

Experimental litterfall manipulation drives large and rapid changes in soil carbon cycling in a wet tropical forest

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Abstract

Global changes such as variations in plant net primary production are likely to drive shifts in leaf litterfall inputs to forest soils, but the effects of such changes on soil carbon (C) cycling and storage remain largely unknown, especially in C-rich tropical forest ecosystems. We initiated a leaf litterfall manipulation experiment in a tropical rain forest in Costa Rica to test the sensitivity of surface soil C pools and fluxes to different litter inputs. After only 2 years of treatment, doubling litterfall inputs increased surface soil C concentrations by 31%, removing litter from the forest floor drove a 26% reduction over the same time period, and these changes in soil C concentrations were associated with variations in dissolved organic matter fluxes, fine root biomass, microbial biomass, soil moisture, and nutrient fluxes. However, the litter manipulations had only small effects on soil organic C (SOC) chemistry, suggesting that changes in C cycling, nutrient cycling, and microbial processes in response to litter manipulation reflect shifts in the quantity rather than quality of SOC. The manipulation also affected soil CO₂ fluxes; the relative decline in CO₂ production was greater in the litter removal plots (−22%) than the increase in the litter addition plots (+15%). Our analysis showed that variations in CO₂ fluxes were strongly correlated with microbial biomass pools, soil C and nitrogen (N) pools, soil inorganic P fluxes, dissolved organic C fluxes, and fine root biomass. Together, our data suggest that shifts in leaf litter inputs in response to localized human disturbances and global environmental change could have rapid and important consequences for belowground C storage and fluxes in tropical rain forests, and highlight differences between tropical and temperate ecosystems, where belowground C cycling responses to changes in litterfall are generally slower and more subtle.

Keywords: carbon dioxide, dissolved organic matter, microbial biomass, net primary productivity, root biomass, soil biogeochemistry, soil carbon chemistry, soil nitrogen, soil phosphorus

Received 11 March 2012 and accepted 19 April 2012

Introduction

Globally, soils store more organic carbon (C) than plants and the atmosphere combined (Schlesinger, 1997), and tropical forests contain roughly 20% of this pool (Jobbagy & Jackson, 2000; Tarnocai *et al.*, 2009). As a result, environmental changes that alter soil C dynamics in tropical forests could have important consequences for the global C cycle. For example, extensive deforestation and cultivation in the tropics has led to decreased plant-derived C inputs to soil with observa-

ble effects on belowground C cycling (e.g., Murty *et al.*, 2002; Holmes *et al.*, 2006). In addition, increasing atmospheric carbon dioxide (CO₂) concentrations and temperature in tropical forests are predicted to alter ecosystem C inputs via changes in net primary production (NPP) with potentially important consequences for soil C storage and losses (Clark *et al.*, 2003; Raich *et al.*, 2006; Hickler *et al.*, 2008). Given the dominant role of tropical forests in regulating global terrestrial-atmospheric CO₂ exchange (Field *et al.*, 1998; Bonan, 2008), it is important to elucidate how predicted changes in litter inputs might affect the fate of soil C in these C-rich and biogeochemically dynamic ecosystems (Townsend *et al.*, 2011).

Unfortunately, logistical constraints, among others, have precluded CO₂ fertilization and/or climate

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warming experiments in tropical forests, limiting our ability to predict how these perturbations may affect the tropical C cycle. In temperate forest ecosystems, free-air CO₂ enrichment (FACE) experiments generally point to at least short-term increases in NPP with increasing atmospheric CO₂ concentrations (Delucia *et al.*, 1999; Norby *et al.*, 2002; Calfapietra *et al.*, 2003; Ellsworth *et al.*, 2012), which could enhance soil C stocks via increased litterfall fluxes. Over longer time scales, however, the effects of elevated CO₂ on belowground C cycling in the temperate zone are less clear, with some suggesting increases in soil C concentrations (Jastrow *et al.*, 2005) but others showing no significant change (Lichter *et al.*, 2005; Hoosbeek & Scarascia-Mugnozza, 2009). Unfortunately, the possible effects of increasing atmospheric CO₂ and temperature on tropical forests remain largely unknown, but some long-term observational data suggest that temperature increases predicted to occur with climate change could drive declines in tropical NPP, and ultimately net losses of soil C (Clark *et al.*, 2003, 2010; Clark, 2004).

In light of uncertainty surrounding the mechanisms driving possible changes in NPP, the direction of those changes, and the difficulties in conducting direct climate and/or CO₂ manipulations, litterfall and plant detritus manipulation experiments offer important clues about the potential effects of changes in plant-derived C inputs on belowground C cycling (*e.g.*, Park & Matzner, 2003; Lajtha *et al.*, 2005; Sulzman *et al.*, 2005; Sayer, 2006; Sayer *et al.*, 2007; Crow *et al.*, 2009; Schaefer *et al.*, 2009; Chemidlin Prévost-Bouré *et al.*, 2010; Vincent *et al.*, 2010; Feng *et al.*, 2011). Not surprisingly, litter removal typically elicits declines in surface soil organic matter through time (SOM; reviewed in Sayer, 2006; Vincent *et al.*, 2010). Results from litter additions have been more inconsistent, showing both positive (Sayer, 2006) and neutral or non-significant increases (Nadelhoffer *et al.*, 2004; Vincent *et al.*, 2010; Sayer *et al.*, 2012) effects on surface soil C pools.

Changes in total soil C pools might simply be predicted to correlate with changes in litter inputs, but several mechanisms could combine to drive more complex shifts in soil C cycling. First, leaching transports dissolved organic C (DOC) from litter into soil, and this process is especially important in wet ecosystems (Neff & Asner, 2001; Cleveland *et al.*, 2006). Increased movement of soluble DOC could stimulate microbial activity and drive associated changes in internal SOM cycling, nutrient cycling, and root dynamics (Neff & Asner, 2001; Cleveland *et al.*, 2006). Similarly, changing litter inputs could affect soil C losses to the atmosphere both directly, via increased mineralization of labile C substrate, and indirectly, via effects on the decomposition of extant soil organic C (SOC). For example, soil CO₂

fluxes would be predicted to vary proportionally with C inputs; accordingly, litter removal treatments often drive proportional declines in soil CO₂ fluxes (Li *et al.*, 2004; Vasconcelos *et al.*, 2004; Sulzman *et al.*, 2005; Sayer *et al.*, 2007; Schaefer *et al.*, 2009). However, CO₂ fluxes often increase disproportionately to litter augmentation, suggesting that elevated C inputs may accelerate decomposition of extant soil C via priming effects (Fontaine *et al.*, 2004; Sulzman *et al.*, 2005; Carney *et al.*, 2007; Fontaine *et al.*, 2007; Sayer *et al.*, 2007; Schaefer *et al.*, 2009; Chemidlin Prévost-Bouré *et al.*, 2010; Sayer *et al.*, 2011).

Thus, while counterintuitive, previous research suggests that increased litterfall could actually drive net soil C losses to the atmosphere (Sayer *et al.*, 2011). Unfortunately, only a handful of studies have directly investigated the effects of varying plant C inputs on soil C cycling in tropical forests (Vasconcelos *et al.*, 2004; Sayer *et al.*, 2007, 2011, 2012), where large soil C pools and ideal climatic conditions combine to promote changes in C cycling that may be fundamentally different from those observed in temperate forests. For example, previous work suggests that relative to temperate forests, soil C in tropical forests turns over much more rapidly, and that tropical soil respiration is dominated by relatively new (*i.e.*, recently fixed) C (Trumbore, 1993, 2000). Therefore, despite varied soil C responses to litter manipulations in temperate ecosystems, there is some reason to expect more rapid and discernible responses to changing C inputs in the tropics.

Here, our overall objective was to assess the effects of different litter C inputs (additions and removals) on soil C pools and fluxes in a wet lowland tropical rain forest. To do so, we initiated a litter manipulation experiment in a lowland tropical forest in Costa Rica to address two hypotheses. First, we hypothesized that increasing and decreasing litter inputs would drive corresponding changes in total soil C pools and shifts in soil C chemistry (*i.e.*, the relative abundances of labile and litter-derived compounds would increase with litter inputs and decline with litter removal). Second, we hypothesized that there would be greater DOC fluxes with increasing litter inputs which would elicit higher CO₂ losses from the soil, while declining DOC fluxes in response to litter removal would drive declines in soil CO₂ efflux.

Materials and methods

Study site

The study was conducted in a lowland primary tropical rain forest site in the Gulfo Dulce Forest Reserve on the Osa Peninsula in southwestern Costa Rica (8°43' N, 83°37' W). This wet

tropical forest site received an average of 4430 mm of rainfall per year during the experiment, and surface soils (0–3 cm) had a mean annual temperature of ~25 °C. At this site, the majority of the precipitation falls during the wet season (April to December), while the dry season typically receives <100 mm month⁻¹ (Cleveland & Townsend, 2006). In addition, litterfall at the site displays strong seasonal patterns with maximum litterfall rates (~90 g C m⁻² month⁻¹) occurring during the dry season. Soil at the site is a highly weathered clay ultisol that formed on the Osa basaltic complex (Berrange & Thorpe, 1988).

Experimental design

In April 2007, we initiated a leaf litter manipulation experiment consisting of 3 × 3 m litterfall removal (0×), control, and litterfall addition (2×) plots (*n* = 10 per treatment; Nemerut *et al.*, 2010; Wieder *et al.*, 2011). At monthly intervals, litter was gently raked, avoiding disturbance, and harvested from the 0× plots, weighed in mesh bags, combined, and evenly distributed onto each of the ten 2× plots. From April 2007 to March 2009, ~450 g C m⁻² yr⁻¹ of litter was removed from the 0× plots and added to the 2× plots. Litter C content was calculated as 0.48× litter biomass (Wieder *et al.*, 2009). Coarse woody debris was not transferred during the experiment. Precipitation was continuously measured using a HOBO data logging rain gauge (Microdaq Inc., Contoocook, NH, USA) placed in a clearing ~400 m from the study site.

Soil characterization

Soil C and nitrogen (N) concentrations, gravimetric soil moisture content, and microbial biomass C and N concentrations were measured in all plots approximately every 4 months by collecting 0–10 cm soil samples with an 8 cm diameter hand corer. Soil samples were transported to the laboratory in sealed plastic bags in coolers on ice. In the laboratory, soils were hand homogenized and a small subsample was removed from each soil sample and oven dried (105 °C for 48 h) to determine gravimetric moisture content and total soil C and N content. For soil C and N analyses, oven-dried soil subsamples were ground to a fine powder and analyzed using a combustion-reduction elemental analyzer (Carlo Erba, Lakewood, NJ, USA). Surface (0–10) cm soil C and N concentrations were converted to areal estimates using mean bulk density for each treatment, assessed using the core method (Culley, 1993) in March 2009.

Soil microbial biomass C and N content was determined on fresh soil samples (stored at 4 °C for less than 72 h) using the chloroform fumigation-extraction method (Brookes *et al.*, 1985). Briefly, for each sample, soil microbial biomass was measured as the difference in 0.5 M K₂SO₄ extractable C and N between fumigated and unfumigated samples. Organic C and N in the extracts were measured using high temperature combustion (Shimadzu TOCvcn, Kyoto, Japan), and soil microbial biomass C and N were calculated as the difference in extractable C and N multiplied by the respective proportionality constants (*K_c* and *K_n*) of 0.45 and 0.54 (Brookes *et al.*, 1985;

Vance *et al.*, 1987). Microbial biomass per unit area was calculated using mean bulk density measurements per litter treatment. Separate surface soil cores (0–10 cm) were collected to determine fine root biomass (<2 mm diameter). After sampling, all roots were removed by hand, rinsed with deionized water, dried at 60 °C for 72 h, and weighed to determine fine root biomass. Fine root biomass C of 0–10 cm was calculated by dividing the weight by the corer sampling area and using a C:biomass ratio of 0.5 (Jackson *et al.*, 1997).

Soil inorganic N and phosphorus (P as phosphate; PO₄³⁻) fluxes were measured *in situ* with ion-exchange resin capsules (Unibest, Bozeman, MT, USA) using a method similar to Cleveland *et al.* (2010). The resin capsules were carefully inserted into each plot at a depth of 5 cm every month using a small hand trowel, making efforts to minimize soil disturbance. Inorganic N (ammonium; NH₄⁺ and nitrate; NO₃⁻) and PO₄³⁻ exchanged on the resin capsules were determined using a 2 M HCl extraction solution and analyzed colorometrically with an autoanalyzer (Seal Analytical Inc., Mequon, WI, USA). Daily N and P fluxes were calculated by dividing the quantities of nutrients bound to the capsules by the number of days they were exposed to the soil.

After 3 years of litter manipulation, we assessed soil organic matter chemistry using pyrolysis-gas chromatography/mass spectrometry (py-GC/MS; Wickings *et al.*, 2011). Soil samples were collected from each plot in April 2010 using a hand corer (0–10 cm), transported to the laboratory, and sieved to 4 mm. Five composites per treatment were created by combining randomly selected pairs. Subsamples from each composite were oven dried (60 °C for 48 h) and finely ground using a shatter box. Soil samples were pulse-pyrolyzed using a Pyroprobe 5150 (CDS Analytical Inc., Oxford, PA, USA) at 600 °C and analyzed using a gas chromatograph (Trace GC Ultra; Thermo Scientific, Waltham, MA, USA) fitted with a fused silica capillary column (60 m, 0.25 mm ID); individual separated compounds were then passed into a mass spectrometer (Polaris Q; Thermo Scientific, Waltham, MA, USA). Peaks were putatively identified by comparing mass spectra to the National Institute of Standards and Technology mass spectral library using the Automated Mass Spectral Deconvolution and Identification System (AMDIS V 2.65).

Litter layer throughfall and DOM fluxes

To quantify dissolved organic matter (DOM) delivery from the litter layer to the soil surface, we constructed zero-tension lysimeters using 10 × 50 cm PVC pipe cut lengthwise. One lysimeter was installed per plot at the soil surface. Lysimeters were equipped with drain valves and a length of nylon tubing that drained collected litter layer DOM into polyethylene collection carboys placed in opaque buckets buried outside the plots. The lysimeters were filled with washed gravel and covered with 0.5 mm mesh to exclude large debris. Leached DOM concentrations were determined every 3–4 d by weighing the carboys, and subsamples from each carboy were immediately frozen for chemical analysis. DOC and total dissolved N (TDN) concentrations in these subsamples were

determined using a Shimadzu TOCvcpn total organic C and total N analyzer. DOC and TDN fluxes were calculated on a per area basis using the open surface area of the lysimeters, and twice weekly observations were summed from each plot to generate monthly averages of DOC and TDN inputs by litter treatment. Cumulative DOC and TDN inputs were calculated by summing inputs over the course of the experiment.

CO₂ fluxes

We measured soil CO₂ fluxes in all plots from April 2007 – March 2009. Initially, a set of permanently deployed ~80 cm² polyvinylchloride plastic collars were randomly placed in each plot (to 6 cm and including soil and surface litter), and CO₂ fluxes were measured weekly using a vented, closed soil chamber system (LI-6400; LI-COR, Lincoln, NE, USA). Following chamber equilibration, CO₂ concentrations were measured for 3–5 min, and fluxes were calculated using linear regression. Cumulative CO₂ production was calculated by linearly interpolating fluxes between measurements.

Statistical analysis

Repeated measures ANOVA and Tukey post hoc tests were used to assess differences in soil pools and fluxes among treatments and control plots. One-way ANOVA was used to test differences in DOM and CO₂ fluxes between treatment plots at the beginning of the experiment. Heterogeneity of variances among treatments was checked using a Levene's test and corrected, if necessary, by log (*ln*) transforming the data. In one case (inorganic N flux), we were unable to meet this assumption even after trying several transformations. Therefore, we used a non-parametric test, and a post hoc test was not conducted for this variable. To determine significant differences in organic

matter chemistry among treatments, we used non-parametric MANOVA with a Bray-curtis distance matrix calculated from the relative abundance of py-GC/MS products for each sample. To assess relationships among average variable measurements across all plots during the second year of the experiment, we calculated Pearson product-moment correlations. In all cases, we checked for non-linear relationships, and transformed data using *ln* transformations as necessary, and we used a significance threshold of $\alpha = 0.05$. Statistical tests were performed in SPSS v. 17 (SPSS, Chicago, IL, USA) except for the non-parametric MANOVA, which was implemented using the Adonis function (Oksanen *et al.*, 2010) in R v. 2.9.2 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Litter input effects on soil C pools and other soil characteristics

Surface soil C concentrations did not differ between treatments at the beginning of the experiment ($P = 0.2$), averaging $5.4 \pm 1.2\%$ (\pm SD), and soil C concentrations remained unchanged in the control plots throughout the experiment ($5.3 \pm 1.7\%$; $P = 0.8$). Yet, after only 8 months of treatment, the 2 \times plots had a significantly greater soil C content ($6.7 \pm 1.9\%$) than the 0 \times plots ($4.5 \pm 0.93\%$; $P = 0.001$), and from March 2008 – March 2009, soil C concentrations were significantly different between each of the litter input treatments and the control plot soils ($P < 0.05$; Table 1). There was also a significant effect of sampling date on surface soil C content as well as a significant time \times treatment interaction ($P < 0.05$) during this period. By March 2009–

Table 1 Soil properties and dissolved organic matter inputs in the litter removal (0 \times), control, and litter addition (2 \times) treatments from March 2008 to March 2009. Soil characteristics are for 0–10 cm

Variable	0 \times	Control	2 \times
C (%)	4.00 ± 0.62^a	5.35 ± 0.78^b	7.12 ± 1.27^c
N (%)	0.35 ± 0.05^a	0.45 ± 0.04^b	0.50 ± 0.1^b
C:N	11.48 ± 0.38^a	11.85 ± 0.94^a	14.09 ± 1.37^b
Gravimetric moisture (%)	38.7 ± 1.4^a	42.5 ± 1.0^b	43.9 ± 2.1^b
[O ₂] [†] (%)	18.0 ± 1.2^a	17.7 ± 1.2^a	17.4 ± 1.4^a
Microbial biomass C ($\mu\text{g g}^{-1}$)	897 ± 150^a	1206 ± 167^b	1397 ± 304^b
Microbial biomass N ($\mu\text{g g}^{-1}$)	169 ± 34^a	198 ± 42^{ab}	246 ± 55^b
Inorganic N flux ($\mu\text{g N d}^{-1}$)	7.15 ± 3.89	18.51 ± 15.08	3.28 ± 1.77
Phosphate flux ($\mu\text{g P d}^{-1}$)	0.07 ± 0.02^a	0.11 ± 0.09^a	0.19 ± 0.14^b
[DOC] (mg L ⁻¹)	3.4 ± 1.2^a	7.3 ± 2.3^b	11.0 ± 2.9^c
[TDN] (mg L ⁻¹)	0.9 ± 0.3^a	1.3 ± 0.7^a	0.9 ± 0.3^a
DOC:TDN	7.17 ± 2.09^a	8.15 ± 2.29^a	14.6 ± 2.92^b
Soil bulk density (g cm ⁻³) [†]	0.65 ± 0.03^a	0.58 ± 0.06^{ab}	0.52 ± 0.07^b

Different superscript letters indicate significantly different mean values between treatments for each variable ($P < 0.05$). Values represent 12 month means \pm 1 SD.

*Calculated using a subset of data from Wieder *et al.* (2011).

†Measured in March 2009 only.

23 months after initiating the litter manipulation – soil C concentrations in the 0× plots ($3.9 \pm 0.64\%$) were 26% lower than in the control plots ($5.2 \pm 1.1\%$), and soil C in the 2× ($6.8 \pm 1.8\%$) plots was 31% higher than in the control plots. Bulk density was greater in 0× plots than 2× plots ($P < 0.05$; Table 1), but on an areal basis, soil C pools were still significantly smaller in 0× plots than in control and 2× plots ($P < 0.05$; Table 2) after accounting for these differences.

We also assessed litter manipulation effects on several other soil properties and processes, and after approximately one year of litter additions and removals, during the 12-month period beginning in March 2008, we observed several significant differences among the treatments ($P < 0.05$; Table 1). Notably, among all variables, differences in PO_4^{3-} fluxes were greatest between the 0× and 2× plots (171% larger in the 2× plots). In addition, the leaf litter manipulation elicited concurrent changes in soil N pools and soil C:N ratios, gravimetric moisture, microbial biomass C and N pools, and fine root biomass—all of which were significantly higher in 2× plots and lower in 0× plots ($P < 0.05$; Tables 1 and 2).

Despite strong experimental effects on soil C pools, we did not observe overall differences in soil organic matter chemistry among treatments after 3 years of treatment. Multivariate analysis of the SOM chemistry showed that while there were some subtle variations in the types and quantities of SOM chemical constituents among experimental plots, this could not be attributed to variation in litter inputs ($P = 0.6$). Across all samples, we identified 239 pyrolysis products that we grouped into compound classes based on their origin. The mean distribution of SOM in these compound classes among all treatments was comprised of: polysaccharides ($34.0 \pm 15\%$), proteins ($16.7 \pm 6.9\%$), lignin ($1.5 \pm 1.1\%$), phenols ($9.4 \pm 3.9\%$), N-bearing compounds ($8.4 \pm 2.4\%$), lipids ($4.9 \pm 2.1\%$), and compounds of unknown origin ($25.1 \pm 4.6\%$).

DOM Fluxes

The DOC and TDN fluxes from the litter layer to soil surface varied among litter treatment and control plots over the course of the entire experiment (Fig. 1). DOC fluxes in the 0× plots ($10.5 \pm 2.9 \text{ g m}^{-2} \text{ yr}^{-1}$) were significantly lower than fluxes in the control plots ($23.0 \pm 6.5 \text{ g m}^{-2} \text{ yr}^{-1}$), and 2× plot fluxes ($31.2 \pm 7.3 \text{ g m}^{-2} \text{ yr}^{-1}$) were significantly greater than fluxes in the control plots ($P < 0.02$ in both cases). Conversely, TDN fluxes were largest in the control plots ($3.19 \pm 1.17 \text{ g m}^{-2} \text{ yr}^{-1}$) and significantly lower in the 2× plots, ($2.29 \pm 0.58 \text{ g m}^{-2} \text{ yr}^{-1}$) and 0× plots ($2.29 \pm 0.78 \text{ g m}^{-2} \text{ yr}^{-1}$; $P < 0.05$). The concentration of DOC and TDN inputs mirrored those of the fluxes: during the second year of the experiment, DOC concentrations were greater in plots with higher litter inputs ($P < 0.05$; Table 1) and TDN concentrations were greatest in control plots, although TDN concentrations were not significantly different among litter treatments ($P > 0.05$; Table 1).

Litter manipulation effects on soil CO_2 fluxes

Soil CO_2 fluxes were not significantly different among the plots at the beginning of the experiment ($P = 0.4$) and averaged $6.8 \pm 2.1 \text{ g CO}_2\text{-C m}^{-2} \text{ d}^{-1}$. However, CO_2 fluxes in 0× plots declined relative to control and 2× plots after only 3 months of litterfall removal (Fig. 2), and over the course of the experiment, CO_2 fluxes in the 2× plots ($1.97 \pm 0.44 \text{ kg CO}_2\text{-C m}^{-2} \text{ yr}^{-1}$) were significantly greater than fluxes in the 0× plots ($1.33 \pm 0.40 \text{ kg CO}_2\text{-C m}^{-2} \text{ yr}^{-1}$; $P < 0.01$). CO_2 fluxes in the 1× and 2× plots were not significantly different than those from the control plots ($1.69 \pm 0.41 \text{ kg CO}_2\text{-C m}^{-2} \text{ yr}^{-1}$; $P > 0.1$). Mean differences amounted to a $21 \pm 24\%$ decline in 0× fluxes and a $17 \pm 26\%$ increase in 2× plot fluxes (Fig. 3a). However, among all plots, there was substantial temporal variation in CO_2 fluxes

Table 2 Cumulative C fluxes measured during the experiment (April 2007 – March 2009), C pools in surface soils (0–10 cm), and changes in treatments relative to controls

C Fluxes (Mg C ha^{-1})	0× plots	Control plots	2× plots	0× change	2× change
Litter inputs	0	8.6 ± 1.6	17.2 ± 3.3	–8.6	+8.6
DOC flux	0.18 ± 0.04	0.42 ± 0.11	0.54 ± 0.09	–0.24	+0.12
Soil CO_2 efflux	26.5 ± 7.6	34.1 ± 7.9	38.0 ± 9.3	–7.6	+3.9
C pools (0–10 cm)					
SOC*	25.1 ± 4.2	30.0 ± 6.6	35.1 ± 9.5	–4.9	+5.1
Fine root biomass C†	0.72 ± 0.43	0.80 ± 0.36	1.4 ± 0.77	–0.08	+0.6
Microbial biomass C†	0.58 ± 0.10	0.70 ± 0.10	0.73 ± 0.16	–0.12	+0.03

Values represent means (± 1 SD). All units are Mg C ha^{-1} .

*Measured in March 2009.

†Values taken from plot means across sampling dates from March 2008 – March 2009.

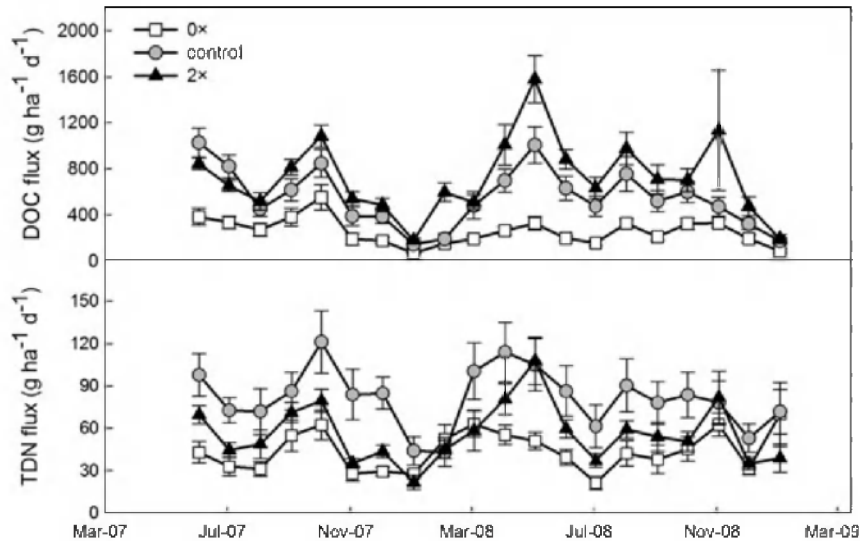


Fig. 1 Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) fluxes in the litter removal (0×), control, and litter addition (2×) litter treatments over the course of the experiment. Values represent monthly means \pm 1 SE.

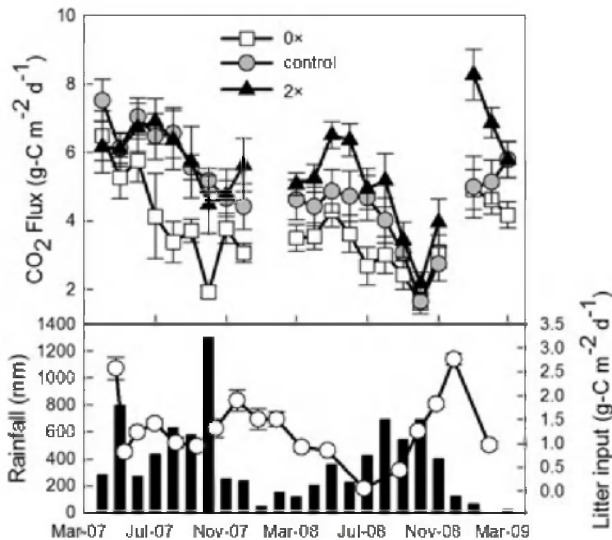


Fig. 2 Monthly mean (\pm 1 SE) CO₂ fluxes from litter removal (0×), control, and litter addition (2×) plots from April 2007 through March 2009. Monthly rainfall (bars) and litter inputs (\pm 1 SE; circles) are shown to illustrate seasonal variations.

with fluxes peaking during the early wet season. This temporal variation coincided with a stronger 0× treatment effect during the first year of the experiment, and a stronger 2× treatment effect during the second year of the experiment (Figs 2 and 3a). During the first year, mean differences caused a $26 \pm 15\%$ decline in 0× plot fluxes and a $0.36 \pm 30\%$ increase in 2× plot fluxes, whereas during the second year, mean differences caused a $17 \pm 32\%$ decline in 0× plot fluxes and a $28 \pm 27\%$ increase in 2× plot fluxes. Mean cumulative

CO₂ respired over the 2 years of data collection showed a similar pattern as fluxes (Table 2; Fig. 3b), and there were significant differences between 0× and 2× plots ($P = 0.006$), but not between treatment and control plots ($P > 0.1$). As with fluxes, the first year showed disproportionately greater declines in the cumulative CO₂ respired when compared to the magnitude of 2× increases, while the second year of litter manipulation exhibited a stronger 2× treatment effect (Fig. 3b).

During the second year of the experiment, across all plots, mean CO₂ fluxes were correlated most strongly with microbial biomass N, microbial biomass C, soil C, and PO₄³⁻ flux ($P < 0.05$ in all cases), but several other variables were also significantly correlated (Table 3). Of the variables we measured, only TDN fluxes and concentrations, gravimetric soil moisture, and O₂ concentrations [calculated with data from Wieder *et al.* (2011)] did not vary significantly with soil CO₂ fluxes ($P > 0.1$; Table 3).

Discussion

Our results indicate that surface soil C cycling in this wet tropical forest ecosystem is extremely sensitive to variations in litter inputs. After only 2 years of experimental manipulation, litter additions drove significant increases and litter removal drove significant declines in surface soil C pools (Tables 1 and 2). These findings differ from those obtained in some temperate forest sites, which generally report non-significant or very subtle effects on surface soil C with changing litter inputs (Nadelhoffer *et al.*, 2004; Sayer, 2006; Hoosbeek & Scarascia-Mugnozza, 2009). Although not always sig-

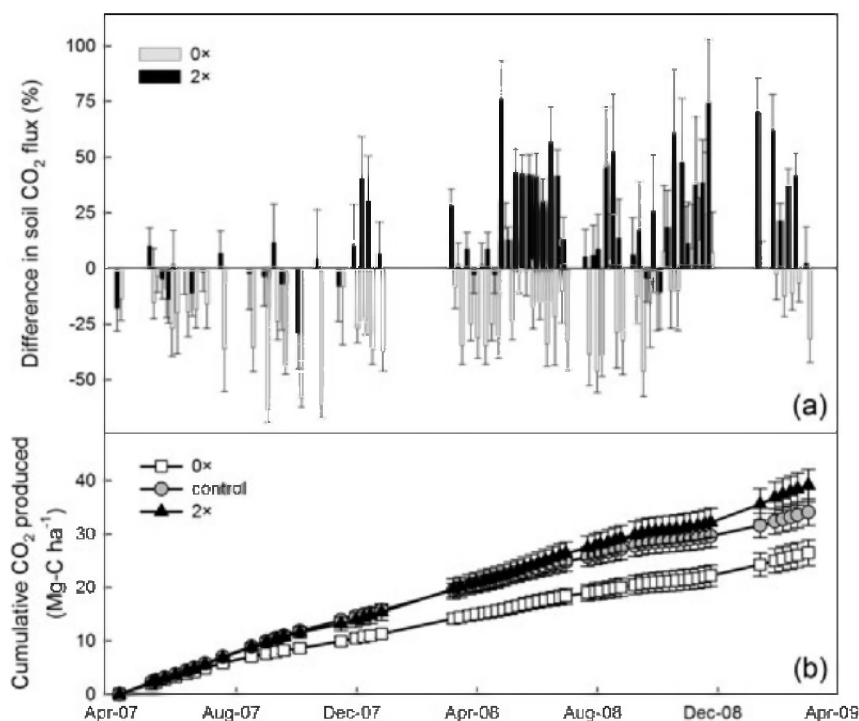


Fig. 3 (a) Mean differences (± 1 SE) between CO_2 fluxes from litter removal ($0\times$) and litter addition ($2\times$) plots and control plots for each sampling date measured. (b) Mean cumulative CO_2 production (± 1 SE) for $0\times$, control, and $2\times$ plots.

nificant, results from another tropical rain forest site in Panama (which receives roughly half the amount of rainfall as the Costa Rica site) suggest litter removal and additions elicit decreases and increases in SOC, respectively (Vincent *et al.*, 2010; Sayer *et al.*, 2012). The apparent differences in both the rate and magnitude of soil C responses among sites are noteworthy because estimates suggest that soils underlying tropical forests contain roughly 20% of global soil organic C, with a significant fraction residing in surface soils (Jobbagy & Jackson, 2000; Tarnocai *et al.*, 2009). Our results suggest that these surface C pools are very sensitive to changes in leaf litter inputs over short timescales, which may have important implications for soil C storage in tropical forests in response to global change.

Beyond changes in soil C pools, changing leaf litter inputs also had strong effects on soil CO_2 fluxes (Fig. 2). Litter removal drove declines in soil respiration similar to those reported in some other tropical ecosystem studies (Vasconcelos *et al.*, 2004; Sayer *et al.*, 2007, 2011). Meanwhile, augmenting litter inputs stimulated soil respiration. Overall, the increases in soil CO_2 fluxes with litter additions were outpaced by the magnitude of the declines in CO_2 fluxes with litter removal, but this was not the case during the second year of the experiment when the treatment effect of litter additions was greater than litter removals (Fig. 3). Previous studies have noted increases in CO_2 produc-

tion rates that cannot be explained by litter C additions alone (Sulzman *et al.*, 2005; Sayer *et al.*, 2007; Schaefer *et al.*, 2009; Chemidlin Prévost-Bouré *et al.*, 2010), and some have suggested that priming effects, whereby additional labile C inputs may stimulate additional SOC mineralization (Kuzuyakov *et al.*, 2000), could explain those results. However, our data do not provide strong evidence suggesting that priming effects played an important role in this study because, overall, litter removals had stronger effects on CO_2 fluxes than litter additions. In addition, if priming did occur in the $2\times$ plots, it did not lead to a decline in soil C pools (Table 2), and other mechanisms such as stimulated roots (discussed below) were likely to have contributed more strongly to elevated CO_2 fluxes in the $2\times$ plots while simultaneously enhancing soil C pools. Furthermore, a lack of strong priming effects may not be unusual in low fertility tropical soils (Nottingham *et al.*, 2011). These results suggest alternative mechanisms may better explain observed changes in soil respiration and total C pools.

Given the relatively modest changes in soil C pools and fluxes observed in response to litter manipulations in some temperate ecosystems (reviewed in Sayer, 2006), how do we explain the rapid changes in C cycling in response to leaf litter manipulation reported here? Overall, the observed declines in soil C in the $0\times$ plots may reflect rapid tropical soil C turnover rates

Table 3 Pearson correlations between measured variables from March 2008 to March 2009 using data from all litterfall manipulation plots. O₂ data were obtained from a subset of those reported in Wieder *et al.* (2011)

Variable	Soil C	Soil N	Soil C:N	DOC flux	[DOC]	DON flux	[DON]	DOC:DON	Gravimetric Soil moisture	[O ₂]	Fine root biomass	Inorganic N flux	Phosphate flux	Microbial biomass C	Microbial biomass N
Soil N	0.87**														
Soil C:N	0.72**	0.31													
DOC flux	0.54**	0.34	0.60**												
[DOC]	0.61**	0.41*	0.64**	0.93**											
DON flux	0.04	0.09	-0.01	0.70**	0.55**										
[DON]	-0.07	0.02	-0.14	0.42*	0.44*	0.80**									
DOC:DON	0.64**	0.31	0.82**	0.63**	0.68**	-0.09	-0.31								
Gravimetric Soil moisture	0.78**	0.71**	0.51**	0.64**	0.72**	0.23	0.09	0.58**							
[O ₂]	-0.30	-0.30	-0.12	-0.03	-0.09	0.07	-0.03	-0.08	-0.41*						
Fine root biomass	0.68**	0.70**	0.38*	0.23	0.37*	-0.17	-0.12	0.47**	0.55**	-0.30					
Inorganic N flux	-0.51**	-0.27	-0.66**	-0.12	-0.15	0.32	0.35	-0.50**	-0.03	0.02	-0.46*				
Phosphate flux	0.68**	0.60**	0.48**	0.46*	0.47**	0.07	-0.12	0.55**	0.51**	-0.24	0.51**	-0.38*			
Microbial biomass C	0.85**	0.84**	0.51**	0.55**	0.66**	0.13	0.00	0.56**	0.71**	-0.23	0.66**	-0.37*	0.59*		
Microbial biomass N	0.84**	0.85**	0.51**	0.40*	0.47**	-0.03	-0.13	0.54**	0.57**	-0.15	0.72**	-0.56**	0.61**	0.90**	
CO ₂ Flux	0.55**	0.43*	0.51**	0.48**	0.44*	0.16	-0.07	0.49**	0.26	-0.03	0.42*	-0.46*	0.53**	0.58**	0.633**

P* < 0.05, *P* < 0.01.

noted by Trumbore (1993). In the absence of new litter-fall C inputs, the favorable climate in tropical forests likely promotes rapid mineralization of the extant soil C pool (Table 2). The observed increases in soil C in the 2× plots may reflect the high clay content of these soils (>40%; Cleveland *et al.*, 2004), which may combine to enhance stabilization of leached litter-derived C via sorption and aggregation (Six *et al.*, 2002). In addition, across all experimental plots, soil C concentrations and rates of soil respiration were both correlated with DOM fluxes, soil moisture, microbial biomass C, and fine root biomass (Table 3), suggesting that those factors largely explain the shifts in soil C pools and fluxes we observed (see below).

The experimental litter manipulation had strong effects on both DOC fluxes and the concentration of DOC moving from the litter layer to the soil (Fig. 2; Table 1). Thus, increases in soil C in the 2× plots may, at least in part, reflect rapid leaching and movement of litter C into SOM pools (e.g., Cleveland *et al.*, 2006). This finding is consistent with previous results from tropical forests showing that litter-derived DOC fluxes correlate with rates of soil respiration (Table 3; Cleveland *et al.*, 2006, 2010). However, while the rapid movement of litter-derived DOC belowground, we observed, may have contributed to the increase in soil C pools and fluxes in 2× plots, the magnitude of these DOC fluxes cannot directly account for more than a small fraction of the observed changes in SOC or soil respiration (Table 2); annual DOC inputs represented only ~5% of total litter C inputs in control plots and 4.9% and 2.4% of observed changes in soil C storage in 0× and 2× plots, respectively (Table 2). Thus, our data suggest that the majority of litter C enters the soil through mechanisms other than leaching and that additional mechanisms were responsible for the large changes in soil C pools.

The litter manipulation may have also elicited indirect changes in C storage via effects on soil microbial biomass C, which decreased by ~26% in the 0× plots and increased by ~16% in 2× plots (Tables 1 and 2). Elsewhere, similar leaf litter manipulation experiments have elicited similar changes in the microbial biomass (Li *et al.*, 2004; Sayer, 2006; Feng *et al.*, 2009), although not consistently (Fisk & Fahey, 2001; Sayer *et al.*, 2007). Here, across the experimental treatments, microbial biomass C concentrations were positively correlated with SOC pools and rates of soil respiration (Table 3), suggesting that changes in microbial biomass in response to litter manipulation could also contribute to variations in C cycling and storage. The py-GC/MS data – which indicate that the SOC in all plots had been heavily transformed by microbial activity – supports this notion. In temperate forests, reported concentra-

tions of lignin-derived compounds are typically an order of magnitude higher than the concentrations we observed (e.g., Grandy & Neff, 2008; Grandy *et al.*, 2009), likely due to the accumulation of partially decomposed particulate fractions that rapidly decompose in the tropics. Furthermore, levoglucosenone, a common pyrolysis product of cellulose, was not detectable in six of fifteen samples, and concentrations averaged <0.3% across all samples, indicating extensive microbial decomposition of plant polysaccharides. Thus, although the direct contribution of changes in microbial biomass C to total surface soil C pools was small, variations in microbial biomass could influence C storage by driving changes in the production and subsequent physicochemical preservation of microbially modified C.

In addition to shifts in DOC fluxes and microbial biomass C, changes in fine root biomass appear to be another important mechanism through which the litter manipulation affected soil C cycling. Although highly variable, the magnitude of the change in fine root biomass may help better explain observed changes in soil C pools and soil respiration in 2× plots (Table 2) and likely reflects experimentally induced changes in soil moisture and/or soil nutrient availability. Leaf litter manipulations may affect fine root biomass via interactions with soil moisture, with thicker litter layers from litter augmentation preventing desiccation, and increasing fine root biomass in surface soils. In addition, treatment-driven shifts in nutrient availability may be responsible for observed changes in fine root biomass. For example, previous work at this site showed that fine root biomass increased following soil N amendments (Cleveland & Townsend, 2006). In our study, litter addition drove increases in soil moisture and soil N concentrations, although these changes were not statistically significant (Table 1). Still, there were strong correlations between fine root biomass and soil moisture, soil N content, and PO_4^{3-} fluxes (which did significantly increase in the 2× plots), suggesting moisture and nutrients were, at least partially, responsible for increased fine root biomass with litter additions (Table 3). Thus, our data suggest that treatment-driven shifts in moisture and soil nutrient availability could, in turn, affect soil C pools and soil CO_2 fluxes.

Whether driven by changes in nutrients, water, or both, changes in fine root biomass almost certainly contributed to the changes in C pools and soil respiration among treatments (Table 2). For example, soil respiration differences between 2× and control plots were lower during the first year of the experiment when fine root biomass differences were more subtle and greater during the second year of the experiment when fine root biomass differences were larger (data not shown).

Differences in fine root biomass could have contributed to differences in soil respiration and C pools through multiple mechanisms including directly through root respiration. In addition, shifts in mycorrhizal biomass – which have been shown to influence decomposition (and hence soil respiration) rates (McGuire *et al.*, 2010) – may have accompanied changes in fine root biomass. The quantity and quality of C delivered by roots could also affect belowground C cycling, with some evidence suggesting that root exudates decompose more slowly than other C compounds (reviewed in Schmidt *et al.*, 2011), thus promoting greater C retention in soils with higher quantities of fine root biomass. Similarly, a synthesis by Rasse *et al.* (2005) argued that roots contribute ~2.5 times more to soil organic matter (SOM) pools than shoots, and observations indicate that microorganisms can process and incorporate root C into biomass four times faster than aboveground plant residues (Kong *et al.*, 2011). Faster cycling of root material suggests that root derived C and nutrients are the most important drivers of microbial activity and microbial biomass turnover. Given current thinking that microbially derived necromass, metabolites, and decomposition products – rather than recalcitrant plant material – account for the majority of stabilized SOM (Simpson *et al.*, 2007; Grandy & Neff, 2008; Kleber & Johnson, 2010; Schmidt *et al.*, 2011), the faster cycling of root material through microbial biomass likely has important implications for SOM formation and may have contributed to the sizable soil C increases in 2× plots. Finally, it is important to note that the relatively small size of the plots used in this experimental manipulation (3 × 3 m) could have influenced our results. For instance, the effect size of the litter manipulation treatments may have been elevated due to the influence of roots from outside of the plots.

Taken together, our results suggest that tropical soil C pools are very sensitive to changes in C inputs, suggesting a fundamental difference between wet tropical forests (where changes were rapid) and temperate forests (where C pools are generally less responsive to litter manipulation). Our results also highlight the role of rainfall-driven DOM fluxes into the microbial-soil system and indicate rapid C turnover rates and extensive microbial processing of SOC. These differences may alter the mechanisms controlling soil C mineralization and stabilization in the lowland tropics. Less understood, however, is the role of roots in mediating soil C responses to changes in litter inputs – especially how root growth and C allocation may change with concurrent alteration in soil moisture and nutrient availability. This study enhances our process-based understanding of how tropical C storage may change with future shifts in tropical primary productivity and

begins to illuminate interactions between above and below ground feedbacks in the terrestrial C cycle. Finally, our results demonstrate the potential for shifts in forest litterfall inputs to impact C cycling on a global scale.

Acknowledgements

We would like to thank W. Combroneiro-Castro for assistance with field work, F. Campos Rivera, the Organización para Estudios Tropicales (OET) and the Ministerio de Ambiente y Energía (MINAE) for assisting with research permits and providing logistical support in Costa Rica, M. Jimenez and the Drake Bay Wilderness Camp for their generous access to field sites; and R. Kimmel, M. Martin, S. Reed, K. Wickings, J. Aylward, and S. Weintraub for additional support in the field and laboratory and reviewing drafts of this manuscript. Grants from the National Science Foundation (DEB-0852916 and DEB-0919080) and the Andrew W. Mellon Foundation supported this work.

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