Chemical and mineral control of soil carbon turnover in abandoned tropical pastures

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Abstract

We investigated changes in soil carbon (C) cycling with reforestation across a long-term, replicated chronosequence of tropical secondary forests regrowing on abandoned pastures. We applied CP MAS ^{13}C NMR spectroscopy and radiocarbon modeling to soil density fractions from the top 10 cm to track changes in C chemistry and turnover during secondary forest establishment on former pastures. Our results showed that inter-aggregate, unattached, particulate organic C (free light fraction) and particulate C located inside soil aggregates (occluded light fraction) represent distinct soil C pools with different chemical composition and turnover rates. The signal intensity of the O-alkyl region, primarily representing carbohydrates, decreased, and alkyl C, attributed to recalcitrant waxy compounds and microbially resynthesized lipids, increased from plant litter to soil organic matter and with incorporation into soil aggregates. The alkyl/O-alkyl ratio, a common index of humification, was higher in the occluded than in the free light fraction. Greater variability in the chemical makeup of the occluded light fraction suggests that it represents material in varied stages of decomposition. Mean residence times (^{14}C-based) of the free light fraction were significantly shorter (4 ± 1 years) than for the heavy fraction. We report two scenarios for the occluded light fraction, one fast-cycling in which the occluded and free light fractions have similar turnover rates, and one slow-cycling, in which the occluded light fraction resembles the heavy fraction. Mean residence times of the occluded light fraction and heavy fraction in active pastures and 10-year old secondary forests in the earliest stage of succession were longer than in older secondary forests and primary forests. This is likely due to a preferential loss of physically unprotected C of more labile composition in the pastures and in the youngest successional forests, resulting in an increase in the dominance of slow-cycling C pools. Soil carbon turnover rates of the mineral-associated C in secondary forests recovering from abandoned pasture resembled those of primary forests in as little as 20 years of succession.
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1. Introduction

Tropical and subtropical forests have the greatest potential to sequester and conserve large amounts of C relative to other forest biomes, as reported by the Third Assessment Report of the Intergovernmental Panel of Climate Change (Metz et al., 2001). Over half of this C sequestration potential could be achieved by slowing deforestation and by allowing for forest regeneration on abandoned agricultural and pasture lands (Brown et al., 1996). Reforestation and afforestation in the tropics result in high rates of C uptake in biomass (Watson et al., 2000). Less is known about the potential for C storage in soil organic matter (SOM), which is also an important contributor to soil fertility (Tiessen et al., 1994; Silver et al., 2000). As the largest terrestrial reservoir of C, soils may exert a strong influence on atmospheric CO_2 concentrations as C sources or sinks (Post et al., 1982; Eswaran et al., 1993; Batjes and Sombroek, 1997).

Biochemical and physical stabilization of SOM are important mechanisms of soil C sequestration (Paul, 1984; Oades, 1988; Sollins et al., 1996; Krull et al., 2003; Mikutta et al., 2006). Biochemical processes include recalcitrant litter chemistries that slow down or inhibit microbial degradation (Scholes et al., 1997), and chemical transformations during humification and other oxidative reactions that lead to the synthesis of new compounds that are more resistant to decay than their precursors (Kögel-Knabner, 1993). In this paper, humification refers to the suite of chemical changes in organic matter resulting from the processes of microbially driven decomposition. Physical protection of C occurs though sorption to mineral and organic surfaces, reactions with Fe and Al oxides to form insoluble complexes, and occlusion inside soil aggregates (Hassink, 1997; Kaiser and Guggenberger, 2000; Baldock and Skjemstad, 2000; Balesdent et al., 2000; Six et al., 2002; Kalbitz et al., 2005). Other factors that affect SOM formation and decomposition include temperature, pH, and the availability of nutrients, O_2, and water (Fog, 1988; Zech et al., 1997; Scholes et al., 1997; Giardina et al., 2004; Swanston et al., 2004), as well as microbial community composition and enzyme availability (Schimel, 2001).

Modeling and experimental studies of SOM decomposition and the effects of cultivation on soil C have provided evidence for at least three distinct pools turning over on different time scales (Jenkinson and Rayner, 1977; Parton et al., 1987; Trumbore, 1993; Townsend et al., 1997): (1) an active or labile pool (one to five years), (2) a slow or intermediate pool (decades), and (3) a passive pool (centuries to millennia). Several approaches have been developed to reconcile these modeled C pools with operational, identifiable pools in soils (e.g., Greenland and Ford, 1964; Trumbore and Zheng, 1996; Christensen, 1996; Falloon and Smith, 2000; Wander, 2004; Ellerbrock and Kaiser, 2005; Kleber et al., 2005; Paul et al., 2006; Zimmerman et al., 2006). Physical fractionation separates SOM by particle-size, -aggregate-size, or density, without chemical modifications (Elliott and Cambardella, 1991; Golchin et al., 1994a,b; Baisden et al., 2002a). Methods that couple density fractionation with physical disruption of aggregates are useful for separating SOM fractions based on location in the soil matrix and degree of association with minerals (Golchin et al., 1995; Christensen, 2001; Swanston et al., 2005).

Recent applications of solid-state cross polarization magic angle spinning (CP MAS) ^{13}C nuclear magnetic resonance (NMR) spectroscopy to soils have increased our understanding of the chemical structure of SOM (Golchin et al., 1995; Kögel-Knabner, 1997, 2000; Preston 2001). ^{13}C NMR provides a first approximation of the relative abundance of organic C functional groups, which can be used as indicators for different compounds. The relative distributions of these broad C categories in SOM vary with decay and microbial processing (Baldock et al., 1992; Golchin et al., 1994b). Further information on specific types of compounds can be gained from ^{13}C NMR spectra by applying a molecular mixing model (Baldock et al., 2004). These techniques, coupled with physical fractionation and radiocarbon (^{14}C) modeling can provide a powerful assessment of the nature and turnover of soil C pools.

Little is known about long-term changes in tropical soil C with reforestation, particularly beyond the first two decades (Silver et al., 2000). Our research takes advantage of a historical trend in post-agricultural natural reforestation in Puerto Rico (Grau et al., 2003) to study long-term soil C dynamics. We used variable-amplitude (VA) CP MAS ^{13}C NMR and ^{14}C-based modeling of soil C turnover times to investigate changes in chemistry and stability of soil density fractions. The transitions from pasture grasses to forest trees, and from early to late successional species allowed us to study how changes in plant cover and litter chemistry affect soil C. We hypothesized that soil C pools, especially the fraction associated with aggregates, would turn over more rapidly in the active pastures compared to primary forests. Conversion of forest to pasture is often associated with a decrease in the amount of C associated with larger-sized aggregates and with their destabilization due to cattle disturbance and combustion during the slash-and-burn clearing process (Garcia-Oliva et al., 1999). We also hypothesized that concentrations of chemically recalcitrant compounds in SOM would increase due to aboveground changes in litter recalcitrance from forage pasture grasses to woody forest species. Plants produce lipids, such as waxes, cutin, suberin, and terpenoids, that are resistant to oxidation and consumption, as protection against herbivory and parasitism (Gleixner et al., 2001). The production of these and other plant secondary compounds is hypothesized to increase during tropical forest succession (Coley and Barone, 1996). We expected that differences in litter chemistry would result in increased soil C turnover time, not only between pastures and forests, but also with increasing forest age. We compared two approaches used to assess litter chemistry, solid-state ^{13}C NMR of whole litter and the more traditional proximate C fraction analysis (Preston et al., 2000).

2. Methods

2.1. Study sites

Our study sites were located between 580 and 700 m above sea level in the subtropical wet forest life zone (Ewel and Whitmore, 1973) in the Sierra de Cayey in southeastern Puerto Rico (18°01′ N, 66°05′ W). The dominant soil type is classified as very-fine, mixed, isothermic Inceptic Hapludox in the Los Guineos series (USDA soil map, Lugo-López et al., 1995). Soils are very deep, well-drained and from sandstone parent material. Mean annual temperature is estimated at 25 °C with little inter-annual variation and mean annual precipitation is 2000 mm (Daly et al., 2003; SERCC, 2006). The land-use chronosequence included three replicate sites each of primary forests, pastures, and secondary forests re-growing on pastures abandoned 10, 30, 60 and 80 years ago, and two replicate forests on pastures abandoned 20 years ago (Marín-Spiotta et al., 2007). Land-use history was identified by use of a time-sequence of aerial photographs and interviews with local landowners. The potential forest vegetation types in the region are lower montane wet evergreen forest, tall cloud forest, and palm breaks (Helmer et al., 2002). Primary forest sites were remnant forest fragments that had not been under pasture cover for at least the last century. Dominant pasture forage species included *Axonopus compressus*, *Panicum laxum* and *Sporobolus jacquemontii* (Sánchez-de León et al., 2003). Pasture sites were actively grazed at the time of sampling.

2.2. Plant samples

Leaf litterfall samples, collected bi-weekly from five litter baskets at each forested site over a period of 12 months, were composited by site ($n=1$ per site) prior to analysis by wet chemistry and ^{13}C NMR spectroscopy. At the pasture sites, grab-samples of rooted (live and dead) aboveground plant material inside 15×15 cm areas were collected with an attempt to sample the heterogeneity of plant cover types (e.g., grasses and forbs). All plant material was oven-dried at 50 °C and a subsample was ground to pass a size 60 mesh on a Thomas Scientific Wiley mill (Philadelphia, PA, USA). Plant litter tissue composition was determined by two methods: ^{13}C NMR and wet chemistry. The latter measures mass recovery after sequential extractions by different solvents (McClaugherty et al., 1985; Ryan et al., 1990) and was performed at the Center for Water and the Environment, Natural Resources Research Institute, University of Minnesota, Duluth, MN. The analysis yields the following fractions: non-polar (NPE), water soluble (WS), and acid soluble extractables (AS), with the final acid-insoluble residue referred to as Klason lignin (KL). The types of compounds represented by each fraction are: NPE: waxes, fats, and chlorophylls; WS: simple sugars, hydroxy phenol groups, and amino acids; and AS: plant polysaccharides (cellulose, hemicellulose, and starch) as well as proteins, polypeptides, some amino acids, and nucleic acids. In addition, polyphenols, measured in tannin equivalents by the Folin–Dennis method (Allen et al., 1974), and which include small poly and mono aromatic compounds of plant resins, are extracted from the WS fraction. Polysaccharides, simple sugars measured in glucose equivalents (Dubois et al., 1956), are extracted from both the WS and AS fractions.

2.3. Soil samples

Soils were collected in duplicate per site from the 0–10 cm depth and stored at 4 °C. We made an *a priori* decision to exclude the 60-y old secondary forest samples from density fractionation based on the similarity of soil C pools and isotope data with other older forest soils, and due to considerable time and expense needed to process the samples. Field moisture was determined on a sub-sample oven dried at 105 °C. In preparation for fractionation, soils were gently broken apart by hand to pass through a 4.75 mm sieve. A 5 g sub-sample was taken to determine initial soil moisture. We fractionated two soil samples per site and report means per site. Our fractionation method separates bulk soils into three fractions: (1) a free light fraction (LF) consisting primarily of inter-aggregate particulate organic matter, (2) an occluded LF, released by the disruption of soil aggregates, and (3) a heavy fraction (HF), comprising organic matter tightly bound or sorbed to minerals. We followed the procedure of Swanston et al. (2005), with slight modifications as described here. The free LF was separated from undisturbed soils by floatation in sodium polytungstate (NaPT, Na_6 [$H_2W_{12}O_{40}$], Sometu–US, Van Nuys, California) at a density of 1.85 g/mL. The occluded LF was floated from the remaining dense material after mixing for 1 min using a bench-top mixer (G3U05R, Lightnin, New York, NY) at 1700 rpm and sonication in an ice bath for 3 min at 70% pulse for a total input of 200 J/mL (Branson 450 Sonifier, Danbury, CT). The HF was collected as the residual, dense soil pellet. We used fresh, moist soils to better mimic field conditions and to minimize pre-processing disturbance to aggregate structure. Before aspiration of the free and occluded LF and after centrifugation, samples were allowed to sit overnight to achieve further separation by floatation of organic debris and settling of clay particles in solution. The LFs were rinsed with 500 mL of distilled, deionized H_2O through a 0.4 μm polycarbonate filter (Whatman Nuclepore Track Etch Membrane) to remove residual NaPT. Visible charcoal fragments were hand-picked from the final oven-dried fractions before grinding to a fine powder for NMR and radiocarbon analyses.

2.4. ^{13}C NMR spectroscopy

Solid-state VACP MAS ^{13}C NMR spectra were acquired on a Varian/Chemagnetics Infinity CMX 300 MHz nuclear magnetic spectrometer (Varian NMR, Fort Collins, CO), located at the High Field Nuclear Magnetic Resonance facility at the Environmental Molecular Sciences Laboratory at Pacific Northwest National Laboratory in Richland, WA. We did not collect ^{13}C NMR spectra on the HF because of paramagnetic ions, such as iron, which can interfere with spectral readings (Schnitzer, 2001) and are very abundant in our soils. Samples were packed in 5 mm diameter Pencil zirconia rotors with boron nitride spacers and spun at 10 kH in a 5 mm HXY MAS probe. This speed was shown to reduce spinning side bands. Preliminary work established the optimal conditions for achieving maximum intensity for all experiments. The optimum *cp* contact time was 1 ms, the proton 90 was 4.5 μs, and the decoupling field was 55.55 kHz. All spectra were referenced to that of adamantane. Spectra were digitally processed using an exponential weighting equation with a line broadening at 100 Hz and a Fourier transformation on MestReC459 software (Universidad de Santiago de Compostela, Spain). The software was used to integrate peak areas under the following seven chemical shift regions (and the general C types they represent): 0–45 (alkyl), 45–65 (methoxyl), 65–95 (O-alkyl), 95–110 (di-O-alkyl and some aromatic C), 110–145 (aromatic), 145–165 (phenolic), and 165–220 ppm (carboxylic and carbonyl C) (Preston et al., 2000; Baldock et al., 2004). The integrated spectral areas were normalized to the total signal intensity for each spectrum.

2.5. Modeling molecular composition

We applied a mixing model (MMM) developed by Nelson et al. (1999) and modified by Baldock et al. (2004) to estimate the molecular composition of our plant litter samples and the soil LFs. The MMM assigns signal intensity across the seven NMR chemical shift regions listed in the previous section to the following types of compounds: carbohydrate, protein, lignin, lipid, carbonyl, and char. Lignin identified by NMR represents plant lignin, and is different from the acid-insoluble residue, Klason lignin (KL), from wet proximate analyses, which likely also includes other non-lignin compounds (Ryan et al., 1990; Preston et al., 1997). The distinction is important because the acid-
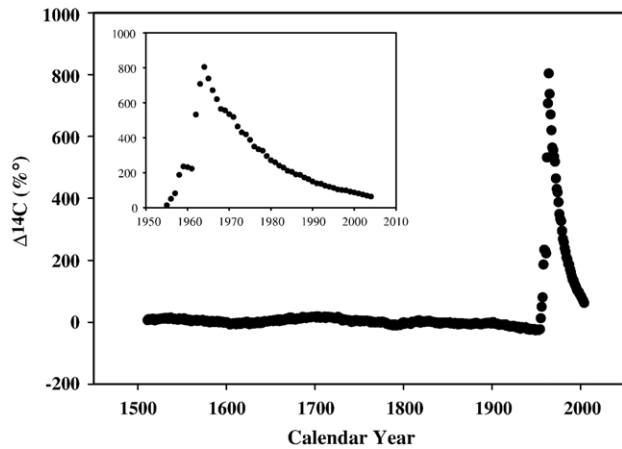
Fig. 1. Atmospheric $\Delta^{14}C$ records dating back to 1511 for Northern Hemisphere Zone 2, which includes the Caribbean region, compiled from Stuiver et al. (1998), Hua and Barbetti (2004), and Levin and Kromer (2004). Inset shows atmospheric $\Delta^{14}C$ records for the years 1955–2004.

insoluble KL has been negatively correlated with litter decay rates (Melillo et al., 1989), and is often used in the ecological literature to represent recalcitrant plant material, whereas NMR identified lignin can undergo considerable microbial transformation in soils (Kögel-Knabner et al., 1991; Preston et al., 2002, 2006). Char is defined as thermally transformed organic material, e.g. from biomass burning or soot, and is relatively resistant to degradation (Skjemstad et al., 1996; Baldock and Smernik, 2002).

2.6. Carbon chemistry statistics

All statistical tests were performed on JMP 5.1 (SAS, Cary, NC), and unless otherwise noted, significance is given at the $p < 0.05$ level. Differences between pasture and forest plant litter chemistry parameters were analyzed by the Wilcoxon–Kruskal Wallis ranks sum test. Pasture soils yielded insufficient free LF

Table 2
Average mean relative contributions (and standard error) of different ^{13}C NMR spectral regions to total peak area for leaf litter, free and occluded light density soil C fractions

NMR spectral region (ppm)	Leaf litter	Free LF	Occluded LF
0–45	18.0 (1.0) a	15.7 (1.2) a	21.4 (2.2) b
45–65	12.0 (0.9) a	14.1 (1.2) a	12.7 (1.2) a
65–95	31.2 (1.6) a	22.4 (2.2) b	21.6 (1.2) b
95–110	8.6 (0.4) a	7.2 (0.3) b	5.5 (0.3) c
110–145	11.6 (0.4) a	16.6 (0.8) b	14.6 (1.0) c
145–165	6.7 (0.5) a	9.3 (0.8) a	8.3 (0.6) a
165–220	12.0 (0.8) a	14.6 (1.8) a	15.8 (1.5) a
Alkyl/O-alkyl	0.36 (0.03) a	0.37 (0.03) a	0.56 (0.08) b

Different lowercase letters following values represent significant differences within rows.

material for successful NMR spectral readings, so pastures were excluded from analyses that compared all fraction types. Differences in normalized contribution of each spectral region to total area and differences in modeled molecular composition by forest age (including primary forests as a separate age class) and fraction type (leaf litter, free LF, occluded LF) were analyzed using a standard least squares ANOVA (SAS, Cary, NC). We also tested for differences in calculated alkyl/O-alkyl ratios (0–45 ppm/45–110 ppm), which have been proposed as an index of humification (Baldock et al., 1997). Pasture samples were included in a standard least squares ANOVA to test for differences in NMR spectral regions and molecular composition by site age and fraction type as leaf litter and occluded LF only. Significant treatment effects ($p < 0.05$) were further explored by means contrasts.

2.7. Radiocarbon analyses and modeling

Bulk soils and density fractions were analyzed for ^{14}C on a Van de Graaff FN accelerator mass spectrometer (AMS) at the Center for AMS at Lawrence Livermore National Laboratory.

Table 1
Analysis of variance (ANOVA) results for main effects and interactions of chemical parameters for leaf litter, free LF, and occluded LF among primary and secondary forests in a wet tropical forest successional chronosequence in Puerto Rico

Variable	Age				Fraction				Age × fraction			
	df	SS	F	p	df	SS	F	p	df	SS	F	p
NMR spectral results (ppm)												
0–45	3	82.1	1.436	0.27	2	151.6	3.978	0.04	6	103.4	0.905	0.90
45–65	3	24.2	0.679	0.58	2	26.8	1.124	0.35	6	54.0	0.756	0.61
65–95	3	7.6	0.074	0.97	2	425.6	5.877	0.01	6	124.8	0.574	0.75
95–110	3	5.9	2.849	0.07	2	36.0	26.240	<0.0001	6	5.2	1.254	0.33
110–145	3	23.5	1.430	0.27	2	111.3	10.170	0.002	6	9.3	0.284	0.94
145–165	3	4.3	0.326	0.81	2	29.4	3.359	0.06	6	7.6	0.287	0.93
165–220	3	4.6	0.067	0.98	2	56.0	1.212	0.33	6	67.1	0.484	0.81
Alkyl/O-alkyl	3	0.1	1.507	0.25	2	0.2	6.234	0.01	6	0.2	1.551	0.23
Molecular mixing-model results												
Carbohydrate	3	16.9	0.120	0.95	2	745.6	7.910	0.01	6	168.8	0.597	0.72
Protein	3	495.8	0.979	0.43	2	21.6	0.064	0.94	6	1231.4	1.215	0.36
Lignin	3	181.4	1.166	0.36	2	810.8	7.819	0.01	6	130.0	0.418	0.86
Lipid	3	231.9	1.752	0.20	2	325.8	3.693	0.05	6	305.5	1.154	0.38
Carbonyl	3	28.7	0.275	0.84	2	44.4	0.638	0.54	6	173.1	0.829	0.57
Char	3	9.4	1.158	0.36	2	4.9	0.903	0.43	6	6.6	0.407	0.86

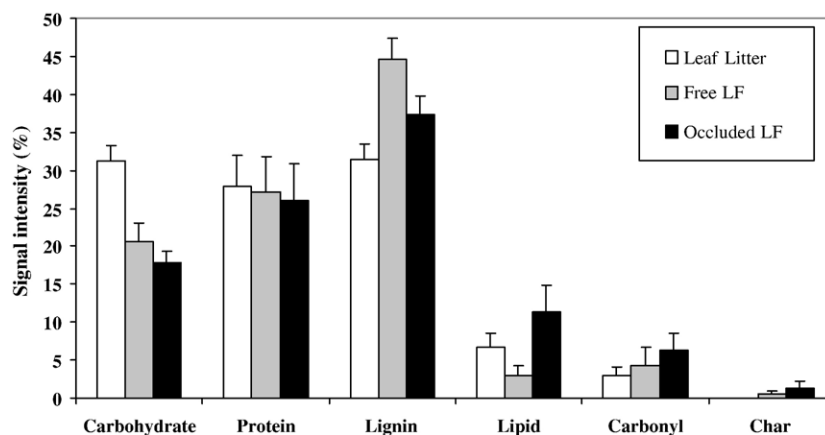df = degrees of freedom; SS = sum of squares.

Fig. 2. Relative mean (and one standard error) proportion in percent of total signal intensity of different compound types as estimated by Baldock et al.'s (2004) molecular mixing model in leaf litter, free, and occluded light fractions.

Prior to analysis, samples were combusted to CO_2 in evacuated, sealed tubes in the presence of CuO and Ag, then reduced onto Fe powder in the presence of H_2 (Vogel et al., 1984). Radiocarbon data are expressed in $\Delta^{14}C$ notation (‰):

$$\Delta^{14}C = [A_{SN}/A_{ABS} - 1] \times 1000 \qquad (1)$$

where A_{SN} is the activity of ^{14}C in the sample normalized for isotopic fractionation and decay-corrected to 1950 and A_{ABS} is the absolute international standard activity of oxalic acid (Stuiver and Polach, 1977). The A_{SN} values were normalized for isotopic fractionation (according to Stuiver and Polach, 1977) using measured $\delta^{13}C$ values analyzed on a Europa 2020 continuous flow mass spectrometer at U.C. Berkeley's Center for Stable Isotope Biogeochemistry. Samples were measured with an average AMS precision of 4‰. We report the radiocarbon and mean residence time values as means of multiple environmental replicates with associated standard errors. We

calculated turnover time for each soil C fraction using a time-dependent steady-state model (Gaudinski et al., 2000; Torn et al., 2002; Sollins et al., 2006). To estimate the ^{14}C of new plant inputs in the model, we used atmospheric $\Delta^{14}C$ records dating back 500 years. The following published sources were used to compile atmospheric $\Delta^{14}C$ records for our sites in Puerto Rico (Fig. 1): 1511–1954 (Stuiver et al., 1998); 1955–1996 (Northern Hemisphere Zone 2, which includes the Caribbean, Hua and Barbetti, 2004); 1997–2004, the year of sampling, (calculated using an 8‰ per year rate of decline in atmospheric $\Delta^{14}C$, Levin and Kromer, 2004).

At steady state conditions, turnover time is equivalent to mean residence time (MRT). The MRT of a pool of soil C atoms is the mean time a C atom has resided in the soil reservoir when it leaves the reservoir (Trumbore, 2000). Our model parameterization did not account for multiple year lag-times between C fixation and inputs into the soil C pool, so MRTs are maximum values (Torn et al., 2005; Sollins et al., 2006). We calculated the

Table 3

Analysis of variance (ANOVA) results for main effects and interactions of chemical parameters for leaf litter and occluded light density soil C fractions across a wet tropical forest land-use chronosequence in Puerto Rico, including pastures, secondary and primary forests

Variable	Age				Fraction				Age × fraction			
	df	SS	F	p	df	SS	F	p	df	SS	F	p
NMR spectral results (ppm)												
0–45	4	329.7	3.231	0.04	1	81.4	3.192	0.10	4	13.1	0.129	0.97
45–65	4	58.6	1.080	0.40	1	1.4	0.102	0.75	4	49.9	0.920	0.48
65–95	4	457.2	3.419	0.04	1	460.5	13.776	0.002	4	18.6	0.139	0.97
95–110	4	35.4	6.205	0.004	1	47.4	33.213	<0.0001	4	1.8	0.307	0.87
110–145	4	22.1	0.806	0.54	1	45.0	6.273	0.03	4	9.6	0.349	0.84
145–165	4	18.6	1.544	0.24	1	17.1	5.662	0.03	4	5.8	0.485	0.75
165–220	4	119.0	2.553	0.09	1	96.2	8.255	0.01	4	13.3	0.286	0.88
Alkyl/O-alkyl	3	0.4	5.452	0.007	2	0.2	10.504	0.006	6	0.0	0.410	0.80
Molecular mixing-model results												
Carbohydrate	4	867.3	4.038	0.02	1	866.1	16.129	0.001	4	26.4	0.123	0.97
Protein	4	431.3	0.525	0.72	1	12.3	0.060	0.81	4	569.1	0.692	0.61
Lignin	4	280.8	1.668	0.21	1	95.1	2.260	0.16	4	101.2	0.601	0.67
Lipid	4	628.6	3.586	0.03	1	125.2	2.857	0.11	4	106.0	0.605	0.67
Carbonyl	4	109.6	1.218	0.35	1	64.3	2.858	0.11	4	50.7	0.564	0.69
Char	4	15.7	0.607	0.66	1	15.9	2.472	0.14	4	15.7	0.607	0.66

df = degrees of freedom; SS = sum of squares.

MRT of each density fraction using a mixing model that incorporated as constraints the amount of C recovered in each fraction as a proportion of bulk C (Marín-Spiotta et al., in review) and the ^{14}C fraction modern (A_{SN}/A_{ABS}) values of bulk soils. Modeling samples that contain significant bomb C is complicated because the same ^{14}C abundance can yield two different MRTs, a longer one reflecting the increasing side of the atmospheric Δ^{14}C curve, and a shorter one reflecting the decreasing side of the curve (Trumbore, 2000). For example, a Δ^{14}C value of 79.2‰ can yield a modeled MRT of 3 years as well as 110 years. Other information about each particular site, such as C input rates, were used to constrain C turnover times, since archived samples were not available (*sensu* Trumbore, 2000; Hahn and Buchmann, 2004). To estimate aboveground C inputs, we used measured annual litterfall rates at the forested sites (Ostertag et al., in preparation), and literature values for aboveground litter production in comparable tropical pastures (Wilsey et al., 2002). To estimate belowground C inputs, we used published values of fine root turnover from tropical forest soils (Ostertag, 2001; Silver et al., 2005). To address some of the uncertainties involved in estimating total C inputs for our sites, we produced a range of input rates under three different scenarios: a low estimate, where all inputs came from roots only with the lowest value of published root turnovers; a medium estimate using an average of root turnover values; and a high estimate assuming the highest value for root turnover and 10% of total aboveground annual litter production contributing to soil C stocks annually. These estimates were then used to ensure that the C inputs calculated from the numerous possible modeled MRTs were reasonable for each site. We measured ^{14}C concentrations in the three fractions generated from one soil sample per site across our chronosequence. Differences in mean ^{14}C concentrations and mean residence times of soil fractions were analyzed as a split-plot nested design using the standard least squares and Restricted Maximum Likelihood Method, with age as the main plot, site as the block, and soil fraction as the subplot using JMP 5.1 software (SAS, Cary, NC). Significant differences ($p < 0.05$) were further explored by means contrasts. Relationships between MRT and NMR spectral regions and modeled compound types were tested by linear regression within each fraction type.

3. Results

3.1. Carbon chemistry

Significant differences in NMR spectral intensities and modeled molecular composition occurred only with fraction type (leaf litter, free LF, or occluded LF) among the forested sites (Tables 1 and 2). Forest age had a marginally significant effect ($p = 0.07$) only on the area between 95–110 ppm, with the main difference occurring between primary and secondary forests. There was no significant interaction between forest age and fraction type. NMR spectra differed significantly with fraction type except for the areas between 45–65 and 165–220 ppm. Alkyl-O/alkyl ratios were significantly higher in the occluded LF than in the free LF and leaf litter (Table 2). Modeled carbohydrate

content was higher in the leaf litter than in the soil fractions (Fig. 2). Lignin content was highest in the free LF. Lipid content was significantly higher in the occluded than in the free LF. There were no significant differences in protein, carbonyl, or char content as determined by the MMM (Fig. 2).

When pastures were included in the ANOVA comparing leaf litter to the occluded LF only, both fraction type and site age had significant effects on many of the C chemistry parameters (Table 3). There was no significant interaction between age and fraction type. Occluded LFs had higher intensities in the 110–145, 145–165, and 165–220 ppm regions than did leaf litter. Occluded LF also had higher intensities in the 0–45 ppm region at the $p = 0.10$ level. Modeled carbohydrate content was significantly higher in leaf litter than in the occluded LF, consistent with greater intensities in the 65–95 and 95–110 ppm regions. Protein content was highly variable and showed no differences between fraction types. Modeled lignin, lipid, carbonyl, and char contents were higher in the occluded LF than in leaf litter at the $p = 0.16$ and $p = 0.11$ levels, consistent with significant differences in the NMR spectral intensities. Only six
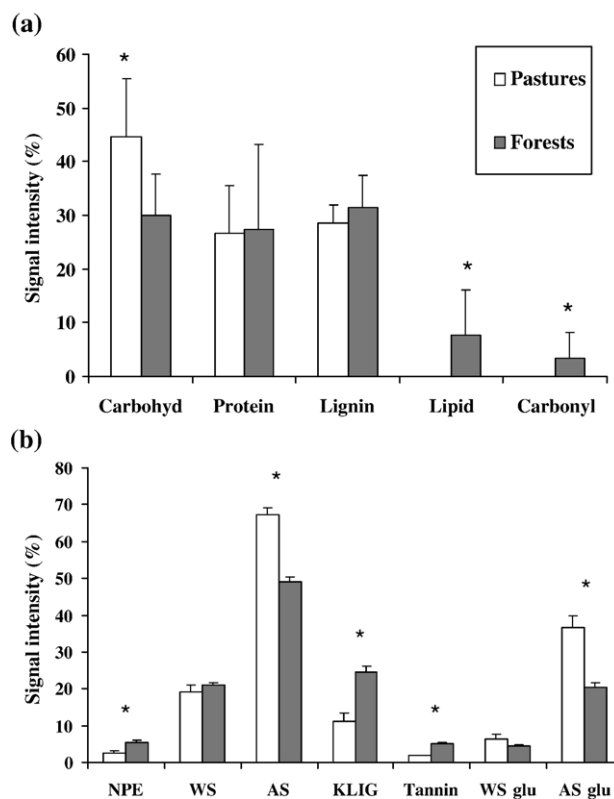

Fig. 3. Aboveground litter chemistry differences between active pastures and wet tropical forests in southeastern Puerto Rico. Top panel (a) represents the relative proportion of main compound types (in % of total signal intensity) estimated by a molecular mixing model which integrates values from ^{13}C NMR spectra (Baldock et al., 2004). Bottom panel (b) represents percent mass recovered in sequential C fractions by a wet chemistry analysis. Abbreviations: carbohydrate (carbohyd), non-polar extractives (NPE), water-soluble (WS), acid-soluble (AS), Klason lignin (KL), water soluble polyphenols as tannins, water-soluble (WS) glucose, and acid-soluble (AS) glucose. All values are means (and one standard error). Forest values are averages across secondary and primary forest sites. All differences shown between land-cover type within each compound type are significant at $p < 0.05$ value.

of the 22 soil C fractions analyzed by ^{13}C NMR spectroscopy showed any evidence for char according to the MMM: two pasture sites, two 10-year old secondary forests, one 20-year old, and one primary forest. The highest concentration of char (10% of total spectral intensity) was in an occluded LF from an active pasture site. All other samples with char had contributions of ≤6% to the total signal intensity. The MMM did not find char in any of the leaf litter samples.

Differences in the NMR spectral intensities of leaf litter and occluded LF with site age were likely driven by differences in plant cover type. Pasture litter had greater intensities in the 65–95 and 95–100 ppm regions, while forest litter had greater intensity in the 0–45 ppm. This is consistent with differences found between pasture and forest litter by the sequential C fraction extractions (Fig. 3). Pasture tissues were dominated by carbohydrates, while forest litter had a greater proportion of NPE, tannin, and KL fractions, as well as modeled lipid fractions. Forest sites showed similar litter chemistries, regardless of successional stage, differing only in WS and WS glucose at the $p = 0.06$ level (data not shown).

Visual comparison of ^{13}C NMR spectra for samples collected from the same site revealed differences in the relative contribution of C types to total signal intensity (Fig. 4). Free and occluded LFs had higher contributions from the 0–45 and 110–160 ppm areas relative to leaf litter spectra, which were dominated by a strong peak at 72–74 ppm, primarily attributed to plant cellulose (Preston et al., 1997). The shoulders on the large peak at 72–74 ppm became more pronounced in the soil fractions, including a peak at 56 ppm, which is an indicator of lignin (Preston et al., 1997). The greatest gain in intensity between soils and litter
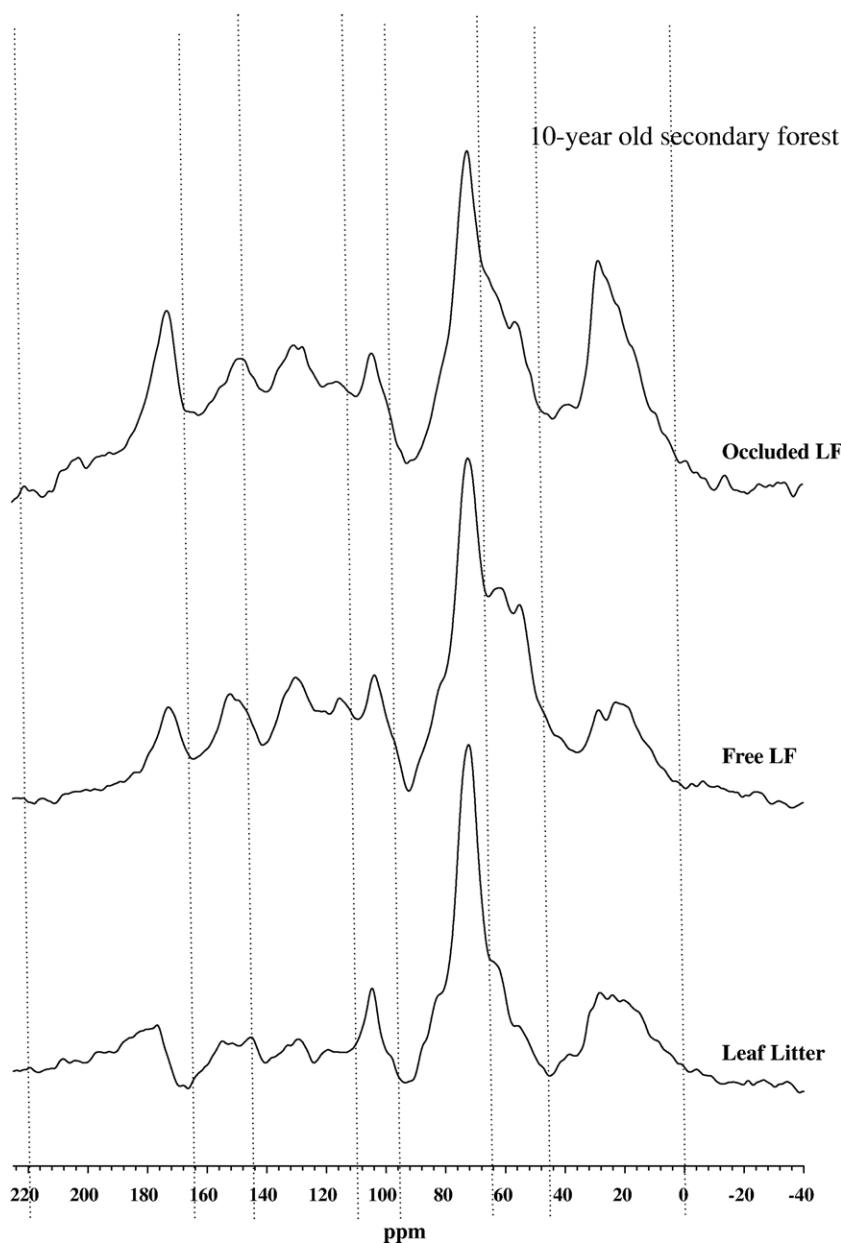

Fig. 4. Representative CP-MAS ^{13}C NMR spectra of leaf litter, free, and occluded light soil C fractions from a 10-year old secondary forest.

Table 4
Radiocarbon concentrations (Δ^{14}C, ‰) of soil C density fractions and bulk soils across a reforestation chronosequence on abandoned pastures in Puerto Rico

Site	Free light	Occluded light	Heavy
Active pastures	69 (13) aA	91 (8) bA	86 (18) bA
Secondary forests			
10-year old	91 (11) aB	87 (7) abA	96 (3) bAB
20-year old	93 (5) aB	131 (15) bB	130 (4) bC
30-year old	78 (5) aB	109 (3) bB	113 (1) bBC
80-year old	93 (5) aB	121 (7) bB	126 (7) bC
Primary forests	99 (7) aB	144 (7) bC	129 (4) bC

Values represent means (and one standard error) of site replicates ($n=3$, except 20-year olds, $n=2$).
Different lowercase letters following values represent significant differences within rows.
Different capital letters following values represent significant differences within columns.

spectra was in the alkyl C region, at 28–30 ppm, a peak attributed to long-chain aliphatics (Kögel-Knabner et al., 1992). Another notable difference between leaf litter and soil fractions, and between the free and the occluded LF, was an increase in the intensity of the peak at around 170 ppm, which has been attributed to carboxyl groups in organic acids or amide groups in peptides (Quideau et al., 2001).

3.2. Radiocarbon

All samples had positive Δ^{14}C, indicating significant contributions of bomb C, i.e., C fixed in the last 45 years (Table 4). Type of density fraction significantly affected ^{14}C concentrations ($p<0.0001$), as did site age ($p=0.003$). There was also a significant interaction between site age and density fraction type ($p=0.03$) (Table 5), indicating that Δ^{14}C changed differently in the fractions as site age increased. Free LF in the pastures had lower Δ^{14}C values than the forests (Table 4). Occluded LF in the active pastures was similar to the 10-year old forests and both were significantly lower than all other forest sites. Heavy fractions followed a similar pattern.

MRT for the free LFs averaged 4 ± 1 years, with the exception of one pasture free LF which had a turnover time of 171 years (Table 6). Based on our model, there were two possible turnover times calculated for the occluded LF, one averaging 7 ± 1 years, hereafter referred to as the fast-cycling occluded LF, and one 77 ± 5 years, or slow-cycling. MRTs for the HFs averaged 73 ± 4 years. We ran two different split-plot ANOVA analyses, one including the free LF outlier and one

Table 6
Average mean residence times (MRT) (and standard error) in years for free light fraction (LF), occluded LF (both slow- and fast-cycling, see text for discussion), and heavy fraction (HF) across a chronosequence of secondary forests regrowing on abandoned pastures

	Free LF	Occluded LF fast	Occluded LF slow	Heavy F
Active Pastures	3.1 (1.3) aA; *59.0 (55.9)*	4.6 (1.1) aA	95.5 (11.9) bA	107.6 (21.9) bA
Secondary Forests				
10 yr	4.4 (1.6) aA	4.1 (1.1) aA	100.8 (8.3) bA	90.1 (3.4) bB
20 yr	4.5 (0.9) aA	8.0 (0.3) aA	70.8 (1.9) bB	63.1 (2.5) bC
30 yr	2.6 (0.9) aA	7.3 (0.3) aA	77.8 (2.7) bB	73.9 (0.8) bC
80 y	5.0 (0.7) aA	8.5 (0.9) aA	67.5 (5.7) bB	64.0 (4.8) bC
Primary Forests	5.7 (0.9) aA	11.5 (1.0) aA	51.1 (4.7) bC	61.4 (3.2) bC

MRT in italics for free LF from active pastures includes a sample with charcoal.
Different lowercase letters following values represent significant differences within rows.
Different capital letters following values represent significant differences within columns.

without it (Table 5). In the first case (MRT 1), only fraction type had a significant effect on MRT ($p<0.0001$). In the second case (MRT 2), all treatment effects were significant. Fraction type had the strongest effect on MRT ($p<0.0001$). Free LFs cycled at a significantly faster rate than did the HF and the slow-cycling occluded LF, and did not differ from the fast-cycling occluded LF. There was also a significant interaction effect between site age and fraction type on MRTs (Table 5), reflecting that free LF MRT did not vary across the chronosequence, but that MRT of the slow-cycling occluded LF and HF did (Table 6). MRT of the fast-cycling occluded LF was not different statistically across sites, although the active pastures and 10-year old secondary forests had MRTs that were on average half that of the occluded LFs in the other forests. The pasture and 10-year old secondary forest slow-cycling occluded LF MRTs were significantly longer than that of the secondary forests ≥20 years, which also differed from the primary forests. Active pasture HF MRTs were significantly longer than all forest sites, and the 10-year old secondary forest HF also differed from the other forests. There were no relationships between density fraction MRT and ^{13}C NMR spectral regions or modeled compound types except for the free LF, where MRTs increased with increasing lignin content (r-square$=0.46$, $p=0.06$).

Table 5
Analysis of variance (ANOVA) results for main effects and interactions of radiocarbon and mean residence times (MRT) across a wet tropical forest land-use chronosequence in Puerto Rico, including pastures, secondary and primary forests

Variable	Age				Fraction				Age × fraction			
	df	SS	*F*	*p*	*df*	SS	*F*	*p*	*df*	SS	*F*	*p*
^{14}C	5	3217	7.153	0.003	3	8212	30.434	<0.0001	15	2970	2.202	0.03
MRT 1	5	3689	1.932	0.17	3	72,981	63.7	<0.0001	15	7968	1.3906	0.21
MRT 2	5	2444	5.13	0.01	3	79,566	278	<0.0001	15	6050	4.24	0.0004

MRT 1 includes a free LF outlier, and MRT 2 excludes this outlier MRT from the analysis. See text for discussion.
df=degrees of freedom; SS=sum of squares.

4. Discussion

4.1. Differences in C chemistry from litter to SOM

Differences observed in the ^{13}C NMR spectra between litter and soil density fractions reflect chemical transformations corresponding to the processes of litter decay and SOM formation. The decreasing contribution of O-alkyl C (65–95 ppm) from fresh leaf litter to the soil C fractions represents a breakdown of plant cellulose and hemicellulose during microbial decomposition. The loss of carbohydrate signature in the soil C fractions was accompanied by a gain in signal intensity in the alkyl C (0–45 ppm), aromatic (110–145 ppm), and phenolic C (145–165 ppm) regions. The accumulation of these C types in SOM may be due to selective preservation of recalcitrant plant compounds, such as waxes, resins, cutin, suberin, and terpenoids, and/or to new production during microbial processing (Kögel-Knabner et al., 1991, 1992; Baldock et al., 1992; Fogel and Tuross 1999). Alkyl C structures, in particular, which represent non-polar compounds generally categorized as soil lipids, are thought to be some of the most recalcitrant components of SOM (Kögel-Knabner et al., 1992; Baldock et al., 1992). It is unknown what proportion of soil lipids is directly derived from plants and how much is produced by microbes and fungi (Braids and Miller, 1975; Dinel et al., 1990; Gleixner et al., 2001). The assignment of lipids by the MMM to some of our soil C fractions at sites where leaf litter did not have measurable lipid content supports *de novo* synthesis during humification. Roots could also contribute lipids to occluded LF, although we did not find differences in concentrations of non-polar extractable fractions between leaf and root tissues (Ostertag et al., in preparation). The trend of decreasing O-alkyl C and increasing alkyl C with decomposition has been reported for many different soil types, suggesting that the ratio between these two NMR regions is a useful index of decomposition (Baldock et al., 1992, 1997; Baldock and Preston, 1995; Golchin et al., 1995; Quideau et al., 2000; Helfrich et al., 2006).

Chemical differences between the free and occluded LFs provide evidence that they represent distinct soil C pools along a continuum of decay (Baldock et al., 1992; Golchin et al., 1994b; Proirier et al., 2005; Sohi et al., 2005). Free LF had higher aromatic C intensities, while the occluded LF had higher alkyl C. Alkyl-to-O-alkyl C ratios were greater and more variable in the occluded LF, suggesting that SOM inside aggregates represents SOM in varying degrees of decay (Golchin et al., 1994a). According to the Golchin et al. (1994a) microaggregate turnover model, the free LF becomes incorporated into aggregates during early stages of decay. Before occlusion, the unattached, inter-aggregate soil C would resemble fresh litter inputs, which show more homogeneous composition within a land-cover type, than the generally more decomposed aggregate-associated SOM. Higher alkyl C and lipid contents in the occluded LF are also consistent with increased microbial and fungal contributions. Higher modeled lignin content in the free LF than the occluded LF could be due to a greater presence of lignified roots in the former, or to progressive loss of plant lignin as SOM becomes more decomposed. Lignin identified by NMR is relatively de-composable in soils but has been shown to contribute significantly to partially decomposed residues, albeit with some modification (Kögel-Knabner et al., 1991; Baldock et al., 1992; Preston et al., 2002). A significant positive relationship between lignin concentrations and MRT for our free LF samples suggests that lignin may be involved in slowing decomposition at least in the initial stages of litter decay, even if it may not persist in later stages.

The modeled protein component did not show any trends with fraction type or site age. Though the controls on organic N cycling are not well understood, there has been recent evidence for stabilization of peptide-like structures by mineral surfaces (Gleixner et al., 1999; Knicker and Skjemstad, 2000; Sollins et al., 2006; Kleber et al., 2007). The increase in peak intensity at about 173 ppm, attributed to carbonyl groups, in our occluded LF samples relative to the free LF and leaf litter could reflect an increase in peptide content in the intra-aggregate fractions (Quideau et al., 2001). Future insights into organic N transformations with decomposition and stabilization may be gained by the application of ^{15}N NMR spectroscopy (Kögel-Knabner, 1997).

4.2. Litter and SOM chemistry with land-cover change

Differences between pasture and forest leaf litter chemistry were detected by both CP MAS ^{13}C NMR and more traditional wet chemistry sequential C extractions. Forage grasses were dominated by carbohydrates, which are relatively easy to decompose, while forest leaf litter had significant amounts of more recalcitrant compounds, such as tannins and lipids. Contrary to our expectations, we did not observe variations in leaf litter chemistry between forest types. Although tree species composition changed during secondary succession (Marín-Spiotta et al., 2007), litter inputs to SOM appeared to be chemically homogeneous once forest cover was established. Soil C chemistry also did not vary with forest age. Differences in soil C chemistry were driven by differences between leaf litter, free LF and occluded LF, suggesting that SOM formation processes are similar across forest sites with different species composition. Pasture and forest SOM differed chemically, reflecting aboveground litter, but fraction type was still the dominant factor. Greater differences between soil C fractions than between land use or land-cover types have also been reported for other soils (Guggenberger et al., 1995).

4.3. Rates of soil C turnover

Given the nature of the bomb curve, soil samples collected after 1964 with Δ^{14}C values greater than those of the contemporary atmosphere can often yield two mathematically viable turnover times (Trumbore, 2000). Combining information on ecosystem productivity and C and ^{14}C mass balance allowed us to make reasonable choices from among the possible turnover times. Using C input estimates, we were able to constrain MRT for the HF from several decades to a century, which is in the same range as mineral-associated fractions in surface horizons of other tropical soils (Trumbore, 1993; de Camargo et al., 1999).

In some instances, information on C inputs were insufficient to discriminate between possible turnover estimates generated from the same $\Delta^{14}C$ value, likely due to the very small size of the light density fractions. Widely different inputs into these two pools appeared to have little effect on the overall C balance, presumedly because more than 80% of the bulk C was associated with the HF (Marín-Spiotta et al., in review). Our model yielded two possible MRT for most free LF samples, <10 years or 100 years. Except for samples where the MMM detected char content, we believe it less likely that the free LF would turn over on average more slowly than the HF. In fact, most studies report free LF material in tropical soils with MRT of <10 years (Trumbore et al., 1995; de Camargo et al., 1999; Trumbore, 2000), consistent with fast decomposition rates in tropical soils. Interactions with mineral surfaces, especially aluminum and iron oxides in highly-weathered acid soils, is a well-documented important mechanism for SOM stabilization, and most studies report slower cycling rates for mineral-associated C than the free, particulate C fraction (Oades, 1988; Trumbore and Zheng, 1996; Zech et al., 1997; Feller and Beare, 1997; Kaiser et al., 2002; Kalbitz et al., 2005; Mikutta et al., 2006). In our soils, a lower positive $\Delta^{14}C$ value was typically more suggestive of more recent atmospheric inputs, while a higher value represented older material. Free LF $\Delta^{14}C$ concentrations were lower than for both the occluded LF and HF, suggesting that SOM exterior to soil aggregates and not attached to mineral surfaces had a shorter MRT. This interpretation is supported by previous studies measuring ^{14}C in soil density C fractions, which showed the isotopic content of the free LF to be most responsive to recent C inputs in temperate forests (Swanston et al., 2005) and in grasslands (Baisden et al., 2002b). One pasture free LF sample, however, yielded only one possible MRT >100 years. This slow turnover rate is probably due to the presence of fine charcoal, or char, which can be resistant to decay (Skjemstad et al., 1996). While we attempted to remove visible pieces of charcoal from our samples before grinding, because we were trying to isolate the fast-cycling soil C pool, the unexpected high MRT for the free LF is evidence that the removal process was inadequate for this particular sample. A more thorough and quantitative chemical removal process (Skjemstad et al., 1996; Krull et al., 2006) should be coupled with density fractionation before radiocarbon analyses in soils where char may be abundant. Another possible explanation for the high MRT in this free LF is the presence of lipids, which can also have very long ^{14}C-based MRTs (Huang et al., 1999).

Using information on total C input rates to the sites did not help us constrain MRT of the occluded LF, also a small contributor to the bulk C pool (Marín-Spiotta et al., in review); thus, we reported two possible scenarios, one fast-cycling and one slow-cycling. The fast-cycling scenario (ranging from 3–14 years) makes the occluded LF equal or similar to the free LF MRT, and both turning over significantly faster than the HF. This would suggest that occlusion within aggregates affords very little protection from decomposition in these soils. The slow-cycling, and more likely, scenario for the occluded LF (40–100 years) would give it similar turnover times to the HF,

and different from the free LF. Our fractionation method was designed to separate light density material in different locations in the soil matrix; the free LF was floated in a dense liquid without disrupting the soil structure, while the occluded LF was floated after vigorous mechanical shaking and sonication to disperse aggregates (Swanston et al., 2005). Chemical differences between the free and occluded LFs, as determined by ^{13}C NMR and radiocarbon concentrations (this study), as well as by stable isotopes and elemental C and N composition (Marín-Spiotta et al., in review), strongly suggest that the occluded LF represents SOM in a later stage of decay than the free LF. Other studies have reported longer turnover times for the occluded LF than for the free LF, but shorter than for the mineral-associated C (Golchin et al., 1995; Swanston et al., 2005; Rasmussen et al., 2005), consistent with a three pool model, in which the free LF represents the active C pool, the occluded LF the slow pool, and the HF or mineral-associated fraction the passive C pool. Baisden et al. (2002b) found no difference in MRT between aggregate and mineral-associated C in a temperate grassland. The importance of soil aggregation in C stabilization is influenced by many factors, including soil type, mineral composition, and disturbance effects (Oades and Waters, 1991; Golchin et al., 1994a; Baldock and Skjemstad, 2000).

4.4. Soil C turnover with land-cover change

Turnover times for the slow-cycling occluded LF and the HF were significantly shorter in the primary forests than in the active pastures. We would have expected the reverse, with potential higher rates of soil surface disturbance in active pastures due to cattle. Higher earthworm densities have been reported in active pastures than forests in this region (Zou and González, 1997; Sánchez-de León et al., 2003). Earthworm density also decreased with secondary forest age on abandoned pastures, with the lowest numbers found in mature forests. This would imply increased physical mixing of the surface horizons, and potential destabilization of aggregates in the youngest forests. High inputs of labile, carbohydrate-rich litter coupled with fast decomposition rates of the organic cores of soil aggregates in the pastures would also be expected to lead to faster rates of aggregate formation and destruction (Golchin et al., 1994a). However, slower radiocarbon MRTs for disturbed, agricultural soils have also been reported (Wang et al., 1999). This is attributed to the preferential loss of labile C in disturbed soils. At our sites, pasture litter was composed of more easily degradable compounds (this study), and we have also found higher C mineralization rates in pasture soils during two separate soil incubation studies (K. Shamieh and F. Hopkins, unpublished data), and lower free LF stocks in pastures compared to forested sites (Marín-Spiotta et al., in review). Faster cycling of the labile pool would leave the more stabilized, slow-turnover SOM pools as the dominant stock, resulting in higher MRT in the active pastures. Turnover times for the occluded LF in the primary forests differed from secondary forests, suggesting there may be long-term effects of changes in land use or species composition on soil aggregate dynamics. However, soil carbon turnover rates for the mineral-associated C in our secondary forests recovering

from abandoned pasture in Puerto Rico resembled those of primary forests in as little as 20 years of succession.

5. Conclusions

Using ^{13}C NMR spectroscopy and more traditional measures of litter chemistry, we showed that grass litter was composed of more chemically labile compounds than forest litter. However, litter chemistry among secondary forests of different ages did not differ, contrary to what might be expected based on theories of plant–herbivore interactions during forest succession. The physical density fractionation scheme we employed yielded soil C fractions with different chemical makeup and residence times in the soil, which represent a progression of decomposition from the free LF, to the occluded LF, to the HF. ^{13}C NMR spectroscopy was a useful tool for tracking chemical transformations during the decomposition of plant litter and formation of SOM. The free and occluded LF were made up of different proportions of C compounds, and in some cases, the occluded LF accumulated compounds, such as lipids, not observed in the leaf litter or free LF, suggesting the incorporation of newly synthesized compounds during microbial decomposition. Soil disturbance from grazing and land-cover change affected C turnover, mostly through the preferential loss of more labile C, and the resulting dominance of residual soil C pools with high mean residence times. Soil C turnover times were shorter than those reported for temperate studies, and support observations of faster C cycling rates in tropical soils. Soil organic matter dynamics of the heavy, mineral-associated pool in abandoned pastures recovered under natural forest regeneration within 20 years.

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