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Litterfall and Decomposition in Relation to Soil Carbon Pools Along a Secondary Forest Chronosequence in Puerto Rico

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Abstract

Secondary forests are becoming increasingly widespread in the tropics, but our understanding of how secondary succession affects carbon (C) cycling and C sequestration in these ecosystems is limited. We used a well-replicated 80-year pasture to forest successional chronosequence and primary forest in Puerto Rico to explore the relationships among litterfall, litter quality, decomposition, and soil C pools. Litterfall rates recovered rapidly during early secondary succession and averaged 10.5 (± 0.1 SE) Mg/ha/y among all sites over a 2-year period. Although forest plant community composition and plant life form dominance changed during succession, litter chemistry as evaluated by sequential C fractions and by ^{13}C-nuclear magnetic resonance spectroscopy did not change significantly with forest age, nor did leaf decomposition rates. Root decomposition was slower than leaves and was fastest in the 60-year-old sites and slowest in the 10- and 30-year-old sites. Common litter and common site experiments suggested that site conditions were more important controls than litter quality in this chronosequence. Bulk soil C content was positively correlated with hydrophobic leaf compounds, suggesting that there is greater soil C accumulation if leaf litter contains more tannins and waxy compounds relative to more labile compounds. Our results suggest that most key C fluxes associated with litter production and decomposition re-establish rapidly—within a decade or two—during tropical secondary succession. Therefore, recovery of leaf litter C cycling processes after pasture use are faster than aboveground woody biomass and species accumulation, indicating that these young secondary forests have the potential to recover litter cycling functions and provide some of the same ecosystem services of primary forests.

Key words: litter; roots; reforestation; secondary succession; soil organic matter; ^{13}C-NMR.

Introduction

Forests recovering after agricultural and pastureland abandonment are dominant landscape features in the tropical biome (Aide and Grau 2004). These secondary forests can provide many ecosystem services, including the potential to be sinks for

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atmospheric carbon (C) in both plant biomass and soils (Brown and Lugo 1990; Silver and others 2004). The chronosequence approach, where time since abandonment varies, but geology, climate, and land-use history are similar, has been useful in documenting changes in vegetation structure during secondary forest development (Saldarriaga and others 1988; Denslow and Guzman 2000). However, only a few studies of litterfall and decomposition use this approach (Xuluc-Tolosa and others 2003; Lawrence 2005). More commonly, studies compare secondary forest of a single age to plantations or to primary forest (for example, Cuevas and others 1991; Lugo 1992; Li and others 2005), or compile data worldwide across forests of different ages (for example, Brown and Lugo 1990). Our understanding of controls on plant C inputs to the forest floor and soil, and rates of decomposition of litter material during tropical secondary succession is therefore limited.

Litter production and decomposition can be impacted both by the structural and floristic changes that occur over secondary succession. Past research has demonstrated that structural variables such as canopy closure, basal area, and stem density generally recover much faster than pre-disturbance species composition (Finegan 1996; Guariguata and Ostertag 2001; Chazdon 2003). If litter production is a function of site occupancy, we might expect litter production and decomposition rates to remain near constant throughout secondary succession following canopy closure. However, if litter production is sensitive to plant C allocation patterns and species composition, we might expect it to change over succession as the dominant life form changes from grasses and ferns to trees and as plants allocate a higher proportion of their photosynthate to woody material. Shifts in plant species composition and biomass allocation may lead to a corresponding change in litter chemical and physical characteristics, which are often correlated with litter decomposition rates (Cornelissen 1996).

Litter chemistry characteristics may be particularly sensitive to secondary successional state. Theories of plant herbivory predict that chemical defense compounds produced for protection from desiccation, herbivory, and parasitism (Coley and Aide 1991) become more important in later successional species (Feeny 1976; Coley 1983; Coley and others 1985). In addition, grasses, which are common in the initial years after pasture abandonment, tend to have fewer waxy compounds and more digestible litter than tropical woody plants (Hunt and others 1988; Cornelissen 1996; Kochy and Wilson 1997; Silver and Miya 2001; Marín-Spiotta and others 2008a). Many secondary compounds are polyaromatics (for example, polyphenols, condensed tannins, terpenoids) or aliphatic hydrocarbons (for example, lipids, waxes, suberin) that are relatively resistant to microbial decay (Gleixner and others 2001). These hydrophobic compounds have been found to accumulate in soil organic matter (SOM) (Köegel-Knabner and others 1991; Lichtfouse and others 1988a, b; Northup and others 1998; Hattenschwiler and Vitousek 2000), establishing an important link among litter production, litter chemistry, litter decomposition, and soil C sequestration.

This study examined litter inputs and leaf and root litter decomposition in relation to bulk soil C pools in a well-replicated, 80-year chronosequence of secondary forests regrowing on abandoned pastures in Puerto Rico. Species composition and plant life forms changed over succession; young secondary forests were dominated by ferns, intermediate sites by pioneers, and older secondary and primary forest sites by other woody species and palms (Marín-Spiotta and others 2007). Given these changes in species composition, we also expected changes in litter chemistry and litter inputs with secondary succession. We expected that the production of leaf litter mass and of hydrophobic compounds would increase with time since pasture abandonment as forests became more dominated by later successional trees and palms. We hypothesized that this change in species composition would be reflected by an increase in both litterfall quantity and litter hydrophobicity across the chronosequence, leading to slower decomposition rates and greater soil C storage in older forests. To test these hypotheses, we measured litter production, decomposition, and litter chemistry over the chronosequence. Litter chemistry can be defined by a wide range of compounds and elemental ratios. Here we used two approaches to assess litter chemistry, solid-state ^{13}C-nuclear magnetic resonance (NMR) spectroscopy, and the more traditional proximate C fraction analysis (Ryan and others 1990; Preston and others 1997, 2000).

Materials and Methods

Study Sites

We conducted this study on private lands in the Sierra de Cayey in southeastern Puerto Rico (18°01′ N, 66°05′ W). The sites are located between 580 and 700 m above sea level in the subtropical wet forest life zone (Ewel and Whitmore 1973). The potential forest

vegetation types in the region are lower montane wet evergreen forest, tall cloud forest, and palm breaks (Helmer and others 2002). The dominant soil type in this region is classified as very fine, mixed, isothermic Inceptic Hapludox in the Los Guineos series (USDA soil map, Lugo-López and others 1995). Soils are very deep, well drained, and from sandstone parent material. Mean annual temperature was estimated at 21–22°C with little annual variation (Daly and others 2003). Mean annual precipitation (1955–2005) was approximately 2,000 mm (Southeast Regional Climate Center 2006).

Our study takes advantage of a historical trend in post-agricultural reforestation in Puerto Rico (Franco and others 1997; Grau and others 2003). Our chronosequence includes three replicate sites each of primary forests, and secondary forests regrowing on pastures abandoned 10, 20, 30, 60, and 80 years ago, for a total of 18 sites, previously described in Marín-Spiotta and others (2007). Land-use history was identified by the use of aerial photographs and interviews with local landowners. We use the term ''primary'' forest to refer to remnant forest fragments that have not been under pasture cover for at least the last century. Forest regeneration has occurred naturally without active management (Aide and others 2000; Pascarella and others 2000). Aboveground forest structure, biomass and soil C inventories are summarized in Table 1. We were unable to correct for length of time each site was under pasture use; however, management practices in this region are such that the organic matter capital of the soil was not depleted by pasture use and soil C pools do not differ among sites (Table 1). The youngest sites had lower basal area and stem density but bulk soil C and nitrogen (N) stocks did not differ among forest ages. The sites differed in plant species composition, with the youngest site being dominated by ferns and the trees *Syzygium jambos* (L.) Alston and *Inga laurina* Willd. *Tabebuia heterophylla* (DC.) Britt. were very abundant in the 20- and 30-year-old sites. The 60- and 80-year-old sites had a more diverse array of tree species as well as retaining species from earlier ages. The primary forests were strongly dominated by the sierra palm, *Prestoea montana*.

Litterfall and Forest Floor

At each site, we set out five litter baskets, stratified by choosing a random angle and distance for each fifth of a 576-m^2 circular plot. Litter baskets were built from laundry baskets with inner dimensions of 42.2 cm × 56.1 cm, lined with window screening (1 mm mesh), and elevated above the ground approximately 0.5 m. Litter was collected biweekly for 2 years beginning in September 2002. All litter was dried at 50°C and sorted into leaves, wood, and miscellaneous (all other) fractions (following definitions in Ostertag and others 2003). The leaf category included all leaf parts from woody and non-woody species (palms, ferns, and dicotyledons). Litter was dried at 50°C so that it could later be used for decomposition experiments and analyzed by wet chemistry. Litter mass data are reported based on a dry weight at 65°C.

Forest floor samples were collected in 2003 by randomly locating five replicate 225 cm^2 templates at each of the forest sites and collecting all loose, dead, recognizable plant material on the soil surface. Samples were dried at 50°C, weighed, and sorted into the same categories as fresh litter. Forest floor leaf and wood fractions from each site were composited separately and ground to pass a size 60 mesh on a Thomas Scientific Wiley mill (Philadelphia, Pennsylvania, USA).

Leaf and Root Litter Decomposition

Leaf decomposition experiments began in July 2003. Senesced leaf litter material collected during the first year of litterfall sampling was bulked by site, excluding leaves with greater than 20% of leaf area loss, and well mixed before filling of litterbags. For palms, only leaflets were used, and not the rachis.

Table 1. Site Characteristics of the Study Plots (with Three Plots per Forest Age)

Age	Basal area (m^2/ha)	Stem density (#/ha)	Total soil C (t/ha)	Total soil N (t/ha)
10	1.4^{a} (1.0)	104^{a} (63)	147 (10)	11.7 (1.2)
20	23.7^{b} (3.5)	854^{b} (93)	112 (25)	10.6 (2.5)
30	16.9^{ab} (1.2)	1048^{b} (113)	100 (19)	9.1 (1.4)
60	32.5^{bc} (1.9)	1076^{b} (44)	98 (5.1)	8.8 (0.5)
80	46.9^{c} (7.1)	1163^{b} (36)	107 (23.1)	8.9 (1.4)
Primary	28.9^{b} (1.5)	926^{b} (95)	109 (17)	8.7 (0.7)

Tree data are from Marín-Spiotta and others (2007) and soil data from Marín-Spiotta and others (unpublished). For soil data, there were three soil pits per plot; values are means and (SE) for 1-m depth. Letters represent significant differences within a column.

Litterbags were 10 cm × 10 cm, made of fiberglass window screening (1 mm mesh), sewn with nylon thread and closed with two plastic rivets. Each litterbag was filled with 5 g of air-dried material, although litter had previously been dried at 50°C to obtain mass of the various litterfall fractions. Initial mass is expressed on a 50°C basis.

In the *in situ* leaf experiment, litter produced at a site was placed back into the site of origin and allowed to decompose for 2, 5, 10, 15, or 20 months. At each time point, six replicate bags were collected, for a total of 540 litterbags. Each of the six replicate bags were tied together with monofilament and five ''strings'' radiated from a randomly chosen point in each plot. Litter was processed by carefully pulling it out of the litterbag, wiping off visible soil, and weighing at 50°C for at least 48 h. Samples were ground in a Wiley mill at 40 mesh and combusted in a muffle furnace at 500°C for a minimum of 4 h. All mass is expressed on an ash-free dry mass basis.

In order to examine root decomposition *in situ*, we collected roots from the top 10 cm of soil at each site, and placed 0.5 g of fine roots (<2 mm in diameter) in 4 cm × 4 cm litterbags made of tent netting (0.3 mm). Soil was washed off roots before they were placed in litterbags. Root litterbags were made of a smaller mesh size than leaf litterbags to avoid losing roots as well as to minimize ingrowth (Ostertag and Hobbie 1999). As in the *in situ* leaf decomposition experiment, roots were placed back into the site in which they were produced. Set-up in the field followed the same procedure as leaves except that the 540 root bags were each buried in a small 45° angle slit in the top 10 cm to allow for good contact with the soil surface. The experiment began in mid March 2004, and six replicate bags were collected at five time points over a 22-month period.

To test for the effects of litter quality on leaf decomposition, we conducted a common site experiment, by placing litterbags with litter collected from one site per age into one primary forest site. If litter quality were having a strong effect, we would expect differences among litter types when decomposed in a common site. To test for the effects of site quality on litter decomposition, a common litter (wooden Popsicle sticks) was decomposed at one site per age, the same litter sites chosen for the common site experiment. These litterbag experiments used the same methods as the *in situ* leaf experiment described above, except that the common litterbags were approximately 4 cm × 18 cm to accommodate the smaller Popsicle sticks. A total of 180 bags were used for both the common litter and common site experiments (6 ages × 5 time points × 6 reps) over a 20-month period.

Litter Quality: Elemental and Proximate C Fraction Analysis

Initial litter chemistry was determined on a subsample of the composited litter that was used for the decomposition experiments. We also collected recently senesced green leaves from the forest floor of seven common species (listed in Table 3) to examine species differences; green leaf litterfall is a common occurrence in these forests due to periodic storm events. Leaf litterfall, forest floor leaf, and wood fractions were analyzed for total C and N concentrations on a CE Instruments NC 2100 Elemental Analyzer (Rodano, Milano, Italy) at U.C. Berkeley. All samples were run in duplicate with replicate error less than 10% for all data reported. Plant tissue composition was determined by a proximate C fraction analysis, which measures mass recovery after sequential extractions by different solvents (McClaugherty and others 1985; Ryan and others 1990), at the Center for Water and the Environment, Natural Resources Research Institute, University of Minnesota, Duluth, Minnesota, USA. This analysis yields the following fractions: non-polar extractable component (NPE), water soluble (WS), and acid soluble (AS) extractables, with the final acid-insoluble residue referred to as Klason lignin. 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 and others 1974), and which include small poly and mono aromatic compounds of plant resins, were extracted from the WS fraction. Polysaccharides, simple sugars measured in glucose equivalents (Dubois and others 1956), were extracted from both the WS and AS fractions. We consider the NPE to represent hydrophobic compounds.

Litter Quality: ^{13}C-NMR Spectroscopy

We compared leaf litter quality as determined by the more traditional sequential C fraction analysis described above (Ryan and others 1990) to the results from ^{13}C-NMR spectroscopy (Preston and others 1997, 2000). Roots were not analyzed by ^{13}C-NMR. Solid-state variable amplitude cross polarization magic angle spinning (MAS) ^{13}C-NMR spectra were acquired on a Varian/Chemagnetics Infinity CMX 300 MHz nuclear magnetic spectrometer (Varian NMR, Fort Collins, Colorado, USA), located at the High Field NMR facility at the

Environmental Molecular Sciences Laboratory at Pacific Northwest National Laboratory in Richland, Washington, USA. 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 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 into four regions that broadly correspond to the following general C types: 0–45 (alkyl C), 45–110 (O-alkyl C), 110–165 (aromatic C), and 165–220 ppm (carboxylic and carbonyl C). The integrated spectral areas were normalized to the total signal intensity for each spectrum. From the ^{13}C-NMR spectral data, two litter quality indices were created. The aliphaticity index was determined as the ratio of the alkyl region divided by the O-alkyl region (also termed A/O-a; Baldock and others 1992, Baldock and Preston 1995). A higher ratio signifies a greater contribution of alkyl C, such as lipids and other aliphatic compounds. A lower ratio indicates a greater contribution of O-alkyl C, mostly represented by plant carbohydrates, including cellulose and hemicellulose, which are considered more labile than alkyl C to microbial decomposers (Baldock and others 1992). The recalcitrance index was determined as (alkyl + aromatic C)/(O-alkyl + carbonyl and carboxyl C). Aromatic compounds include tannins and lignin phenols, whereas the carbonyl and carboxyl NMR region represent compounds such as peptides. Plant hydrophobic compounds can contribute to signal intensities in both the alkyl and aromatic NMR regions.

Soil C Pools

Bulk soils were collected in 10 cm intervals to a 1-m depth from three pits dug at each site. Soils were air-dried and a sub-sample was ground to fine powder in a mortar and pestle for C analyses. No carbonates were detected after treatment with 5% HCl (Nelson and Sommers 1996), so all C was assumed to be organic. Bulk density was measured by a quantitative core (6.1-cm diameter) and soil bulk density values were used to convert C concentrations to C content (Mg/ha).

Data Analyses

Differences in litterfall and forest floor mass among ages were analyzed using one-way ANOVA; total forest floor mass was log-transformed to meet the equal variance assumption. Litter decomposition rates were examined using linear and exponential models based on the relationship between log mass remaining and time (Wieder and Lang 1982). Decomposition rate constants (k) were calculated as the slope of this relationship. For the *in situ* leaf and root litter experiments, one-way ANOVA was used to examine differences in k values among ages, after checking for normality and equal variances. Because the common site and common litter experiments occurred at only one site per age, it was not possible to do statistical analyses on those rates. However, comparisons of *in situ*, common litter, and common site k values within a site provide insights on the importance of site versus litter quality controls on decomposition. A series of non-parametric correlations were done to determine if the *in situ* k values for leaves and roots were related to (1) bulk soil C or N, (2) forest floor C or N, or (3) any of the litter quality variables (proximate fractions and ^{13}C-NMR data). All statistics were done with JMP v. 3.17 (SAS Institute 1995).

Results

Litterfall and Forest Floor Mass

Litterfall averaged 10.4 (± 0.6 SE) Mg/ha/y in year 1 and 10.6 (± 0.5 SE) Mg/ha/y in year 2 across all forest ages. Total litterfall varied significantly among ages in year 1 (Figure 1A). The 10- and 30-year-old secondary forests and the primary forests had the lowest values, averaging around 8 Mg/ha/y ($F_{5,12}$ = 5.39, $P < 0.01$). In year 2, there were no significant differences among ages (Figure 1B). The standing crop of all forest floor components was 7.6 (± 1.0) Mg/ha across sites. Forest floor leaf mass did not vary among ages, but total forest floor mass was lower in the 20- and 30-year-old sites relative to the primary forest ($F_{5,12}$ = 4.37, $P < 0.01$) (Figure 1C).

Leaf and Root Litter Decomposition

An exponential model appropriately described decomposition rates at all sites and leaf types, with R^2 values ranging from 70.9 to 99.6% (Table 2). Final leaf mass remaining ranged from 23 to 37% for the *in situ* litter (Figure 2A), and there were no statistically significant differences in leaf k values among sites (Figure 1D). Final leaf mass for the common site ranged from 27 to 42% (Figure 2B) and 14 to 48% for the common litter (Figure 2C).

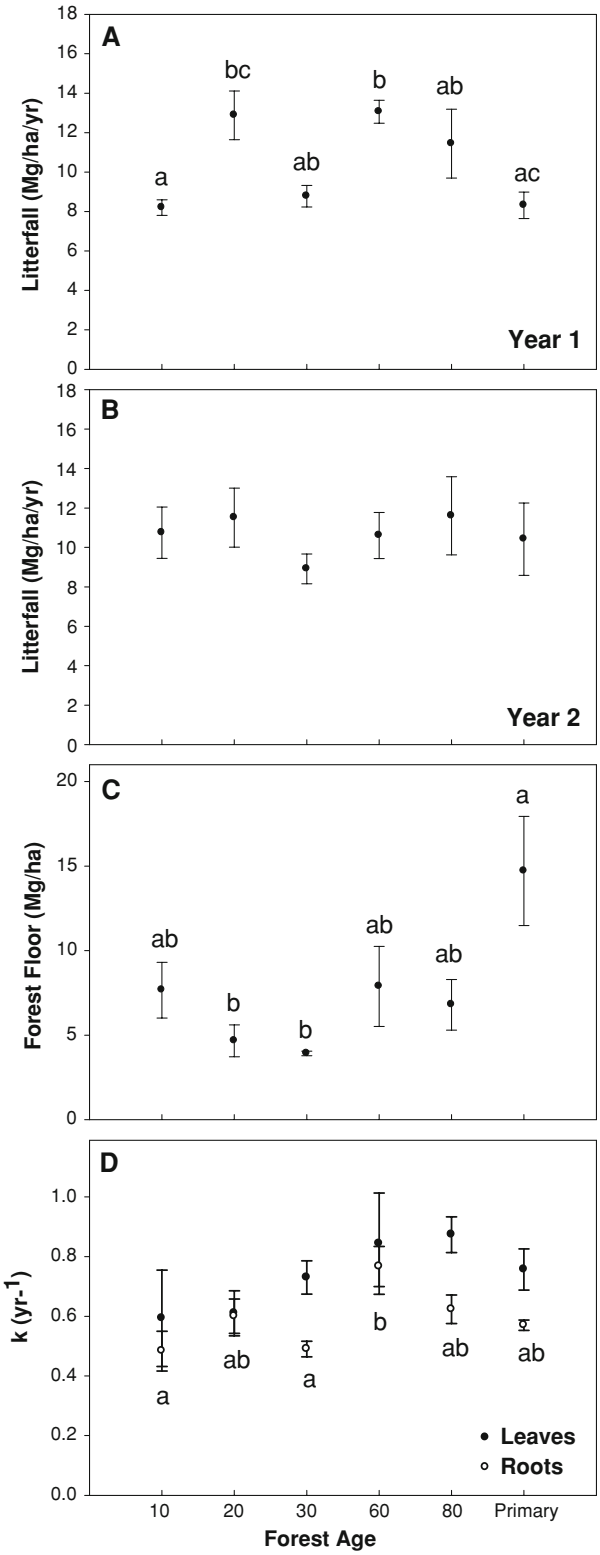

Figure 1. (**A**) Total litterfall for the first year of sampling. *Points* represent the average and standard errors of three plots, each of which contained five litter traps. *Letters* represent significant differences across forest ages. (**B**) Total litterfall for the second year of sampling, similar to figure above. There were no significant differences among forest ages. (**C**) Total of all forest floor components. *Points* represent the average and standard errors of three plots per age. (**D**) Decomposition rate constants for roots and leaves across the chronosequence. *Points* represent the average and standard errors of three plots per age. *Letters* represent significant differences for roots with forest age; there were no significant differences for leaves.

The common litter and common site experiments tested the influence of leaf litter quality versus site quality, and both factors were found to be important, depending on site age. For litter decomposed at the common site, k values were relatively similar to each other (Table 2), suggesting that litter quality differs little among sites. Common litter decomposed at all sites was more variable, suggesting the importance of a given site's environmental conditions in controlling decomposition (Table 2). Comparison of all three k values within a site showed that at the 10- and 30-year-old sites, *in situ* decomposition was slower than what would be expected based on litter quality (from the common site experiment), suggesting site, or environmental controls on decomposition. At the 20-, 60-, and 80-year-old sites, the *in situ* decomposition rate was slower than expected based on site effects, highlighting the role of litter quality. The primary forest site had similar *in situ*, common litter, and common site decomposition rates (Table 2).

Exponential decay described root decomposition well (Table 2), with final mass remaining ranging from 26 to 39% after 22 months (Figure 2). Root litter decomposition was significantly faster at the 60-year-old sites and slower at the 10- and 30-year-old sites, with the other sites being intermediate ($F_{5,12} = 4.16$, $P < 0.05$, Figure 1D). A paired t-test conducted on the k values for roots and leaves *in situ* at each site showed that within a site roots decomposed slower than leaves ($P < 0.005$).

Litter Quality Measurements

Mixed-leaf litter chemistry differed significantly from root litter chemistry. Leaf litter had a higher percentage of C ($P < 0.0001$), water-soluble compounds ($P < 0.0001$), and tannins (WS polyphenols, $P < 0.0001$). Root litter had significantly more Klason lignin ($P = 0.03$) and AS polysaccharides than leaves ($P < 0.0001$, Table 4). In addition, roots had significantly higher lignin-to-N ratios ($P < 0.0001$). There were no statistically significant differences in the percentage of N, NPE, AS compounds, water-soluble polysaccharides, or C-to-N ratios between leaves and roots.

Table 2. Decomposition Rate Constants for the Secondary Forest Sites and Primary Forest

Site	Leaves *in situ*			Leaves common site		Leaves common litter		Roots *in situ*	
	Age (year)	k (y^{-1})	R^2 (%)	k (y^{-1})	R^2 (%)	k (y^{-1})	R^2 (%)	k (y^{-1})	R^2 (%)
1	10	0.91**	83.6					0.60***	95.5
2	10	0.38*	80.0	0.69**	92.7	0.45***	99.2	0.48**	85.9
3	10	0.49*	81.3					0.37**	90.9
4	20	0.76**	92.2					0.50*	82.6
5	20	0.55*	81.8	0.47**	86.3	1.21***	99.6	0.70	87.2
6	20	0.52*	70.9					0.60**	85.8
7	30	0.66*	79.5	0.76**	93.3	0.64	60.6	0.53*	76.9
8	30	0.84**	86.1					0.44***	94.8
9	30	0.69**	84.2					0.50*	73.5
10	60	1.17***	97.8					0.87*	86.7
11	60	0.60*	76.1	0.64**	94.0	1.16**	93.6	0.79***	96.9
12	60	0.76*	80.6					0.64**	87.5
13	80	0.79**	91.4					0.69**	88.8
14	80	0.84**	87.2	0.62**	89.3	1.14***	98.3	0.65**	86.6
15	80	0.99**	91.5					0.53**	89.7
16	Primary	0.89**	91.9					0.54	99.0
17	Primary	0.66**	86.2					0.57***	96.9
18	Primary	0.72**	94.7	0.72**	94.7	0.74***	95.3	0.60**	90.5

*Values of k were calculated for each site using an exponential decay model, and R^2 values represent fit of the model, with *P < 0.05, **P < 0.01, ***P < 0.001. Leaves were decomposed in situ in litterbags at all sites and in a common site (site 18) and with a common Litter (popsicle sticks).*

Individual species varied in their litter quality characteristics; for example, there was a threefold difference in lignin among species (Table 3). For mixed leaf and root litter, only ash and percent C differed significantly among forest ages (Table 4). The forested sites showed similar leaf litter chemistries over the successional chronosequence, with no differences in ^{13}C-NMR spectral regions or proximate C fractions.

Bulk soil C followed no distinct trend over the successional sequence. Soil C was positively correlated with the leaf litter aliphaticity index (r = 0.86, Spearman rho, $P < 0.01$) and the recalcitrant index (r = 0.83, Spearman rho, P = 0.01), suggesting that there is greater soil C accumulation if leaf litter contains more tannins and waxy compounds relative to more labile compounds. Root chemistry did not correlate with soil C pools. Root decomposition rates were best predicted by percent N ($P < 0.05$) and C-to-N ratios (P = 0.07).

Discussion

Successional Age and the Functioning of Secondary Forests

Secondary forests are a dominant land-cover type in the tropics. Understanding the recovery of ecosystem processes such as C cycling in abandoned agricultural and pasturelands is critical to the valuation of these forests. Although structural changes in species composition and biomass accumulation along chronosequences are well studied (for example, Saldarriaga 1988; Tucker and others 1998; Guariguata and others 1997; Denslow and Guzman 2000; Martin and others 2004), there are many fewer investigations on the functioning of secondary forests (for example, Hughes and others 1999; Feldpausch and others 2004), and very few studies that have examined forests greater than 20 years old (Finegan 1996; Silver and others 2000; Chazdon 2003).

This 80-year-old replicated chronosequence demonstrated that although dramatic changes in species composition can occur during secondary succession (Marín-Spiotta and others 2007), changes in processes related to C cycling are less pronounced. Litterfall varied somewhat among the forest ages in the first, but not the second, year of collection, and decomposition rates of leaf litter were comparable over succession (Figure 1). Similar results were reported in three successional Mexican dry forests (3, 13, and >50 years), where the decay rates of three dominant species decomposed singly did not vary with forest age (Xuluc-Tolosa and others 2003). Species richness and basal area can recover after a few decades of secondary

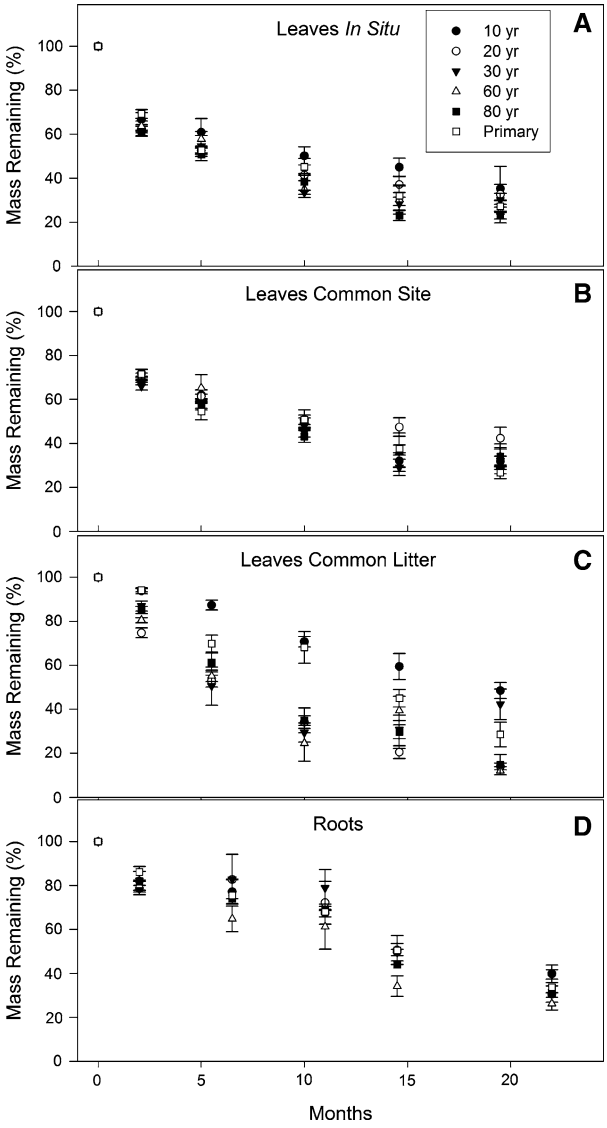

Figure 2. (**A**) Mass loss of leaves in litterbags placed at different aged sites along the successional chronosequence. *Points* represent the average and standard errors of three plots per age. In the *in situ* experiment root litter was placed back into the plot in which it was produced. (**B**) Mass loss from litterbags for leaves collected at one site per age and placed into a common site (primary forest). *Points* represent the average and standard error of six replicate litterbags per time point. (**C**) Mass loss from litterbags for popsicle sticks placed at one site per age. *Points* represent the average and standard error of six replicate litterbags per time point. (**D**) Mass loss of roots in litterbags placed at different aged sites along the successional chronosequence. *Points* represent the average and standard errors of three plots per age. In the *in situ* experiment root litter was placed back into the plot in which it was produced.

succession despite the fact that it may take centuries to return to the original floristic composition (Aide and others 2000; Pascarella and others 2000;

Table 3. Leaf Chemical Properties of Some of the Dominant Species in Secondary Forests of the Cayey Region of Puerto Rico

Species	Family	Age most important	Proximate analyses						Elemental analyses			
			Ash (%)	NPE (%)	WS (%)	AS (%)	Lignin (%)	Tannin (%)	WS polysaccharides (%)	N (%)	C (%)	C:N	Lignin:N
S. jambos L. Alston	Myrtaceae	10 years	7.5	17.9	29.4	35.7	17.0	13.9	4.3	1.2	50.7	43.3	14.6
C. schreberiana Miq.	Cecropiaceae	20 years	12.5	7.9	11.7	48.2	32.2	3.1	1.8	1.4	49.1	36.4	23.8
T. heterophylla (D.C.) Britton	Bignoniaceae	30 years	7.2	9.3	28.1	51.7	10.9	6.9	9.1	1.1	49.3	43.6	9.6
Myrcia deflexa (Poir.) DC.	Myrtaceae	60 years	8.6	7.7	15.8	48.2	28.3	3.8	2.2	1.5	50.5	34.6	19.4
Ocotea leucoxylon (Sw.) Mez	Lauraceae	60 years	7.9	14.0	20.3	45.7	20.0	4.1	3.4	2.1	53.4	25.2	9.4
Schefflera morototoni (Aubl.) Maguire, Steyerm. & Frodin	Araliaceae	60 years	5.4	8.2	28.5	43.7	19.6	7.6	6.1	1.5	54.5	35.4	12.7
P. montana (R. Grah.) Nichols	Aracaceae	Primary	13.7	7.5	9.9	58.2	24.4	1.5	1.3	1.4	46.0	33.3	17.7

Samples were taken from one site (#23), which represented 80-year-old secondary forest. See section ''Methods'' for description of chemical analyses. The age class that a species is most important in comes from importance values presented in Marín-Spiotta and others (2007).

Table 4. Measurements of Mean Initial Litter Quality (SE) for Leaves and Roots at the Sites Along the Chronosequence (n = 3)

Age	Proximate analyses							
	Ash (%)	Non-polar Extractable (%)	Water soluble (%)	Acid soluble (%)	Lignin (%)	Tannin (%)	Water soluble soluble polysaccharides (%)	Acid soluble polysaccharides (%)
Leaves								
10	8.4 (1.2)	6.2 (0.8)	17.5 (1.7)	50.5 (1.6)	25.7 (3.7)	2.9 (0.4)	3.1 (0.1)	16.8 (1.3)
20	8.4 (0.9)	6.2 (0.5)	20.5 (2.7)	51.2 (3.9)	22.1 (5.3)	4.6 (1.1)	4.4 (1.5)	22.7 (6.1)
30	6.4 (0.4)	4.6 (0.7)	23.2 (0.4)	54.5 (3.1)	17.6 (3.6)	4.7 (0.2)	6.5 (0.5)	26.4 (2.7)
60	5.2 (0.5)	5.5 (1.5)	17.9 (1.2)	44.8 (2.6)	31.8 (1.6)	4.1 (0.8)	3.6 (0.6)	19.1 (1.6)
80	6.4 (1.1)	5.5 (0.5)	21.9 (0.6)	45.1 (1.7)	27.5 (2.5)	5.9 (0.7)	4.0 (0.4)	17.2 (1.9)
Primary	6.1 (0.3)	5.4 (1.1)	22.6 (1.6)	47.1 (2.9)	25.0 (0.7)	6.9 (1.5)	3.9 (0.5)	18.2 (2.2)
Roots								
10	11.1 (1.0)	5.6 (0.4)	17.0 (3.0)	51.4 (2.6)	26.1 (2.3)	2.2 (0.2)	3.9 (0.7)	30.0 (0.9)
20	12.3 (0.2)	8.0 (1.9)	15.8 (2.3)	49.7 (2.2)	26.5 (2.2)	2.3 (0.3)	4.5 (1.5)	28.3 (1.9)
30	10.0 (0.3)	8.1 (1.8)	15.9 (0.6)	49.3 (1.1)	26.7 (1.4)	2.3 (0.1)	4.7 (0.1)	29.3 (0.8)
60	8.9 (1.0)	6.2 (1.7)	15.2 (1.2)	49.4 (1.2)	29.2 (1.5)	2.7 (0.1)	3.5 (0.2)	30.1 (1.3)
80	7.4 (0.5)	6.1 (1.7)	14.4 (0.6)	46.4 (1.5)	33.1 (2.2)	3.5 (0.8)	3.6 (0.2)	27.6 (1.4)
Primary	5.1 (0.6)	4.5 (0.4)	13.6 (0.8)	49.8 (0.2)	32.2 (0.5)	3.3 (0.4)	3.2 (0.4)	33.9 (3.2)

Age	Elemental analyses				^{13}C-NMR analyses					
	N (%)	C (%)	C:N	Lignin:N	Alkyl (0–45)	*O*-alkyl (45– 110)	Aromatic (110–160)	Carbonyl (160–220)	Recalcitrant index	Aliphaticity index
Leaves										
10	1.3 (0.04)	45.4 (0.6)	33.9 (1.6)	19.3 (3.4)	0.2 (0.01)	0.5 (0)	0.2 (0)	0.1 (0.01)	0.6 (0.01)	0.4 (0.01)
20	1.3 (0.26)	44.6 (0.2)	39.0 (9.1)	17.2 (0.7)	0.2 (0.03)[1]	0.5 (0.05)[1]	0.2 (0)[1]	0.1 (0.02)[1]	0.6 (0.08)[1]	0.4 (0.10)[1]
30	0.9 (0.1)	45.7 (0.9)	48.9 (3.4)	18.3 (2.1)	0.2 (0.02)	0.5 (0.04)	0.2 (0.1)	0.1 (0.01)	0.5 (0.05)	0.3 (0.05)
60	1.4 (0.1)	48.4 (1.0)	33.7 (1.4)	22.2 (2.0)						
80	1.3 (0.1)	46.8 (1.0)	37.4 (0.9)	22.0 (1.9)						
Primary	1.1 (0.3)	47.1 (0.9)	41.7 (1.7)	22.1 (1.1)	0.2 (0.02)	0.5 (0.06)	0.2 (0.02)	0.1 (0.03)	0.5 (0.09)	0.3 (0.1)
Roots										
10	0.9 (0.1)	41.0 (0.2)	45.4 (2.9)	28.6 (1.4)						
20	1.1 (0.2)	43.8 (0.2)	47.2 (12.8)	27.5 (5.3)						
30	0.9 (0.1)	43.1 (0.3)	45.7 (1.8)	28.2 (0.6)						
60	1.4 (0.1)	44.1 (0.5)	32.2 (3.2)	21.5 (2.9)						
80	1.1 (0.1)	44.3 (0.2)	41.5 (3.5)	32.7 (4.5)						
Primary	1.2 (0.1)	46.1 (0.2)	38.2 (1.6)	26.7 (1.7)						

Leaf and root material described here was used for the decomposition experiments. See Methods for explanation of the different types of chemical analyses. [1]*n = 2.*

Guariguata and Ostertag 2001; Marcano-Vega and others 2002; Chazdon 2003). Our results show that litter dynamics also recover quickly. In this region, forest cover, rather than species composition, appears to be the key to re-establishing rates of litter inputs and decomposition.

Controls over Litter Decomposition

Results of the common litter and common site experiments for leaf litter suggest that site effects may be more important than litter quality in determining decomposition rates. Similarly, mass loss of a common litter was greater in a young secondary forest (25 years) than in bush fallow (4 years) or 12-year-old secondary forest in Cameroon (Hauser and others 2005), also suggesting site effects. In contrast, in a comparison between a mid-successional forest (ca. 50 years) and an adjacent mature tabonuco forest in Puerto Rico, decomposition rate was slightly higher in the secondary forest, and this difference was related to litter quality, but not site quality (Zou and others 1995).

Two competing forces may be affecting decomposition rates over succession, and therefore leading to no detectable patterns over time. Abiotic constraints, such as higher temperatures and lower soil moisture in younger forests under a developing canopy may contribute to slower decomposition rates (Martius and others 2004). Our data generally fit this pattern, where common litter decomposed slowest at the 10- and 30-year-old sites, and comparisons with the *in situ k* values suggest that environmental or edaphic conditions of these sites depressed decomposition rate. The 10-year-old sites did not have a closed canopy, and the 30-year-old sites showed differences from the other secondary forests, which may reflect a successional change (Marín-Spiotta and others 2007). Specifically, the 30-year-old secondary forests were most heavily dominated by one species, *T. heterophylla*, and had the most homogeneous stem size distribution. In contrast, the 60- and 80-year-old sites had much higher decay rates for the common litter, suggesting that these sites provided better microsites for decay (Table 2). This pattern also fits the root decomposition data; roots tended to decompose slower at the 10- and 30-year-old sites, and fastest at the 60-year-old site (Figure 1D). The 20-year-old site used in the common litter experiment did not fit into this pattern because common litter decomposed at similar rates as in the older forests, but litter collected from these sites decomposed slower than other litter types at the common site, suggesting a litter quality effect (Table 2). This site is dominated by *C. schreberiana* (Table 3), a genus that has been shown to decompose slowly at other tropical sites (Mesquita and others 1998), and which we found to have the highest Klason lignin concentrations of our survey species (Table 4).

Linkages Between Litter and Soil C Storage

We had originally hypothesized that the change in species composition would be reflected in an increase in the hydrophobicity of litter across the chronosequence. Primarily, we expected to see an increase in NPE fractions and in the alkyl C region with forest age, corresponding to increased production of secondary compounds during succession (Coley and Barone 1996). Recently senesced leaves collected from branches did differ in their tissue quality among species, but we did not detect species effects in the composited samples of annual mixed leaf litterfall, either with ^{13}C-NMR spectroscopy or by sequential C fraction analyses. Most decomposition studies do not focus on the heterogeneous mixture of different leaf species that occurs in forests, but instead focus on individual species (Ewel 1976; Mesquita and others 1998; Xuluc-Tolosa and others 2003; Cleveland and others 2006). However, a review of the chemistry of litter mixtures reported frequent non-additive effects that can be either stimulatory or inhibitory, and noted that there can be considerable nutrient transfer among leaves of different species (Gartner and Cardon 2004). In our study of mixed forest litter inputs, chemistries were remarkably homogeneous, independent of forest community composition. Understanding mixed species litter decomposition is important from a C cycling perspective due to potential non-additive effects, and other species-specific factors such as genotype and herbivory damage that also appear to affect the decomposition rate of litter mixtures (Schweitzer and others 2005). Further research is needed to shed light on this apparent contradiction between differences in individual species foliage quality and chemical diversity in litter inputs (Epps and others 2007).

We included root decomposition in our study because it has been suggested that roots may contribute a greater proportion of C to soil organic matter than leaves (Bayala and others 2006; Kalyn and Van Rees 2006; Johnson and others 2007). Within a site, roots differed in tissue quality (for example, lignin and polyphenols) and decomposed significantly more slowly than leaves, and slower decay may mean that roots contribute more to the

soil C pool than leaves. This assertion is supported by other studies in the literature. For example, in a ^{14}C labeling experiment, root litter was found to have greater longevity than leaf litter or root exudates in arctic tundra soil (Loya and others 2004). Similarly, four times more root-derived C remained in the soil than leaf-derived C after 1 year in an experiment using $^{13}CO_2$ labeled roots and leaves (Puget and Drinkwater 2001). In Costa Rican plantations, once litter inputs reached a threshold level, roots were more responsible for soil C accumulation than leaf litter, and this contribution of soil C was controlled mainly by root litter chemistry and not the quantity of root litter inputs (Russell and others 2004). All of these studies point to the need to examine root characteristics and decomposition rates in conjunction with soil C studies.

The two NMR indices were strongly correlated with soil C pools, suggesting that sites with leaf litter with more recalcitrant compounds such as tannins and lipids also have greater storage of soil organic C (SOC). This would point to an indirect effect of litter quality, whereby these more recalcitrant materials decompose more slowly than more labile C sources and contribute to the formation of stable SOM. Tannins can form protein complexes in the soil that are fairly resistant to decomposition, as well as inhibit microbial growth (Kraus and others 2003). Lipids, which contribute mostly to the alkyl C region in the NMR spectra of plant litter (Almendros and others 2001), have been shown to be relatively resistant to decomposition and to persist in soils (Braids and Miller 1975; Dinel and others 1990; Gleixner and others 2001).

The lack of a direct link between litter inputs and bulk soil C content (also reported by Loranger and others 2002; Hauser and others 2005) does not preclude other potential differences in the forms of C and its turnover that may not be detected by static or one time sampling. For example, in a study with ^{14}C-labeled litter placed on soil cores, C from leaf and root decomposition did not result in greater total soil C, but differences in the chemistry of the soil C pool, in particular the distribution of sequential C fractions (WS, NPE, AS, and acid insoluble) were observed (Loya and others 2004). In studies of single-species plantations, species effects (via their litter quality) were much easier to detect in the particulate organic matter than in bulk SOM (Lehmann and others 2001).

In our chronosequence, sites varied in the turnover rates of different soil C fractions composing the bulk pool (Marín-Spiotta and others 2008a), even though there were no differences in bulk soil C contents with forest age (Table 1). In fact, Marín-Spiotta and others (unpublished) found no significant differences among primary forests, secondary forests, or active pastures. Conversion of forests to grasslands often results in an accumulation of soil C, likely due to higher belowground C inputs (Fisher and others 1994). However, many studies also report losses in soil C with conversion of tropical forests to pasture, especially with increasing length of pasture use (Veldkamp 1994; Rasiah and others 2004; Asner and others 2004; Elmore and Asner 2006). Multiple factors in addition to decomposition can affect the direction of change in soil C pools after land-use conversion, including soil type (mineralogy), type of disturbance, clearing method, use of fire, duration and intensity of land use, management type, and vegetation type (Marín-Spiotta and others 2008b).

In conclusion, our data show that forest C cycling processes can recover quickly after pasture use and abandonment in this region, suggesting that ecosystem functions can be restored in secondary forests even if species composition remains distinct from primary forests. Species differences in litter quality appear to have little influence on litter decomposition rates or on bulk soil C formation when considered as mixed species litter. As abandoned agricultural and pasture lands become reforested, new species assemblages are emerging, and although these species combinations may strongly influence aboveground patterns and trophic interactions, their influence on litter inputs and soil C cycling may be less important. These ''new forests'' (*sensu*) have the potential to recover ecosystem function and provide some of the same services of primary forests.

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