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Carbon Isotope Characterization of Vegetation and Soil Organic Matter in Subtropical Forests in Luquillo, Puerto Rico[1]

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ABSTRACT

We examined natural abundances of ^{13}C in vegetation and soil organic matter (SOM) of subtropical wet and rain forests to characterize the isotopic enrichment through decomposition that has been reported for temperate forests. Soil cores and vegetative samples from the decomposition continuum (leaves, new litter, old litter, wood, and roots) were taken from each of four forest types in the Luquillo Experimental Forest, Puerto Rico. SOM $\delta^{13}C$ was enriched 1.6‰ relative to aboveground litter. We found no further enrichment within the soil profile. The carbon isotope ratios of vegetation varied among forests, ranging from −28.2‰ in the Colorado forest to −26.9‰ in the Palm forest. Isotope ratios of SOM differed between forests primarily in the top 20 cm where the Colorado forest was again most negative at −28.0‰, and the Palm forest was most positive at −26.5‰. The isotopic differences between forests are likely attributable to differences in light regimes due to canopy density variation, soil moisture regimes, and/or recycling of CO_2. Our data suggest that recalcitrant SOM is not derived directly from plant lignin since plant lignin is even more ^{13}C depleted than the bulk vegetation. We hypothesize that the anthropogenic isotopic depletion of atmospheric CO_2 (*ca* 1.5‰ in the last 150 years) accounts for some of the enrichment observed in the SOM relative to the more modern vegetation in this study and others. This study also supports other observations that under wet or anaerobic soil environments there is no isotopic enrichment during decomposition or with depth in the active profile.

Key words: carbon dioxide; carbon isotope; decomposition; enrichment; fractionation; litter; rain forest; soil organic matter; $\delta^{13}C$.

RESUMEN

Examinamos la abundancia natural de ^{13}C en la vegetación y la materia orgánica del suelo (SOM) de bosques subtropicales húmedos y lluviosos para caracterizar el enriquecimiento isotópico, debido a descomposición, que ha sido reportado en bosques templados.

Muestras de planta y suelo representando diversas fases del proceso de descomposición (hojas verdes, hojas recién caídas, hojarasca, madera y raíces) se tomaron en cada uno de cuatro tipos de bosque en el Bosque Experimental Luquillo, Puerto Rico. El $\delta^{13}C$ de la SOM mostró un enriquecimiento de 1.6‰ en comparación con la hojarasca. No encontramos enriquecimiento adicional en el perfil del suelo. Las proporciones isotópicas de carbono de la vegetación variaron entre los diferentes bosques desde −28.2‰ en el Bosque Colorado hasta −26.5‰ en el Bosque Palma. Las proporciones de isotópos de la SOM, principalmente en los 20 primeros cm., también variaron entre los bosques, siendo el Bosque Colorado el del valor más negativo con −28.0‰, y el Bosque Palma el del valor más positivo con −26.5‰. Las diferencias isotópicas entre los bosques podrían atribuirse a diferencias en los regímenes de luz debido a variaciones en la densidad del dosel, a diferencias en los regímenes de humedad del suelo y/o al reciclaje de CO_2. Nuestros datos sugieren que la SOM recalcitrante no se deriva directamente de la lignina de las plantas debido a que ésta última está aún más empobrecida en ^{13}C que la vegetación en su totalidad. Planteamos la hipótesis de que la degradacion isotópica de CO_2 atmosférico debido a la acción del hombre (1.5‰ en los últimos 150 años), daría cuenta en parte del enriquecimiento observado en la SOM en relación con la vegetación actual analizada en éste y en otros estudios. Este estudio también apoya otras observaciones de que, bajo condiciones de suelo anaeróbico o húmedo, no existe enriquecimiento isotópico durante la descomposición ni al profundizar en el perfil activo.

Palabras clave: dióxido de carbono; isotópos de carbono; descomposición; enriquecimiento; fraccionamiento; hojarasca; bosque lluvioso; materia orgánica del suelo; $\delta^{13}C$.

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PRIMARY PRODUCERS FIX CARBON AND ISOTOPICALLY LABEL the organic matter they create. The stable carbon isotope ratios ($\delta^{13}C$ values) of most plants fall into two broad ranges based on their photosynthetic metabolism: those plants which use a C_4 (Hatch-Slack) mechanism have an average $\delta^{13}C$ near $-12.5‰$ to $-14.0‰$, while those which use the C_3 (Calvin cycle) mechanism are significantly more negative near $-26.5‰$ to $-28.0‰$ (O'Leary 1988, Tieszen 1994). This isotopic label is incorporated into other compartments in the ecosystem or returned to the atmosphere with respired CO_2. Because isotopic compositions integrate ecosystem processes (see Ehleringer *et al.* 1993), understanding the isotopic development of soil organic matter (SOM) has become especially useful to document vegetation change (Dzurec *et al.* 1985, Schwartz *et al.* 1986, Volkoff & Cerri 1987, Vitorello *et al.* 1989, Tieszen 1991, Andreux *et al.* 1992, Balesdent *et al.* 1993, and Mariotti & Peterschmitt 1994) and to reconstruct paleoenvironments (Krishnamurthy *et al.* 1982, Cerling 1991, Sukumar *et al.* 1993). The quantification of these vegetation changes will be aided by a clearer understanding of any fractionation which may occur between plant material and SOM formation.

If there were no fractionation or selective decomposition during the decay process, SOM would contain the same carbon isotope value as the vegetation that gave rise to it. Because SOM exists in pools characterized by different turnover rates (Parton *et al.* 1987), the isotopic signals of these pools integrate the vegetation histories which produced the SOM. These differences in turnover rates were shown clearly by Balesdent (Balesdent *et al.* 1987) where the C_3 isotopic signals derived from forest soil were almost totally replaced in the more labile organic matter pools by a C_4 signal following 23 years of maize cultivation. More recalcitrant pools were largely unaffected. Furthermore, the study showed less organic matter turnover with greater depth in the profile.

We would expect ecosystems which have existed for long periods (thousands of years) to possess uniform carbon isotope ratios throughout the profile and in all SOM pools. However, some results suggest that under long-term forestation there is an enrichment ($\delta^{13}C$ becoming more positive) both between litter and soil and with depth in the soil profile. Some New Zealand soils under deciduous forests showed a 1.4‰ to 1.5‰ enrichment between the surface and 75 cm (Goh *et al.* 1976). Similar enrichment within the profile was reported in German forests where deeper soils were enriched 2‰ relative to surface soils (Schleser *et al.* 1981). North American forests have also exhibited enrichment within the soil profile: soils under old oak forests in Wisconsin were enriched 1.6‰ between the top 10 cm of mineral soil and the deeper soils (Nadelhoffer & Fry 1988), and soil beneath a pine forest in Massachusetts was enriched 1.6‰ from the forest floor to 45 cm deep (Melillo *et al.* 1989). These enrichments have generally been attributed to preferential microbial degradation of ^{13}C-depleted biochemical components.

In contrast to the above ^{13}C enrichment of SOM, Ember *et al.* (1987) reported that soils developed under marsh grass, *Spartina alterniflora,* were depleted 2‰ relative to fresh litter. Stout *et al.* (1981, Stout & Rafter 1978) showed that the carbon isotope value of equilibrated aboveground vegetation in New Zealand grasslands did not differ from that of the upper soil horizons. Similarly, Stout detected no fractionation with depth in peats or in *Agathis* forests in New Zealand (Stout *et al.* 1975) where decomposition was restricted in wet soils. The studies by Stout and Ember have linked the isotopic depletion with depth to restricted degradation due to anaerobic conditions. Under these conditions, presumably, ^{13}C depleted biochemicals like lignin are retained.

This study was designed to complement similar studies in temperate forests by characterizing the isotopic composition of some tropical forests and investigating the potential and magnitude of the reported enrichment of ^{13}C associated with soil depth. We describe the stable carbon isotope composition of plant compartments and SOM in four forests in the Luquillo Experimental Forest, Puerto Rico. We establish the relationships between carbon isotopic signals in SOM and vegetation in the forest ecosystems that have existed for long periods.

MATERIALS AND METHODS

The Luquillo Experimental Forest, an NSF Long Term Ecological Research (LTER) site, is located in the Luquillo Mountains of northeastern Puerto Rico (18°18'N, 65°47'W). We sampled the four life zones (forests) defined by Ewel and Whitmore (1973) which typify the range of diversity found with the Luquillo forest. Based on climate and vegetation, the Colorado forest is classified as a subtropical rain forest, the Dwarf forest as a lower montane rain forest, the Palm forest as a lower montane wet forest, and the Tabonuco forest as a subtropical wet forest. The Colorado and Dwarf forests receive over 4 m of precipitation annually

and Palm and Tabonuco receive about 2 m, keeping all forests wet throughout the year. Soil characteristics of the Luquillo Experimental Forest are reviewed and described as quite diverse (Brown *et al.* 1983). The Ultisols at our sites were generally deep, weathered, leached, and low in pH. As altitude increases from the lower and well drained Tabonuco sites, soil moisture and organic matter increase. The Palm and Dwarf soils are poorly drained and saturated with stronger reducing conditions in the Dwarf forest. The Tabonuco forest occupies the largest fraction of Luquillo and is found mostly on better-drained ridges below the 600 m cloud condensation level. Colorado and Palm forests are both found above 600 m, but Palm forests usually occupy wetter soils and steeper slopes than the Colorado. The Dwarf forest exists at the highest elevations in Luquillo and is characterized by wind and nearly constant cloud cover (Brown *et al.* 1983).

We analyzed soil and vegetation samples from four sites within each forest. Within each 5 × 5 m site we pooled two soil cores (2.5 cm diameter) ranging in depth from 40 to 60 cm. The cores were stratified and pooled by depth at 0 to 10 cm, 10 to 20 cm, and greater than 20 cm. Prior to analyses, soil samples from each strata were picked free of macroscopic roots which were then sorted into roots greater than 2 mm in diameter and roots less than 2 mm. Both roots and soil were treated with 1 N HCl at room temperature and shaken to remove carbonates, occasionally present, before being ground to homogeneity.

We collected four categories of composited vegetation at each site including: fully developed green "leaves" from trees and undergrowth surrounding the core area, leaves which had recently fallen from the trees and had not yet lost their green color (hereafter "new litter"), "old litter" which was partially decomposed identifiable organic matter from the forest floor, and dead "wood," including proportional amounts of twigs, branches and boles, that had fallen to the ground. Each vegetation sample consisted of >500 g of plant material. All vegetation samples were dried and ground to pass a 40 mesh sieve. Lignin and cellulose extracts were taken from some of the vegetation according to Fagre *et al.* (1991). A soluble fraction was removed by extracting the ground sample in pH 7.0 water, for 24 hours at room temperature, and with constant agitation.

The leaves sampled from one site in each forest were separated and identified by species to describe isotopic variation among species. Species were identified by the LTER field vegetation technician. For statistical purposes, we calculated the green value for this intensively analyzed site from the mean of the values for these species.

Vegetation and soil samples were analyzed for C and N by combustion (1.5 mg sample size for vegetation, 5 to 20 mg for soil) on the Carlo Erba 1500 CN analyzer. Sample CO_2 from elemental analysis was then cryogenically purified and analyzed for carbon isotope ratios on a VG SIRA 10 isotope ratio mass spectrometer (IRMS) (Tieszen & Fagre 1993). The $\delta^{13}C$ values were calculated by the following standard equation: $\delta^{13}C = (R_s - R_p)R_p^{-1} \times 1000$ where R_s = ratio of ^{13}C to ^{12}C in the sample, and R_p = ratio of ^{13}C to ^{12}C in PDB standard. The precision of the instrument including sample preparation and analysis is better than 0.2‰. Periodic replicates were run, and internal standards were interspersed with every 10th sample. SOM was radiocarbon dated by conventional counting at Geochron Inc.

Statistical analyses were performed on SuperANOVA (Abacus Concepts, Inc., Berkeley, California). When models were significant ($P < 0.05$), we grouped similar means by the statistically conservative Tukey-Kramer test.

RESULTS

VEGETATION.—The mean carbon isotope ratios of vegetation compartments fell into a narrow range from −30.7‰ for leaves in the Tabonuco forest to −26.9‰ for roots in the Dwarf and Palm forests (Table 1). Roots did not differ isotopically with depth in the soil profile ($F = 0.5$, $P = 0.6$), or by size ($F = 3.9$, $P = 0.53$), and were thus pooled within forests for statistical comparisons. Isotope ratios of vegetation compartments differed consistently between the more negative leaves and more positive roots. New litter, old litter, and wood were isotopically similar and intermediate, differing as a group from leaves and roots. Vegetation in the Tabonuco and Colorado forests possessed the more negative isotope ratios, and Dwarf and Palm the more positive.

The patterns of C:N ratios were similar in all forests. Leaves had the lowest C:N ratio, averaging 28. Litter and finer roots were intermediate while large roots and wood had typically high C:N ratios together averaging 78 (Table 1). Vegetation C:N ratios did not differ significantly among forests.

Leaves sampled from individual species in the understory ranged from −26.6‰ for *Daphnopsis philippiara* in the Colorado forest to −33.3‰ for

TABLE 1. *Mean of carbon isotope ratios and carbon to nitrogen ratios ($g \cdot g^{-1}$) and results of two-factor ANOVAs for vegetation compartments in the Luquillo Experimental Forest, Puerto Rico. Letters group similar means within compartments or forests as determined by the Tukey-Kramer test at 0.05 significance level.* N = *pooled samples from four sites in each forest. Isotope data from roots of different depths, roots <2 mm, and roots >2 mm were pooled because they were not statistically different.*

	Forest				
	Colorado	Dwarf	Palm	Tabonuco	ANOVA
$\delta^{13}C$ ‰ ± SE					$F = 43.7$, $P < 0.0001$
Leaves	−30.5 ± 0.2	−29.8 ± 0.4	−29.3 ± 0.2	−30.7 ± 0.2	A
New litter	−28.4	−28.9 ± 0.1	−29.3 ± 0.5	−29.3 ± 0.1	B
Old litter	−29.6 ± 0.4	−28.3 ± 0.2	−27.6 ± 0.5	−30.0 ± 0.1	B
Wood	−28.9 ± 0.4	−27.4 ± 0.6	−28.5 ± 1.1	−29.6 ± 0.5	B
Roots	−28.2 ± 0.1	−26.9 ± 0.2	−26.9 ± 0.2	−27.8 ± 0.2	C
ANOVA $F = 11,7$, $P < 0.0001$	A	B	B	A	
C/N ± SE					$F = 44.7$, $P < 0.0001$
Leaves	25.1 ± 4.8	31.3 ± 2.1	21.4 ± 2.5	26.6 ± 0.8	A
New litter	55.5	36.0 ± 2.9	32.6 ± 3.6	33.1 ± 3.3	AB
Old litter	38.3 ± 0.9	36.1 ± 1.9	24.8 ± 2.6	26.7 ± 1.4	AB
Wood	79.3 ± 13.6	102.1 ± 7.6	85.5 ± 5.5	69.2 ± 5.6	C
Roots <2 mm	57.8 ± 5.6	36.2 ± 1.4	27.0 ± 2.6	52.6 ± 5.6	B
Roots >2 mm	78.2 ± 5.1	57.8 ± 5.4	62.0 ± 5.4	89.9 ± 5.1	C
ANOVA $F = 2.6$, $P = 0.054$					

two species in the Dwarf (*Miklania fragilis*) and Tabonuco (*Palicourea riparia*) forests (Table 2). Several species *e.g., Alchornea latifolia, Calicogonium squamulosum,* and *Prestoea montana,* differed by more than 3.3‰ between forests.

The isotope ratios of lignin and cellulose departed predictably from the bulk signal (Table 3). Lignin was depleted by an average of 1.4‰, relative to the bulk, while cellulose was enriched an average 1.3‰. The soluble fraction was small in mass and was enriched relative to the bulk.

SOIL ORGANIC MATTER.—The isotope ratios of SOM differed between forests with Colorado generally the most negative and Palm the most positive. SOM from the top 10 cm was most negative in the Colorado forest, averaging −28.0‰ and most positive in the Palm at −26.4‰ (Table 4). Even at the greatest depths sampled, the Palm forest retained this relative enrichment. There were no differences in the $\delta^{13}C$ values of SOM with profile depth in any forest.

The soil C:N ratios differed strongly among forests. SOM in the Dwarf forest possessed the highest C:N ratio at 22.3, while Tabonuco contained the lowest at 10.4. There was no significant change in C:N ratio with depth. Soil organic C concentration differed between Dwarf and the other forests. The Colorado, Palm, and Tabonuco forests did not differ in C concentration, and averaged 4.1 percent C through the top 10 cm. Dwarf soil averaged 14.7 percent C for its top 10 cm. Soil C concentration decreased through the soil profile to roughly half that of the surface layers below 20 cm.

Radiocarbon dating of SOM from the Colorado and Dwarf forests indicated that while surface soils contained modern (perhaps "bomb" enriched) ^{14}C concentrations, soils >10 cm had a ^{14}C age of 2570 years b.p. in the Colorado and 910 years b.p. in the Dwarf (Table 5).

SOM was isotopically different from litter and wood (the aboveground SOM-forming vegetation compartments). The SOM was enriched relative to litter in all forests by an average 1.7‰ (Table 6 and Fig. 1), relative to the recent by an average of 1.9‰, and enriched relative to forest floor wood by 1.4‰. The isotope ratios of soils were not different from those of roots.

TABLE 2. *Isotope ratios of fully expanded leaves of species sampled in four forests in the Luquillo Experimental Forest, Puerto Rico. Selections include the most common species in the vicinity of the soil cores and vegetation samples. Leaves were pooled from several specimens in the lower part of the canopy (<5 m).*

Species	Forest			
	Colorado	Dwarf	Palm	Tabonuco
Alchornea latifolia	−32.8			−29.3
Alsophyla bryophyla		−28.9		
Anthurium sp.		−29.2		
Ardisia laquillensis		−30.7		
Begonia decandra			−28.3	
Calicogonium squamulosum	−33.1	−30.1		−29.8
Calyptranthes portoricensis		−31.7		
Cecropia peltata			−29.9	
Chionanthus dominguensis				−30.5
Croton poecilanthus			−29.5	−28.7
Cyathea portoricensis	−30.6	−30.7	−29.5	
Cyrilla racemiflora			−28.1	
Dacryoides excelsa				−31.7
Danea nodosa		−29.1		
Daphnopsis philippiara	−26.6			
Dillenia viridifolia		−30.7	−28.8	
Dioscorea sp.				−30.9
Euginea boringuensis		−29.8		
Gleichina bifida	−29.2			
Gonocalyx sp.		−30.3		
Guarea guidonea				−32.0
Guarea ramiflora			−30.5	
Guzmania biomeleid		−29.4		
Icanthus pallens			−28.3	
Ilex sp.		−30.9		
Manilkara bidentata				−31.7
Marcgravia rectiflora		−30.3		−29.8
Mectanium amigdalinum				
Miconia faveloata		−30.1		
Miconia pachyphyla		−29.6		
Micropholis chrysophylloides	−32.7			
Micropholis garciniefolia		−30.9		
Miklania fragilis		−33.3		
Miklania racemosa				−32.2
Ocotea leucoxylum				−30.8
Ocotea spathulata		−30.0		
Palicourea riparia				−33.3
Phylodendron sp.				−30.0
Pilea sp.		−29.2	−29.5	−31.1
Piper hispidum				−31.4
Pitcarnia sp.	−30.1			
Prestoea montana		−27.7	−29.2	−31.4
Psychotria albide				−30.6
Psychotria berteriana	−31.1	−30.0	−30.2	
Psychotria maleolans	−31.8			
Rourea surinamensis				−30.6
Sloanea berteriana				−29.4
Tabebaria rigida	−29.8	−29.7		
Tetragastris balsimifera				−32.0
Wallenia yunguensis		−30.7		
Mean ± SE	−30.8 ± 0.6	−30.0 ± 0.2	−29.2 ± 0.2	−30.9 ± 0.3

DISCUSSION

VARIATION BETWEEN FORESTS.—The organic material for all species and compartments in these four forests tend to be isotopically depleted relative to systems with more open canopy structures; however, there are isotopic differences which are consistent among the forests. These differences in the leaf compart-

TABLE 3. *Mean and SE of differences between $\delta^{13}C$ values (‰), of biochemical extracts and Colorado forest vegetation compartments, Luquillo Experimental Forest, Puerto Rico. Positive values indicate isotopic enrichment of the extract relative to the bulk, while negative values indicate depletion.*

Compartment	Chemical fraction		
	Cellulose	Lignin	Soluble
Litter	1.51 ± 0.04	−1.40 ± 0.08	1.73 ± 0.37
Wood	1.90 ± 0.23	−1.59 ± 0.32	1.23 ± 0.21
Roots	0.71	−1.24 ± 0.22	3.11 ± 0.26

TABLE 5. *Results of radiocarbon dating on root- and carbonate-free soils from two forests in the Luqillo Experimental Forest, Puerto Rico.*

	Years b.p. ± SD	% Modern ± SD
Colorado		
0–10 cm	Modern	100.6 ± 1.5
>10 cm	2570 ± 125	72.6 ± 125
Dwarf		
0–10 cm	Modern	108.3 ± 1.4
>10 cm	910 ± 155	89.3 ± 1.8

ments can be attributed primarily to different canopy densities which affect light levels, recycling of respired CO_2, or elevated CO_2 concentrations (Broadmeadow & Griffiths 1993, Jackson *et al.* 1993). Decomposition on the forest floor and in the soil produces ^{13}C-depleted CO_2 (relative to ambient CO_2) which is photosynthetically reincorporated into the vegetation (Schleser & Jayasekera 1985, Medina *et al.* 1991). As a result, isotope ratios of leaves growing in the lower canopy should be 3 to 5‰ more negative than the leaves at the tree tops. This effect will exist in all closed canopy systems where air circulation is diminished and the respired CO_2 is not rapidly mixed with the bulk atmosphere. As a result of this effect, Ducatti *et al.* (1991) observed that plants found in a closed canopy were 1.6‰ more negative than the same plants found in an open canopy. However, gaps may experience

TABLE 4. *Mean and SE of carbon isotope ratios, percent carbon, carbon to nitrogen ratios ($g \cdot g^{-1}$), and results of two-factor ANOVAs for soils and forests in the Luquillo Experimental Forest, Puerto Rico. Letters group similar means among forests or depths as determined by the Tukey-Kramer test at 0.05 significance level.* N = *4 sites of 2 pooled cores from each forest.*

Depth	Forest				ANOVA
	Colorado	Dwarf	Palm	Tabonuco	
$\delta^{13}C$ ‰ ± SE					$F = 1.2$, $P = 0.3$
0–10 cm	−28.0 ± 0.3	−27.7 ± 0.1	−26.4 ± 0.4	−27.0 ± 0.2	
10–20 cm	−28.1 ± 0.3	−27.8 ± 0.2	−26.6 ± 0.2	−26.7 ± 0.4	
>20 cm	−27.5 ± 0.4	−27.7 ± 0.3	−26.0 ± 0.7	−26.7 ± 0.4	
ANOVA $F = 12.8$, $P < 0.0001$	A	A	B	B	
% C ± SE					$F = 26.0$, $P < 0.0001$
0–10 cm	4.6 ± 0.4	14.7 ± 1.7	4.9 ± 1.1	3.2 ± 0.9	B
10–20 cm	3.2 ± 0.3	11.6 ± 1.9	3.2 ± 1.4	3.1 ± 0.5	B
>20 cm	1.4 ± 0.5	5.4 ± 2.4	1.9 ± 0.5	1.9 ± 0.4	A
ANOVA $F = 10.5$, $P = 0.0003$	A	B	A	A	
C/N ± SE					$F = 0.4$, $P = 0.6$
0–10 cm	20.5 ± 1.3	22.3 ± 0.5	15.1 ± 1.0	10.4 ± 0.3	
10–20 cm	20.5 ± 2.3	24.8 ± 1.4	18.1 ± 3.3	11.3 ± 0.6	
>20 cm	17.6 ± 3.1	24.9 ± 4.2	18.2 ± 4.1	10.7 ± 0.4	
ANOVA $F = 15.2$, $P < 0.0001$	BC	C	B	A	

TABLE 6. *Mean and SE of carbon isotope ratios and results of a two-factor ANOVA between carbon isotope ratios of SOM and selected vegetation compartments in the Luquillo Experimental Forest, Puerto Rico. Soils did not differ with depth and were pooled for these analyses. Roots did not differ by size or depth and were also pooled. Letters group similar means among samples as determined by the Tukey-Kramer test at 0.05 significance level. The interaction term on the ANOVA was also significant* ($F = 4.6$, $P < 0.0001$).

	Forest				
	Colorado	Dwarf	Palm	Tabonuco	ANOVA
δ ^{13}C ‰ ± SE					$F = 24.4$, $P < 0.0001$
Soil	−27.9 ± 0.2	−27.7 ± 0.1	−26.3 ± 0.3	−26.8 ± 0.2	B
Old litter	−29.6 ± 0.4	−28.3 ± 0.2	−27.6 ± 0.5	−30.0 ± 0.1	A
Wood	−28.9 ± 0.4	−27.4 ± 0.6	−28.5 ± 0.9	−29.6 ± 0.5	A
Roots	−28.2 ± 0.1	−26.9 ± 0.2	−27.0 ± 0.2	−27.8 ± 0.2	B
ANOVA $F = 12.8$, $P < 0.0001$	A	B	B	A	

CO_2 accumulation similar to that of nearby closed forests and result in similar isotope values as shown for Luquillo (Medina *et al.* 1991).

The forests we studied vary from dense vegetation in the Colorado, Tabonuco, and Dwarf forests to the relatively open canopy of the Palm forest. The Dwarf forest, however, differs from the others because its canopy is only a few meters high as opposed to 20 m in Colorado and 30 m in Tabonuco (Smith 1970). The more positive values for the leaves in the Dwarf and Palm forests can thus be explained by diminished CO_2 recycling as a result of greater air circulation; higher irradiances, resulting in a lower c_i/c_a ratio could produce the same effect. Discrimination by C_3 plants also decreases with altitude (Korner *et al.* 1991). The 500 m difference between our upper and lower sites could account for about 0.5‰ of these differences. As suggested by Jackson *et al.* (1993), the distinction between direct environmental effects on isotopic fractionation and the recycling of CO_2 requires simultaneous measurements of at least CO_2 concentrations if not isotopic composition. We attribute the variation between the leaves and new litter in our study to our sampling technique; since green leaves were hand picked from near the forest floor, their carbon isotope values are more negative because of this canopy effect. This is supported by our analysis of the leaf data secured by Medina in natural Luquillo forests where leaves derived from the canopy were significantly ($P = .0001$) more positive (4‰) than those from the understory. Our samples of recent leaves should adequately represent litter-producing organic matter since this sample pooled random and recent transfers from all heights in the canopy contributing to the litter fall.

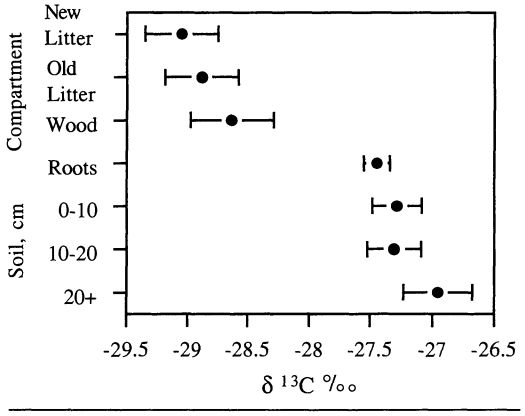

FIGURE 1. Mean (bar represents SE) isotope ratios of SOM and vegetation compartments across four forests in Luquillo, Puerto Rico. Roots were pooled by depth and size.

VARIATION WITHIN FORESTS.—Several studies have suggested that carbon is fractionated during decomposition and during the formation of SOM. The fractionation process can be divided into two stages: an initial stage in which recently dead aboveground inputs are progressively decomposed to unidentifiable litter and new SOM in the surface soil (surface fractionation), and a later stage of physical and chemical transformations which preserve the recalcitrant, structural fractions of the SOM (soil fractionation). Because the input for SOM is primarily in the surface layers, it is assumed that aboveground plant organic matter is the chief source of SOM (see

Anderson & Swift 1983), and comparisons between the isotope ratio of those soil-forming plant materials and SOM are therefore appropriate. Nevertheless, the isotopic values of roots are often more positive than the aboveground inputs as also shown by others (Medina *et al.* 1991). We need a better understanding of the quantitative contributions, both in terms of mass and isotopic signal, of aboveground and belowground inputs to the SOM.

Our results from all four Luquillo forests support a pattern of surface enrichment (soil enriched relative to vegetation) as observed in temperate forests (Nadelhoffer & Fry 1988, Balesdent *et al.* 1993) but not any associated soil enrichment (enrichment within the soil profile). In Luquillo, there is potential for the large volumes of precipitation to move water soluble organic matter through the soil profile. However, we find no evidence to support substantial illuvial translocation of isotopically distinct soil-forming material. Substantial movement of SOM within the soil profile is not supported by the ^{14}C data. Also, Hall *et al.* (1992) use a carbon balance hydrological model to suggest that only small amounts of C are transported out of these systems except during extreme hydrological events. Additionally, although the water soluble fractions of plant material are slightly enriched, and in terrestrial systems have not been studied adequately (Andreux *et al.* 1992), they probably do not comprise a substantial proportion of SOM since they likely are readily decomposed to CO_2. Thus, we believe that lateral or horizontal translocation cannot account for enrichment between aboveground inputs and SOM.

Although the composition and concentration of lignin are both important determinants of decomposition processes (see Moran *et al.* 1989, Melillo *et al.* 1989), it is not clear how, or even if, plant lignin becomes incorporated into SOM. On the molecular level, the Century model for decomposition and SOM formation (Parton *et al.* 1987) suggested that lignin was preserved relative to cellulose and that as cellulose was microbially consumed, lignin was released and became a structural part of the soil. Preservation of lignin, an isotopically depleted material by our results and others (Benner *et al.* 1987, Fagre *et al.* 1991), would lead to depletion in SOM relative to current vegetation. This selective preservation of isotopically depleted lignin does account for the depletion described by Ember (Ember *et al.* 1987) in SOM relative to current vegetation in an anaerobic salt marsh. Ember did not report depletion under aerobic surface conditions. Similarly, Stout and Rafter (1978) attributed isotopic depletion in subsurface organic matter to selective preservation of lipids, which are also isotopically depleted.

Because all four forests in Luquillo are enriched in SOM relative to aboveground inputs by more than 1‰, our data do not support the direct incorporation of lignin into SOM. The transformation of these compounds during decomposition, however, is complex. Melillo's experimental work (Melillo *et al.* 1989) with pine needles showed some isotopic enrichment of litter during early stages followed by a loss of cellulose and a retention of lignin with a subsequent slight depletion only after 29 months. It is also known that during litter decomposition the early-formed light SOM fractions contain lignins chemically similar to those found in plants (Kögel 1986, Kögel-Knabner & Ziegler 1993) but quite different from the strongly modified lignins found in heavier, older, and more recalcitrant SOM fractions. However, microbial breakdown of cellulose and the subsequent microbial synthesis of "lignoid" material could account for the origin of recalcitrant SOM and its isotopic composition. Furthermore, Moran (Moran *et al.* 1989) points out that degradation rates vary for the major subclasses of lignin and that some substituents, *e.g.,* methoxyl groups, are easily removed prior to other cleavages. This early modification of lignin could alter its isotopic composition and allow lignin to still become stabilized in SOM that is not depleted in ^{13}C. Critical experimental analyses under laboratory and field conditions should be undertaken to clarify these events during the formation of SOM. In addition, experimental evidence to describe the quantitative contribution to SOM of aboveground and belowground sources is needed, especially because of the different isotopic compositions.

We propose that the major reason the isotopic composition of the bulk SOM is enriched relative to modern vegetation inputs is because modern vegetation has become isotopically depleted during historical time. This is because of the anthropogenic depletion of CO_2 resulting largely from burning fossil fuels. This depletion of ^{13}C in the atmosphere (Keeling *et al.* 1979, Friedli *et al.* 1986, Marino & McElroy 1991, Tieszen & Fagre 1993) during the last few decades and since 1800 has accounted for a depletion around 1.5‰. Because the compartments into which carbon is transferred (*i.e.,* new leaves, litter, wood, relatively labile and recalcitrant SOM, *etc.*) vary in turnover rate, isotopic signals should differ predictably as the anthropogenic signal changes. Thus, we could expect ancient SOM to depart in a systematic way: it should appear enriched, relative to modern carbon. Recent vegeta-

tion, as expressed in new leaf fall and litter, should be around 1.5‰ more negative than SOM older than 100 or 200 years and also cause older, deeper soils to appear enriched relative to more recent, surface soils. Soil organic matter in Luquillo forests is enriched an average 1.5‰ relative to litter. Enrichment, however, is not observed from the top to the bottom of the soil profile despite modern radioisotope dates for surface soils. This may be a result of slow accumulation of SOM in the tropics due to rapid decomposition of most new plant litter (Anderson & Swift 1983, Lugo *et al.* 1990).

We have undertaken a test of this explanation for the difference between litter inputs and SOM values by incorporating isotopic information in a STELLA version of the Century model (Parton *et al.* 1987). Although details will be presented elsewhere, we simulated a weekly input of litter; typical lignin : nitrogen ratios; and reasonable half-lives for active (1.5 yr), slow (25 yr), and passive (1000 yr) soil organic matter with a content of 15 kg C/m^2. This is less than the potential 40 kg/m^2 for undisturbed forest (Brown & Lugo 1982) and more than 9 kg/m^2 for secondary forests (Weaver *et al.* 1987). We used a ramp function to simulate the estimated curve of atmospheric depletion since 1800 and quantified isotopic changes in various pools. Metabolic and structural pools of plant litter are 95 to 100 percent equilibrated with the modern signal and the active pool of SOM contains 75 percent of this new signal. However, the pools accounting for the bulk of SOM, slow and passive, change isotopically relatively little (35% and 2%, respectively). These simulations suggest that less than 20 percent of the bulk carbon in the profile possesses the new signal. A detailed comparison of the incorporation of this anthropogenic signal in soils of various textures and in divergent climates could help validate turnover rates incorporated in various SOM and decomposition models.

Our data and initial simulations provide strong support for the suggestion of Balesdent *et al.* (1990, 1993) that apparent "enrichments" between litter and near surface SOM less than 1.5‰ can be accounted for directly by this anthropogenic effect. Application of Century-type models under different climates should establish the variations in the rates at which the anthropogenic signal becomes incorporated under a wide range of ecological conditions. This perturbation of a steady-state isotopic relationship between plant and SOM fractions must be understood as we develop isotopic approaches to assess vegetation change in response to climate change or human intervention and as we develop our understanding of the roles of tropical forests in global fluxes and sequestering of CO_2 (Brown *et al.* 1992, 1993; Lugo 1992).

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