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Soil organic matter dynamics during 80 years of reforestation of tropical pastures

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Abstract

Our research takes advantage of a historical trend in natural reforestation of abandoned tropical pastures to examine changes in soil carbon (C) during 80 years of secondary forest regrowth. We combined a chronosequence approach with differences in the natural abundance of ^{13}C between C3 (forest) and C4 (pasture) plants to estimate turnover times of C in the bulk soil and in density fractions. Overall, gains in secondary forest C were compensated for by the loss of residual pasture-derived soil C, resulting in no net change in bulk soil C stocks down to 1 m depth over the chronosequence. The free light fraction (LF), representing physically unprotected particulate organic matter, was most sensitive to land-use change. Reforestation replenished C in the free LF that had been depleted during conversion to pastures. Turnover times varied with model choice, but in general, soil C cycling rates were rapid for the 0–10 cm depth, with even the heavy fraction (HF) containing C cycling in decadal time scales. Turnover times of C in the free LF from the 0–10 cm depth were shorter than for the occluded and HFs, highlighting the importance of physical location in the soil matrix for residence time in the soil. The majority of the soil C pool (82 ± 21%) was recovered in the mineral-associated density fraction. Carbon-to-nitrogen ratios and differences in natural abundance ^{15}N of soil organic matter (SOM) showed an increasing degree of decomposition across density fractions with increasing mineral association. Our data show that the physical distribution of C in the soil has a large impact on soil C turnover and the ability of soils to maintain SOM stocks during land-use and land-cover change.

Keywords: carbon-13, density fractionation, nitrogen-15, Puerto Rico, secondary forests, succession

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Introduction

Secondary forests are a dominant forest cover type in the tropics (Brown & Lugo, 1990a; Aide & Grau, 2004). Reforestation of former agricultural and pasturelands provides opportunities for carbon (C) sequestration and for the restoration of forest ecosystem goods and services (Lugo & Brown, 1992; Metz *et al*., 2001). Although secondary forests can achieve structural characteristics of old-growth forests in as little as 20 years (Guariguata & Ostertag, 2001), less is known about long-term controls on soil C dynamics during succession. The world's soils store two to three times more C than is found in aboveground biomass, thus changes in soil C have the potential to greatly impact atmospheric CO_2 concentrations (Post *et al*., 1982; Detwiler, 1986; Trumbore *et al*., 1996; Batjes & Sombroek, 1997).

Effects of reforestation on soil C pools are varied. Studies have reported accumulation (Rhoades *et al*., 2000; Guo & Gifford, 2002; de Koning *et al*., 2003; Silver *et al*., 2004; López-Ulloa *et al*., 2005; Lemma *et al*., 2006), losses (de Koning *et al*., 2003; Bautista-Cruz & del Castillo, 2005; López-Ulloa *et al*., 2005), or no net change (Hughes *et al*., 1999; Guo & Gifford, 2002). Most studies have focused on the first 20 years of succession (but see Hughes *et al*., 1999), which may not be long enough to capture the trends in long-term soil C dynamics (Silver

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et al., 2000). Detecting changes in the large stock of soil C typical of tropical forests can be difficult. A number of physical and chemical methods have been developed to separate the bulk pool into fractions that differ in chemical composition and/or location in the soil matrix (e.g., Elliott & Cambardella, 1991; Golchin *et al.*, 1994a, b; Trumbore & Zheng, 1996; Christensen, 2001). Turnover times of these fractions can range from a few years to centuries or millennia due to differences in their chemical and physical stability (Jenkinson & Rayner, 1977; Parton *et al.*, 1987; Sollins *et al.*, 1996).

Natural abundance stable C isotopes, coupled with a chronosequence approach, can be used to estimate rates of soil C accumulation and loss when land-use change results in a shift in the photosynthetic strategy of the dominant plant species between C4 and C3 photosynthesis (Balesdent *et al.*, 1987; Vitorello *et al.*, 1989; Bernoux *et al.*, 1998; López-Ulloa *et al.*, 2005). These pathways differ in their C isotope fractionation and result in litter, and ultimately soil organic matter (SOM), with distinct isotopic signatures. Stable C and nitrogen (N) isotopes can also be used to estimate the degree of decomposition and humification of SOM. Bulk soils in undisturbed, well-drained profiles typically exhibit enrichment in ^{13}C and ^{15}N with depth, which can be attributed to isotopic fractionation during litter decomposition (Connin *et al.*, 2001) and SOM formation (Nadelhoffer & Fry, 1988; Ehleringer *et al.*, 2000).

In this study, we combined a long-term chronosequence approach with soil density fractionation and stable isotope analyses to examine belowground C dynamics after pasture abandonment and during 80 years of secondary succession in Puerto Rico. We hypothesized that soil C derived from forest trees accumulates rapidly in the free light fractions (LFs) and occluded LFs during secondary succession, and helps offset losses of pasture-derived C. We also predicted that the proportion of soil C stored in the pools with longer turnover times (>10 years) would increase with time since reforestation, due to the expected increase in litter quantity and production of more recalcitrant litter with tropical secondary succession (Feeny, 1976; Coley, 1983; Coley *et al.*, 1985; Coley & Aide, 1991), resulting in a net accumulation of soil C.

Methods

Site description

We conducted this study on private land in the Sierra de Cayey in southeastern Puerto Rico (18°01′N, 66°05′W), between 580–700 m above sea level, in the subtropical wet forest life zone (Ewel & Whitmore, 1973). Sites were chosen to control for parent material and soil type. Soils are well-drained silty clay loams classified as very fine, mixed, isothermic Inceptic Hapludox in the Los Guineos series (Lugo-López *et al.*, 1995). Mean annual temperature is estimated at 21–22 °C with little annual variation, and mean annual precipitation is approximately 2000 mm (Daly *et al.*, 2003; SERCC, 2006). Potential forest vegetation types in the region are lower montane wet evergreen forest, tall cloud forest, and palm breaks (Helmer *et al.*, 2002).

Our study takes advantage of a historical trend in postagricultural natural reforestation in Puerto Rico (Pascarella *et al.*, 2000; Grau *et al.*, 2003). The mountainous regions of Puerto Rico represent a shifting mosaic of land uses due to the typically small size of land holdings and multiple waves of human migration, which have led to a network of patches of land in different stages of cultivation or abandonment (Thomlinson *et al.*, 1996; Helmer, 2004). Because of the small size of farmsteads, ranching practices have changed little over the last century and involve little or no mechanization (Domínguez Cristóbal, 2000). With the use of a time sequence of aerial photographs and interviews with local landowners and neighbors, we identified a chronosequence consisting of replicate sites of primary forests, pastures, and secondary forests regrowing on pastures abandoned 10, 20, 30, 60, and 80 years ago ($n = 3$ sites for all ages, except $n = 2$ for 20-year secondary forests). We use the term 'primary' forest to refer to remnant forest fragments that in the very least had not been under pasture cover for the last century (Pascarella *et al.*, 2000), if ever, instead of the term 'mature' as we have previously reported similar forest structure for all forests older than 20 years (Marin-Spiotta *et al.*, 2007). Reforestation in this area has occurred through natural regeneration. Tree species composition across the study chronosequence differed greatly, with the youngest secondary forests dominated by ferns and the trees *Syzygium jambos* and *Inga laurina* (Marin-Spiotta *et al.*, 2007). The 20- and 30-year-old sites were dominated by *Tabebuia heterophylla*, and the 60- and 80-year-old secondary forests had a more diverse community of tree species as well as retaining species from earlier ages. The primary forests were dominated by the sierra palm, *Prestoea montana*. Pasture sites were actively grazed at the time of sampling. Dominant forage grass species in the study region included *Axonopus compressus*, *Panicum laxum*, and *Sporobolus jacquemontii* (Sánchez-de León *et al.*, 2003).

Soil sampling and fractionation

Bulk soils were collected at 10 cm intervals to a 1 m depth from three pits dug at each site in 2001–2002 ($n = 3$ soil samples per depth per site). At one pit per site, a quantitative core (6.1 cm diameter) was taken from each depth for bulk density. Soils were air-dried and a subsample was ground to fine powder in a mortar and pestle for chemical analyses. No carbonates were detected after treatment with 5% HCl (Nelson & Sommers, 1996), so all C was assumed to be organic. Soil texture was analyzed using the hydrometer method for one air-dried bulk soil sample (0–10 cm depth) per site, distinguishing the following particle sizes: clay (<0.002 mm), silt (0.002–0.05 mm), and sand (0.05–2 mm) (Gee & Bauder, 1986).

Soils for density fractionation were collected in duplicate in 2004 from the 0–10 cm soil depth at each site ($n = 2$ soil samples per site). We made an *a priori* decision to exclude the 60-year-old secondary forest samples from density fractionation analyses 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 from each soil sample after oven-drying at 105 °C. Soils were stored at 4 °C until processed. The fractionation scheme we employed separates the bulk SOM into (1) a free LF, composed of interaggregate, unattached, particulate OM, representing physically unprotected SOM pool, turning over on short time scales; (2) an occluded LF released from the physical disruption of soil aggregates, representing C protected from decomposition by incorporation into aggregates, typically with intermediate turnover times; and (3) a mineral-associated heavy fraction (HF) representing physically stabilized SOM, turning over on longer time scales. We followed the procedure described by Swanston *et al*. (2005) with slight modifications, provided below. We used fresh, moist soils instead of air-dried soils to better mimic field conditions and to minimize preprocessing disturbance to aggregated structure. In preparation for fractionation, soils were passed through a 4.75 mm sieve. A 5 g subsample was taken to correct initial soil masses for moisture content. The free LF was separated from undisturbed soils by floatation in a heavy liquid, the occluded LF was floated after mechanical mixing and sonication to break apart soil aggregates, and the HF was collected with the remaining dense soil pellet. We used sodium polytungstate (NaPT, $Na_6[H_2W_{12}O_{40}]$, Sometu-US, Van Nuys, CA, USA) at a density of $1.85\,g\,mL^{-1}$ as the heavy liquid. After centrifugation, and before aspiration of the supernatants, samples were allowed to sit overnight. 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. The fractions were oven-dried overnight at 105 °C and ground to fine powder for chemical analyses. We recovered approximately 93% of the original soil mass; the residual mass 'loss' was more likely derived from variability in the dry weight conversion of the aggregated soil than from actual soil loss during sample processing. To correct for this, we calculated individual fractions as percentages of the final soil mass (i.e., sum of the three recovered fractions) (Swanston & Myrold, 1997). The mass correction increased C and N recoveries significantly to $92 \pm 0.01\%$ and $87 \pm 0.01\%$, respectively. Losses of C and N during physical fractionation procedures are commonly reported in the literature (Sollins *et al*., 1984; Swanston & Myrold, 1997; Shang & Tiessen, 2000; Swanston *et al*., 2004; Crow *et al*., 2007).

Plant sampling

Above- and belowground plant tissues were collected and analyzed for $\delta^{15}N$ and $\delta^{13}C$. Leaf litter was sorted from litterfall samples collected biweekly from five baskets at each forested site over a period of 12 months (Ostertag *et al*., 2008) and composited by site before chemical analyses. In the pastures, five grab samples of rooted live and dead aboveground plant material were collected and analyzed separately. Litter samples were oven-dried at 50 °C for further chemical analyses. Subsamples were ground to pass through a size 60 mesh on a Thomas Scientific Wiley mill (Philadelphia, PA, USA). Fine roots (<2 mm diameter) were collected using a 6.1 cm diameter soil core at 10 cm depth intervals to 50 cm at one soil pit per site. Root material was sorted by washing soil through successively smaller screens. Roots were oven-dried at 65 °C, weighed, and ground to fine powder in steel canisters on a Dentsupply-Rinn Crescent wig-l-bug Model C32-003A (Elgin, IL, USA).

Elemental and stable isotope analyses

Bulk soils, density fractions, leaf litter, and roots were analyzed for total C, N, $\delta^{13}C$, and $\delta^{15}N$. Total C and N concentrations were measured on a CE Instruments NC 2100 Elemental Analyzer (Rodano, Milano, Italy) at UC Berkeley. All samples were run in duplicate with replicate error $<10\%$. Stable C and N isotopes were analyzed on a Europa 2020 continuous flow mass spectrometer at UC Berkeley's Center for Stable Isotope Biogeochemistry. Analytical error was ± 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$ for soils with $>0.5\%$ C. Soil samples with $<0.5\%$ C were run separately for $\delta^{13}C$ with sucrose standards. We report isotopic data to 80 cm depth; C concentrations below this depth were too low for reliable $\delta^{13}C$ values. We did not have $\delta^{15}N$ values for all 0–10 cm depth root samples, and as we found no systematic differences in root $\delta^{15}N$ values with depth (data not shown), we averaged our root samples ($n = 12$) across depths for comparison with the aboveground inputs ($n = 21$) and soils. Stable isotopic compositions are expressed in δ notation (‰):

$$\delta^{13}\mathrm{C}\ \text{or}\ \delta^{15}N = [(R_{\mathrm{sample}} - R_{\mathrm{std}})/R_{\mathrm{std}}] \times 1000, \qquad (1)$$

where R_{sample} is the ratio of the heavy to the light isotope ($^{13}\mathrm{C}/^{12}\mathrm{C}$ or $^{15}\mathrm{N}/^{14}\mathrm{N}$) of a sample and R_{std} is the ratio of a standard (Dawson & Brooks, 2001). Differences in $\delta^{13}\mathrm{C}$ isotopic composition due to photosynthetic pathways allow for the proportion of soil C derived from forest (C3) or pasture grass (C4) sources to be calculated using a two-compartment mixing-model (Balesdent & Mariotti, 1996):

$$\mathrm{FC_B} = (\delta_X - \delta_{\mathrm{A}})/(\delta_{\mathrm{vegB}} - \delta_{\mathrm{A}}), \qquad (2)$$

where $\mathrm{FC_B}$ is the fraction of new soil C, δ_X is the $\delta^{13}\mathrm{C}$ of the soil sample in question, δ_{A} is the average $\delta^{13}\mathrm{C}$ of the corresponding soil from the same depth under the initial land use, and δ_{vegB} is the average $\delta^{13}\mathrm{C}$ value of plant inputs to the SOM pool in the new land use (B). Balesdent & Mariotti (1996) proposed a revised equation for systems where isotopic differences between SOM and plant inputs are large due to fractionation with decomposition (Nadelhoffer & Fry, 1988; Ehleringer *et al*., 2000), or where multiple land cover conversions have occurred:

$$\mathrm{FC_B} = (\delta_X - \delta_{\mathrm{A}})/(\delta_{\mathrm{vegB}} - \delta_{\mathrm{vegA}}), \qquad (3)$$

where δ_{vegA} is the average $\delta^{13}\mathrm{C}$ value of plant inputs to the SOM pool in the initial land use (A). We report results using both equations.

The fraction of C derived from the old vegetation, $\mathrm{FC_A}$, is calculated by

$$\mathrm{FC_A} = 1 - \mathrm{FC_B}. \qquad (4)$$

Using these approaches, pasture soils below 10 cm had lower C4-C contents than the youngest secondary forests, likely due to residual forest-derived C as well as mixed-litter inputs. To account for this, we also report results for bulk soils setting the fraction of new (pasture) C in the pastures to be 1. Finally, we also report results using (a) the mean $\delta^{13}\mathrm{C}$ of aboveground (leaf) litter for the primary forests and pastures, and (b) the mean $\delta^{13}\mathrm{C}$ of belowground litter (roots) for each depth from the primary forests and pastures as the vegetation inputs.

Rates of pasture-derived C loss were calculated using the following equation:

$$\ln(x_t/x_0) = -kt. \qquad (5)$$

where x_t is the fraction of C4-C remaining in the soil at time t, x_0 is the initial fraction of C4-C in the soil at time 0 (pasture), k is the decomposition rate constant, and t is the age of the secondary forest site in years. This approach assumes that the size of the C4-C pool was relatively similar within an age class at the time of abandonment. Our replicate sites and depth samples suggest that this is a valid assumption for this region. Turnover times (years) were calculated as $1/k$. Standard errors (SEs) of turnover times are reported using the ratio of SE of the slope to the slope of the decay curves.

We calculated accumulation rates of new C3-C (t C3-C ha yr^{-1}) in secondary forest soils as the amount of C3-C gained above and including the soil depth of the greatest proportion of C4-C, divided by the years since pasture abandonment for each soil pit, and averaged by site and by age. There was considerable overlap in C accumulation rates calculated by the different methods, so we only report results using Eqn (2) and leaf litter inputs.

Statistics

All statistical analyses were performed using JMP 5.1 software (SAS, Cary, NC, USA). All data were tested for normality and homogeneity of variance. The effect of site age or time since abandonment on mean bulk soil C and N pools was tested three ways because of uncertainty in the age of the primary forests. First, we used a conservative age estimate of 200 years for the primary forests and used a one-way analysis of variance (ANOVA), with active pastures set at 0 years. Then, we ran the same analysis excluding the primary forests. Finally, we used a nonparametric Wilcoxon/Kruskal–Wallis test which ranks each site category (age). Density fraction percent C and N and $^{13}\mathrm{C}/^{12}\mathrm{C}$ ratios were transformed to the power of 0.3 to reduce variance heterogeneity and analyzed as a split-plot design using the standard least squares and restricted maximum likelihood method, with age as the main plot, site as the block, and fraction as the subplot. C : N and $^{15}\mathrm{N}/^{14}\mathrm{N}$ ratios of density fractions were also analyzed as a split-plot, but data did not require transformation. Isotope data, reported as δ (‰), was converted to $^{13}\mathrm{C}/^{12}\mathrm{C}$ and $^{15}\mathrm{N}/^{14}\mathrm{N}$ ratios standardized to reference ratios before statistical analyses. Significant differences among ages within each fraction were further explored by Tukey Kramer honest significant difference tests. Differences in the contribution of the three density fractions to the bulk soil C and N stocks and mass were also transformed to the power of 0.3 and analyzed using Wilcoxon/Kruskal–Wallis test due to heterogeneous variances. Significance levels were set at $P < 0.05$ unless otherwise noted. Values reported in the text and tables are means ± 1 SE with error propagation where appropriate.

Results

Belowground C and N stocks

Soil C down to 1 m depth averaged 107 ± 29 t C ha^{-1} in active pastures, 109 ± 19 t C ha^{-1} in primary forests,

Table 1 Mean (and standard error) carbon (C) and nitrogen (N) content in bulk soils and fine roots, bulk density, and soil texture across a land-use chronosequence in the wet subtropical life zone of southeastern Puerto Rico ($n = 3$ sites for each land use and forest age, except $n = 2$ for 20-year forests)

	Active pastures	Secondary forests					Primary forests
		10 years	20 years	30 years	60 years	80 years	
Bulk soils							
0–100 cm							
t C ha^{-1}	106.7 (29.3)	146.8 (51.1)	134.9 (24.3)	99.9 (18.3)	97.8 (19.0)	106.9 (32.1)	109.1 (19.0)
t N ha^{-1}	8.9 (2.2)	11.7 (4.0)	12.3 (2.1)	9.1 (1.3)	8.8 (1.8)	9.0 (1.9)	8.7 (1.5)
0–10 cm							
t C ha^{-1}	31.9 (4.1)	39.9 (14.1)	39.3 (6.8)	26.6 (7.5)	31.7 (6.1)	36.1 (17.5)	31.0 (5.8)
t N ha^{-1}	2.6 (0.3)	3.1 (1.1)	3.3 (0.5)	2.1 (0.6)	2.7 (0.3)	2.7 (0.7)	2.4 (0.4)
10–20 cm							
t C ha^{-1}	16.9 (4.1)	26.1 (12.7)	27.3 (5.1)	20.5 (10.0)	18.2 (4.9)	19.6 (7.0)	18.0 (4.8)
t N ha^{-1}	1.4 (0.3)	2.1 (0.8)	2.4 (0.5)	1.6 (0.5)	1.7 (0.5)	1.6 (0.4)	1.5 (0.4)
20–50 cm							
t C ha^{-1}	32.8 (8.2)	46.9 (14.6)	41.5 (5.8)	28.7 (5.0)	27.3 (5.4)	28.8 (6.2)	32.4 (4.5)
t N ha^{-1}	2.8 (0.6)	3.8 (1.1)	3.8 (0.6)	2.8 (0.4)	2.6 (0.5)	2.6 (0.5)	2.7 (0.3)
Fine roots (0–50 cm)							
t C ha^{-1}	1.4 (0.5)	1.9 (0.2)	2.2 (0.9)	3.1 (0.1)	2.4 (0.5)	2.2 (0.9)	3.3 (0.6)
t N ha^{-1}	0.03 (0.01)	0.05 (0.004)	0.06 (0.03)	0.08 (0.04)	0.08 (0.01)	0.05 (0.02)	0.08 (0.01)
Bulk density (g cm^{-3})							
0–10 cm	0.80 (0.09)	1.00 (0.07)	0.90 (0.15)	0.77 (0.09)	0.67 (0.12)	0.73 (0.04)	0.70 (0.12)
Soil texture							
% sand	3 (1)	10 (5)	19 (2)	15 (4)	9 (4)	13 (5)	4 (2)
% silt	62 (4)	61 (2)	56 (1)	58 (4)	62 (4)	57 (3)	60 (5)
% clay	34 (3)	29 (4)	25 (0.2)	27 (0.1)	29 (1)	30 (2)	36 (5)

and 117 ± 70 t C ha^{-1} in secondary forests, pooled across all ages. Time since abandonment (forest age) or land cover had no significant effect on total soil C or N content in the top 1 m of soil or at any single depth sampled. There were also no effects of age or cover type on fine root C to 50 cm depth (Table 1). Across all pits, 30% of the total C content down to 1 m was found in the top 10 cm of soil, and 77% in the top 50 cm. Average soil bulk density and texture did not differ among forest age or land cover type (Table 1). There were no significant relationships between percent sand, silt or clay, and total C or N content in the 0–10 cm depth.

Site age significantly affected C concentrations of density fractions ($P = 0.01$), as did the type of density fraction ($P < 0.0001$), but there was no significant interaction between site age and soil density fraction. Free LF C concentrations were lower (23.7 ± 4.2%) in the pastures than in the primary forests (32.4 ± 5.7%), with secondary forests having intermediate values ($P = 0.08$) (Table 2). Occluded LF C concentrations were lower in the 10-year-old secondary forests (20.6 ± 12.0%) than in the primary forests (33.6 ± 2.1%). Averaged across all sites, the free and occluded LF had similar C concentrations, which were more than six times higher than the HF (Table 3). N concentrations and C : N ratios were not affected by site age, but differed significantly among density fractions ($P < 0.0001$). The occluded LF had the highest percent N, followed by the free LF, then the HF. Average C : N ratios decreased significantly from the free LF to the occluded LF to the HF ($P < 0.0001$) (Table 3).

C and N concentrations of the free LF increased significantly with time since pasture abandonment (C: $r^2 = 0.31$; N: $r^2 = 0.30$). The proportion of total C and N recovered in the free LF also increased with time since pasture abandonment (C: $r^2 = 0.61$, $P < 0.001$; N: $r^2 = 0.65$, $P < 0.001$). The mass of soil and proportion of bulk C recovered in the free LF were significantly lower in the pasture sites than in the 80-year secondary and primary forests (Table 2). The majority of soil mass was recovered in the HF (98 ± 2%) (Table 3). The free and occluded LFs together made up less than 2% of the bulk soil by mass, but up to 10% of the soil C content.

Stable isotope natural abundance

Mean aboveground litter δ^{13}C differed significantly between pastures (−16.1 ± 1.1‰) and primary forests (−29.5 ± 0.7‰). Fine root δ^{13}C in the 0–10 cm depth averaged −15.3 ± 1.4‰ in the pastures and

Table 2 Mean (and standard error) mass recovery, carbon (C) and nitrogen (N) concentrations, contributions to bulk C and N, and stable isotopes in density soil C fractions ($n = 2$ per site) from 0–10 cm depth averaged across three sites per age class, except 20-year forests with two sites

Density fraction	% of bulk soil	gC/100g soil	gN/100g soil	C/N	Distribution of C (% of bulk soil C)	Distribution of N (% of bulk soil N)	δ^{13}C (‰)	δ^{15}N (‰)
Free light								
Active pastures	0.2 (0.1) a	23.7 (4.2) a	0.9 (0.1)	25.8 (4.0)	1.0 (0.2) a	0.5 (0.2) a	−18.8 (6.9) a	2.2 ab
Secondary forests								
10 years	0.6 (0.5) ab	26.7 (2.8) ab	1.1 (0.2)	23.9 (2.5)	4.3 (2.3) ab	2.4 (1.6) ab	−27.4 (1.0) b	2.8 (0.8) a
20 years	0.7 (0.3) ab	28.7 (3.5) ab	1.3 (0.1)	23.1 (1.8)	5.3 (2.3) ab	2.7 (1.3) ab	−28.5 (0.4) b	1.4 (0.5) ab
30 years	0.6 (0.2) ab	25.2 (2.1) ab	1.0 (0.1)	25.9 (3.7)	3.4 (1.0) a	1.8 (0.5) a	−29.2 (0.8) b	2.0 (0.7) ab
80 years	1.5 (1.0) b	30.4 (2.9) ab	1.4 (0.3)	21.9 (4.3)	7.5 (3.2) b	4.4 (2.0) b	−29.0 (0.4) b	1.2 (0.3) b
Primary forests	1.0 (0.8) b	32.4 (5.7) b	1.3 (0.1)	26.5 (5.0)	4.7 (1.1) ab	2.4 (0.8) ab	−28.5 (0.7) b	2.0 (0.4) ab
Occluded light								
Active pastures	0.4 (0.2)	24.6 (5.2) ab	1.5 (0.1)	16.5 (2.8)	2.6 (1.3)	2.0 (0.9)	−19.8 (2.4) a	4.8 (0.6)
Secondary forests								
10 years	2.4 (1.5)	20.6 (12.0) a	1.1 (0.6)	18.2 (1.8)	10.9 (2.4)	8.0 (1.7)	−26.9 (0.7) b	4.1 (0.6)
20 years	0.5 (0.3)	26.3 (0.3) ab	1.9 (0.0)	14.6 (0.1)	3.2 (1.3)	2.5 (0.9)	−27.1 (0.3) b	4.4 (0.0)
30 years	1.3 (1.1)	21.8 (3.0) ab	1.3 (0.2)	17.0 (1.7)	7.2 (5.8)	5.23 (3.9)	−28.0 (0.7) b	2.7 (1.2)
80 years	0.5 (0.2)	30.3 (3.6) ab	1.9 (0.3)	16.5 (2.7)	2.7 (0.5)	2.03 (0.4)	−27.9 (0.3) b	3.7 (0.6)
Primary forests	1.3 (0.8)	33.6 (2.1) b	1.6 (0.2)	21.3 (2.7)	6.8 (2.7)	4.3 (2.2)	−27.9 (0.4) b	3.4 (0.1)
Heavy								
Active pastures	99.5 (0.2)	2.9 (0.3)	0.2 (0.02)	12.8 (0.5)	83.1 (11.0)	80.9 (11.0)	−20.2 (2.2) a	6.3 (1.1)
Secondary forests								
10 years	97.0 (1.4)	3.0 (1.5)	0.2 (0.1)	12.8 (0.9)	78.3 (7.8)	80.4 (7.8)	−25.6 (1.3) b	5.5 (1.5)
20 years	98.8 (0.3)	3.4 (0.4)	0.3 (0.03)	12.1 (0.2)	85.7 (2.2)	81.7 (2.2)	−25.9 (0.6) b	5.0 (0.5)
30 years	98.1 (0.9)	3.5 (0.9)	0.3 (0.1)	13.4 (0.6)	83.7 (12.6)	81.4 (12.6)	−27.0 (0.9) bc	4.6 (0.3)
80 years	98.0 (1.2)	4.5 (0.4)	0.4 (0.1)	12.3 (1.2)	79.5 (6.5)	81.3 (6.5)	−27.7 (0.4) c	4.4 (0.2)
Primary forests	97.7 (1.4)	5.3 (2.6)	0.4 (0.2)	12.5 (0.8)	80.9 (7.8)	82.2 (7.8)	−27.3 (0.2) bc	5.1 (1.0)

Different letters down the columns within density fraction type represent significant differences between sites.
Values reported are untransformed, although statistical analyses for most properties were performed on transformed data. See the text for details.

Table 3 Means (and standard error) of bulk soils, density fractions and fine roots from 0–10 cm, and leaf litter averaged across all sites

	Free LF	Occluded LF	HF	Bulk soils	Fine roots	Leaf litter
% of bulk soil	0.8 (1.4) a	1.1 (2.1) a	98.2 (2.5) b	na	na	na
gC/100 g soil (% C)	27.8 (9.1) a	26.2 (14.1) a	3.8 (3.2) b	4.3 (3.2) b	na	na
gN/100 g soil (% N)	1.2 (0.5) a	1.5 (0.7) a	0.3 (0.2) b	0.4 (0.2) b	na	na
C:N	24.5 (9.0) a	17.3 (5.4) b	12.7 (1.96) c	12.6 (1.7) b	na	na
Distribution of C (% of bulk soil)	4.4 (4.8) a	5.6 (7.1) a	81.9 (21.2) b	na	na	na
Distribution of N (% of bulk soil)	2.4 (3.0) a	4.0 (5.0) a	81.3 (17.8) b	na	na	na
δ^{15}N (‰)	1.9 (0.7) a	3.8 (0.9) a	5.3 (1.1) b	5.2 (1.0) b	3.0 (0.5) a	−0.4 (0.4) c

Different letters across the rows represent significant differences between fractions.
Values reported are untransformed, although statistical analyses for % C and % N were performed on transformed data.
na, not applicable; LF, light fraction; HF, heavy fraction.

−27.8 ± 0.9‰ in the primary forests. Secondary forest leaf litter and root δ^{13}C (data not shown) did not differ from the primary forests. Depth profiles of the δ^{13}C in bulk soils differed among the pastures and forested sites, especially in the top 30 cm (Fig. 1). Pastures averaged −19.3 ± 0.5‰ in the 0–10 cm soil and became progressively more depleted in ^{13}C with depth. Bulk soils from 10-, 20-, and 30-year-old forests showed

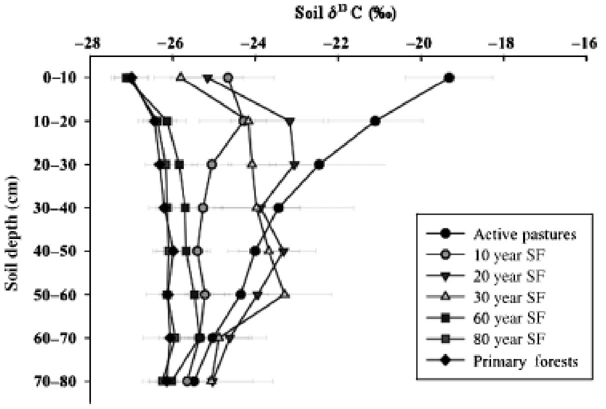

Fig. 1 Average (and standard error) δ^{13}C values for soil organic carbon with depth across a land-use chronosequence in Puerto Rico. SF, secondary forest.

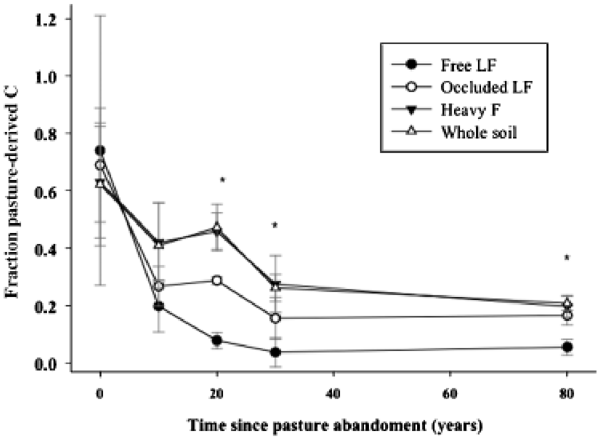

Fig. 2 Contribution of pasture-derived carbon (C) to soil C pools (as a fraction of total soil C) in the 0–10 cm soil depth with time since pasture abandonment using Eqn (2) and aboveground litter inputs. The symbol '*' represents significant differences among soil density fractions within each age group. LF, light fraction.

intermediate δ^{13}C values relative to the pasture and primary forest. The 20- and 30-year-old forest soils became more enriched in δ^{13}C with depth and overlapped with the active pasture soils in the 30–60 cm depth, below which the δ^{13}C composition shifted back toward a C3-dominated system. Soil δ^{13}C profiles in the 60- and 80-year-old secondary forests paralleled those of the primary forests. Below 60 cm, there was considerable overlap in the δ^{13}C values of all sites, regardless of the land cover or forest age.

Site age significantly affected ^{13}C/^{12}C concentrations of density fractions ($P<0.0001$), as did the type of density fraction ($P<0.0001$), and there was also a significant interaction between site age and soil density fraction ($P=0.0078$). All density fractions in the pastures were significantly enriched in ^{13}C compared with the forested sites (Table 2). Soil C pools in the forests became progressively more enriched in δ^{13}C from roots, to free LF, to occluded LF, and finally HF (Table 2). The pattern was reversed in the pastures, with δ^{13}C enrichment appearing to gradually increase from HF to roots.

Average leaf litter was significantly more depleted in ^{15}N than roots and LFs, which in turn were significantly more depleted than the HF or bulk soils (Table 3). Aboveground plant litter δ^{15}N values were significantly depleted in 20- and 30-year-old secondary forests than in the pastures. Forest age had no effect on N isotopic composition of density fractions, but fraction type did ($P<0.0001$). Density fractions showed enrichment in δ^{15}N in the order: free LF ($+1.9 \pm 0.7$‰), occluded LF ($+3.8 \pm 0.9$‰), and HF ($+5.3 \pm 1.1$‰) (Table 3). Free LF δ^{15}N was significantly higher than the δ^{15}N values of leaf litter ($+0.4 \pm 0.5$‰) but lower than those of fine roots ($+3.5 \pm 0.4$‰) averaged across all sites ($P<0.0001$).

Soil C turnover

Once pastures were abandoned, the loss of pasture-derived C from the soil was rapid, with the greatest losses of C occurring in the first 10 years of reforestation [results using Eqn (2) and leaf litter inputs shown in Fig. 2]. Losses were greater in the LFs than in the HF and bulk soil. After 80 years of reforestation, only about $5 \pm 3\%$ of pasture-derived C remained in the free LF, while the HF still contained $20 \pm 4\%$. Contributions of pasture-derived C were significantly lowest in the free LF relative to all other fractions in the 30-year-old sites, and lower than the HF and bulk soil in the 20- and 80-year-old secondary forest sites (Fig. 2).

Turnover times for bulk soil C in the top 10 cm ranged from 35 ± 4 to 78 ± 10 years using Eqn (2) and from 113 ± 20 to 276 ± 67 years using Eqn (3) (Table 4). Calculated turnover times were shortest when using root δ^{13}C inputs and Eqn (2). Regardless of the model used, turnover times increased with soil depth. Free LF had the shortest turnover times, while the occluded and HF did not differ from each other (Table 5). Estimates for free LF turnover times using Eqn (2) increased 10-fold from 3 ± 1 to 33 ± 1 years depending on whether above- or belowground litter inputs were used. Equation 3 gave much higher estimates for free LF turnover times (Table 5). Forest-derived C accumulation slowed exponentially with secondary forest age, with rates of $5.9 \pm 3.4\,\mathrm{t\,C\,ha\,yr^{-1}}$ in the first 10 years, and 1.9 ± 0.8, 1.2 ± 0.4, 1.0 ± 0.2, and $0.8 \pm 0.1\,\mathrm{t\,C\,ha\,yr^{-1}}$ after 20, 30, 60, and 80 years, respectively.

Table 4 Results from log-linear fits to fractions of pasture-derived carbon (C) remaining in bulk soils with depth across an 80-year secondary forest chronosequence using root and leaf litter inputs

	Decay rate (k)	Turnover time ($1/k$) (years)	r^2
(a)			
Using root inputs			
0–10	−0.028 (0.004)	40 (5)	0.84
10–20	−0.019 (0.002)	77 (17)	0.58
20–30	ns		
Using leaf litter inputs			
0–10	−0.015 (0.002)	78 (10)	0.8
10–20	−0.011 (0.002)	165 (58)	0.35
20–30	ns		
Setting pasture fC4 = 1			
Using root inputs			
0–10	−0.028 (0.004)	35 (4)	0.81
10–20	−0.019 (0.002)	52 (6)	0.82
20–30	−0.011 (0.002)	91 (16)	0.68
30–40	−0.008 (0.003)	132 (29)	0.58
40–50	−0.010 (0.002)	98 (23)	0.54
50–60	−0.009 (0.002)	107 (23)	0.59
60–70	−0.005 (0.001)	194 (41)	0.6
70–80	−0.006 (0.001)	174 (38)	0.58
Using leaf litter inputs			
0–10	−0.015 (0.002)	65 (10)	0.74
10–20	−0.011 (0.002)	89 (12)	0.79
20–30	−0.008 (0.001)	124 (22)	0.67
30–40	−0.006 (0.001)	163 (36)	0.57
40–50	−0.005 (0.001)	196 (48)	0.53
50–60	−0.005 (0.001)	218 (48)	0.58
60–70	−0.002 (0.001)	409 (86)	0.6
70–80	−0.002 (0.001)	420 (91)	0.59
(b)			
Using root inputs			
0–10	−0.005 (0.001)	276 (67)	0.6
10–20	ns		
20–30	ns		
Using leaf litter inputs			
0–10	−0.004 (0.001)	213 (40)	0.66
10–20	ns		
20–30	ns		
Setting pasture fC4 = 1			
Using root inputs			
0–10	−0.007 (0.002)	137 (31)	0.56
10–20	−0.005 (0.001)	201 (30)	0.75
20–30	−0.003 (0.001)	335 (64)	0.64
30–40	−0.002 (0.0004)	511 (116)	0.56
40–50	−0.002 (0.0004)	597 (147)	0.52
50–60	−0.001 (0.0003)	693 (154)	0.58
60–70	−0.001 (0.0002)	1418 (302)	0.6
70–80	−0.001 (0.0001)	1605 (350)	0.58
Using leaf litter inputs			
0–10	−0.009 (0.002)	113 (20)	0.68
10–20	−0.006 (0.001)	181 (27)	0.75
20–30	−0.003 (0.001)	290 (55)	0.65
30–40	−0.002 (0.001)	434 (99)	0.56
40–50	−0.002 (0.0003)	558 (137)	0.52
50–60	−0.002 (0.0003)	649 (144)	0.58
60–70	−0.001 (0.0002)	1332 (284)	0.6
70–80	−0.001 (0.0001)	1443 (289)	0.63

Standard errors are given in parentheses ($n = 17$ sites). Panel (a) reports results using Eqn (2) and panel (b) reports results using Eqn (3).
ns, not significant.

Table 5 Results from log-linear fits to fractions of pasture-derived carbon remaining in density fractions (0–10 cm) across an 80-year secondary forest chronosequence using root and leaf litter inputs

	Decay rate (k)	Turnover time ($1/k$) (years)	r^2	n
(a)				
Using root inputs				
Free light	−0.304 (0.084)	3 (1)	0.76	6
Occluded light	−0.039 (0.009)	26 (6)	0.68	11
Heavy	−0.033 (0.006)	31 (6)	0.72	13
Using leaf litter inputs				
Free light	−0.030 (0.009)	33 (10)	0.47	14
Occluded light	−0.014 (0.005)	74 (26)	0.40	14
Heavy	−0.013 (0.002)	74 (13)	0.73	14
(b)				
Using root inputs				
Free light	−0.013 (0.004)	78 (26)	0.43	14
Occluded light	−0.006 (0.002)	176 (68)	0.36	14
Heavy	−0.005 (0.001)	220 (54)	0.58	14
Using leaf litter inputs				
Free light	−0.010 (0.003)	101 (34)	0.43	14
Occluded light	−0.004 (0.002)	243 (103)	0.31	14
Heavy	−0.003 (0.001)	324 (122)	0.37	14

Values are means (and 1 standard error). Sample sizes used in the regressions differ due to different numbers of samples with zero C4-C content. Panel (a) reports results using Eqn (2) and panel (b) reports results using Eqn (3).

Discussion

Reforestation effects on bulk soil C pools and N isotopes

Secondary succession following pasture abandonment at our study sites was characterized by increased biomass, distinct changes in floristic composition, and rapid recovery of litterfall rates (Marin-Spiotta *et al.*, 2007; Ostertag *et al.*, 2008). Differences in litter inputs and changes in species dominance during reforestation did not increase bulk soil C content or change the distribution of soil C with depth. Global and pantropical reviews of soil C dynamics have reported increased soil C pools with forest regrowth and plantations (Post & Kwon, 2000; Silver *et al.*, 2000), although results from individual studies across the Neotropics vary (Hughes *et al.*, 1999; Saynes *et al.*, 2005; Schedlbauer & Kavanagh, 2008). The response of soil C pools to reforestation is controlled by multiple factors, including land-use history (type and intensity), frequency of disturbance, soil mineralogy, and nutrient status (Hughes *et al.*, 1999; de Koning *et al.*, 2003; Rasiah *et al.*, 2003; López-Ulloa *et al.*, 2005; Zarin *et al.*, 2005; Davidson *et al.*, 2007). Tree species composition can also affect whether reforestation will result in an accumulation or loss of soil C (Kaye *et al*., 2000; Neufeldt *et al*., 2002; Paul *et al*., 2002). The amount of time a plot has been in pasture use can also be a determining factor in whether a reforested site will gain or lose C relative to the former land use (de Koning *et al*., 2003).

Similar to our results, Brown *et al*. (1984) also found no significant differences between pasture and forest soil C pools across different life zones in Puerto Rico. The loss of soil C during conventional cultivation has been well documented (Detwiler, 1986; Mann, 1986; Brown & Lugo, 1990b), but the effects of pasture use on soil C are less predictable. Pasture soils are often expected to have greater soil C than forests due to higher rates of belowground productivity and greater allocation to root biomass (Fisher *et al*., 1994), although over-grazing can decrease soil C content (Elmore & Asner, 2006; Schipper *et al*., 2007). Thus, pasture establishment on deforested soils can have negative (Veldkamp, 1994; Van Dam *et al*., 1997; Fearnside & Barbosa, 1998), positive (Trumbore *et al*., 1995; Neill *et al*., 1997; Rhoades *et al*., 2000), or no net effect (Cleveland *et al*., 2003) on bulk soil C pools, likely due, in part, to differences in soil type, land use history, and intensity. We found no significant decrease in soil bulk density with succession, similar to other studies in the region (Brown & Lugo, 1990b). Highly weathered clay soils can be relatively resistant to change when soil structure is not severely disrupted (Brown & Lugo, 1990b; Uehara, 1995). The low stocking densities and infrequent use of mechanization typical of our study region likely contributed to minimizing soil disturbance and associated net C losses. The scenario may be entirely different in degraded pastures resulting from more intensive, large-scale cattle ranching (Rasiah *et al*., 2003; Asner *et al*., 2004).

We had originally hypothesized that soil C storage would increase with the production of recalcitrant plant secondary compounds during secondary succession. However, while species dominance changed across the chronosequence (Marin-Spiotta *et al*., 2007), and individual species chemistry differed (Ostertag *et al*., 2008), we detected no chemical differences as measured by ^{13}C-nuclear magnetic resonance (^{13}C-NMR) and traditional proximate analyses in mixed-species litterfall across a year of sampling or in fine roots (Ostertag *et al*., 2008). We previously reported significant positive correlations between hydrophobic indices of litter chemistry and bulk soil C stocks (Ostertag *et al*., 2008), but there was no pattern in litter chemistry with forest age.

Disturbances that occur during deforestation can result in nutrient limitation, which may affect rates of forest recovery and soil C accumulation (Zarin *et al*., 2005; Davidson *et al*., 2007). Differences in the natural abundance of δ^{15}N can be used as indicators of nutrient status (Martinelli *et al*., 1996; Austin & Vitousek, 1998; Davidson *et al*., 2007). Bulk soil and leaf litter δ^{15}N at our forested sites were on the low end for tropical forests, and more similar to other montane forests and lowland, oligotrophic forests (Martinelli *et al*., 1996; Austin & Vitousek, 1998), but there were no significant differences between forest ages. Forest structure (basal area and stem density) was recovered quickly in our chronosequence, in as little as 20 years after pasture abandonment (Marin-Spiotta *et al*., 2007), suggesting that differences in nutrient limitation were not controlling forest recovery rates.

Changes in soil density fractions with reforestation

Although there was no net change in the bulk soil C pool during reforestation, there was a significant loss of residual pasture-derived C that offset the accumulation of new, forest-derived C. Similarly, in *Eucalyptus* plantations growing on abandoned sugarcane in Hawaii, soil C gains in the first 10 cm were balanced by losses of residual C from lower depths (Bashkin & Binkley, 1998). In our study, density fractions and C isotopes were dynamic over time, providing insights into the mechanisms responsible for the maintenance of soil C stocks during secondary succession. We hypothesized that forest C accumulation would be greatest in the free and occluded LF fractions during early secondary succession. Land-use change affected the mass of soil recovered in the free LF, as well as C concentration and distribution of C in the free LF, which was depleted in pastures, relative to primary forests and 80-year-old secondary forests. Reforestation quickly replenished this physically unprotected, particulate C fraction, in as little as 10 years, likely due to increased inputs of more recalcitrant litter in the forests than in pastures. While litterfall rates were low in the 10-year-old secondary forests, slow litter decay rates (Ostertag *et al*., 2008) would also contribute to the replenishment of the free LF. Leaf litter from secondary and primary forests was characterized by greater concentrations of more recalcitrant compounds than pasture litter as determined both by wet chemistry and ^{13}C-NMR spectroscopy (Marin-Spiotta *et al*., 2008). Pastures in Ecuador also showed lower contributions of free LF to C storage than primary and secondary forests (Paul *et al*., 2008). On a deforested site in Brazil, the free LF C pool was recovered by planting nitrogen-fixing trees (Macedo *et al*., 2008).

The free and occluded LFs are dominated by particulate organic matter and are often the most sensitive to cultivation and other land-use change (Alvarez *et al*., 1998; Guggenberger & Zech, 1999; Compton & Boone, 2000; Baisden *et al*., 2002; John *et al*., 2005; Helfrich *et al*., 2006). Few studies have followed soil C pools over such long periods of succession; our results indicate that in soils where the capacity for minerals to sorb C are not disrupted, total soil C pools may be resilient to land-use change, but are still dynamic with regard to source–sink relationships.

Dynamics of C and N in density fractions

The soil C fractionation method we employed yielded SOM fractions with different chemical make-up and turnover times that corresponded with increasing degree of decomposition. The C : N ratios of the free LF reflected more recent litter inputs, while the occluded LF had much lower mean C : N ratios, although not as low as the mineral soil. Decreasing C : N ratios in soil C fractions have been shown to be coupled with increasing decomposition of SOM and mineral association (Nadelhoffer & Fry, 1988; Baldock *et al*., 1992; John *et al*., 2005). Greater enrichment of ^{13}C and ^{15}N across density fractions also reflects microbial processing during litter decomposition and SOM formation. In the forested sites, fractions were progressively enriched in δ^{13}C from the free LF, to the occluded LF, to the HF, representing increasing presence of residual pasture-derived C in the physically protected fractions. In the active pastures, soil density fractions were depleted in δ^{13}C relative to plant litter inputs, due to the presence of residual forest SOM.

Patterns in δ^{15}N of soil density fractions provided further evidence to support the conclusion that the free LF, occluded LF, and HF represent distinct soil C pools along a continuum of decay. Values of δ^{15}N integrate across N cycling processes in the soil and can be used as indicators of the degree of microbial transformation (Robinson, 2001; Amundson *et al*., 2003; Kramer *et al*., 2003). SOM decomposition typically results in an enrichment in ^{15}N (Nadelhoffer & Fry, 1988), so higher δ^{15}N for the occluded LF relative to the free LF in our soils would suggest that the organic matter inside soil aggregates is more decomposed than the free LF. An alternative explanation for the higher δ^{15}N in the occluded LF is a greater contribution of fine roots vs. leaf litter precursors in this fraction relative to the free LF. However, turnover times estimated from the changes in δ^{13}C across the chronosequence suggest that organic matter within soil aggregates is older and has undergone further decomposition than the free, particulate C. Positive relationships between soil δ^{15}N and turnover times provide further evidence that changes in the natural abundance of δ^{15}N may represent differences in degree of SOM humification (Baisden *et al*., 2002; Billings, 2006; Liao *et al*., 2006; Sollins *et al*., 2006). In cultivated soils, δ^{15}N values of the light and HFs were more similar to each other than the same fractions in forested soils (Compton & Boone, 2000), presumably due to faster soil aggregate turnover rates and accelerated cycling of N through the fractions as a consequence of soil disturbance during cultivation.

Soil C turnover

Even though there were no significant net changes in bulk soil C pools with reforestation, isotopic measurements and turnover time estimates suggest rapid C cycling rates. Free LF typically had much shorter turnover times than the occluded LF and HF, which did not differ greatly from each other, suggesting that occlusion within aggregates provided an important physical protection mechanism in these highly weathered soils. The fact that the majority of the soil C pool (82 ± 21%) was recovered in the HF points toward direct associations with minerals as the dominant control of soil C turnover in these soils.

The choice of litter inputs and mixing-model affected estimates of turnover times. Cycling times for the free LF using root inputs and Eqn (2) (3 ± 1 years) were very similar to those reported for physically unprotected light density or particulate organic matter in other tropical soils (Martin *et al*., 1990; Trumbore *et al*., 1995; de Camargo *et al*., 1999; Trumbore, 2000), and mean residence times estimated for the same samples using radiocarbon (Marin-Spiotta *et al*., 2008). Using leaf litter inputs and the modified mixing-model [Eqn (3)] yielded much slower cycling rates for all fractions, and in particular for the free LF, but also increased variability in the estimates. The presence of charcoal or other chemically recalcitrant particulate organic matter external to soil aggregates could yield much longer turnover times than would be expected if physical protection were the only mechanism controlling dynamics of the free LF.

Given the uncertainties around the mean turnover times, these latter models still yielded estimates for the occluded LF and HF that fell within the range of 50–100 years typically reported for corresponding soil fractions in other tropical surface soils (Desjardins *et al*., 1994; de Camargo *et al*., 1999; Schwendenmann & Pendall, 2006). Radiocarbon-based mean residence times for the same density fraction samples (Marin-Spiotta *et al*., 2008), averaged across all forested sites in our chronosequence, were significantly and strongly correlated only with turnover times from the natural abundance ^{13}C technique using Eqn (2) and leaf litter values ($r^2 = 0.99$, $P = 0.03$).

Turnover times based on natural abundance stable isotope methods tend to be more sensitive to recent C inputs and C pools associated with the C3/C4 vegetation type conversion (Six & Jastrow, 2002), whereas radiocarbon methods may be more sensitive to longer term pools that are less affected by recent inputs (Trumbore, 2000). Physical fractionation methods, such as ours, which used density and physical disruption of soil aggregates, are an attempt to better understand soil C dynamics by acknowledging that the bulk SOM pool is not a homogeneous pool, but in fact represents a continuum of substrates with different turnover times. There is evidence, in addition, that these pools may also themselves be heterogeneous. Recent studies have shown that the mineral-associated C, or HF, is in fact composed of fast-cycling and slow-cycling pools (Swanston *et al*., 2005; Crow *et al*., 2007), as we show by the rapid replacement of pasture-derived C in our soils.

Conclusions

The bulk soil C pool was maintained during conversion of pasture to forest and the subsequent 80 years of secondary succession that followed. The gain of secondary forest C was rapidly compensated for by the loss of pasture-derived C4-C, resulting in no net change down to 1 m depth during reforestation. We observed changes, however, in the distribution of soil C across physical density fractions. Reforestation replenished the amount of C in the free LF that had been depleted during conversion to pastures. This physically unprotected fraction was the most sensitive to land cover change and had the fastest turnover times estimated using both stable and radiocarbon (Marin-Spiotta *et al*., 2008) isotope techniques. Our elemental and isotopic composition data support a model of older, more decomposed SOM with increasing mineral association from the free LF, to the occluded LF, to the HF.

Understanding the types of disturbance and land-use activities that disrupt or maintain a soil's capacity to physically protect and store C is crucial for selecting sites for C sequestration projects. This knowledge will help predict the fate of these soils as they are affected by future human activities and climatic change. In Puerto Rico, as in much of the rapidly urbanizing tropics, secondary forests are threatened by a new deforestation trend for residential and commercial development (Thomlinson & Rivera, 2000; Helmer, 2004). How this new land-use driver will affect soil C stocks, and the resiliency of these soils, remains to be seen.

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