

Effects of nutrient additions on ecosystem carbon cycle in a Puerto Rican tropical wet forest

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Abstract

Wet tropical forests play a critical role in global ecosystem carbon (C) cycle, but C allocation and the response of different C pools to nutrient addition in these forests remain poorly understood. We measured soil organic carbon (SOC), litterfall, root biomass, microbial biomass and soil physical and chemical properties in a wet tropical forest from May 1996 to July 1997 following a 7-year continuous fertilization. We found that although there was no significant difference in total SOC in the top 0–10 cm of the soils between the fertilization plots ($5.42 \pm 0.18 \text{ kg m}^{-2}$) and the control plots ($5.27 \pm 0.22 \text{ kg m}^{-2}$), the proportion of the heavy-fraction organic C in the total SOC was significantly higher in the fertilized plots (59%) than in the control plots (46%) ($P < 0.05$). The annual decomposition rate of fertilized leaf litter was 13% higher than that of the control leaf litter. We also found that fertilization significantly increased microbial biomass (fungi + bacteria) with $952 \pm 48 \text{ mg kg}^{-1}$ soil in the fertilized plots and $755 \pm 37 \text{ mg kg}^{-1}$ soil in the control plots. Our results suggest that fertilization in tropical forests may enhance long-term C sequestration in the soils of tropical wet forests.

Keywords: decomposition, forest floor mass, litterfall, long-term carbon, microbial biomass, soil carbon

Received 19 November 2004; revised version received 12 September 2005 and accepted 21 September 2005

Introduction

Increasing scientific attention has been paid to the role of nutrient additions, such as N, P and K, on C sequestration in terrestrial ecosystems (Fenn *et al.*, 1998; Hagedorn *et al.*, 2003; Hall & Matson, 2003; Giardina *et al.*, 2003). Nutrient inputs from both human fertilization and natural atmospheric deposition have considerably altered ecosystem processes, such as productivity, soil fertility, and decomposition; thus, nutrient additions may also change ecosystem C sequestration quantitatively and qualitatively (Aber *et al.*, 1995; Hall & Matson, 1999; Chapin *et al.*, 2002; Neff *et al.*, 2002; Guo & Gifford, 2002).

Wet tropical forests are among the most productive terrestrial ecosystems and exert a disproportionately large influence on global C cycling (Jobbágy & Jackson,

2000). However, C allocation and the response of various C pools to nutrient addition in these forest ecosystems are far from clear. For instance, the effect of N deposition on C sequestration in forest ecosystems, at a global scale, has been estimated to range from 0.1 to 2.3 Pg C yr^{-1} (Nadelhoffer *et al.*, 1999). This substantial range of uncertainty is primarily because of the lack of mechanistic understanding of the interactions between C and N cycles and associated biological and biophysical processes, especially for belowground processes. Soils are highly complex media with various substrates that feature different organic C and nutrient contents. As a result, nutrient addition to soils could increase the decomposition rate for some soil organic C (SOC) pools and, meanwhile, decrease the decomposition rate for other C pools (Neff *et al.*, 2002). For example, Hagedorn *et al.* (2003) reported that increased N deposition retarded mineralization of old soil organic matter. Giardina *et al.* (2004) found that nutrient additions decreased belowground C cycling in a humid tropical forest. Although the responses of forest ecosystems to nutrient additions have been studied extensively

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during the past decades, most previous studies mainly focused on the effects of nutrient addition on above-ground productivity. Studies on the effect of fertilization on belowground C cycle have been rarely reported, especially in forest ecosystems (Giardina & Ryan, 2002; Hall & Matson, 2003).

At a global scale, 70% of land biomass is stored in forest and 53% of soil C is in forest soils (Dixon *et al.*, 1994; IPCC, 2000). Therefore, the study of soil C dynamics is of vital importance to understand the C balance in forests and their response to future global change (Davidson *et al.*, 2000). More C can be stored belowground by increasing the input rate of organic matter, increasing the size of C stock, boosting the C density in the soils, and decreasing the C turnover rate in soils (Post & Kwon, 2000). Carbon turnover rate varies considerably among different forests depending on climate, vegetation type, and litter quality. Differentiation of the total SOC into labile C (with a resident time ranging from months to several years) and decay-resistant C (with a resident time varying from 25 years to hundreds of years) is indispensable in understanding the mechanisms controlling the overall turnover rate of SOC in forest ecosystems (Sun *et al.*, 2004). Separation of soil C based on density flotation has been widely used to separate animal and plant debris in 'a light fraction' (LF-OC) and to separate organo-mineral associations, usually in heavy-fraction organic C (HF-OC) (Stevenson & Elliot, 1989). HF-OC, in general, has a longer resident time than LF-OC because the LF-OC pool is usually linked to macroaggregates and the HF-OC pool is involved in silt- or clay-sized organo-mineral complexes which protect SOC from decomposition. Post & Kwon (2000) pointed out that the turnover of LF-OC in agricultural ecosystems has a bulk resident time from months to a few years, while the HF-OC is stabilized through microaggregation and its resident time is on the order of decades.

In this study, we examined the effects of nutrient addition on SOC pool both quantitatively and qualitatively in an old growth tabonuco forest in the wet tropics in Puerto Rico. Our overall aim is to examine soil C quantity and quality as a result of continuous long-term nutrient addition. The specific objectives of this study are to examine: (1) whether nutrient addition changes litter production, microbial biomass, and soil C stock; and (2) whether nutrient addition affects the quality of SOC, such as the composition of long-term C vs. short-term C.

Methods

This study was conducted in a wet tropical forest in the Luquillo Experimental Forest in north-eastern Puerto Rico (18°18'N, 65°50'W), the north-west flank of Luquil-

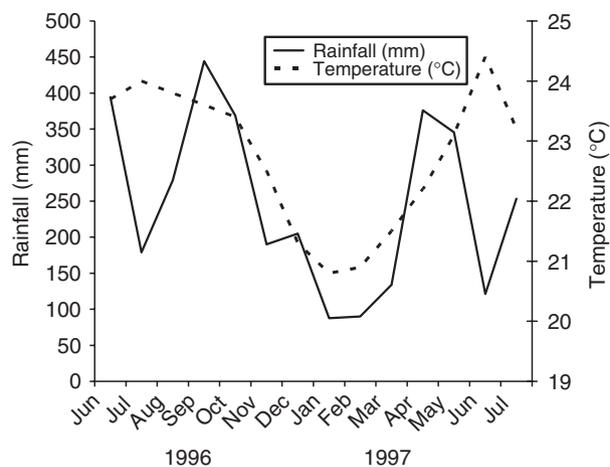


Fig. 1 Monthly precipitation (mm) and air temperature (°C) in the Luquillo Experimental Forest in Puerto Rico from June 1996 to July 1997.

lo Mountains. The study sites, on average, receive an annual precipitation of 3500 mm (Brown *et al.*, 1983), with an annual mean temperature of 22.1 °C (Fig. 1). The vegetation in the study area is characterized by tabonuco forest with *Dacryodes excelsa* Vahl as the dominant tree species. The other frequently seen tree species include *Manilkara bidentata* A. Cher., *Sloanea berteriana* Choisy, *Prestoea montana* Nichols, and *Cecropia schreberiana* Mig. Soils are a complex of upland Ultisols and Oxisols (Soil Survey, 1995). The general topography is mountainous with deeply dissected drainage with elevations between 340 and 460 m above sea level.

The experimental treatments were first initiated in November 1989 shortly after hurricane Hugo passed the study area (Zimmerman *et al.*, 1995). The experiment was conducted in four complete blocks with a randomly assigned fertilization plot and a control plot in each block. The four blocks were located in Sonodora, Prieta, and Tornja watersheds of the El Verde research area. All blocks were located within the tabonuco forest zone, although there were minor differences both within and among blocks in tree species composition, tree density, and stem basal area (Parrotta & Lodge, 1991). A prefertilization investigation for all trees (>2 m in height), tree density, and stem basal area among the blocks was conducted. The mean number of tree, mean tree density, and stem basal area of the four blocks were 8.5, 0.25 m⁻², and 23.6 m² ha⁻¹, respectively. The plot for each treatment was 20 m × 20 m in size with an interior area of 10 m × 10 m for measurement, leaving the rest of the plot as a buffer. The plots were located on slopes in such a way as to preclude the possibility of surface and subsurface water flow between plots. Fertilization was implemented by quarterly addition of fertilizers at an annual rate of 300 kg N ha⁻¹, 100 kg P ha⁻¹, 100 kg K

Table 1 Initial leaf chemical content in the leaf litter decomposition experiment in the Luquillo Experimental Forest in Puerto Rico

	C (%)	S (%)	N (%)	Ca (mg g ⁻¹)	P (mg g ⁻¹)	Al (mg g ⁻¹)	Fe (mg g ⁻¹)	Mn (mg g ⁻¹)	K (mg g ⁻¹)	Mg (mg g ⁻¹)
Fertilization	54.3 a (6.6)	0.57 a (0.08)	1.49 a (0.17)	7.89 a (0.22)	0.41 a (0.04)	0.83 a (0.05)	0.45 a (0.06)	1.43 a (0.08)	3.29 b (0.18)	2.47 a (0.28)
Control	45.7 a (6.8)	0.51 a (0.03)	0.72 b (0.05)	6.43 b (0.14)	0.21 b (0.03)	0.35 b (0.08)	0.37 b (0.03)	1.36 a (0.17)	1.45 b (0.22)	1.53 b (0.23)

Common letters within a column indicate no significant difference between the treatments according to Tukey's test at $\alpha = 0.05$. Numbers in the parentheses are standard error ($n = 4$).

ha⁻¹, 8 kg B ha⁻¹, 15.4 kg Cu ha⁻¹, 2.2 kg Fe ha⁻¹, 25 kg Mn ha⁻¹, 26 kg Zn ha⁻¹, and 19 kg Mg ha⁻¹. Five baskets (0.50 m² in surface area, 0.65 m aboveground) were placed in each plot for litterfall collection. The litterfall was collected and measured every 2 weeks from July 14, 1996 to June 30, 1997. The collected materials were separated into leaves, wood, and miscellaneous (misc.) materials and then oven-dried at 50 °C for 1 week before determining the dry weight. Forest floor materials were collected every 2 months from July 1996 to May 31, 1997 by sampling an area of 0.5 m × 0.5 m in each plot. The samples of forest floor materials were processed in the same way as litterfall.

Leaf decomposition rate was measured using nylon litterbags with 1.0 mm mesh openings. Four types of litterbags were constructed in considering the effects of fertilization and leaf litter composition on decomposition: (1) leaf materials from fertilization plots with a species composition ratio of *D. excelsa* : *M. bidentata* : *B. capitata* : misc. = 50.5 : 19.8 : 6.4 : 23.3 as in the fertilization plots; (2) leaf materials from fertilization plots with a species composition ratio of *D. excelsa* : *M. bidentata* : *B. capitata* : misc. = 38.2 : 23.8 : 7.4 : 30.6 as in the control plots; (3) leaf materials from the control plots with the same species composition ratio as in type 1 litterbags; and (4) leaf materials from control plots with the same species composition ratio as in type 2 litterbags. It could be noted that the leaf litter composition (proportion of different species) has changed after 6-year continuous fertilization because of different responses of species to nutrient addition. The species composition ratios for filling the litterbags were determined by analyzing the annual litterfall data collected in the fertilization and control plots in a prior project managed by the Minority Research Center of Excellence. We constructed a total of 192 litterbags for the litter decomposition experiment. Each litterbag contained 5 g of air-dried leaf tissue. We placed 24 litterbags for each type in the fertilization plot and 24 in the control plot in each experimental block. Four litterbags for each treatment were retrieved to determine the amount of remaining litter in the bags after leaving in the plots for 2, 4, 6, 8, 10, and 13 months, respectively. Initial leaf chemical content was also de-

termined by measuring similar leaf materials used for filling the litterbags. These leaf materials were oven-dried to a constant mass at 50 °C and then ground with a Wiley mill to pass a 0.85 mm mesh sieve. Total C and N were determined by combustion in an LECO C-H-N elemental analyzer (Foremost Equipment, Inc., Rochester, NY, USA). Elemental concentrations of P, S, Na, K, Ca, Mg, Mn, Fe, and Al were analyzed using a Thermal Jarell Ash Inductively Coupled Plasma Analyzer (ICP) (Scientific Instrument Services, Inc., Ringoes, NJ, USA) after samples were digested with H₂O₂ and concentrated HNO₃ (Liu & Zou, 2002). Total C and N in soils were obtained using a Perkin-Elmer CHN analyzer (Life & Analytical Sciences, Inc., Boston, MA, USA). Initial leaf chemistry in both the fertilization and the control plots is shown in Table 1.

Soil sampling was conducted in August 1996 and in March 1997. Soil samples (0–10, 10–25 cm) were collected from four cores using a soil corer (68.9 mm diameter) in each plot without including forest floor. After separation of the two layers, the four cores (sub-samples) were mixed and separated into two plastic bags (0–10, 10–25 cm). These soil samples were used to measure microbial biomass, soil pH, soil moisture, SOC, bulk density, and roots. Data illustrated in the result section are the means of the two sampling dates. Four samples (combined from four pseudoreplicates) in each soil layer in each treatment were used for data analysis.

Soil pH was measured at a 1 : 1 ratio of water to soil fresh weight with a combination electrode. Soil moisture contents were determined by oven-drying 10 g fresh soil at 105 °C for 48 h. Root samples were separated according to size (<5, >5 mm diameter), and dried at 70 °C to a constant mass for conversion of total fresh mass in to dry mass equivalents.

We used the agar film techniques (Lodge & Ingham, 1991) and the fluorescein isothiocyanate technique (Babiuk & Paul, 1970; Zou & Bashkin, 1998) to estimate fungal and bacterial biomass, respectively. Although these two methods have the limitations of being selective in targeting dominant groups of soil microbial community, they can separate fungi and bacteria into dead and active tissues, thus providing information on

the active groups of microbial community in soils. For fungal estimates, 1 g wet soil was placed in 9 mL of sterile tap water (1/10 dilution) and shaken manually for 5 min. A 1/100 dilution was prepared by transferring 1 mL of the 1/10 dilution to 9 mL of sterile diluent. One milliliter aliquots from each dilution (1/10 and 1/100) were transferred to test tubes and stained for 5 min by adding 1 mL of fluorescein diacetate in buffer. One milliliter of fresh molten agar was then mixed with the stained soil suspension, and an aliquot was transferred to the well of a coverslip well slide. Coverslip wells were prepared by taping two coverslips of known thickness to a microscope slide approximately 1 cm apart. A drop of agar suspension was placed on the slide between the two coverslips, and another coverslip was immediately pressed down on the agar to produce a film of known thickness. Active hyphal length was estimated on fresh agar film using epifluorescent microscopy. The total length of hyphae was estimated on fresh films using phase-contrast microscopy. At least 20 fields were viewed along a vertical transect across the coverslip, and three transects were scanned on each slide. The hyphal length was calculated by multiplying the length of hyphae in one field by the number of fields needed to equal 1 cm^{-3} and then multiplying by the dilution of soil in the agar suspension (mL g^{-1} soil).

For bacterial estimates, 20 g of soil samples were placed in a Waring Blender containing 190 mL of sterile-distilled water and shaken for 15 min manually. Subsamples were removed for either plate counting or direct microscopy. Prepared soil smears were stained for 4 min with fluorescein isothiocyanate solution and then washed in 0.5 M sodium carbonate buffer for 10 min and in 5% sodium pyrophosphate for 2 min. The smears were mounted in glycerol (pH 9.6) and observed with a microscope equipped with a mercury lamp and a barrier filter. The dispersed soil was diluted to the required dilution (10^{-4} , 10^{-5} , and 10^{-6} g soil mL^{-1} water), and 0.1 mL portions were spread on the solidi-

fied agar. Five plates were used for each dilution. These plates were incubated for 2 weeks at 21°C before counting the bacterial and actinomycete colonies.

Carbon in the LF was determined using the density isolation method (Sollins *et al.*, 1984). Air-dried soil was passed through a 2 mm mesh sieve, and then 1 g of the soil was suspended in 20 mL of sodium iodide solution adjusted gravimetrically to 1.85 g mL^{-1} density. The suspension was sonicated for 2 min, placed under vacuum (70 kPa) for 10 min, and then settled for 24 h at room temperature to separate LF and HF. The LF at the surface of the density liquid was aspirated and trapped onto a Whatman glass fiber filter paper (GF/A), rinsed with deionized water, and then analyzed for C and N using a Perkin-Elmer CHN analyzer. All plant litter samples were dried to a constant mass at 70°C for determination of mass and chemical analysis.

Decay constant (k), the average rate of litter loss, was determined using the data from the litterbag decomposition experiment by the formula: $M_t = M_0 e^{-kt}$ (Olson, 1963). The bulk densities were calculated using the formula: bulk density = (dry weight of sample in grams)/(volume of the sample in cubic centimeters). We used a two-way analysis of variance (ANOVA) to detect differences in decomposition between treatments (fertilization vs. control) and between litter types. Treatment effect on soil C, fungal, and bacterial biomass, and soil parameters were analyzed using one-way ANOVA. Significant differences in seasonal variation of litterfall, forest floor mass, and litter decomposition were tested by repeated measurement analysis. Significance difference levels were set at $\alpha = 0.05$.

Results

Fertilization effect on SOC and soil physical properties

Fertilization significantly enhanced long-term C sequestration, indicated by the HF-SOC in the top 0–10 cm soil

Table 2 Soil pH, gravimetric moisture, bulk density, total soil organic carbon (SOC) and SOC in heavy fraction OC (HF-OC) in the fertilization and control plots

	Total OC (kg m^{-2})	HF-OC (kg m^{-2})	Bulk density (g cm^{-3})	pH	Soil moisture (%)
0–10 cm					
Fertilization	5.42 (0.20) a	3.22 (0.19) a	0.64 (0.06) a	4.39 (0.03) b	62 (9.4) a
Control	5.27 (0.28) a	2.42 (0.15) b	0.72 (0.07) a	5.23 (0.04) a	64 (4.8) a
10–25 cm					
Fertilization	4.13 (0.22) a	2.61 (0.21) a	0.69 (0.07) a	4.53 (0.04) b	67 (8.4) a
Control	3.92 (0.18) a	2.38 (0.19) a	0.74 (0.08) a	5.39 (0.05) a	59 (11.2) a

Common letters within a column indicate no significant difference between the treatments according to Tukey's test at $\alpha = 0.05$. Numbers in the parentheses are standard error ($n = 4$).

layer ($P < 0.05$), while fertilization had little effect on total SOC in both the 0–10 and 10–25 cm soil layers (Table 2). The total SOC did not differ statistically between the fertilized and the control plots in both the top 0–10 and the 10–25 cm soil layers (Table 2). In addition, we did not find a significant fertilization effect on the HF-SOC in the 10–25 cm layer soils (Table 2). Soil pH value was significantly lower in the fertilization plots than the control plots in both the 0–10 and the 10–

25 cm layers of the soils. Fertilization had little effect on soil moisture and soil bulk density in both the 0–10 and the 10–25 cm soil layers (Table 2).

Litterfall and forest floor mass

Fertilization significantly enhanced total litter production, especially leaf and miscellaneous litterfall, in the forest (Fig. 2a). The total annual litterfall in the fertiliza-

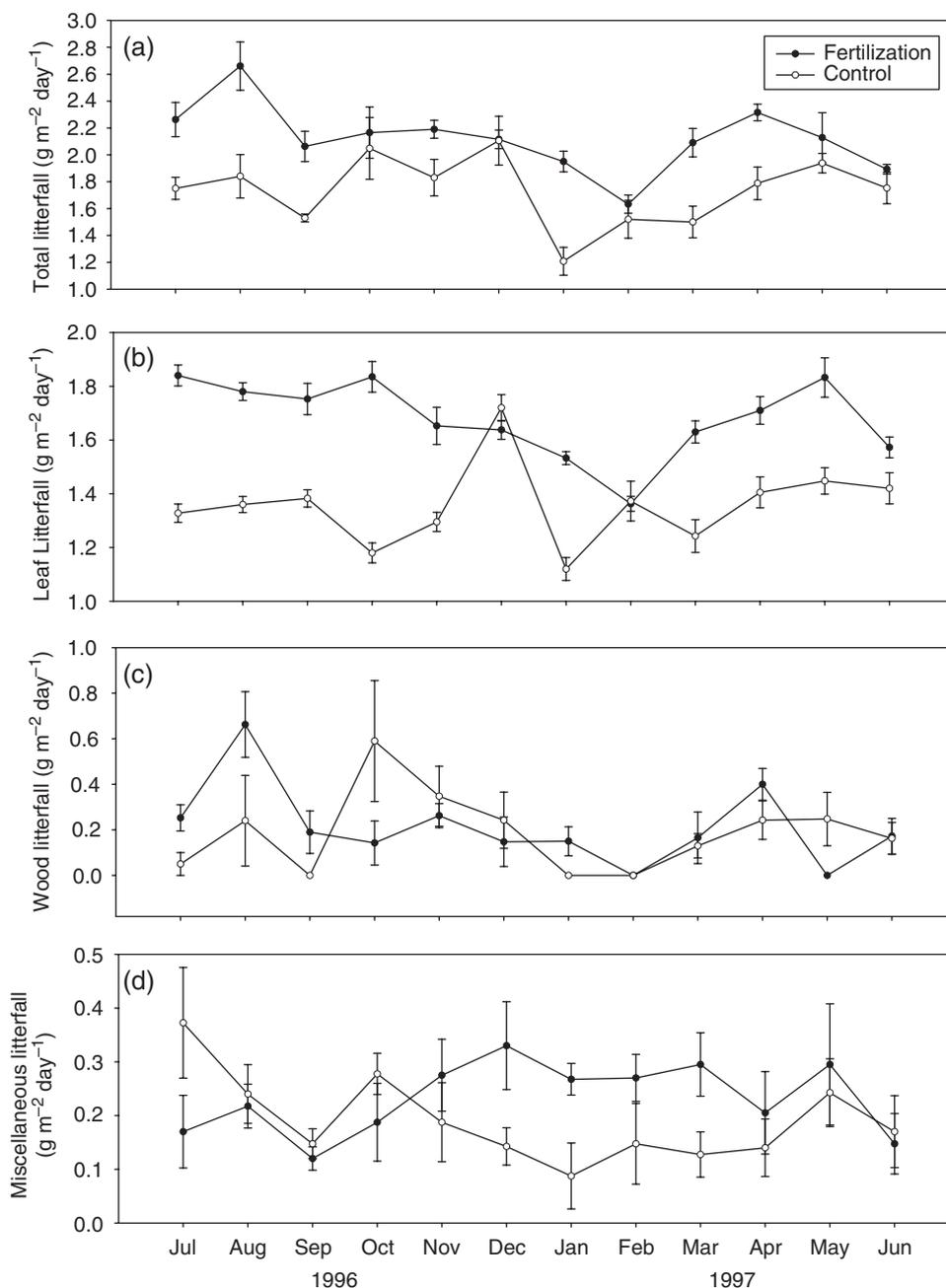


Fig. 2 Mean monthly litterfall (mean \pm standard error) in the fertilization plots and the control plots from July 1996 to June 1997. The lines join the points of same treatments.

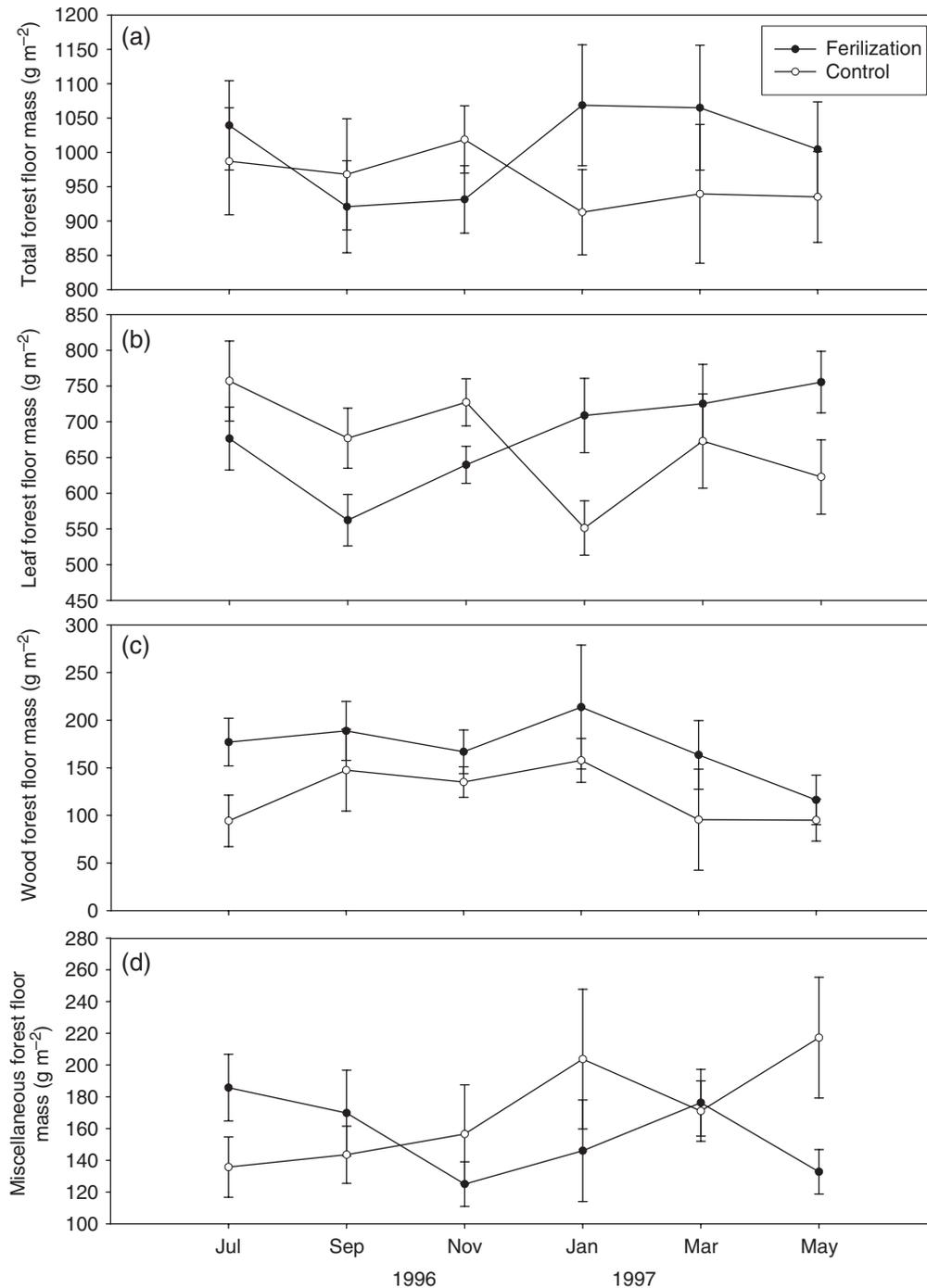


Fig. 3 Bimonthly forest floor mass (mean \pm standard error) of the fertilization and the control plots from July 1996 to May 1997. The lines join the points of same treatments.

tion plots and the control plots was 766.5 and 627.8 g m^{-2} , or 416 and 341 g C m^{-2} , respectively. In addition, fertilization also changed aboveground C allocation, with proportionally more C allocated in leaves in the fertilization plots than the controls. Specifically, leaf, wood, and miscellaneous litter accounted for 81%, 10%, and 9% of the total litterfall in the

fertilization plots, respectively, while the corresponding values were 78%, 11%, and 11%, respectively, in the control plots (Fig. 2). Total forest floor mass (e.g. sum of leaf litter, wood, and miscellaneous litter, mean \pm SE) did not differ significantly between the fertilization plots ($1004.3 \pm 55.8 \text{ g m}^{-2}$) and the control plots ($956.5 \pm 33.7 \text{ g m}^{-2}$) (Fig. 3a), suggesting a greater C

output (decomposition or transfer to soil C) on the forest floors in the fertilization plots. Leaf litter, wood and miscellaneous litter in the fertilization plots accounted for 67%, 17%, and 16% of the total forest floor mass respectively, while the corresponding proportion in the control plots was 69%, 12%, and 19%, respectively (Fig. 3).

Seasonally, the rate of total litterfall was significantly lower in February 1997 than other months of the year in the fertilization plots. Leaf and wood litterfall demonstrated seasonal patterns similar to the total litterfall in the fertilization plots. In the control plots, the rate of total litterfall was significantly lower in January 1997 than other months (Fig. 2). In addition, total forest floor mass, leaf, wood, and miscellaneous litter did not differ significantly between the dry and the wet seasons in both the fertilization and the control plots (Fig. 3).

Leaf litter decomposition

Leaf litter from fertilized plots decomposed faster than that from the control plots, while the incubation site had little effect on decomposition (Table 3). The decay constant, k , of the leaves from control plots was 1.36 when incubated in the control plots and 1.45 when incubated in the fertilization plots. The value of k of the leaves from fertilization plots was 1.79 when placed in the control plots and 1.87 in the fertilization plots.

As expected, the mass loss rate declined with the incubation time (Fig. 4). The leaf litter decayed fast during the first 3 months regardless of leaf source and incubation sites. No significant difference in the decomposition rate was found during the first 2 months between the treatments (fertilization vs. control). The most striking difference of mass loss rate between bags of different leaf litter quality was detected from the third to sixth month of the incubation (Fig. 4). After 8 months of incubation, the leaf mass loss slowed down and approached a constant value through the next 4 months of the study period.

Root biomass and microbial biomass

Fertilization had little effect on root biomass in this study because the root biomass of both medium roots (diameter > 5 mm) and fine roots (diameter < 5 mm) did not significantly differ between the fertilization and control plots (Table 4). The total microbial biomass in the fertilization plots (952 mg kg⁻¹ soil) was significantly greater than in the control plots (755 mg kg⁻¹ soil), among which fungal biomass was significantly higher in the fertilization plots, while bacterial biomass was not statistically different between the fertilization and the control plots.

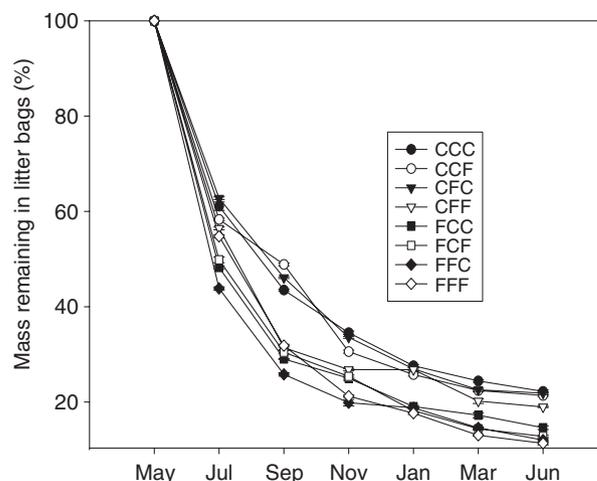


Fig. 4 Leaf litter decomposition (represented by the percentage of leaf litter mass remaining in the litterbags) (mean \pm standard error) in the fertilization plots and the control plots from July 1996 to June 1997. The first letter in the legends indicates leaf litter source, the second letter indicates species composition, and the third letter indicates incubation site. C, control and F, fertilization. For example, CFC means that leaf litter materials came from control plots, litterbags were filled according to the species composition in fertilization plots, and litterbags were incubated in control plots.

Discussion

Giardina *et al.* (2004) studied C cycling in a young humid tropical forest in Hawaii, and found that fertilization increased aboveground plant production but reduced belowground C cycling. Binkley *et al.* (2004) studied soil C and N in a *Eucalyptus* plantation in Hawaii and concluded that the gains in new soil C through increased root biomass were entirely offset by losses of old soil C, even with heavy fertilization. However, Hagedorn *et al.* (2003) reported that N additions retarded mineralization of old soil organic matter in a study of forest ecosystems of beech and spruce. Neff *et al.* (2002) found that N fertilizer increased stable C and decreased labile C in an alpine soil. Similarly, in the present study, we found that the HF (stable part) of SOC increased with fertilization, although fertilization had little effect on total SOC. The higher litter decomposition and turnover in the fertilization plots could result in more HF-OC accumulation in the fertilization plots. The same indication (i.e. litter turnover rate is higher in the fertilization plots) could be reached by the comparison of microbial biomass, which is significantly higher in the fertilization plots.

The higher litter nutrient concentration in the fertilization plots may stimulate litter decomposition, which explains why the forest floor mass was not significantly different between the fertilization and control plots,

Table 3 Mean (standard error) annual decay constant, k , in the fertilization and control plots of the forest

Leaf source	Incubation site	k	R^2	P	Mass loss year
Control	Control	1.36	0.67	<0.01	77.8 (0.42) b
Control	Fertilization	1.45	0.55	<0.03	78.7 (0.28) b
Fertilization	Control	1.69	0.75	<0.00	87.9 (0.76) a
Fertilization	Fertilization	1.87	0.73	<0.00	88.6 (0.47) a

R^2 presents the regression of decay rate over time. Common letters in each column indicate no significant difference by Tukey's test at $\alpha = 0.05$.

Table 4 Root biomass and microbial biomass at 0–25 cm soil depth in the fertilization and control plots

	> 5 mm root (g)	< 5 mm root (g)	Fungal biomass (mg kg ⁻¹ soil)	Bacterial biomass (mg kg ⁻¹ soil)	Litter production (g m ⁻¹ day)
Fertilization	351.6 (52) a	185.7 (16) a	806 (79) a	146 (27) a	2.11 (0.18) a
Control	341.2 (44) a	168.7 (29) a	622 (56) b	133 (33) a	1.72 (0.11) b

Common letters within a column indicate no significant difference between the treatments according to Tukey's test at $\alpha = 0.05$, ($n = 4$).

although the aboveground litter input was greater in the fertilization plots than in the control plots. Vitousek (1998) reported that adding P to a forest dominated by a native tree *Metrosideros polymorpha* in Hawaiian increased foliar P concentrations threefold and litter P concentrations up to 10-fold, and the decomposability of leaf litter increased from 32% to 46% mass loss in the first year. In the same study, adding N to the forest increased leaf and litter N concentrations by only 15–20%, with little or no effect on the decomposability of tissues. However, Tanner (1991) reported that the leaf decomposition rate was significantly correlated with leaf N content in a decomposition experiment (15 species studied) in tropical forests in Jamaica. In our study the leaf litter from the fertilization plots decayed faster than the control leaf litter in both the fertilization and the control plots based on the litter bag reciprocal experiment, which suggests that litter quality, rather than soil chemical and biological factors, might dominate the decomposition processes in these forests.

Microbial biomass has been shown to be a sensitive indicator responding to environmental impacts in the tropics. Smolander *et al.* (1994) and Lee & Jose (2003) reported that soil microbial biomass decreased with higher rates of N fertilization. In contrast, Zhang & Zak (1998) found that N fertilization increased microbial biomass and root growth in a semiarid environment in West Texas. In the present study, we found that soil fungal biomass significantly increased in the fertilization plots, while the fertilization effect on bacterial biomass was not statistically significant. These conflicting results may be explained by the different initial

status of microbial composition, soil pH, and other soil chemical and physical properties. Binkley & Högberg (1997) reported that the responses of forest soils to nutrient addition vary with soil pH. Our result that soil fungal biomass increased and soil pH decreased after continuous 7 years fertilization indicates that fertilization may alter soil microbial communities and, thus, affect SOC composition (labile C vs. decay-resistant C) by selecting different feeding substrates by different microbes. An increased level of fungal hyphae may enhance the formation of organo-mineral aggregate association.

The percentage of 1 year weight loss from the forest leaf litter (77.8% in the control plots; 78.7% in the fertilization plots) in our study was in agreement with the results reported by Fonte & Schowalter (2004) in a decomposition study at the same site. An initial faster decomposition rate of leaf materials, followed by a subsequent slower rate (after 6 months), is in line with the results reported by others (Kumar & Deepu, 1992; Sundarapandian & Swamy, 1999). This decomposition pattern over time could be because of a higher initial nutrient concentration and water-soluble materials at the earlier stage and more recalcitrant constituents in the residue litter mass at the later stage.

In conclusion, a 7 year continuous nutrient addition significantly enhanced decay-resistant SOC (HF-OC) sequestration in the tropical forest soils although it has minor effect on total SOC pool. Our results indicate that nutrient addition had significantly increased the fraction of HF-OC from 46% to 59% in the top 0–10 cm soil layer, but this increase was less significant in the

deeper soils. We believe that longer-term (>7 years) nutrient addition may increase the fraction in deeper soils as the HF-OC continuously moves down from the surface soil. In addition, we found that nutrient addition significantly increased aboveground litter production, leaf litter nutrient concentration, increased fungal biomass, and stimulated litter decomposition. These results may provide insights into effective C management of tropical forests in mitigating CO₂-induced global warming.

Acknowledgements

This research was supported by grants BSR-8811902, DEB 9411973, DEB 0080538, and DEB 0218039 from NSF to the Institute for Tropical Ecosystem Studies, University of Puerto Rico, and to the International Institute of Tropical Forestry USDA Forest Service, as part of the Long-Term Ecological Research Program in the Luquillo Experimental Forest, and grants were also given by the US National Aeronautics and Space Administration (NAGW 4059) to the University of Puerto Rico. The US Forest Service (Department of Agriculture) and the University of Puerto Rico gave additional support. Rutgers University provided facilities for data analysis. We are grateful to the two anonymous reviewers for their thorough and sharp reviews, which improved the manuscript significantly.

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