



Factors affecting the molecular structure and mean residence time of occluded organics in a lithosequence of soils under ponderosa pine



Katherine Heckman ^{a,*,1}, Heather Throckmorton ^{b,2}, Christopher Clingensmith ^{a,1},
Francisco Javier González Vila ^{c,3}, William R. Horwath ^{b,2}, Heike Knicker ^{c,3},
Craig Rasmussen ^{a,1}

^a Dept. of Soil, Water & Environmental Science, University of Arizona, PO Box 210038, Tucson, AZ 85721-0038, USA

^b Dept. Land, Air and Water Resources, University of California, Davis, One Shields Ave, Davis, CA 95616, USA

^c Instituto de Recursos Naturales y Agrobiología de Sevilla, Consejo Superior de Investigaciones Científicas, Avenida Reina Mercedes 10, 41080 Seville, Spain

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ABSTRACT

Occluded, or intra-aggregate, soil organic matter (SOM) comprises a significant portion of the total C pool in forest soils and often has very long mean residence times (MRTs). However, occluded C characteristics vary widely among soils and the genesis and composition of the occluded organic matter pool are not well understood. This work sought to define the major controls on the composition and MRT of occluded SOM in western U.S. conifer forest soils with specific focus on the influence of soil mineral assemblage and aggregate stability. We sampled soils from a lithosequence of four parent materials (rhyolite, granite, basalt, and dolostone) under *Pinus ponderosa*. Three pedons were excavated to the depth of refusal at each site and sampled by genetic horizon. After density separation at 1.8 g cm⁻³ into free/light, occluded and mineral fractions, the chemical nature and mean residence time of organics in each fraction were compared. SOM chemistry was explored through the use of stable isotope analyses, ¹³C NMR, and pyrolysis GC/MS. Soil charcoal content estimates were based on ¹³C NMR analyses. Estimates of SOM MRT were based on steady-state modeling of SOM radiocarbon abundance measurements. Across all soils, the occluded fraction was 0.5–5 times enriched in charcoal in comparison to the bulk soil and had a substantially longer MRT than either the mineral fraction or the free/light fraction. These results suggest that charcoal from periodic burning is the primary source of occluded organics in these soils, and that the structural properties of charcoal promote its aggregation and long-term preservation. Surprisingly, aggregate stability, as measured through ultrasonic dispersion, was not correlated with occluded SOM abundance or MRT, perhaps raising questions of how well laboratory measurements of aggregate stability capture the dynamics of aggregate turnover under field conditions. Examination of the molecular characteristics of the occluded fraction was more conclusive. Occluded fraction composition did not change substantially with soil mineral assemblage, but was increasingly enriched in charcoal with depth relative to bulk SOM. Enrichment levels of ¹³C and ¹⁵N suggested a similar degree of microbial processing for the free/light and occluded fractions, and molecular structure of occluded and free/light fractions were also similar aside from charcoal enrichment in the occluded fraction. Results highlight the importance of both fire and aggregate formation to the long-term preservation of organics in western U.S. conifer forests which experience periodic burning, and suggest that the composition of occluded SOM in these soils is dependent on fire and the selective occlusion of charcoal.

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* Corresponding author. Current address: USDA Forest Service, Northern Research Station, Center for Accelerator Mass Spectrometry (CAMS), Lawrence Livermore National Lab (LLNL), L-397, 7000 East Ave, Livermore, CA 94550, USA. Tel.: +1 925 422 9556; fax: +1 925 423 7884.

E-mail addresses: kaheckman@fs.fed.us, kheckman14c@gmail.com (K. Heckman), hthrockmorton@lanl.gov (H. Throckmorton), c.clingensmith@ufl@gmail.com (C. Clingensmith), fjgon@irnase.csic.es (F.J. González Vila), wrhorwath@usdavis.edu (W.R. Horwath), knicker@irnase.csic.es (H. Knicker), crasmuss@cals.arizona.edu (C. Rasmussen).

¹ Tel.: +1 520 621 1646; fax: +1 520 621 1647.

² Tel.: +1 754 6185; fax: +1 752 1552.

³ Tel.: +34 95 462 4711; fax: +34 95 462 4002.

1. Introduction

Temperate forest soils contain a substantial portion of the global terrestrial C pool, and gaining the ability to accurately model forest soil C dynamics is important for the improvement of both regional and global C cycle models. The soil organic C cycle has been the subject of intense study in recent decades (e.g. Kögel-Knabner and Kleber, 2011), but considerable knowledge gaps remain. One such knowledge gap is the identification of the mechanisms and/or characteristics of occluded organic matter which lead to its persistence in soils. Here we specifically address this knowledge gap through quantifying the physical partitioning, chemistry, and residence time of soil C in a range of temperate conifer forests in the Southwest U.S.

The SOM pool encompasses a high degree of heterogeneity in both molecular structure and mean residence time (MRT). Particulate organics found within the mineral soil matrix, but outside aggregate structures, strongly resemble local plant inputs, and tend to have a comparably short MRT. Organic compounds intimately associated with mineral particles are often compositionally more similar to microbial necromass than plant inputs, and usually have comparably longer MRTs due to chemical protection against biodegradation. Occluded SOM, or aggregate-protected particulate organic matter, is assumed to be physically protected from biodegradation through association with soil minerals, but occluded organics vary widely in both composition and MRT (von Lützow et al., 2006, 2007; Kögel-Knabner and Kleber, 2011).

Isolation of occluded organics from mineral-associated (mineral/heavy fraction) and particulate (free/light) organics is usually achieved through density separation in combination with aggregate disruption (Golchin et al., 1994; Sohi et al., 2001; Swanston et al., 2003). Density separation allows for the separation of organics adhered to mineral surfaces (mineral fraction) from organics unassociated with the mineral component (free/light fraction). The incorporation of aggregate disruption into density separation methods (achieved through sonication or crushing) allows for the isolation of the occluded fraction of SOM, or SOM protected through occlusion within aggregate structures and/or coating with mineral grains. Though these methods have their limitations (Crow et al., 2007), they have been shown to effectively isolate unique pools of SOM with distinct differences in MRT (e.g., Rasmussen et al., 2005).

The occluded fraction comprises a significant portion of the total organic matter pool in forest soils, with some estimates as high as 40–50% of total soil C (Rasmussen et al., 2005; Wagai et al., 2008). However, the genesis and composition of occluded organics are not well understood. In a summary of contemporary investigations, Wagai et al. (2009) noted several trends in occluded SOM characteristics. Occluded organics typically have a smaller particle size than organics in the free/light fraction, ranging from 10 to 100 μm . Occluded organics generally lack recognizable features of plant fragments, and the occluded fraction tends to accumulate structurally recalcitrant compounds such as pollen, fungal spores and charred materials. Wagai et al. (2009) reported C:N ratios of occluded organics ranging from 13 to 88, and MRTs of ~50 to >1000 years.

This heterogeneity in composition and MRT of occluded C across soil and ecosystem types creates difficulty in developing a consistent understanding and prediction of occluded C dynamics. Models which partition organics according to structural properties can't account for the presence of a wide variety of compounds within a single functional pool, whereas models which regard SOM pools as homogenous black boxes lack the mechanistic parameters necessary for linking shifts in environmental parameters with shifts in SOM MRT (Derrien and Amelung, 2011; Kuzyakov, 2011; Manzoni

and Porporato, 2009). Thus occluded organics, though functionally very different than other pools of SOM, are not represented accurately in current modeling efforts.

In order to gain a better understanding of the mechanisms regulating occluded SOM genesis and turnover in forest soils, we density separated soils from a lithosequence under *Pinus ponderosa*. The use of a lithosequence under a single vegetation type was used to examine the specific influence of mineral assemblage on occluded fraction characteristics while constraining other highly influential soil forming factors such as climate and plant inputs. Four parent materials were examined (dolostone, basalt, granite, and rhyolite) that encompass a wide range of soil texture, mineral assemblage, and acidity (Heckman and Rasmussen, 2011). Samples from each genetic horizon were density separated, and isolated fractions were examined using a combination of ^{13}C , ^{15}N , total organic C and N, ^{14}C abundance, pyrolysis gas chromatography/mass spectrometry (GC/MS), and cross polarization magic angle spinning (CPMAS) ^{13}C nuclear magnetic resonance (NMR) spectrometry. We sought to define the major controls on the composition and MRT of occluded SOM with specific focus on the following questions: i) How is the occluded fraction distinguishable from organics associated with mineral surfaces (mineral fraction) and organics neither associated with mineral surfaces nor occluded within aggregates (free/light fraction)? ii) What is the influence of soil aggregate stability on occluded SOM composition and MRT? and iii) How does the occluded fraction vary in MRT and composition both with changes in depth and among soils of differing mineral assemblage? We hypothesized that mineral assemblage would exert significant control over occluded SOM composition and MRT indirectly through its influence on aggregate stability and turnover rates. We also hypothesized that occluded SOM composition and MRT would shift significantly with depth in the profile, following variation in the composition and lability of plant inputs.

2. Methods

2.1. Sample sites & soil physiochemical properties

Sample site characteristics and soil mineral assemblages are discussed in detail in Heckman and Rasmussen (2011). Briefly, three replicate soil pedons were sampled in Arizona, USA, from a lithosequence comprising four different parent materials: rhyolitic ash, granite, basalt, and dolostone. Soils from the dolostone site were developed from dual parent materials: the underlying dolomitic dolostone bedrock and mafic ash deposited during the formation of nearby cinder cones. Climate and vegetation characteristics were nearly equivalent among sites, so that differences in soil and organic matter characteristics can reasonably be attributed to differences in parent material characteristics. All soils were sampled from north-facing backslope positions with *P. ponderosa* as the dominant canopy species. Mean annual temperature ranged from 9 to 12 $^{\circ}\text{C}$. Mean annual precipitation ranged from 675 to 815 mm yr^{-1} . Previously measured soil characteristics including soil texture, specific surface area, quantitative mineral composition, pH, EC, and exchangeable cations were used in correlation analyses in the current study. These mineral and physiochemical characteristics and methods are given in Heckman and Rasmussen (2011).

2.2. Density separation, elemental & isotope analysis

Soils from each horizon of all three replicate pedons of each parent material were density separated at 1.8 g cm^{-3} , following the methods of Rasmussen et al. (2005). Briefly, the free/light fraction was floated off after mixing 30 g bulk soil with 150 cm^{-3} of a sodium polytungstate solution adjusted to a density of 1.8 g cm^{-3} .

Soils were then subjected to sonication at a rate of 1500 J g⁻¹ soil in order to disrupt aggregates and liberate occluded organics. Following sonication, the occluded fraction was floated off. Losses of mass, C, and N during density separation are given in [Supplementary Table 1](#).

Samples were analyzed for %C, %N, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ by dry combustion at 1000 °C with an elemental analyzer (Costech Analytical Technologies, Valencia, CA, USA) coupled to a continuous-flow mass spectrometer (Finnigan Delta PlusXL, San Jose, CA, USA) at the University of Arizona Stable Isotope Laboratory.

Radiocarbon abundance of the density/aggregate fractions and bulk soils was measured by Accelerator Mass Spectrometry (AMS) at the NSF – Arizona AMS Laboratory at the University of Arizona (Tucson, AZ) ([Donahue et al., 1990](#)), the Centro Nacional de Aceleradores (Seville, Spain) ([Arévalo et al., 2009](#)), and the Center for AMS at Lawrence Livermore National Laboratory (Livermore, CA) ([Vogel et al., 1987](#); [Davis et al., 1990](#)). For each horizon, density/aggregate fractions and bulk soils from each site were composited for AMS analysis.

2.3. CP/MAS ¹³C NMR analysis

For the NMR spectroscopic analysis, composite samples were obtained from all three pedons sampled at each site, yielding one composite sample per genetic horizon. Prior to NMR analysis, bulk soil samples were treated four times with 10% hydrofluoric acid (HF) to remove iron oxides and concentrate the organic fraction following [Gonçalves et al. \(2003\)](#). Mass, C, and N recovery percentages of soils following HF digestion are given in [Supplementary Table 2](#). In addition the occluded C fraction of each genetic horizon was analyzed.

The ¹³C NMR spectra were acquired with a Bruker DSX 200 spectrometer operating at a ¹³C resonance frequency of 50.32 MHz. Samples were confined in a zirconium oxide rotor with an external diameter of 7 mm. The variable amplitude cross polarization magic angle spinning technique was applied with a contact time of 1 ms, a spinning speed of 6.8 kHz and a pulse delay of 1 s. For quantification, the spectra were subdivided into different chemical shift regions according to [Fründ and Lüdemann \(1989, 1991\)](#) and [Knicker and Lüdemann \(1995\)](#), and spinning side band corrections were applied ([Knicker et al., 2005](#)). The relative intensity distribution of ¹³C was determined by integrating the signal intensity in different chemical shift regions with an integration routine supplied with the instrument software. From these data, the degree of aromaticity (%) was calculated ([Hatcher et al., 1981](#)), as well as the ratio of alkyl to O-alkyl C, as an indicator of the degree of OM decomposition ([Baldock et al., 1997](#)). Studies have shown that NMR spectroscopy has good reproducibility, with a standard deviation of <10% for the relative intensities determined for the various chemical shift regions ([Dieckow et al., 2005](#); [Knicker, 2011](#)).

2.4. Pyrolysis gas chromatography/mass spectrometry (Py GC/MS)

To achieve a more detailed molecular characterization of the samples, selected density/aggregate fractions and bulk soil samples were analyzed by pyrolysis GC/MS. Selected subsurface density/aggregate fractions were analyzed at the Biogeochemistry and Nutrient Cycling Lab at the University of California, Davis. Samples were pyrolyzed at 590 °C in pyrofoils (Pyrofoil F590, Dychrom, Sunnyvale, CA) within a Curie Point pyrolyzer (Japan Analytical Industry Co.) following methods detailed in [Throckmorton \(2012\)](#).

Selected surface density/aggregate fractions were analyzed at the Institute of Natural Resources and Agrobiological, National Research Council, in Seville, Spain. Samples were pyrolyzed 500 °C (pyrolyzer model 2020, Frontier Laboratories Ltd. Fukushima,

Japan) in platinum cups. Detailed experimental conditions are given in the [Supplementary methods](#).

Pyrolysis products were identified through peak comparison with published and stored (NIST and Wiley libraries) data. The sum of all identified pyrolysis products' total ion signal integrated area was defined as 100%, from which the relative amount of each pyrolysis product was calculated. Results were expressed as the relative abundance of total identifiable compounds and grouped into 7 chemical classes: benzene, lignin, lipid, phenol, polysaccharide, and nitrogenous.

2.5. Aggregate stability

Aggregate abundance and stability was measured by horizon for each pedon, following procedures modified from [Fuller and Goh \(1992\)](#), [Rasmussen et al. \(2005\)](#), and [White et al. \(2009\)](#). This method of measuring aggregate stability uses the amount of clay released per unit of energy applied as a metric of aggregate disruption and assumes that the greater the amount of clay measured in a sonicated sample corresponds to the amount of aggregates that were broken apart. Aggregate stability then is negatively correlated with clay release. Data analysis followed [Rasmussen et al. \(2005\)](#). Briefly, a four parameter exponential growth model was fitted to plots of energy (J) versus clay release (% relative to clay release at 1500 J):

$$\% \text{ clay released} = a \left(1 - e^{-b(J(\text{g soil})^{-1})} \right) + c \left(1 - e^{-d(J(\text{g soil})^{-1})} \right),$$

where *a* and *c* represent pool sizes in units % clay released, *b* and *d* are constants for each pool in units of $[J(\text{g soil})^{-1}]^{-1}$. The stability of the aggregate pool was estimated by the inverse of the exponential constant, estimating the Joules per gram of soil required to disaggregate the aggregate structures in that pool. Model parameters were fitted by a least squares regression technique ([Raine and So, 1993](#)).

2.6. Data and statistical analyses

The Molecular Mixing Model published by [Nelson and Baldock \(2005\)](#) was applied to the NMR data. This model uses ratios of peak areas from NMR spectra to estimate the relative abundance of organic compound classes in the sample. Organic compound classes included: carbohydrate, nitrogenous compounds, lignin, aliphatic material, carbonyl, and charcoal.

Mean residence times (MRT) of bulk soils and density/aggregate fractions were calculated using the measured radiocarbon abundance and a time-dependent steady-state model ([Trumbore, 1993](#); [Torn et al., 2002](#)). This model assumes all new organic inputs to each modeled fraction bear an atmospheric radiocarbon signature and does not account for lag times associated with the transfer of organics among fractions, so calculations likely overestimate MRTs. Due to the incorporation of “bomb” radiocarbon into soil fractions at different rates, some fractions had two possible MRTs. In all cases where two MRT solutions were possible, the shorter MRT was eliminated as a possible solution due the unreasonably large predicted yearly output of C from the fraction (>30% of total pool respired per year).

A total of three replicate pedons were sampled per study site. Significant differences between density/aggregate fraction characteristics across sites were determined by one-way ANOVA followed by Tukey–Kramer *post hoc* test at a 95% confidence limit (*n* = 3 for each site). Simple linear least-squares regression and multiple linear regression were used to examine relationships between soil

physicochemical variables, aggregate stability, estimated MRTs, and occluded SOM properties ($n = 14$). Principal components analysis (PCA) of the compound classes was used to succinctly illustrate variance in Py GC/MS data across sites. To avoid issues of collinearity, explanatory variables with correlation coefficients greater than 0.70 were not concurrently included in regression models (Zar, 1999).

3. Results

3.1. Elemental data ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, C-to-N, ^{14}C)

Elemental composition differed significantly among density fractions (Table 1). C-to-N ratios, ^{13}C enrichment, and ^{15}N enrichment were similar among free/light and occluded fractions. Mineral fractions had significantly lower C-to-N ratios and were enriched in both ^{13}C and ^{15}N in comparison to both free/light and occluded fractions. Occluded fraction elemental composition did not differ significantly among mineral assemblages, but did vary with depth (Table 2). The occluded fraction accounted for the smallest total amount of C among the three fractions (Fig. 1), and its relative abundance generally decreased with depth. Relative abundance of occluded C was highest in surface horizons of the dolostone and basalt soils (~30%), and lowest at the soil/saprolite boundary of the rhyolite, granite and basalt soils (~10%).

The modeled MRT of the occluded fraction generally increased with depth and was longer than any other fraction of SOM in all but the BCr horizon of the rhyolite soils. In the deeper soils, rhyolite and basalt, the occluded fraction decreased in MRT at the soil/saprolite boundary. The free/light fraction consistently had the shortest MRT of all the fractions, and varied little with depth. Mineral/heavy fractions generally had MRTs intermediate between free/light and occluded fractions (Fig. 2, Table 2). Detailed data associated with radiocarbon measurements is given in Supplementary Table 3.

3.2. ^{13}C CPMAS-NMR spectroscopy

Due to the analysis time and cost associated with NMR analyses, only bulk SOM and occluded fractions were examined by NMR spectroscopy. O-alkyl/alkyl ratios increased with depth in both the occluded and bulk SOM, an indication of increasing degree of decomposition with depth (Baldock et al., 1997). A comparison of the occluded to the bulk SOM across all soils revealed consistent and significant differences. Bulk SOM had a significantly higher concentration of proteins ($P = 0.0082$), lignin ($P = 0.0120$), and carbohydrates (0.0038) than the occluded SOM. However, the most striking difference between bulk SOM and occluded SOM was the large enrichment of charcoal in the occluded fractions ($P = 0.0099$). High intensity in the chemical shift region between 140 and 110 ppm is indicative of charcoal content (Supplementary Fig. 1), especially when it is unaccompanied by low intensity in the

140–160 ppm range. Both the Molecular Mixing Model (Nelson and Baldock, 2005) and the C-H-aryl C/O-aryl intensity ratio (Dieckow et al., 2005) were used as metrics of charcoal content (Table 3), and the two estimates of charcoal content were highly correlated ($r^2 = 0.80$, $P < 0.0001$). The occluded fraction was significantly enriched in charcoal in comparison to bulk SOM at all depths and in all soils, regardless of mineral assemblage, though the absolute charcoal content of the occluded fraction tended to decrease in the deepest horizons (Fig. 3).

Comparison of SOM molecular composition on the basis of soil parent material type was confounded by differences in depth among the soils and the lack of replication (only composite samples were analyzed). However, qualitative examination of the NMR spectra revealed both similarities and differences among soils (Supplementary Fig. 1). Bulk SOM was similar in molecular composition among soils aside from a large enrichment in aliphatic C (alkyl peak, 0–45 ppm) in the surface of the granite soil. Occluded SOM appeared to vary both with depth and among soils. In the rhyolite soils occluded fraction charcoal content was highest in the surface horizon and decreased with depth, whereas the opposite trend was observed in the basalt soils. The occluded fraction of the granite surface soil showed the same enrichment in aliphatic C as the granite bulk SOM.

3.3. Pyrolysis GC/MS

Composition of free/light, occluded, and mineral/heavy OM fractions are easily distinguished from one another based on pyrolysate abundance. Most notably, the mineral/heavy fraction was enriched in nitrogenous compounds and depleted in lignin and lipids relative to the free/light and occluded fraction (Table 1). Other significant differences among fractions included enrichment of lipids and benzenes in the free/light fraction, and enrichment of phenol-based pyrolysates in the occluded fraction. Free/light and occluded fractions were similar among parent materials, but the mineral/heavy OM fractions show substantial variation among parent materials (Fig. 4a, Table 2). Variation in occluded fraction molecular composition followed variation in bulk C composition (Table 4).

Fig. 4a shows a PCA plot of compound class abundance (benzene, phenol, lignin, polysaccharide, and nitrogenous) for each fraction from the two soil horizons (surface versus subsurface) analyzed for each soil parent material. Samples grouped according to fraction type (free/light, occluded, mineral) along principal component axis 1 (accounting for 44% of total variance), and grouped according to depth along principal components axis 2 (accounting for 27% of total variance). Regression of compound class data against the principal component values indicated that nitrogenous compound content accounted for the bulk of the variation in samples along axis 1 ($r^2 = 0.77$, $P < 0.0001$), with nitrogenous compound content increasing from right to left along

Table 1
Comparison of density/aggregate fraction characteristics, C/N ratios, $\delta^{13}\text{C}\text{‰}$, and $\delta^{15}\text{N}\text{‰}$ values are the depth-weighted average across all sites ($n = 12$). Pyrolysis GC/MS compound abundances are the average of one subsurface and one surface horizon across all sites ($n = 8$). Standard errors are given in parentheses.

Fraction	Elemental data			Pyrolysis GC/MS compound abundance (% of total)					
	C/N	$\delta^{13}\text{C}\text{‰}$	$\delta^{15}\text{N}\text{‰}$	Benzene	Lignin	Lipid	Phenol	Polysaccharide	Nitrogenous
Free/Light	44.1 (± 6.7) ^A	−24.7 (± 0.3) ^B	−0.10 (± 0.54) ^A	11.9 (1.8) ^B	31.1 (4.3) ^A	4.8 (1.2) ^A	11.8 (1.8) ^{AB}	33.7 (3.2) ^A	6.4 (0.9) ^B
Occluded	48.4 (± 8.8) ^A	−24.4 (± 0.3) ^B	0.19 (± 0.50) ^A	23.8 (1.9) ^A	27.6 (4.6) ^A	4.0 (1.0) ^{AB}	15.5 (2.7) ^A	21.5 (2.9) ^A	7.6 (0.6) ^B
Mineral/Heavy	12.2 (± 1.9) ^B	−22.2 (± 0.5) ^A	1.89 (± 2.12) ^A	25.0 (2.7) ^A	10.2 (2.1) ^B	0.9 (0.5) ^B	6.3 (2.1) ^B	25.9 (5.0) ^A	30.6 (5.4) ^A

Significance was determined using one-way ANOVA by treatment followed by Tukey's HSD *post hoc* test ($\alpha = 0.05$). Within each column, different letters indicate statistically significant differences among variables. ($n = 12$ or $n = 8$) for each fraction type. The sum of all identified pyrolysis products' total ion signal integrated area was defined as 100%, from which the relative amount of each pyrolysis product was calculated. Results were expressed as the relative abundance of total identifiable compounds and grouped into 7 chemical classes: benzene, lignin, lipid, phenol, polysaccharide, and nitrogenous.

Table 2
Average C-to-N ratio, $\delta^{13}\text{C}\text{‰}$, $\delta^{15}\text{N}\text{‰}$, $\Delta^{14}\text{C}\text{‰}$, and modeled mean residence time (MRT) of each fraction, by horizon for each soil. Values are the average of 3 pedons per site, except for ^{14}C and MRT which were composite samples. Standard errors are given in parentheses ($n = 3$).

Parent material	Horizon	$\delta^{13}\text{C}\text{‰}$			$\delta^{15}\text{N}\text{‰}$			$\Delta^{14}\text{C}\text{‰}$			Modeled MRT (years)		
		Bulk	Free/Light	Occluded	Mineral	Bulk	Free/Light	Occluded	Mineral	Bulk	Free/Light	Occluded	Mineral
Rhyolite	A1	18.3 (2.9)	23.4 (0.9)	27.5 (4.8)	10.9 (1.9)	-25.0 (0.3)	-25.1 (0.1)	-25.0 (0.2)	-23.5 (0.3)	-0.3 (0.5)	1.2 (0.5)	3.9 (1.0)	94 46.7
	A2	17.7 (1.2)	28.0 (1.8)	37.4 (2.4)	10.5 (0.8)	-24.6 (0.1)	-24.6 (0.3)	-24.1 (0.4)	-22.9 (0.2)	1.2 (0.7)	1.0 (0.3)	3.9 (0.2)	12 -6.8
	A3	17.2 (3.2)	31.8 (2.2)	41.3 (0.2)	8.4 (0.9)	-23.9 (0.2)	-24.9 (0.0)	-24.7 (0.2)	-22.7 (0.2)	1.1 (0.7)	0.8 (0.3)	4.4 (0.1)	-52 17.9
	B _w 1	14.5 (1.4)	35.1 (2.3)	31.2 (3.9)	21.1 (10.5)	-23.7 (0.3)	-25.4 (0.1)	-24.6 (0.0)	-23.0 (0.3)	1.2 (0.4)	1.4 (0.5)	-0.7 (1.1)	-13 40
Granite	B _w 2	13.2 (2.4)	36.9 (2.6)	31.6 (1.0)	18.0 (9.0)	-23.3 (0.3)	-25.3 (0.2)	-24.7 (0.1)	-22.5 (0.2)	0.9 (0.5)	1.6 (0.3)	1.0 (2.7)	-71 59.8
	BC _t	11.5 (0.8)	41.2 (3.7)	26.3 (6.6)	11.0 (3.5)	-23.1 (0.1)	-25.2 (0.1)	-24.9 (0.0)	-22.2 (0.1)	-0.3 (0.4)	1.1 (0.2)	0.2 (5.7)	-78 79.0
	A1	20.0 (1.0)	33.9 (3.3)	44.8 (5.0)	14.7 (0.3)	-24.4 (0.1)	-25.0 (0.1)	-24.9 (0.2)	-23.9 (0.1)	-1.5 (1.0)	-0.7 (0.7)	-0.7 (1.8)	60 100.4
	A2	17.3 (0.7)	40.6 (4.6)	51.7 (6.8)	10.4 (0.7)	-23.9 (0.1)	-25.0 (0.2)	-24.4 (0.0)	-23.5 (0.2)	0.5 (1.1)	-0.6 (0.9)	1.3 (2.5)	29 44.1
Basalt	AC	19.0 (0.9)	47.8 (9.2)	62.0 (8.3)	14.5 (1.1)	-23.8 (0.1)	-24.4 (0.4)	-24.8 (0.2)	-22.9 (0.2)	-0.7 (0.7)	-0.3 (0.8)	-4.5 (5.5)	35 65
	A	18.8 (0.1)	36.8 (2.2)	43.8 (1.3)	11.6 (0.8)	-24.0 (<0.1)	-24.9 (0.1)	-24.5 (0.1)	-22.7 (0.1)	-1.6 (0.7)	-0.4 (0.8)	2.4 (0.2)	66 91.3
	B ₁	15.6 (1.7)	48.6 (8.2)	60.1 (5.4)	9.6 (1.5)	-22.7 (0.4)	-24.3 (0.4)	-24.0 (0.1)	-21.6 (0.3)	0.4 (0.1)	0.5 (1.0)	5.6 (1.0)	27 80.2
	B ₂	12.6 (0.3)	69.3 (4.7)	83.4 (0.6)	10.7 (0.6)	-21.9 (0.2)	-24.1 (0.2)	-23.7 (0.3)	-21.0 (0.1)	0.8 (0.5)	1.7 (0.4)	2.6 (2.3)	-68 104
Dolostone	B ₃	11.6 (0.3)	69.3 (1.0)	81.9 (10.5)	9.9 (2.7)	-21.7 (0.3)	-24.2 (0.2)	-23.9 (0.1)	-23.8 (2.6)	-0.6 (0.6)	-1.5 (0.7)	4.4 (3.4)	-143 43.7
	A	20.0 (1.7)	33.7 (3.8)	38.7 (0.8)	14.2 (1.7)	-23.0 (0.6)	-24.4 (0.5)	-24.6 (0.4)	-22.2 (0.4)	-2.6 (0.3)	-0.8 (0.7)	1.7 (0.8)	56 103.7
	B _t	18.3 (1.0)	39.1 (1.1)	45.8 (2.7)	11.8 (0.4)	-21.4 (0.2)	-24.5 (0.6)	-24.2 (0.4)	-21.2 (0.1)	-0.4 (0.8)	-0.04 (0.7)	4.5 (0.7)	17 106
													13
													52
													271
													157

the axis. Benzene and phenol abundance accounted for variance along axis 2 ($r^2 = 0.87$, $P < 0.0001$). Within occluded fractions (Fig. 4b), surface and subsurface samples differed based on lignin, polysaccharide, and phenol content. Principal components axis 1 accounted for 37% of total variance. Principal components axis 2 accounted for 26% of total variance.

3.4. Aggregate stability

Aggregate stability analyses excluded the rhyolite BCr and granite AC horizons due to the abundance of saprolitic materials in these horizons. Aggregate stability did not vary consistently among the soils or mineral assemblages examined, but did show relationships with soil % clay and depth (Table 5). Abundance of weak aggregates increased with depth and strong aggregate abundance decreased with depth in all soils except those derived from granite, likely driven by organic matter content (strength of aggregates estimates based on the amount of energy required for disruption). Increasing clay content increased the abundance of weak aggregates, but decreased the abundance and stability of strong aggregates. Weak aggregates decreased in strength with depth in all soils, likely due to the lower abundance of organics at depth.

3.5. Correlation analysis

A simple statistical exploration of the NMR data revealed that after accounting for the influence of depth, both indices of occluded SOM charcoal content (modeled % char and (C-, H-aryl)/O-aryl ratio) were strongly positively correlated with occluded C MRT (depth + % char: $r^2 = 0.85$, $P < 0.0001$; depth + (C-, H-aryl)/O-aryl: $r^2 = 0.77$, $P = 0.0002$). Depth and charcoal content accounted for similar amounts of variation in occluded SOM MRT. Bulk SOM MRT was not significantly correlated with charcoal content. Though the MRT of both the bulk and occluded SOM showed variance among soils, they did not vary predictably according to soil parent material. Bulk SOM MRT was highly correlated with depth ($r^2 = 0.63$, $P = 0.0004$), but occluded MRT was not ($r^2 = 0.21$, $P = 0.085$). Neither occluded C abundance nor MRT were correlated with aggregate stability after accounting for the influence of depth.

4. Discussion

The most notable quality of the occluded fraction was the selective preservation of pyrogenic materials. The high intensity of the signal at around 130 ppm in the ^{13}C NMR spectra, the elevated C-to-N ratio and the high concentration of benzene- and phenol-containing compounds noted in the pyrolysis GC/MS data all support a large polyaromatic content in occluded fractions in comparison to bulk soil and other density fractions. The enrichment of charcoal in occluded fractions in comparison to bulk soils was present in all soils regardless of mineral assemblage and generally increased with depth, reaching nearly five times the concentration of charcoal in the subsurface of the basalt soil (Fig. 3a). This same trend has been observed by other authors (Skjemstad et al., 1992) and most notably by Brodowski et al. (2006). Stable isotope enrichment levels suggested a similar degree of decomposition among free/light and occluded fractions, while elemental data and molecular structure data indicate mineral/heavy fractions were substantially more processed, and bore a microbial signature in comparison to the more plant-like character of free/light and occluded fractions (Baisden et al., 2002).

Occluded C characteristics changed consistently with depth, generally corresponding to changes in free/light and bulk SOM characteristics, suggesting that relatively undecomposed

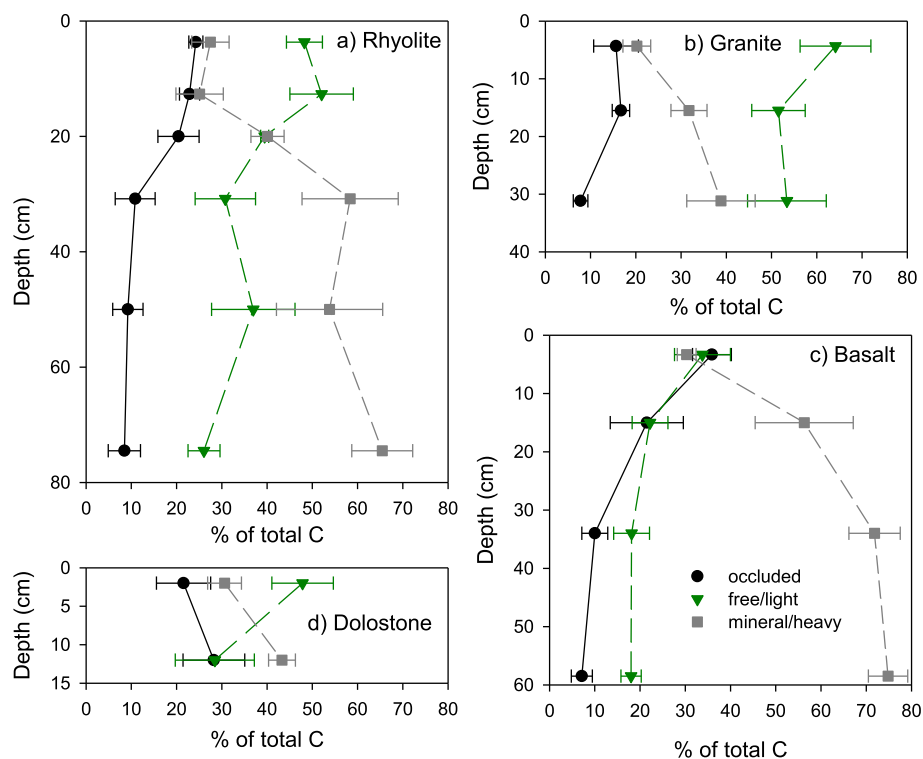


Fig. 1. Distribution of organic C among occluded, free/light, and mineral/heavy fractions, expressed as the percentage of bulk soil organic C contained in each fraction. Bars represent the standard error of three replicates per soil type.

particulate organic matter is occluded within aggregates during their formation and turnover. The fact that occluded C MRT increased with depth, while free/light C did not may indicate a mostly one-way flow of organics from the free/light to occluded

fraction in these soils and may additionally suggest that aggregate turnover at depth operates at timescales reaching or exceeding thousands of years. If this is indeed the case, it lends new insight into the modeling of SOM transfer among distinct SOM pools. The

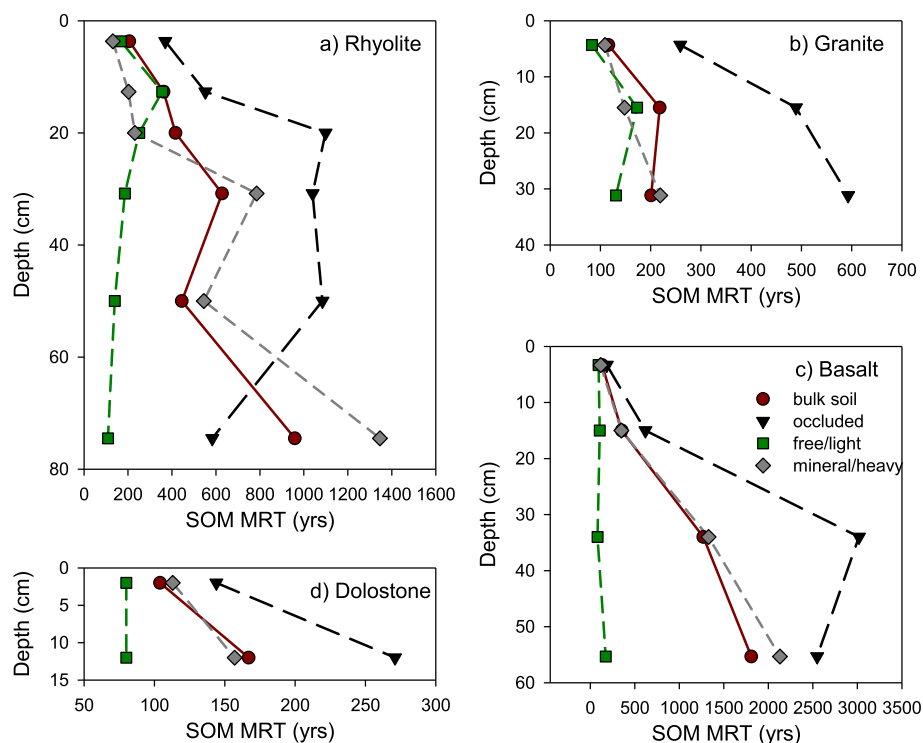


Fig. 2. Calculated mean residence times for bulk soil organic matter and density/aggregate fractions for each of the four soils examined. Each point represents one sample composited from three pedons.

Table 3

Results of the molecular mixing model by Nelson and Baldock (2005). The relative abundance of compound types in the occluded fractions and bulk SOM. Also, ratios of peak areas that serve as indices for soil organic matter quality.

Soil and fraction	Horizon	Relative % abundance of compound type						Alkyl-to-O-Alkyl ^a	C-,H-Aryl-to-O-Aryl ^b	% Aromaticity ^c
		Carbohydrate	Nitrogenous	Lignin	Aliphatic material	Carbonyl	Char			
Rhyolite	A1	8	11	8	11	7	56	0.9	3.0	10
Occluded OM	A2	8	7	8	17	8	51	0.8	3.0	9
	A3	10	6	5	12	7	59	1.1	3.4	6
	B _w 1	17	9	10	9	7	49	2.0	3.2	13
	B _w 2	21	9	14	11	6	39	2.0	3.0	20
	B _C r	22	9	17	15	8	29	1.7	2.8	25
Rhyolite	A1	13	11	24	17	7	28	1.0	2.1	32
Bulk SOM	A2	14	10	23	12	10	32	1.4	2.1	30
	A3	14	10	23	12	10	32	1.9	1.8	30
	B _w 1	19	12	39	4	11	15	2.3	1.6	57
	B _w 2	14	14	46	4	9	13	1.9	1.6	64
Granite	A1	11	6	14	33	5	30	0.6	2.6	17
Occluded OM	A2	9	5	7	21	6	52	0.8	3.3	8
	AC	13	5	5	19	8	50	1.0	3.3	6
Granite	A1	18	7	22	28	5	21	1.0	2.2	29
Bulk SOM	A2	18	10	26	15	5	27	1.5	2.4	36
	AC	26	10	16	20	8	20	1.5	2.0	25
Basalt	A	18	6	19	20	6	31	1.2	2.5	25
Occluded OM	B _t 1	11	5	13	12	8	52	1.3	3.0	16
	B _t 2	11	2	5	11	8	63	1.5	3.4	6
	B _t 3	16	3	9	12	7	52	1.8	3.2	12
	A	25	9	26	19	4	16	1.5	2.0	40
Basalt	A	25	9	26	19	4	16	1.5	2.0	40
Bulk SOM	B _t 1	17	11	39	9	9	14	1.7	1.7	54
	B _t 2	27	15	31	13	2	13	1.8	2.1	52
	B _t 3	26	16	29	11	6	12	1.8	1.9	50
	A	19	7	16	23	5	29	1.1	2.7	22
Dolostone	A	19	7	16	23	5	29	1.1	2.7	22
Occluded OM	B _t	17	6	16	18	5	38	1.3	2.9	21
	A	25	10	18	24	5	19	1.2	2.3	27
Dolostone	A	25	10	18	24	5	19	1.2	2.3	27
Bulk SOM	B _t	21	11	20	18	8	22	1.4	2.6	30
	A	21	11	20	18	8	22	1.4	2.6	30

^a Decomposition is associated with an increase in alkyl C content and a decrease in O-alkyl C content. A higher alkyl/O-alkyl ratio indicates a greater degree of decomposition (Baldock et al., 1997).

^b The contribution of condensed aromatic structures (not lignin) to the overall content of aromatics can be demonstrated by the C-, H-aryl/O-aryl ratio. The larger the ratio, the more charcoal is present (Dieckow et al., 2005).

^c degree of aromaticity (%) was calculated as [aromatic C × 100/(alkyl C + N-alkyl/methoxyl C + O-alkyl C + aromatic C)] (Hatcher et al., 1981).

occluded fraction is often assumed to be intermediate in degree of decomposition and stability between the free/light and mineral/heavy fractions (Lützow et al., 2007), with active transport among all three fractions but most notably between free/light and occluded fractions as aggregates disassociate and reform as soils weather and experience seasonal climatic changes (Kelly et al.,

1997). However a clear understanding of the links and fluxes between SOM pools is still mostly lacking (Kuzyakov, 2011), and the identification of a relatively passive and chemically distinct occluded particulate fraction in western U.S. conifer forest soils may help clarify the direction and magnitude of between-pool fluxes in these systems.

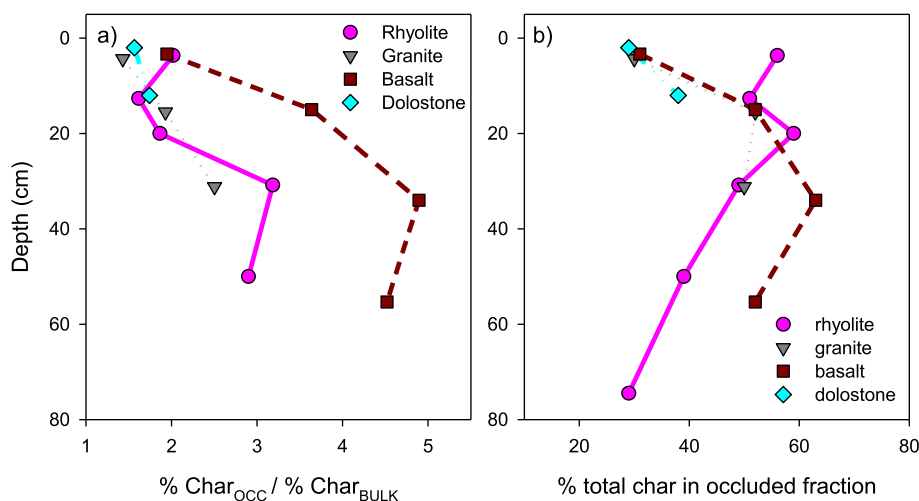


Fig. 3. a) The percentage of charcoal in the occluded fraction divided by the percentage of charcoal in the bulk soil. Charcoal was enriched in the occluded fraction in comparison to bulk soil organic matter in all soils. b) The percentage of total bulk soil charcoal included in the occluded fraction. Note that this graph implies that charcoal is present in all density/aggregate fractions, and at depth the majority of charcoal is not in the occluded fraction.

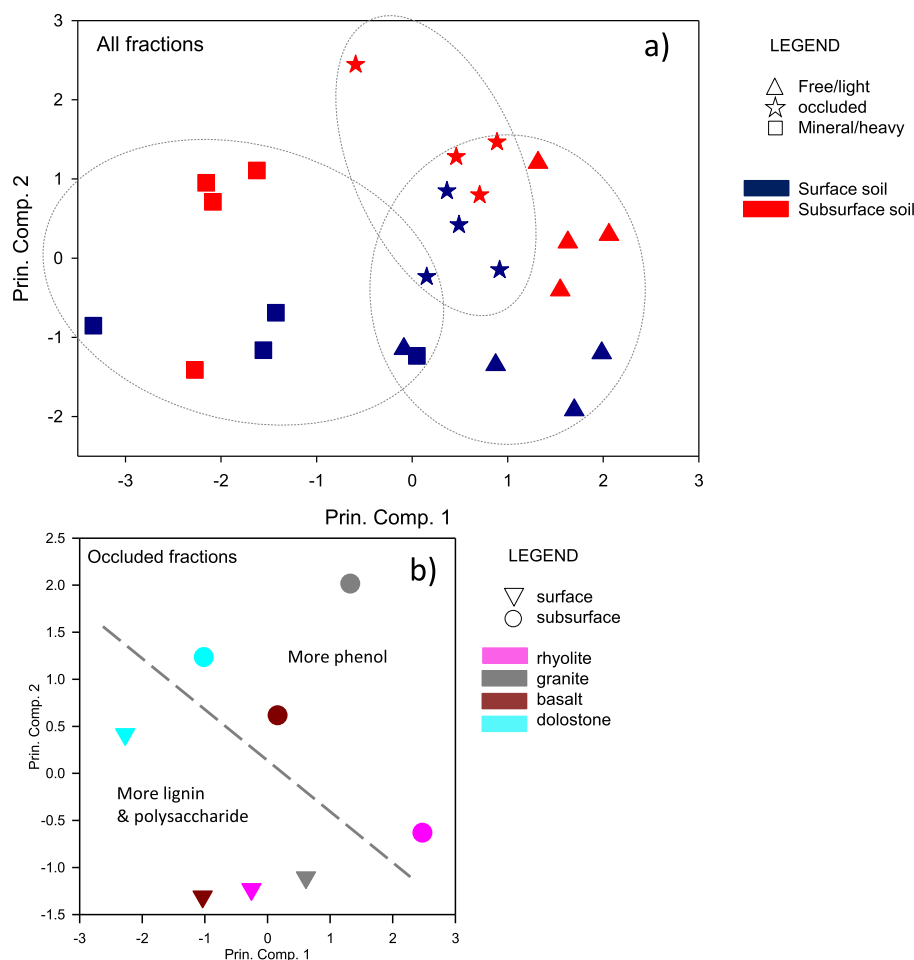


Fig. 4. Principal components analyses of pyrolysis GC/MS data. a) Plot shows the relationship among the molecular compositions of density/aggregate fractions. b) Plot shows the relationship among occluded soil organic matter fractions from soils of differing mineral assemblages. Composition of occluded fractions roughly groups according to depth.

Table 4

Twenty most abundant soil organic matter pyrolysis products and their abundance in bulk soil and density/aggregate fractions, listed in order of abundance in the bulk soil. Standard errors are given in parentheses. Values are the average of 8 soil horizons (one surface and one subsurface from each site).

Compound	Class	% Abundance			
		Free/Light	Occluded	Mineral/Heavy	Bulk soil
Methylbenzene	Benzene	5.8 (0.7)	11.7 (1.2)	10.6 (2.9)	13.5 (2.7)
Furan-2-carbaldehyde	Polysaccharide	9.9 (0.9)	7.4 (1.5)	4.7 (1.4)	9.5 (2.2)
Phenol	Phenol	6.4 (0.8)	8.9 (0.8)	4.5 (1.2)	7.8 (1.5)
Pyridine	Nitrogenous compound	1.7 (0.7)	1.6 (0.8)	11.3 (3.8)	6.6 (2.6)
Styrene	Benzene	3.7 (0.7)	5.8 (0.7)	4.7 (1.2)	5.6 (0.4)
Ethanoic acid	Polysaccharide	1.7 (0.5)	0 (0)	3.3 (2.5)	4.7 (2.3)
Methylpyridine	Nitrogenous compound	1.3 (0.5)	3.0 (0.9)	11.8 (5.9)	4.1 (1.3)
Benzene	Benzene	1.6 (1.1)	4.2 (1.2)	8.2 (2.9)	3.8 (0.9)
Methylphenol	Lignin	4.1 (0.8)	4.2 (1.5)	1.9 (0.7)	3.7 (1.0)
Dimethylbenzen	Benzene	0.4 (0.4)	1.7 (0.9)	2.7 (1.5)	3.1 (1.3)
2-Methylfuran	Polysaccharide	2.2 (0.9)	2.2 (0.9)	3.5 (2.0)	3.0 (1.8)
Furan, 2,5-dimethyl-	Polysaccharide	0.3 (0.3)	1.7 (1.3)	2.6 (1.3)	2.7 (1.5)
2-furancarboxaldehyde, 5-methyl-	Polysaccharide	5.7 (0.5)	3.4 (1.4)	1.5 (0.8)	2.5 (0.9)
Furan	Polysaccharide	1.0 (0.6)	1.4 (0.8)	2.3 (1.1)	2.3 (1.2)
1-Benzofuran	Polysaccharide	0.7 (0.5)	4.1 (2.1)	1.4 (0.4)	1.7 (0.4)
Phenol, 2-methoxy-	Lignin	8.1 (0.9)	8.6 (1.3)	0 (0)	1.2 (0.9)
Phenol, 4-ethenyl-2-methoxy-	Lignin	7.5 (1.0)	4.3 (1.2)	0 (0)	0.3 (0.3)
Alkene	Lipid	3.0 (1.2)	3.0 (0.8)	0.2 (0.2)	0.2 (0.1)
Phenol, 2-methoxy-methyl-	Lignin	3.3 (1.0)	2.6 (0.8)	0 (0)	0 (0)
Phenol, 4-allyl-2-methoxy-	Lignin	1.7 (1.0)	0.8 (0.6)	0 (0)	0 (0)

Table 5

Correlations among aggregate stability model parameters and soil physiochemical characteristics.

Parameter	R^2	P-value	Model
a	0.78	<0.001	$a = 40.15 + 0.53 \cdot \% \text{clay}$
c	0.58	0.001	$c = 77.41 - 0.94 \cdot \% \text{clay}$
1/b	0.74	<0.001	$1/b = 127.88 - 1.75 \cdot \text{depth (cm)}$
1/d	0.46	0.010	$1/d = 10666.03 - 16.82 \cdot \% \text{clay}$

Contrary to our hypotheses, occluded SOM characteristics were not linked to variance in soil mineral assemblage or aggregate abundance/stability. If turnover of occluded C was dependent on the dissolution and reformation of aggregates, occluded C MRT should show a correlation with aggregate stability/turnover. The lack of correlation among aggregate stability parameters and occluded C characteristics indicates that either aggregate stability is not a good predictor of occluded C protection against biodegradation or the laboratory methods employed here to gauge aggregate stability are not a good proxy for aggregate turnover under field conditions. It's likely that application of sonic energy to disrupt aggregate structures does not mimic the natural processes governing aggregate dissolution and reformation such as wet/dry cycles, clay flocculation, and root/mycelium growth. It may also be that the actual bond strength among aggregate components is less important than the amount of disturbance a particular soil horizon experiences, and weakly bonded aggregates may persist at depth but experience high turnover in surface horizons. The lack of relationships among aggregate parameters, mineral assemblage and occluded fraction abundance and MRT suggest that persistence of occluded organics in these soils is not strongly dependent on the presence of specific mineral phases, soil texture, or the aggregation qualities associated with specific clay types/abundance.

We hypothesized that aggregate stability would vary considerably and consistently among soils of differing mineral assemblage,

with oxyhydroxide phases and 1:1 phyllosilicate content driving increases in aggregate abundance and stability (Six et al., 2002). Correlation analysis indicated that aggregate stability and abundance had significant relationships only among percent clay and depth. Increasing depth was associated with a decrease in the stability of weaker aggregates, perhaps due to a decrease in organic coatings on mineral surfaces and a lower level of biological activity. This explanation is consistent with previous research indicating a strong dependence of microaggregate stability on organic binding agents in a variety of soils (Tisdall and Oades, 1982; Amézketa, 1999). Increases in clay content had an inconsistent effect on aggregate parameters, increasing the abundance of weak aggregates but decreasing the abundance and stability of stronger aggregates. This is possibly due to the relative abundance of illite and vermiculite clays in these soils, phases which render aggregates most sensitive to dispersion (Amézketa, 1999 and references therein). The abundance of Fe and Al oxyhydroxide phases is often implicated in driving a large amount of variation in SOM turnover (Torn et al., 1997; Parfitt et al., 2002), but did not show a relationship with occluded C MRT in these soils. Likewise, oxyhydroxides did not account for any variation in aggregate parameters. However this may be due to the relatively low abundance of oxyhydroxides in the majority of the soils examined in this study.

Charcoal content of the occluded fraction was highly correlated with both the abundance and MRT of C in this fraction, indicating that fire exerts a large degree of control over the formation and preservation of occluded organics in soils of temperate forests that include wildfire as a component of ecosystem processes. A compilation of available data from forested systems (including the data from this study) indicates significant variation in occluded fraction MRT depending upon wildfire regime (Fig. 5). Specifically, data from western U.S. (Arizona and California) conifer forests prone to burning (Rasmussen et al., 2005; Heckman et al., 2009; Lybrand et al., 2013) indicates that the occluded C tends to be the oldest fraction of soil C (Figs. 2 and 5a) with mean residence times

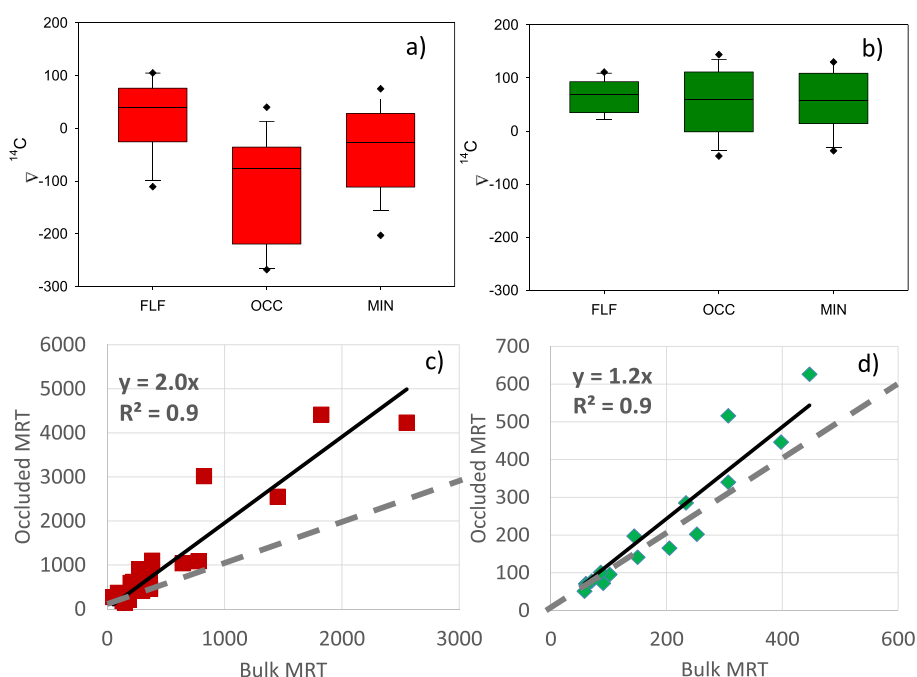


Fig. 5. Radiocarbon content of free light (FLF), occluded (OCC), and mineral (MIN) fractions for a mix of surface and subsurface samples for [a] western conifer forests prone to wildfire ($n = 27$) and [b] wet temperate and tropical forests ($n = 18$). Data in [c] and [d] indicate the mean residence time (MRT, years) calculated for the occluded fractions relative to bulk soil for the fire prone systems and wet forest systems, respectively. The dashed lines are the 1:1 line and the solid black lines are best fit regressions with the intercept set equal to zero.

generally twice that of the bulk soil (Fig. 5c). In contrast, wet forested ecosystems (Marin-Spiotta et al., 2008; McFarlane et al., 2012) exhibit little variation in radiocarbon content among free light, occluded, and mineral fractions (Fig 5b), with occluded C MRT roughly equivalent to that of the bulk C (Fig. 5d). Thus, differences in fire regime may partially account for the high degree of heterogeneity in occluded fraction composition and MRT among forested ecosystems.

The physiochemical properties of charcoal are a probable mechanism to explain the preservation of occluded C in these systems, due both to the inherent structural recalcitrance of pyrogenic materials and the reactivity of charcoal structures (Schmidt and Noack, 2000; Brodowski et al., 2005, 2006; Czimczik and Masiello, 2007; Keiluweit and Kleber, 2009; Knicker, 2011). Condensed polyaromatic structures are metabolically unfavorable in comparison to other microbial growth substrates, and the strongly sorbing aromatic π -systems of charcoal can form strong bonds with other soil constituents (Keiluweit and Kleber, 2009), properties long recognized to promote the long-term preservation of charcoal in soils. The hydrophobicity of charcoal surfaces also plays a role in its preservation though reducing wettability which effectively limits enzymatic access. Hydrophobic organic domains also promote strong interactions with phyllosilicates, especially kaolinite (Puget et al., 2000; Ellerbrock et al., 2005; Bachmann et al., 2008). How much of a part the actual formation of soil aggregates plays in protecting these organics from biodegradation isn't clear, but radiocarbon-based MRT estimates indicate occlusion within aggregates or coating of occluded OM surfaces with mineral particles does contribute significantly to the persistence of these organics (Wagai et al., 2009). This observation is additionally supported by the comparatively fast turnover and lack of charcoal observed in the free/light fractions of these soils, and previous research describing rapid mineralization of unprotected charcoal (Marschner et al., 2008; Hilscher and Knicker, 2011). It can be said that fire is the origin of a large fraction of occluded C in these soils, and the molecular properties of the pyrogenic materials promote their occlusion within aggregates.

Though the occluded fraction contained the least amount of C relative to free/light and mineral/heavy fractions, it generally had the longest MRT in soils regardless of mineral assemblage indicating that this fraction is an important repository of stable C in western U.S. conifer forest soils. In forested soils which experience periodic fire events, it appears that it is only through the combination of chemical recalcitrance and occlusion within aggregates that organics can attain "passive" MRTs. Additionally, these results indicate that fire regime may be a good indicator of the abundance of the passive C fraction in temperate forest soils, though how charcoal degradation rates will be modified by changes in climate has not been resolved (Liski et al., 1999; Nguyen et al., 2010).

4.1. Conclusions

Data describing the molecular composition of occluded organics in these soils indicated that the fraction is dominated by pyrogenic materials regardless of the particular soil mineral assemblage or estimated aggregate stability. Radiocarbon-based MRTs of occluded organics revealed that occluded C has a greater resistance to biodegradation than C stabilized through bonding to mineral surfaces (mineral/heavy fraction), and that this stabilization can be directly linked to the abundance of charcoal in the occluded fraction. Results point to the importance of fire as a driver of occluded C formation, and the structural recalcitrance of pyrogenic materials as a major mechanism of occluded C stabilization in these systems. These findings suggest that understanding the fire regime of

temperate forest ecosystems may be important in modeling the abundance and behavior of passive SOM pools.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2014.05.028>.

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