, there does not appear to be a difference between sites commensurate to very long SOC_R MRT's at the SP sites. Differences in clay minerology between MLRAs could contribute to the enhanced SOC_R residence time observed at SP, though our study did not examine minerology, nor was it designed to capture the diversity of clay minerology across the range of longleaf pine. There is also the possibility that geogenic C, which is >50,000 years old and has no ^{14}C activity, was present in small quantities and affected the MRT (Helfrich et al., 2007).

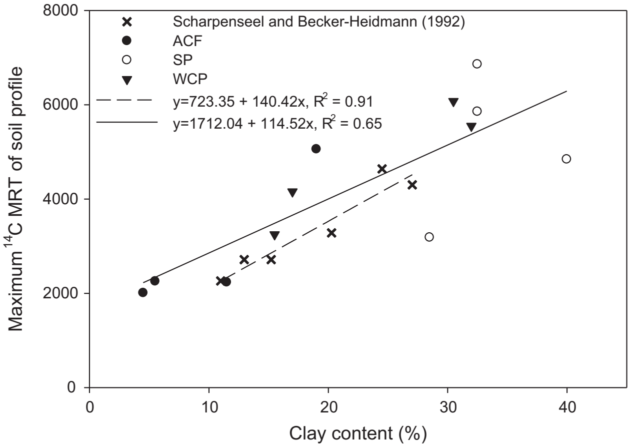

Fig. 6. Relationship between maximum ^{14}C MRT and clay content observed in the present study (solid line) from sites in the Atlantic Coast Flatwoods (ACF), Southern Piedmont (SP) and Western Coastal Plain (WCP) and six Alfisol profiles presented by Scharpenseel and Becker-Heidmann (1992) (dashed line).

4.4. Role of PyC

PyC can play important roles in soil C sequestration and stabilization, as well as in enhancing fertility by improving nutrient retention and cation exchange capacity in soil (Glaser et al., 2001; Liang et al., 2006). Considering the importance of frequent low intensity fires in longleaf pine ecosystems, we expected PyC to contribute to long-term sequestration, however the results did not support this hypothesis. PyC represented 5–7% of SOC and the majority of PyC was in the least persistent SOC_{OX} fraction with residence times one tenth that of SOC_R. Possible explanations for this observation include rapid degradation of PyC under environmental conditions and offsite transport of PyC. We used a conservative multiplier (2.27) to account for the loss of PyC during the formation of BPCAs (Glaser et al., 1998). A recent study by Cotrufo et al. (2016a) reports a correction factor of approximately 4 for mineral soil from 0 to 5 and 5 to 15 cm depths, based on comparisons of BPCA, hydrogen pyrolysis and mid infrared spectroscopy. As soils in the present study were from warm, humid environments conducive to oxidation and collected from depths to 1 m where remaining PyC may exhibit increases in aromatic condensation, we chose to present the conservative estimates.

While pyrogenic transformation of C can lead to biochemical stability for millennia, PyC is not immune to decomposition. A synthesis by Singh et al. (2012) concluded that PyC MRT ranged on the order of centuries, similar to the MRT of slow cycling PyOM (500–600 years) described by Knicker et al. (2013) and the MRT of SOC_{OX} in the present study. Hammes et al. (2008) demonstrated after the cessation of grassland fires in a Russia Steppe in 1900, the stocks of

PyC in soil declined by 25% over the next century and the turnover time was 293 years. In a subsequent study of the forest fire southern Spain reported by Knicker et al. (2013), repeated forest fire events had little impact on soil C contents and physical properties, though significant, albeit small, increases in aromaticity were observed (Lopez-Martin et al., 2016). Based on the loss of charcoal content over time, Lopez-Martin et al. (2016) concluded that the impact of the fires on SOM is limited to the decadal time scale. Several studies have documented factors affecting PyC decomposition rates under laboratory and field conditions e.g. Cheng et al. (2006), Liang et al. (2008), Nguyen and Lehmann (2009), Zimmerman (2010), Foereid et al. (2011). The potential recalcitrance of PyC that does remain on site in forest soil is highly dependent on feedstock, temperature and aeration during formation, environmental conditions favoring decomposition, binding with soil minerals as well as physical protection after formation, i.e. charcoal left on the surface is susceptible to re-ignition in subsequent fires or erosion. In general, low temperature combustion yields PyC with less aromatic condensation e.g. Soucemarianadin et al. (2013) and Schneider et al. (2010), that is more susceptible to decomposition than PyC formed at higher combustion temperatures (Nguyen and Lehmann, 2009; Zimmerman, 2010). Soucemarianadin et al. (2015) found that early-season fires in boreal black spruce forests produce PyC with low intrinsic recalcitrance and this phenomenon may be occurring in longleaf pine forests. The majority of fires in longleaf pine stands are not stand replacing events and result in very different combustion conditions and pyrogenic products than wildfires in the western United States (Campbell et al., 2007).

Most newly formed PyC is concentrated in the lightest fraction on the soil surface and is susceptible to rapid offsite transport via surface flow and leaching (Velasco-Molina et al., 2016) following precipitation unless it mixed with mineral soil (Cotrufo et al., 2016b). The material may be biochemically stable and sequestered from the atmosphere, but much of it may be removed and ultimately deposited in marine systems instead of the point of production in forests (Masiello and Druffel, 1998; Jaffe et al., 2013; Bird et al., 2015). In the longleaf pine stands sampled, there was little mixing of PyC with mineral soil and when combined with 1000–1500 mm annual precipitation typical of the region, PyC would receive minimal protection. Decomposition and offsite transport are both reasonable mechanisms to explain why less PyC is retained in forest soils than would be expected from PyC production estimates. Little to no research has been conducted to distill the contribution of the two. To better assess the contribution of each mechanism, new research where high frequency sampling of soil surfaces, soil, surface flow and ground water is required.

4.5. Edaphic factors associated with SOC stocks

While depth explained most of the variation in each C fraction, clay and extractable Fe were positively associated with C retention. Mehlich-3 extractable Fe (M-3) comprised the collective pool of exchangeable Fe (from cation exchange sites) as well as Fe readily soluble at low pH. This easily soluble pool is most likely sourced from poorly crystalline Fe hydroxide phases as evidenced by weak correlations of M-3 extractable Fe with oxalate extractable Fe (Marcos et al., 1998). Together clay and Fe (M-3) give some measure of potential mineral association related to C stabilization. Poorly crystalline Fe hydroxides have been specifically linked to the retention of microbial sugars in subsoil horizons (Schoening et al., 2005; Spielvogel et al., 2008). Fe and clay stabilize C via mineral association in forms that may persist for thousands of years (Scharpenseel and Becker-Heidmann, 1992; Eusterhues et al., 2005). C is moving from biomass pools to soil, but the capacity to stabilize and retain that C is limited. Richter et al. (1999) described a similar situation in a loblolly pine plantation at the Calhoun Experimental Forest where C retention in soil was not source limited, but rather storage limited in coarse soils with little absorptive capacity for C.

5. Conclusions

Total soil C stocks (0–1 m) in longleaf pine stands ranged from 44.1 to 98.1 ($\bar{x}$ = 77.0) Mg C ha^{-1}, and variation in soil C concentration was largely dependent on depth, though positive association with clay and extractable Fe content were found. PyC was a minor component of bulk SOC, comprising 5–7% of the total SOC stock. The majority of PyC was in the oxidizable fraction, which was found to have an estimated MRT almost a magnitude lower than the resistant fraction. This indicates that PyC is not contributing substantially to long-term C accumulation in soil within the forest stand where it was produced. There was no enhanced accumulation or deposits of PyC with depth (to 1 m), PyC declined in proportion with SOC, conversely the proportion of SOC$_R$ in SOC increased with depth. The estimated MRT of SOC in surface soils was hundreds of years, and at depths >50 cm estimated MRT increased to thousands of years. Despite the flow of C from biomass in the form of decay products, litter fall, root turnover and pulses of PyC, soils retained little of the new C, which may be rapidly oxidized, lost to the atmosphere from periodic fires or in the case of PyC may be transported out of the system. Our results indicate that soils are not strong sinks for atmospheric CO$_2$ compared to C accumulation in biomass in longleaf pine forests.

Acknowledgements

This research was supported wholly (or in part) by the U.S. Department of Defense through the Strategic Environmental Research and Development Program (SERDP). The authors wish to thank Marianne Farris, Jake Blackstock, Justin Rathel, Michael Ramirez, and John Lewis for assistance in sample collection. We would also like to thank James Parker and Brian Waldrep from Fort Benning, Land Management Division, Lisa Lewis, Lyn McDonald, Barbara Bell and Kathryn Duncan from the USDA Forest Service, Kisatchie National Forest Calcasieu Ranger District, Susan Cohen and Austin Powell from Camp Lejeune, Forest Management Program for their assistance. The Radiocarbon Collaborative, a joint program of US Forest Service, NRS, the Center for Accelerator Mass Spectrometry at Lawrence Livermore National Lab (CAMS, LLNL), and Michigan Technological University (MTU) supported radiocarbon analysis in part. We appreciate the technical support provided by Joel Burley, Thomas Christensen, Robert Eaton, Shelly Hooke, Karen Sarsony and Rodney Simpson.

References

Ascough, P.L., Bird, M.I., Brock, F., Higham, T.F.G., Meredith, W., Snape, C.E., Vane, C.H., 2009. Hydropyrolysis as a new tool for radiocarbon pre-treatment and the quantification of black carbon. Quat. Geochronol. 4, 140–147.

Ascough, P.L., Bird, M.I., Francis, S.M., Thornton, B., Midwood, A.J., Scott, A.C., Apperley, D., 2011. Variability in oxidative degradation of charcoal: influence of production conditions and environmental exposure. Geochim. Cosmochim. Acta 75, 2361–2378.

Ball, P.N., MacKenzie, M.D., DeLuca, T.H., Holben, W.E., 2010. Wildfire and charcoal enhance nitrification and ammonium-oxidizing bacterial abundance in dry montane forest soils. J. Environ. Qual. 39, 1243–1253.

Batjes, N.H., 1996. Total carbon and nitrogen in the soils of the world. Eur. J. Soil Sci. 47, 151–163.

Bird, M.I., Wynn, J.G., Saiz, G., Wurster, C.M., McBeath, A., 2015. The Pyrogenic Carbon Cycle. In: Jeanloz, R., Freeman, K.H. (Eds.), Annual Review of Earth and Planetary Sciences, vol. 43, pp. 273–298.

Boot, C.M., Haddix, M., Paustian, K., Cotrufo, M.F., 2015. Distribution of black carbon in ponderosa pine forest floor and soils following the High Park wildfire. Biogeosciences 12, 3029–3039.

Bouyoucos, G., 1962. Hydrometer method improved for making particle size analyses of soil. Agro. J. 54, 464–465.

Brodowski, S., Rodionov, A., Haumaier, L., Glaser, B., Amelung, W., 2005. Revised black carbon assessment using benzene polycarboxylic acids. Org. Geochem. 36, 1299–1310.

Campbell, J., Donato, D.C., Azuma, D., Law, B., 2007. Pyrogenic carbon emission from a large wildfire in Oregon, United States. J. Geophys. Res.-Biogeosci. 112, 11.

Cheng, C.-H., Lehmann, J., Thies, J.E., Burton, S.D., Engelhard, M.H., 2006. Oxidation of black carbon by biotic and abiotic processes. Org. Geochem. 37, 1477–1488.

Churchill, D.J., Larson, A.J., Dahlgreen, M.C., Franklin, J.F., Hessburg, P.F., Lutz, J.A., 2013. Restoring forest resilience: from reference spatial patterns to silvicultural prescriptions and monitoring. Forest Ecol. Manag. 291, 442–457.

Cotrufo, M.F., Boot, C., Abiven, S., Foster, E.J., Haddix, M., Reisser, M., Wurster, C.M., Bird, M.I., Schmidt, M.W.I., 2016a. Quantification of pyrogenic carbon in the environment: an integration of analytical approaches. Org. Geochem. 100, 42–50.

Cotrufo, M.F., Boot, C., Kampf, S., Nelson, P., Brogan, D.J., Covino, T., Haddix, M.L., MacDonald, L., Rathburn, S., Ryan-Bukett, S., Schmeer, S., Hall, E., 2016b. Redistribution of pyrogenic carbon from hillslopes to stream corridors following a large montane wildfire. Glob. Biogeochem. Cycles 30, 1348–1355.

Davis, J.C., Proctor, I.D., Southon, J.R., Caffee, M.W., Heikkinen, D.W., Roberts, M.L., Moore, T.L., Turteltaub, K.W., Nelson, D.E., Loyd, D.H., Vogel, J.S., 1990. LLNL/US AMS facility and research program. Nucl. Instrum. Meth. B 52, 269–272.

Dick, D.P., Goncalves, C.N., Dalmolin, R.S.D., Knicker, H., Klamt, E., Kogel-Knaber, I., Simoes, M.L., Martin-Neto, L., 2005. Characteristics of soil organic matter of different Brazilian Ferralsols under native vegetation as a function of soil depth. Geoderma 124, 319–333.

Eusterhues, K., Rumpel, C., Kleber, M., Kogel-Knabner, I., 2003. Stabilisation of soil organic matter by interactions with minerals as revealed by mineral dissolution and oxidative degradation. Org. Geochem. 34, 1591–1600.

Eusterhues, K., Rumpel, C., Kogel-Knabner, I., 2005. Stabilization of soil organic matter isolated via oxidative degradation. Org. Geochem. 36, 1567–1575.

Foereid, B., Lehmann, J., Major, J., 2011. Modeling black carbon degradation and movement in soil. Plant Soil 345, 223–236.

Frost, C., 1993. Four centuries of changing landscape patterns in the longleaf pine ecosystem. Proc. Tall Timbers Fire Ecol. Conf. 18, 17–44.

Frost, C., 2006. History and Future of the Longleaf Pine Ecosystem. In: Jose, S., Jokela, E., Miller, D. (Eds.), The Longleaf Pine Ecosystem. Springer, New York, pp. 9–48.

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

Glaser, B., Haumaier, L., Guggenberger, G., Zech, W., 2001. The 'Terra Preta' phenomenon: a model for sustainable agriculture in the humid tropics. Naturwissenschaften 88, 37–41.

Gleixner, G., Poirier, N., Bol, R., Balesdent, J., 2002. Molecular dynamics of organic matter in a cultivated soil. Org. Geochem. 33, 357–366.

Hammes, K., Torn, M.S., Lapenas, A.G., Schmidt, M.W.I., 2008. Centennial black carbon turnover observed in a Russian steppe soil. Biogeosciences 5, 1339–1350.

Helfrich, M., Flessa, H., Mikutta, R., Dreves, A., Ludwig, B., 2007. Comparison of chemical fractionation methods for isolating stable soil organic carbon pools. Eur. J. Soil Sci. 58, 1316–1329.

Inoue, J., Inoue, Y., 2009. Comparison of the reflectances of black plant fragments in melanic Andisols with those of fresh charcoal from modern fires. Soil Sci. Plant Nutr. 55, 358–362.

Jaffe, R., Ding, Y., Niggemann, J., Vahatalo, A.V., Stubbins, A., Spencer, R.G.M., Campbell, J., Dittmar, T., 2013. Global charcoal mobilization from soils via dissolution and riverine transport to the oceans. Science 340, 345–347.

Johnsen, K.H., Samuelson, L.J., Sanchez, F.G., Eaton, R.J., 2013. Soil carbon and nitrogen content and stabilization in mid-rotation, intensively managed sweetgum and loblolly pine stands. Forest Ecol. Manag. 302, 144–153.

Johnsen, K.H., Wear, D., Oren, R., Teskey, R.O., Sanchez, F., Will, R., Butnor, J., Markewitz, D., Richter, D., Rials, T., Allen, H.L., Seiler, J., Ellsworth, D., Maier, C., Katul, G., Dougherty, P.M., 2001. Carbon sequestration and southern pine forests. J. Forest. 99, 14–21.

Johnson, D.W., Knoepp, J.D., Swank, W.T., Shan, J., Morris, L.A., Van Lear, D.H., Kapeluck, P.R., 2002. Effects of forest management on soil carbon: results of some long-term resampling studies. Environ. Pollut. 116, S201–S208.

Johnson, D.W., Todd, D.E., Tolbert, V.R., 2003. Changes in ecosystem carbon and nitrogen in a loblolly pine plantation over the first 18 years. Soil Sci. Soc. Am. J. 67, 1594–1601.

Knicker, H., Gonzalez-Vila, F.J., Gonzalez-Vazquez, R., 2013. Biodegradability of organic matter in fire-affected mineral soils of Southern Spain. Soil Biol. Biochem. 56, 31–39.

Kurth, V.J., MacKenzie, M.D., DeLuca, T.H., 2006. Estimating charcoal content in forest mineral soils. Geoderma 137, 135–139.

Law, B., Arkebauer, T., Campbell, J.L., Chen, J., Sun, O., Shwartz, M., van Ingen, C., Verma, S., 2008. Terrestrial carbon observations: protocols for vegetation sampling and data submission. Terrestrial Carbon Observations (TCO) Panel of the Global Terrestrial Observing System (GTOS). FAO, Rome.

Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'Neill, B., Skjemstad, J. O., Thies, J., Luizao, F.J., Petersen, J., Neves, E.G., 2006. Black Carbon increases cation exchange capacity in soils. Soil Sci. Soc. Am. J. 70, 1719–1730.

Liang, B., Lehmann, J., Solomon, D., Sohi, S., Thies, J.E., Skjemstad, J.O., Luizao, F.J., Engelhard, M.H., Neves, E.G., Wirick, S., 2008. Stability of biomass-derived black carbon in soils. Geochim. Cosmochim. Acta 72, 6069–6078.

Lopez-Martin, M., Velasco-Molina, M., Knicker, H., 2016. Variability of the quality and quantity of organic matter in soil affected by multiple wildfires. J. Soils Sediments 16, 360–370.

Marcos, M.L.F., Alvarez, E., Monterroso, C., 1998. Aluminum and iron estimated by Mehlich-3 extractant in mine soils in Galicia, northwest Spain. Commun. Soil Sci. Plant Anal. 29, 599–612.

Markewitz, D., Sartori, F., Craft, C., 2002. Soil change and carbon storage in longleaf pine stands planted on marginal agricultural lands. Ecol. Appl. 12, 1276–1285.

Masiello, C.A., 2004. New directions in black carbon organic geochemistry. Mar. Chem. 92, 201–213.

Masiello, C.A., Druffel, E.R.M., 1998. Black carbon in deep-sea sediments. Science 280, 1911–1913.

Mehlich, A., 1984. Mehlich-3 soil test extractant - a modification of the Mehlich-2 extractant. Commun. Soil Sci. Plant Anal. 15, 1409–1416.

Nguyen, B.T., Lehmann, J., 2009. Black carbon decomposition under varying water regimes. Org. Geochem. 40, 846–853.

NRCS, 2006. Land Resource Regions and Major Land Resource Areas (MRLAs) of the United States, the Caribbean, and the Pacific Basin. U.S. Department of Agriculture Handbook 296. USDA Natural Resources Conservation Service.

Oades, J.M., 1988. The retention of organic-matter in soils. Biogeochemistry 5, 35–70.

Preston, C.M., Schmidt, M.W.I., 2006. Black (pyrogenic) carbon: a synthesis of current knowledge and uncertainties with special consideration of boreal regions. Biogeosciences 3, 397–420.

Richter, D.D., Hofmockel, M., Callaham, M.A., Powlson, D.S., Smith, P., 2007. Long-term soil experiments: keys to managing Earth's rapidly changing ecosystems. Soil Sci. Soc. Am. J. 71, 266–279.

Richter, D.D., Markewitz, D., Trumbore, S.E., Wells, C.G., 1999. Rapid accumulation and turnover of soil carbon in a re-establishing forest. Nature 400, 56–58.

Rumpel, C., Chaplot, V., Chabbi, A., Largeau, C., Valentin, C., 2008. Stabilisation of HF soluble and HCL resistant organic matter in sloping tropical soils under slash and burn agriculture. Geoderma 145, 347–354.

Rumpel, C., Kogel-Knabner, I., 2011. Deep soil organic matter-a key but poorly understood component of terrestrial C cycle. Plant Soil 338, 143–158.

Samuelson, L.J., Stokes, T., Butnor, J.R., Johnsen, K., Gonzalez-Benecke, C.A., Martin, T. A., Cropper, W.P., Anderson, P.H., Ramirez, M.R., Lewis, J.C., 2017. Ecosystem carbon density and allocation across a chronosequence of longleaf pine forests. Ecol. Appl. 27, 244–259.

Samuelson, L.J., Stokes, T.A., Butnor, J.R., Johnsen, K.H., Gonzalez-Benecke, C.A., Anderson, P., Jackson, J., Ferrari, L., Martin, T.A., Cropper, W.P., 2014. Ecosystem carbon stocks in *Pinus palustris* forests. Can. J. Forest Res. 44, 476–486.

Santin, C., Doerr, S.H., Kane, E.S., Masiello, C.A., Ohlson, M., de la Rosa, J.M., Preston, C.M., Dittmar, T., 2016. Towards a global assessment of pyrogenic carbon from vegetation fires. Glob. Change Biol. 22, 76–91.

SAS Institute Inc., 2013. SAS/STAT 13.1 User's Guide. SAS Institute Inc., Cary, NC.

Scharpenseel, H.W., Becker-Heidmann, P., 1992. 25 Years of radiocarbon dating soils - paradigm of erring and learning. Radiocarbon 34, 541–549.

Schmidt, M.W.I., Noack, A.G., 2000. Black carbon in soils and sediments: analysis, distribution, implications, and current challenges. Global Biogeochem. Cycles 14, 777–793.

Schmidtling, R.C., Hipkins, V., 1998. Genetic diversity in longleaf pine (Pinus palustris): influence of historical and prehistorical events. Can. J. Forest Res. 28, 1135–1145.

Schneider, M.P.W., Hilf, M., Vogt, U.F., Schmidt, M.W.I., 2010. The benzene polycarboxylic acid (BPCA) pattern of wood pyrolyzed between 200 degrees C and 1000 degrees C. Org. Geochem. 41, 1082–1088.

Schoening, I., Knicker, H., Kogel-Knabner, I., 2005. Intimate association between O/N-alkyl carbon and iron oxides in clay fractions of forest soils. Org. Geochem. 36, 1378–1390.

Singh, N., Abiven, S., Torn, M.S., Schmidt, M.W.I., 2012. Fire-derived organic carbon in soil turns over on a centennial scale. Biogeosciences 9, 2847–2857.

Snowdon, P., 1991. A ratio estimator for bias correction in logarithmic regressions. Can. J. Forest Res. 21, 720–724.

Soil Survey Staff, 2000. Soil Inorganic Carbon Map. Natural Resources Conservation Service, United States Department of Agriculture, Washington, DC.

Soucemarianadin, L.N., Quideau, S.A., MacKenzie, M.D., Bernard, G.M., Wasylishen, R.E., 2013. Laboratory charring conditions affect black carbon properties: a case study from Quebec black spruce forests. Org. Geochem. 62, 46–55.

Soucemarianadin, L.N., Quideau, S.A., Wasylishen, R.E., Munson, A.D., 2015. Early-season fires in boreal black spruce forests produce pyrogenic carbon with low intrinsic recalcitrance. Ecology 96, 1575–1585.

Spielvogel, S., Prietzel, J., Kogel-Knabner, I., 2008. Soil organic matter stabilization in acidic forest soils is preferential and soil type-specific. Eur. J. Soil Sci. 59, 674–692.

Spokas, K.A., 2010. Review of the stability of biochar in soils: predictability of O:C molar ratios. Carbon Manage. 1, 289–303.

Stambaugh, M.C., Guyette, R.P., Marschall, J.M., 2011. Longleaf pine (*Pinus palustris* Mill.) fire scars reveal new details of a frequent fire regime. J. Veg. Sci. 22, 1094–1104.

Stuiver, M., Polach, H.A., 1977. Reporting of C-14 data - discussion. Radiocarbon 19, 355–363.

Torn, M.S., Swanston, C.W., Castanha, C., Trumbore, S., 2009. Storage and Turnover of Organic Matter in Soil. In: Huang, P.M., Senesi, N. (Eds.), Biophysico-Chemical Processes Involving Natural Nonliving Organic Matter in Environmental Systems. Wiley, Hoboken, pp. 219–272.

Trumbore, S.E., 1993. Comparison of Carbon dynamics in tropical and temperature soils using radiocarbon measurements. Global Biogeochem. Cycles 7, 275–290.

Velasco-Molina, M., Berns, A.E., Macias, F., Knicker, H., 2016. Biochemically altered charcoal residues as an important source of soil organic matter in subsoils of fire-affected subtropical regions. Geoderma 262, 62–70.

Vogel, J.S., Southon, J.R., Nelson, D.E., 1987. Catalyst and binder effects in the use of filamentous graphite for AMS. Nucl. Instrum. Methods Phys. Res. Section B-Beam Interact. Mater. Atoms 29, 50–56.

von Lutzow, M., Kogel-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. Eur. J. Soil Sci. 57, 426–445.

Wiedemeier, D.B., Abiven, S., Hockaday, W.C., Keiluweit, M., Kleber, M., Masiello, C. A., McBeath, A.V., Nico, P.S., Pyle, L.A., Schneider, M.P.W., Smernik, R.J., Wiesenberg, G.L.B., Schmidt, M.W.I., 2015. Aromaticity and degree of aromatic condensation of char. Org. Geochem. 78, 135–143.

Wiedemeier, D.B., Hilf, M.D., Smittenberg, R.H., Haberle, S.G., Schmidt, M.W.I., 2013. Improved assessment of pyrogenic carbon quantity and quality in environmental samples by high-performance liquid chromatography. J. Chromatogr. A 1304, 246–250.

Wiedemeier, D.B., Lang, S.Q., Gierga, M., Abiven, S., Bernasconi, S.M., Fruh-Green, G. L., Hajdas, I., Hanke, U.M., Hilf, M.D., McIntyre, C.P., Scheider, M.P., Smittenberg, R.H., Wacker, L., Wiesenberg, G.L., Schmidt, M.W., 2016. Characterization, Quantification and Compound-specific Isotopic Analysis of Pyrogenic Carbon Using Benzene Polycarboxylic Acids (BPCA). J. Vis. Exp.

Wiesmeier, M., Schad, P., von Lutzow, M., Poeplau, C., Sporlein, P., Geuss, U., Hangen, E., Reischl, A., Schilling, B., Kogel-Knabner, I., 2014. Quantification of functional soil organic carbon pools for major soil units and land uses in southeast Germany (Bavaria). Agri. Ecosyst. Environ. 185, 208–220.

Zimmerman, A.R., 2010. Abiotic and Microbial Oxidation of Laboratory-Produced Black Carbon (Biochar). Environ. Sci. Technol. 44, 1295–1301.

Zimmermann, M., Leifeld, J., Schmidt, M.W.I., Smith, P., Fuhrer, J., 2007. Measured soil organic matter fractions can be related to pools in the RothC model. Eur. J. Soil Sci. 58, 658–667.