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Experimental litterfall manipulation effects on soil bacterial community structure and soil carbon cycling in a wet tropical forest

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EXPERIMENTAL LITTERFALL MANIPULATION EFFECTS ON SOIL BACTERIAL COMMUNITY STRUCTURE AND SOIL CARBON CYCLING IN A WET TROPICAL FOREST

By

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ABSTRACT

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Global changes such as increasing atmospheric carbon dioxide (CO_2) concentrations or climate change are likely to drive shifts in plant-derived carbon (C) inputs to terrestrial ecosystems via changes in litterfall and plant net primary production (NPP). However, the effects of shifting detrital C inputs on belowground microbial community function, C cycling and fluxes remain largely unknown, especially in tropical forest ecosystems. To investigate how shifts in bacterial community composition resulting from differences in C availability affect organic matter decomposition and how soil C pools and fluxes respond to shifts in C inputs, I utilized an *in situ* litter manipulation experiment in a tropical rain forest in Costa Rica. In one study, I assessed whether changes in bacterial community composition and diversity were related to changes in microbial community function. To do this I used bar-coded pyrosequencing and a series of laboratory incubations to test the potential functional significance of community shifts on organic matter decomposition. In another study, I assessed the effects of the litterfall manipulation on *in situ* dissolved organic matter (DOM) fluxes, internal C and nutrient cycling, and soil CO_2 fluxes. The manipulation had clear effects on soil bacterial community composition but mixed effects on microbial community function. These results show that while resource-driven shifts in soil bacterial community composition have the potential to influence decomposition of specific C substrates, those differences may not translate to differences in mixed DOM decomposition rates *in situ*. In the second study, results showed that increasing and decreasing litterfall inputs drove rapid and significant shifts in belowground C cycling, suggesting that shifts in litterfall inputs in response to global environmental change could have important consequences for belowground C storage and fluxes in tropical rain forests. Furthermore, the observed responses highlight the potential for marked differences between tropical ecosystems and temperate ecosystems, where the effects of forest litter on belowground C cycling are typically much more subtle. Taken together, these studies demonstrate the strong potential impacts of shifts in plant-derived C inputs on C cycling and bacterial community structure while having complex effects on microbial community function.

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INTRODUCTION

The global carbon (C) cycle is mediated by a suite of fundamental ecosystem processes that control rates of C transfer between atmospheric and terrestrial pools. Autotrophic organisms (e.g., plants) incorporate atmospheric carbon dioxide (CO_2) into their tissues through photosynthesis and heterotrophic decomposer organisms (e.g., soil microorganisms) decompose plant-derived organic matter, returning CO_2 to the atmosphere. Understanding the biogeochemical factors controlling the balance between C uptake and losses is essential to generating accurate predictions of how the terrestrial C cycle will respond to environmental change. For example, increasing concentrations of atmospheric CO_2 may cause shifts in plant productivity and alter the amount of C delivered to soils. Shifts in plant C inputs could, in turn, have important consequences for soil C storage and fluxes, potentially causing feedbacks to the global C cycle.

For my master's thesis, I addressed two unanswered questions regarding how soils will respond to shifts in plant-derived C inputs: (1) Does soil microbial community composition affect organic matter decomposition rates?; and (2) Do shifts in leaf litter inputs drive changes in soil C pools and fluxes? My early review of the literature for work conducted in chapter one indicated that a relatively large number of studies have documented shifts in microbial community composition shift in response to natural or experimentally-induced changes in environmental conditions (e.g., temperature, land use change, etc.). However, surprisingly few studies have directly tested whether shifts in microbial community composition affect ecosystem process rates, and only a handful have been conducted in tropical forests. Similarly, few studies have documented how

soil C pools may respond to changes in aboveground productivity. To address these shortcomings, I used an experimental litterfall manipulation experiment in Costa Rica and conducted two studies to investigate how changes in C inputs may alter soil C cycling. In the first, I used a set of laboratory incubation experiments to address possible links between microbial community composition and organic matter decomposition. Next, I analyzed a suite of biogeochemical data obtained *in situ* in an attempt to understand how changes in aboveground C inputs may alter soil C pools and fluxes. Together, my two studies provide a valuable contribution to our understanding of soil C cycling. For example, my work is among the first to show that soil C pools may respond rapidly to shifting plant detrital inputs in the tropics, and provides early evidence suggesting that while soil bacterial community composition is likely to be linked to decomposition rates following shifts in resource availability, process rates are influenced by other biotic and abiotic controls more strongly than by microbial community composition itself. Overall, this work takes a valuable first step in providing a clearer understanding of how specific environmental perturbations will affect soil processes at multiple scales, from soil microbial metabolism to the global C cycle.

CHAPTER 1

The effects of soil bacterial community structure on decomposition in a tropical rain forest

ABSTRACT

Soil microorganisms are key drivers of terrestrial biogeochemical cycles, yet it is still unclear how variation in soil microbial community composition influence many ecosystem processes. To investigate how shifts in bacterial community composition resulting from differences in carbon (C) availability affect organic matter decomposition, I utilized an *in situ* litter manipulation experiment in a tropical rain forest in Costa Rica to assess if changes in microbial community function were related to bacterial community composition and diversity. I used bar-coded pyrosequencing to characterize soil bacterial community composition in litter manipulation plots and performed a series of laboratory incubations to test the potential functional significance of community shifts on organic matter decomposition. Despite clear effects of the litter manipulation on soil bacterial community composition, the treatments had mixed effects on microbial community function. Distinct communities varied in their ability to decompose a wide range of C compounds, and functional differences were related to both the relative abundance of the two most abundant bacterial sub-phyla (Acidobacteria and Alphaproteobacteria) and to variation in bacterial alpha-diversity. However, distinct communities did not differ in their ability to decompose native dissolved organic matter (DOM) substrates that varied in quality or quantity. My results show that while resource-driven shifts in soil bacterial community composition have the potential to influence decomposition of specific C substrates, those differences may not translate to differences in DOM decomposition rates *in situ*. Taken together, the results suggest that microbial communities may be either functionally dissimilar or equivalent during decomposition depending on the nature of the organic matter being decomposed.

INTRODUCTION

Microorganisms have been aptly described as the "engines that drive biogeochemical processes" (Falkowski et al. 2008), yet important questions about the potential effects of changes in microbial community composition on ecosystem function remain (Groffman and Bohlen 1999; Tiedje et al. 1999; Nannipieri et al. 2003; O'Donnell et al. 2005; Condron et al. 2010). Decomposition is among the most fundamental of biogeochemical processes, and a large body of research has explored the factors that regulate decomposition rates (Meentemeyer 1978; Couteaux et al. 1995; Gholz et al. 2000; Cornwell et al. 2008). While the combined importance of litter chemical composition (Gholz et al. 2000; Cornwell et al. 2008; Grandy and Neff 2008), nutrient availability (Melillo et al. 1982; Taylor et al. 1989), and climate (Meentemeyer 1978; Aerts 1997; Gholz et al. 2000) on decomposition has been clearly established, the effects of variation in microbial community composition have been largely unexplored (Bardgett et al. 2008; McGuire and Treseder 2010). As a result, most current ecosystem models implicitly treat soil as a "black box" where microbial function is solely determined by abiotic constraints (Parton et al. 1994; Tiedje et al. 1999). Yet, soil microbial communities are incredibly diverse (Fierer et al. 2007b), and many macro-ecological studies provide evidence that community composition can influence ecosystem processes (Hooper et al. 2005).

Two competing hypotheses have been proposed to describe the effects of microbial community composition shifts on ecosystem processes: The first – functional equivalence – suggests that functional redundancy across phylogenetically distinct microbial communities should minimize the effects of community shifts on

biogeochemical processes. By contrast, the second hypothesis – functional dissimilarity – suggests that variation in community composition will be reflected by differences in either the ability of a community to carry out a specific process, or in the rates of specific processes (Strickland et al. 2009). Cavigelli and Robertson (2000) provided some direct evidence for the functional dissimilarity of soil microorganisms involved in denitrification, and others have documented the influence of soil microbial community structure on other N cycling processes (Balser and Firestone 2005) and methane production and consumption (Schimel and Gulledge 1998). Some authors have suggested that functional dissimilarity is more likely for processes that are restricted to relatively few microbial taxa (e.g., Schimel 1995; Schimel et al. 2005), but there is evidence suggesting that soil microbial community structure has the potential to influence more basic ecosystem processes like decomposition (Waldrop et al. 2000; Carney and Matson 2005; Strickland et al. 2009; Keiser et al. 2011).

Recently, links between the availability of decomposable organic matter and the relative abundance of bacterial subphyla and phyla have been shown (Smit et al. 2001; Fierer et al. 2007a; Nemergut et al. 2010) supporting the notion that higher bacterial taxa can be ecologically distinct (Philippot et al. 2010). For example, Fierer et al. (2007a) showed that soil C availability was positively correlated with the relative abundance of Bacteroidetes and Betaproteobacteria. These taxa could generally be described as copiotrophic (or r-selected) bacteria, while the relative abundance of Acidobacteria – a generally oligotrphic, or K-selected group – was inversely related to C availability. This ecological classification scheme provides a testable and tractable framework for assessing relationships between soil microbial community composition and ecosystem function.

Indeed, in past work at the study site described here, Cleveland et al. (2007) showed that laboratory C additions to soil drove increases in putative copiotrophic bacteria that correlated with an increase in soil CO_2 flux. Similarly, Nemergut et al. (2010) showed that increasing C inputs (by experimentally manipulating leaf litter inputs *in situ*) drove a relative decrease in the abundance of putative oligotrophic soil bacteria (Acidobacteria) and relative increases in putative copiotrophic soil bacteria (Alphaproteobacteria).

Together, the Cleveland et al. (2007) and Nemergut et al. (2010) studies not only suggest that changes in C availability altered microbial community structure in predictable ways, but they provide a possible mechanistic link between changes in community structure and the decomposition process. However, neither study directly investigated whether changes in microbial community structure caused differences in decomposition. Experiments directly testing the functional effects of variation in soil microbial communities are rare because experimentally manipulating microbial community composition *in situ* it is very difficult. As a result, most studies have relied on correlations between community composition and processes to infer structure-function relationships. However, such approaches often cannot distinguish between the effects of community composition and other confounding variables (Reed and Martiny 2007). In addition, very few studies have been conducted in tropical rain forests (Balser et al. 2010) despite the fact that they play a dominant role in the global C cycle (Zhao and Running 2010).

However, the observation made by Nemergut et al. (2010), which showed that litter manipulations in a tropical rain forest in Costa Rica drove significant shifts in microbial community composition, provided us with a rare opportunity to examine

whether previously quantified, resource-driven shifts in bacterial community composition are paralleled by changes in microbial function. I addressed this question using a series of laboratory incubation experiments with soil samples obtained from the same litter manipulation plots described in Nemergut et al. (2010). To do this, I first assessed *potential* differences in the ability of distinct soil microbial communities to decompose a wide array of C substrates that vary in their overall chemistry and quality. Given that C input quantity can influence soil C chemistry (Kiem et al. 2000; Grandy and Neff 2008), I hypothesized that the litter manipulation would alter soil C chemistry, and that this would lead to shifts in microbial communities and their ability to degrade a wide array of C substrates.

Next, I assessed the possible effects of bacterial community composition on the decomposition of a native C source: litter-leached dissolved organic matter (DOM). In any ecosystem, movements of DOM from the litter layer to soil represent important C fluxes (Currie and Aber 1997; Neff and Asner 2001; Cleveland et al. 2004), but they are especially important in this wet tropical forest ecosystem (Cleveland and Townsend 2006). Given that the relative abundance of copiotrophic bacteria varied positively with C inputs in the study soils (Nemergut et al. 2010), I hypothesized that the decomposition rates of DOM would be highest in soil that had received the largest litter inputs. Furthermore, I hypothesized that soils receiving high litter inputs would decompose high quality DOM more rapidly than soils exposed to low C inputs, and that low-C soils would decompose low quality DOM more rapidly than high-C soils. Finally, I assessed the effects of DOM quantity on decomposition rates by adding several known concentrations of DOM to soil samples and assessing relationships between soil type, DOM

concentration, and soil CO_2 production rates. Cleveland et al. (2010) showed that soil CO_2 fluxes increased with DOM concentration, and Nemergut et al. (2010) observed that bacterial communities exposed to similar DOM concentrations had similar compositions. Therefore, I hypothesized that soil microbial communities in litter addition plots would decompose high concentrations of DOC more rapidly than communities in soils exposed to litter removal and that these differences would be more subtle at lower concentrations.

METHODS

Study site

The study was conducted in a diverse lowland tropical rain forest in the Golfo Dulce Forest Reserve (8°43' N, 83°37' W) on the Osa Peninsula in southwestern Costa Rica. Mean annual temperature (MAT) at the site is ~ 26°C and mean annual precipitation (MAP) averages > 5,000 mm yr^{-1}, but the site has a distinct dry season (December – April) when precipitation averages < 100 mm $month^{-1}$ and litterfall and standing litter mass are at annual maxima (Cleveland and Townsend 2006). Soil at the site is clay (Wieder et al. 2011) and classified as an Ultisol that developed on a steeply dissected landscape in the Osa basaltic complex (Berrange and Thorpe 1988). A complete site description including soil physical and chemical properties can be found in Cleveland et al. (2006).

Litterfall manipulation experimental design

To test the effects of the quantity of leaf litter inputs on soil microbial community structure and function, I utilized an existing set of *in situ* litter manipulation plots

described by Nemergut et al. (2010). In April 2007, 30 randomly assigned litter manipulation plots (3 × 3 m) were established. Since their establishment, litter was collected at monthly intervals from ten litter removal (0×) plots, weighed and distributed evenly to ten litter addition (2×) plots, and the remaining ten plots were not manipulated (controls). On average, the control and 2× plots received 0.90 ± 0.05 kg litter m^{-2} y^{-1} and 1.79 ± 0.11 kg litter m^{-2} y^{-1}, respectively, over the course of the experiment.

Soil sampling and analysis

Soil samples (0-10 cm) were collected from each of the 30 litterfall manipulation plots double-bagged, and transported on ice to the laboratory at the University of Montana. There, soil samples were sieved to 4 mm, stored at 4°C (except subsamples for microbial community analysis which were stored at -80°C), and analyzed within one week. Soils were sampled in April 2010 for the catabolic response profile analysis, 16S rRNA gene sequencing, and soil chemistry analysis; in October 2009 for the native DOM quality incubation experiment; and in January 2010 for the native DOM quantity experiment. Previous 16S rRNA gene data showed no major seasonal differences in bacterial community composition at the site (Nemergut et al. 2010), and relationships between variables were only assessed for measurements taken on the same samples or subsamples (i.e., same collection dates).

I determined soil moisture content on all samples gravimetrically after drying soil samples for 48 h at 105°C. pH was determined on air-dried soils in a soil:deionized water slurry (1:5). Total soil C and N were determined on ground samples (0.5 mm) using a combustion-reduction elemental analyzer (Carlo Erba, Lakewood, NJ, USA). Soil microbial biomass C in fresh soil samples was determined using the chloroform

fumigation-extraction method (Brookes et al. 1985). Briefly, fumigated (5 d) and unfumigated samples (4.5 g dry mass) were extracted in 40 ml of 0.5 mol L^{-1} K_2SO_4 for 1 h, centrifuged for 5 min (5,000 rpm), and filtered. Organic C in extracts was analyzed using a TOC-VCPN total organic C analyzer (Shimadzu Inc., Columbia, MD, USA). I calculated microbial biomass C as the difference between the extractable C in fumigated and unfumigated samples using a proportionality constant (K_c) of 0.45 (Vance et al. 1987). Finally, compound specific soil C content on soil subsamples was assessed from the catabolic potential assay (see below) using pyrolysis-gas chromatography/mass spectrometry (GCMS) following a method similar to Wickings et al. (2011). Soil samples were finely ground and pulse-pyrolyzed using a Pyroprobe 5150 (CDS Analytical Inc., Oxford, PA, USA) at 600 °C. The pyrolysis products were separated 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), delivered to a mass spectrometer (Polaris Q, Thermo Scientific, Waltham, MA, USA), and ionized at 200 °C. The chromatogram peaks were identified by comparing the mass spectra of compounds with the National Institute of Standards and Technology mass spectral library using the Automated Mass Spectral Deconvolution and Identification System (AMDIS V 2.65). The relative abundances of compounds were calculated as the peak area for each compound divided by the sum of the areas of all identified peaks for a given sample.

Microbial community analysis

To verify and quantify differences in bacterial community composition between litterfall inputs, I subsampled a set of composited soil samples (by treatment) collected in April 2010 and used in the catabolic potential assay (see below). Briefly, DNA was

extracted and the 27-338 region of 16S rRNA gene was sequenced using bar-coded pyrosequencing following protocols from Nemergut et al. (2010). A modified PCR amplification was used and the sequencing procedure used Titanium chemistry (454 Life Sciences, Bradford, Connecticut, USA). PCR reactions were performed in triplicate and consisted of 10 µl of sterile water, 10 µl of 5 PRIME hot master mix (5 PRIME, Gaithersburg, MD, USA), 2 µl (5 µM) of the reverse primer, 1 µl (10 µM) of the forward primer, and 2 µl of the sample DNA. Samples were initially denatured for 3 min at 94 ºC followed by 25 cycles at 94 ºC for 45 sec, 50 ºC for 30 sec, 72 ºC for 90 sec and a final elongation step at 70 ºC for 10 min. After sequencing, I conducted all downstream sequence analyses prior to statistical analysis using the QIIME pipeline (Caporaso et al. 2010). This pipeline assigns sequences to samples and filters out both low quality reads and reads of unexpected lengths. All samples were denoised using the provided denoising step to reduce the number of erroneous sequences. I determined operational taxonomic units (OTUs) at the 97% sequence similarity level, assigned taxonomic identities using the RDP database, and all samples were rarefied at 620 sequences per sample before performing final analyses to account for differences in sampling effort. I assessed alpha-diversity in the communities using three metrics: the observed number of OTUs in a sample, the Shannon index (Hill et al. 2003), and the phylogenetic diversity index (Faith 1992). I calculated phylogenetic distances between communities using the weighted UniFrac distance metric (Lozupone and Knight 2005).

Catabolic potential assay

I assessed soil microbial community metabolic capabilities using community response profiles (CRPs). CRPs have been used to characterize soil microbial

communities and assess differences in their catabolic diversity (Degens and Harris 1997). After sieving, I bulked randomly selected pairs of soil samples within each treatment to form a total of five composite samples per treatment. CRPs of the composite soil samples were assessed using a protocol modified from Degens and Harris (1997). Briefly, 2 g subsamples of each of the fifteen soil composites were placed in 60 ml vials fitted with septa (25 vials per composited sample). Next, 2 ml C aliquots (900 mM) of twenty-four C substrate solutions were added to the vessels. Substrates consisted of three simple sugars (fructose, glucose, and sucrose), four polysaccharides (amylopectin, amylase, cellulose, and glycogen), five amino acids (glutamic acid, glutamine, glycine, histidine, and lycine), one amino sugar (glucosamine), two proteins (bovine serum albumen and casein), two carboxylic acids (citric acid and lactic acid), two fatty acids (linoleic acid and oleic acid), one non-amino acid amine (urea), one nucleic acid (DNA), and three recalcitrant compounds (chitin, humic acid, and lignin). Another sample from each composite received a water-only addition to assess incidental wet-up effects when adding the C substrates. All substrate solutions and the added water were adjusted to a pH of 6.0 using HCl or NaOH prior to additions.

Twenty-four h after the C additions, soil responses to substrate additions were determined by removing a 3 ml headspace sample from each vial using a syringe/needle. CO_2 in the headspace was analyzed using an infrared gas analyzer (CA-10a, Sable Systems Inc., Las Vegas, NV, USA) with N_2 as the carrier gas. CO_2 flux rates were calculated and adjusted to account for the dry soil weight equivalent of soil samples, and the amount of CO_2 produced in the water-only treatments was subtracted from the substrate treated samples. To control for differences in total microbial activity, adjusted

CO_2 production rates were then summed across all substrates for each sample, and further analysis was performed on the CO_2 fluxes for each substrate divided by this sum. I excluded two substrate responses (cellulose and oleic acid) from consideration since they were undetectable in more than one third of all samples. In addition, I used normalized CO_2 fluxes from each substrate to calculate Simpson's index of diversity (1-D) for each sample (Magurran 2004).

Native DOM incubation experiment: The effects of DOM quality

I further examined the potential effects of litter-driven changes in microbial composition on decomposition dynamics using two laboratory incubation experiments. First, I investigated differences in the ability of the microbial communities to degrade two types DOM leached from two common tree species from the study site: *Schizolobium parahyba* and *Manilkara staminodella*. Wieder et al. (2008) showed that *S. parahyba* leachate (relatively low C:N and C:P ratios; high quality) decomposes more rapidly than *M. staminodella* (relatively high C:N and C:P ratios; low quality), allowing us to examine variation in microbial community responses to C quality. DOM solutions were made by leaching 25 g air-dried litter from each species in 500 ml of deionized water at 25°C. After 24 h, leachate was filtered to 0.2 µm using nylon filters, and leachate DOC concentrations (~ 900 mg l^{-1}each) were measured using a TOC-VCPN (Shimadzu, Columbia, MD, USA) total organic C analyzer.

After collecting leachate, a set of fresh soil samples (25 g each) were placed in glass Mason jars fitted with lids containing septa and adjusted to 50% water holding capacity (WHC) with deionized water. 2 ml of each DOM type were added to samples, and respired CO_2 was measured at regular intervals (with samples being vented in

between samplings) for 159 h by evacuating the headspace and analyzed using a gas chromatograph (Shimadzu, Columbia, MD, USA). CO_2 fluxes were calculated as a rate of CO_2 respired per dry weight equivalent of soil, and I calculated the cumulative CO_2 produced by each sample by linearly interpolating fluxes between sampling events.

Native DOM incubation experiment: The effects of DOM quantity

I conducted a second incubation experiment to assess differences in the ability of the microbial communities from the different litter input treatments to degrade varying concentrations of DOM. Nine soil samples from each litter input treatment were randomly selected to generate three composite soil samples per treatment, each consisting of three individual samples. 70 g of mixed litter was leached in 700 ml of deionized water for one hour, sterile filtered to 0. 2 µm, and DOC concentrations were measured using a TOC analyzer. The leached DOC stock was then used to generate a set of solutions with varying DOC concentrations (2, 10, 50, 250, and 1000 mg C l^{-1}). Equal volumes (4 ml) of each solution were then added to 20 g of soil from each composite in glass jars (N = 3 per DOM concentration). Following DOM additions, samples were incubated at 21°C for 12 h, and CO_2 concentrations in the incubation vessels were assessed using gas chromotography. Initial respiration rates (rates at 1.7 h) were normalized by both the soil dry weight equivalent and soil microbial biomass C content.

Statistical analysis

With the exception of soil C chemistry, analysis of variance (ANOVA) and Tukey's HSD post-hoc tests were used to test for significant differences in soil characteristics, relative abundances of individual bacterial taxa, bacterial diversity, and

catabolic diversity. Differences in C chemistry, bacterial community composition, and CRPs were assessed using variance partitioning with nonparametric MANOVA (McArdle and Anderson 2001) using the Adonis function (Oksanen et al. 2011) on Bray-Curtis dissimilarity matrices calculated separately for soil C substrates and CRPs and the UniFrac matrix for bacterial community composition. To visualize differences in bacterial community composition and CRPs between litter input treatments, I created principal coordinates analysis (PCoA) plots based on the distance matrices. To visualize relationships between the relative abundances of Acidobacteria and Alphaproteobacteria and bacterial community composition or individual C substrate decomposition rates and CRPs, I used vector fitting, which uses multiple linear regression (using the first two principal coordinates, or the axes in the PCoA plots) as the explanatory variables and the variable of interest (in this case, bacterial taxon relative abundance or C substrate decomposition rate) as the dependent variable (Jongman et al. 1995). Only vectors representing significant relationships between the first two principal coordinates and the relative substrate decomposition rates for individual compounds were plotted on the CRP PCoA.

To assess the relationships between bacterial community composition and soil characteristics and CRPs, I used Mantel tests with Spearman's rank correlations (10,000 permutations) and multiple regression on distance matrices (MRM; 10,000 permutations), an extension of the partial Mantel test, which allows testing several explanatory distance matrices concurrently (Lichstein 2007). For the Mantel tests and the MRM analysis, I used the distance matrices previously mentioned and Euclidean distance matrices for all

other variables. Relationships among metrics of catabolic diversity and bacterial community alpha-diversity were assessed using Pearson correlations.

To analyze CO_2 responses to additions of two different types of DOM (DOM quality experiment), I used analysis of covariance (ANCOVA) with litter input treatment and DOM type as fixed factors and soil C, soil N, and microbial biomass C as covariates. Prior to analysis, cumulative CO_2 production data were log (*ln*) to meet the assumptions of normality and the heterogeneity of variances. To analyze soil CO_2 responses to additions of varied DOM concentrations, I used ANOVA.

ANOVA, Tukey HSD tests, ANCOVA, and simple linear regression tests were performed using SPSS v. 17 (SPSS, Chicago, Illinois, USA), and PCoA, vector fitting, Mantel tests, and MRM analyses were performed using the pco, vf, mantel, and MRM functions in the ecodist package (Goslee and Urban 2007) in R v. 2.9.2 (The R Foundation for Statistical Computing, Vienna, Austria). Adonis analyses were conducted using the vegan package in R. For all statistical tests, significance was determined when $P < 0.05$.

RESULTS

Soil Functional Responses to C Substrate Additions: Catabolic Response Profiles

Soils exposed to varying litter treatments differed in their ability to degrade the range of C compounds used in the CRP incubation experiment ($P = 0.02$; Figure 1-1). While the soil microbial communities from all litter treatments could decompose all substrates, the proportional decomposition response (i.e., the individual substrate decomposition rate relative to the sum of the decomposition rates of all substrates for a

given sample) varied between litter treatments. For instance, soils from the 2× plots showed greater proportional decomposition responses to glucose, lactic acid, glycine, glutamic acid, and glucosamine, and lower proportional decomposition responses to DNA, urea, and lignin than 0× soils (Figure 1-1; Table 1-1; Appendix 1-1).

To assess possible drivers of the observed differences, I explored relationships between a number of soil properties and CRPs. Consistent with previous observations from this litter manipulation experiment (Nemergut et al. 2010), the manipulation drove differences in soil nutrient pools, as I observed significantly greater proportions of total soil C and N and greater microbial biomass C in 2× than in 0× plots in the October 2009 samples (Table 1-2). However, the litter manipulation did not result in broad-scale changes in soil C chemistry. 239 distinct pyrolysis products were identified, and multivariate analysis of the soil organic matter (SOM) chemical characteristics indicated there was considerable variation in the types and quantities of C compounds among experimental plots, but this could not be attributed to treatment effects.

Nemergut et al. (2010) showed that the *in situ* litter manipulations drove significant differences in soil bacterial community composition ($P = 0.001$; Figure 1-2). For example, Nemergut et al. (2010) showed that Acidobacteria and Alphaproteobacteria were the most abundant higher-level taxa across treatments, the relative abundances of these taxa significantly differed between treatments, and variation in total soil C significantly explained variation in bacterial community composition. My results confirmed that at the time of the experiments, these differences in community composition were still present. In addition, the results also showed that variation in soil C chemistry among the different treatments did not explain variation in microbial

community composition. Moreover, I found the 2× plots contained a significantly higher number of OTUs than the 0× plots, but there were no differences in the Shannon or phylogenetic diversity measurements between soil samples from different litter treatments ($P > 0.1$; Table 1-3).

Among all samples, bacterial community composition, soil C chemistry, soil C, soil N, soil C:N ratios and microbial biomass C were all significantly related to variation in CRPs ($P < 0.05$), but the relationship between CRPs and bacterial community composition was the strongest (Table 1-4). In addition, the MRM analysis indicated that including the other soil properties (i.e., moisture, pH, total C, total N, C composition, microbial biomass C, and C chemistry) did not significantly improve the explanatory power of the model over what was observed when including bacterial community composition alone. Finally, I found significant relationships with CRPs for both Acidobacteria ($\rho = 0.27$; $P = 0.013$) and Alphaproteobacteria ($\rho = 0.39$; $P = 0.003$; Figure 1-2) relative abundances.

I also observed differences in soil catabolic diversity in response to the litter manipulation. For example, soil from the 0× plots had significantly lower catabolic diversity than other soils (Appendix 1-1). In addition, catabolic diversity was significantly correlated with two bacterial diversity metrics – the number of observed OTUs per sample ($r = 0.71$; $P = 0.005$) and the Shannon index ($r = 0.72$; $P = 0.004$). However, catabolic diversity did not significantly correlate with bacterial phylogenetic diversity (Figure 1-3).

Soil Functional Responses to C Substrate Additions: Native DOM Quality

Over the course of the 159 h incubation, the 2× soils had significantly higher respiration rates than either the 1× or 0× soils, irrespective of the DOM type added ($P < 0.001$). Relative to soils from the 1× plots, soil samples from the 0× plots produced 33% less CO_2 and 2× soils produced 76% more CO_2 over the course of the incubation. Soil samples also significantly varied in their response to DOM type ($P < 0.001$). Among all samples, high quality DOM leached from *S. parahyba* elicited a 13% increase in soil respiration relative to soil respiration rates following addition of DOM leached from low quality *M. staminodella*. Yet, soils from different treatments did not demonstrate different trends in their CO_2 production for different DOM types as there was no statistical interaction between the litter input treatment and the DOM type ($P > 0.1$). Furthermore, after accounting for variation in soil C, soil N, and microbial biomass C (using ANCOVA), litter input treatment did not significantly contribute to variation in CO_2 produced over the course of the incubation, and all other variables significantly explained 91.5% of the variation in the cumulative CO_2 produced ($P = 0.001$).

Soil Functional Responses to C Substrate Additions: Native DOM Quantity

To assess whether shifts in bacterial community composition in the litter input treatments caused soils receiving greater inputs to decompose higher concentrations of DOM more quickly, I calculated CO_2 fluxes and evaluated whether the relative fluxes between treatments were different among the various DOM concentrations. Soil samples from different litter input treatments varied in their response to the range of DOM concentrations added in the native DOM quantity incubation experiment ($P < 0.001$), and initial soil respiration rates increased both with litter input (i.e., 0× < control < 2×) and

DOM concentration. Yet, while the overall magnitude of the responses to higher DOM concentrations increased with litter input, the differences in CO_2 fluxes were similar between litter input treatments regardless of the concentration of DOM added, and there was not a significant interaction between DOM concentration and litter input treatment (Figure 1-4).

DISCUSSION

Strickland et al. (2009) proposed two competing hypotheses to describe the possible effects of divergent microbial communities on ecosystem processes. The first – functional equivalence – suggests that microbial communities contain many functionally redundant members and/or they can quickly adapt to shifting conditions. The second – functional dissimilarity – suggests that differences in community composition also affect processes. Using a full-factorial (litter × soil inoculum) experiment, Strickland et al. (2009) showed that decomposition rates varied depending on the soil inoculum used, and suggested that this was evidence of functional dissimilarity.

Here, I first asked how litter-driven changes in microbial community composition contribute to differences in organic matter decomposition. My analyses confirmed that the microbial communities differed between treatments in ways similar to those previously described (Figure 1-2; Nemergut et al. 2010). I took advantage of these community composition differences in the CRP experiments, in which I explored soil responses to an array of specific C compound additions. Overall, the results supported my initial hypothesis – soil microbial communities from the 0×, control, and 2× plots differed in their ability to respire the compounds added in the CRP analysis (Figure 1-1).

Moreover, among all samples, a substantial amount ($\rho = 0.46$) of the variation in CRPs could be explained by specific variation in bacterial community composition, and including an array of common biogeochemical variables in the multivariate analysis did not strengthen the fit of the modeled results to the data. Finally, the two most abundant bacterial taxa, Acidobacteria and Alphaproteobacteria, were strongly related to differences in CRPs ($\rho = 0.27$ and 0.39, respectively).

These findings suggest that variation in community composition, and in particular, the relative abundance of the bacterial taxa Acidobacteria and Alphaproteobacteria, are important in explaining the observed variation in decomposition rates of C substrates. Moreover, the results not only confirm previous work showing that variation in litterfall C inputs drove predictable shifts in microbial community composition, but the data also suggest that changes in community composition correspond to changes in the overall ability of the resulting communities to decompose added C substrates. Overall, these results support the functional dissimilarity hypothesis, and are consistent with other studies that have shown variation in decomposition rates across different soil microbial communities taken from a single ecosystem (Carney and Matson 2005; Brant et al. 2006).

There are several possible explanations for the observed changes in microbial community function between treatments, and the results may actually reflect the effects of multiple interacting mechanisms. For example, the litterfall manipulation enhanced soil C in the 2× plots, and decreased soil C in the 0× plots (Table 1-3; Nemergut et al. 2010). Thus, I predicted that variation in the delivery of labile C would also alter soil C chemistry (e.g., Kiem et al. 2000), which could in turn alter community-specific

responses to any specific compound array. However, I did not observe differences in the overall soil C chemistry between litter input treatments that are typically associated with variation in soil decomposer communities (Grandy et al. 2009; Wickings et al. 2011). Soil C chemistry did not explain soil bacterial community structure differences, and soil C quantity corresponded more strongly to differences in CRPs than soil C chemistry (Table 1-4). These findings suggest that soil C quantity was more important to microbial community structure and function than soil C chemistry in the experimental plots.

Next, it is also possible that overall changes in bacterial diversity could help explain differential responses across treatments (Zhou et al. 2002; Bell et al. 2005; Waldrop et al. 2006; Langenheder and Prosser 2008). At first glance, such variation in diversity does not seem to exist in the samples: bacterial alpha-diversity among soils did not vary significantly across soils receiving different litter inputs in two of the three metrics I used (Table 1-3). Thus, the results are more consistent with others showing no detectable links between soil C and total bacterial diversity across a wide variety of ecosystems (Lauber et al. 2009). However, while the treatments did not seem to drive changes in Shannon or phylogenetic diversity, among all samples combined, there were strong positive correlations between bacterial diversity and catabolic diversity for two of the three diversity metrics (Figure 1-3). Thus, the results provide some additional evidence that catabolic diversity may vary with bacterial diversity in soil. I also observed a positive relationship between catabolic diversity and litter inputs, which is consistent with Degens et al. (2000), who showed that catabolic evenness declined with lower soil organic C content. Thus, although inconclusive, evidence from this experiment and

others suggest that increases in soil C might drive increases in bacterial diversity that, in turn, lead to increases in catabolic diversity.

While the CRPs effectively illustrate the *potential* effects of varying community composition on the decomposition of individual C substrates, it is important to note that the native DOM experiments seem to present an entirely different picture of the connections between microbial community structure and decomposition. I conducted the DOM experiments in an effort to assess the effects of phyla and sub-phyla differences in bacterial community composition on ecosystem function in a way that is more representative of *in situ* decomposition processes. In contrast to the CRP experiment (which assessed microbial metabolic responses to additions of single, pure substrates), leached DOM is a heterogeneous mixture of plant-derived C compounds. I hypothesized that soil from the 2× plots (with a higher proportion of copiotrophic bacteria) would decompose DOM more rapidly than soil from the litter removal plots (with a higher proportion of oligotrophic bacteria). Although I observed differences in the amount of respired CO_2 produced following DOM additions, I saw no evidence to suggest that these differences were driven by differences in bacterial community composition. For example, when manipulating DOM quality, differences in CO_2 fluxes could be explained by variation in soil C, soil N, microbial biomass C, and litter quality, and other possible differences between litter input treatment soils, including differences in bacterial community composition, could not significantly explain additional variation in CO_2 fluxes. These four biogeochemical variables combined explained the vast majority (91.5%) of the variation in the total CO_2 produced over the course of the incubation. Thus, the results do not support the hypothesis that leaf litter-driven differences in

microbial community composition would be reflected by differences in decomposition rates between treatments, and are consistent with other studies that found subtle, if any effects of microbial community composition on the decomposition of either low molecular weight C (Rousk et al. 2011) or SOM (Kemmitt et al. 2008).

Similarly, neither of my other two hypotheses regarding the functional dissimilarity in DOM decomposition rates was supported by the incubation data. First, I hypothesized that differences in decomposition rates would vary between microbial communities based on the biodegradability of the added DOM (Wieder et al. 2008). However, I saw no evidence for this in the native DOM quality manipulation experiment: The *S. parahyba* DOM decomposed more quickly than *M. staminodella* DOM, but microbial community composition did not explain the overall patterns. Next, given the known links between DOM concentration and soil respiration rates in this site (Cleveland et al. 2010), I predicted that 2× communities would decompose high concentrations of DOM more rapidly than the 0× communities. Thus, I evaluated whether the difference in CO_2 fluxes between treatments varied across experimental concentrations and found there was no significant statistical interaction between litter input treatment and DOM concentration (Figure 1-4) indicating this was not the case. This result suggests that differences in soil respiration rates between treatments receiving different concentrations of DOM were not related to differences in microbial community composition, and thus, the observed differences in CO_2 flux rates with increasing DOM concentration observed previously (Cleveland et al. 2010) are not driven by variation in microbial community composition per se, but rather other biogeochemical and/or microbial physiological factors (i.e., soil C, soil N, and microbial biomass).

Although past work has demonstrated links between microbial community structure and ecosystem function (e.g., Carney and Matson 2005; Strickland et al. 2009; Keiser et al. 2011), it has also been suggested that a process such as C mineralization is so common (and heterotrophic microorganisms are so diverse) that microbial community structure should have little bearing on the rate at which organic C compounds are decomposed (functional equivalence; Schimel 1995; Groffman and Bohlen 1999; Nannipieri et al. 2003). This is the essence of the functional equivalence hypothesis discussed above (Strickland et al. 2009). However, there is growing support for the idea that functional dissimilarity among microbial communities may drive variation even in organic matter decomposition (e.g., Condron et al. 2010). Results from the native DOM experiments supported the functional equivalence hypothesis: differences in native DOM decomposition rates could not be attributed to differences in microbial community composition. While similar results have been reported in the literature (e.g., Rousk et al. 2011), Strickland et al. (2009) concluded that decomposer microbial communities were functionally dissimilar. This inconsistency may reflect the fact that Strickland et al. (2009) investigated the effect of communities from vastly different ecosystems on the decomposition of non-native litter, thus maximizing the potential effects of community composition. By contrast, my experiment may have more effectively mimicked the type of variation that biotic and/or environmental changes might drive within a single ecosystem. My results suggest that even when such changes are large (e.g., a doubling or removal of litter), resultant shifts in the microbial community may not have significant direct effects on the mineralization of dissolved organic matter pools.

Nonetheless, my experiments did show inconsistent effects of microbial community composition on decomposition. For example, while the results of the DOM experiments support the functional equivalence hypothesis, the CRP analysis suggests that variation in community composition could drive variation in decomposition. My findings are consistent with Carney and Matson (2005) who found that soil microbial communities varied in their ability to degrade individual C substrates, but differences in litter decomposition were more strongly related to variation in microbial biomass than community composition. Schimel et al. (2005) suggested that organic matter decomposition represents an "aggregate" process, meaning that it consists of multiple individual biochemical pathways, and rates might not be strongly influenced by shifts in microbial communities. The contrasting results could reflect the fact that DOM decomposition is an aggregate process, whereas the decomposition of pure substrates in the CRP experiment reflects variation in individual processes. For instance, the litter input-driven differences in bacterial community composition may have actually driven undetected differences in the decomposition rates of some DOM constituents, but the measured response to DOM additions actually reflect the combined community response to a suite of C compounds. However, the results from these two different experiments illustrate the potential complexity of soil microbial community composition influence on organic matter decomposition and suggest that while variation in community composition may not influence rates of aggregate processes over short time scales, differences in relative decomposition rates of individual compounds could potentially influence soil C chemistry and SOM pools over the long term.

CHAPTER 2

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

ABSTRACT

Current and future global changes such as increasing atmospheric carbon dioxide (CO_2) concentrations or climate change are likely to drive shifts in plant-derived carbon (C) inputs to forest soils via changes in litterfall and plant net primary production (NPP). However, the effects of shifting detrital C inputs on belowground C cycling and fluxes remain largely unknown, especially in tropical ecosystems. I assessed the effects of experimentally manipulating aboveground litterfall inputs in a tropical rain forest site in Costa Rica on dissolved organic matter (DOM) fluxes, internal C and nutrient cycling, and soil CO_2 fluxes. I then compared the factors driving differences in CO_2 fluxes across the treatments with those driving seasonal variation in CO_2, which enabled me to assess the potential contribution of seasonal variation in bacterial community structure. The results showed that increasing and decreasing litterfall inputs drove rapid and significant increases and decreases in dissolved organic C (DOC) fluxes and total soil C concentrations, respectively, but had only subtle effects on soil C chemistry. Additionally, CO_2 fluxes were significantly greater in litter addition plots when compared to removal plots. My analysis also showed that variation in CO_2 fluxes across the treatments were strongly correlated with microbial biomass pools, soil C and nitrogen (N) pools, soil inorganic P fluxes, and DOC fluxes while seasonal variation in CO_2 fluxes were more strongly related to variation in O_2 concentrations. Furthermore, there were only subtle seasonal shifts in bacterial community structure, suggesting it plays a small role in seasonal CO_2 flux variability. Collectively, the data suggest that shifts in litterfall inputs in response to global environmental change could have important consequences for belowground C storage and fluxes in tropical rain forests. Furthermore, the responses

I observed highlight the potential for marked differences between tropical ecosystems and temperate ecosystems, where the effects of forest litter on belowground C cycling are typically much more subtle.

INTRODUCTION

Globally, soils store more carbon (C) than plants and the atmosphere combined (Schlesinger 1997), and thus changes in detrital C inputs, soil C pools or soil carbon dioxide (CO_2) fluxes could all have important consequences for the global C cycle. These changes could result from increasing atmospheric CO_2 concentrations, climate change, or atmospheric deposition of limiting plant nutrients (e.g., DeLucia et al. 1999, Clark et al. 2003, Galloway et al. 2004, Okin et al. 2004). For instance, free-air CO_2 enrichment (FACE) experiments conducted in temperate forest ecosystems indicate that increasing atmospheric CO_2 concentrations are likely to elicit increases in both litterfall and overall net primary productivity (NPP; e.g., DeLucia et al. 1999, Norby et al. 2002, Calfapietra et al. 2003). However, the effects of elevated CO_2 on belowground C cycling have been mixed, with some suggesting increases (Jastrow et al. 2005) but others showing no significant change (e.g., Lichter et al. 2005, Hoosbeek and Scarascia-Mugnozza 2009). Unfortunately, these inconsistent responses limit our ability to predict how elevated CO_2-induced changes in litterfall inputs may affect the global C cycle.

The potential effects of changes in plant-derived C inputs on belowground C cycling are most commonly directly investigated by experimentally manipulating litterfall and plant detritus (e.g., Park and Matzner 2003, Lajtha et al. 2005, Sulzman et al. 2005, Sayer 2006, Sayer et al. 2007, Crow et al. 2009, Schaefer et al. 2009, Feng et al. 2011), but those experiments, too, have shown inconsistent results. In some cases, litter removal elicits declines in surface soil organic C (SOC; reviewed in Sayer 2006). However, fewer studies have assessed the impacts of litter inputs on soil C

concentrations, with some suggesting they may increase with litter additions (Sayer 2006), but others showing no significant change (e.g., Nadelhoffer et al. 2004).

In addition to the potential effects of shifting litter inputs on soil C pools, they may also affect internal C cycling via changes to the movement of dissolved organic carbon (DOC) and other nutrients into soil. Some studies have documented increases in dissolved organic matter (DOM) fluxes with litter addition (Sayer 2006), especially following substantial plant C additions (e.g., wood additions; Lajtha et al. 2005). Yet, others suggest only subtle (if any) effects on soil dissolved organic carbon (DOC; Park and Matzner 2003, Nadelhoffer et al. 2004, Lajtha et al. 2005) or on mineral soil labile organic C (Feng et al. 2011). Nonetheless, previous work suggests that leaching is an important mechanism for the transport of soluble C from litter into soil and is especially important in ecosystems that receive large amounts of precipitation (Neff and Asner 2001, Cleveland et al. 2006). Thus, changes in litter inputs could also affect both the amount and concentration of DOC inputs that drive associated changes in internal soil organic matter (SOM) cycling, microbial activity, nutrient availability, and root dynamics. Microbial activity, in particular, may be an important mediator of SOM cycling responses, as labile C delivered by litterfall can stimulate decomposer organisms to mineralize C contained in extant SOM (e.g., via priming effects; Kuzyakov et al. 2000).

Changes in litterfall could also affect internal soil C cycling beyond the direct effects of simply altering C inputs. For example, low soil phosphorus (P) availability has been shown to limit soil microbial respiration rates in the tropics (Cleveland and Townsend 2006), and thus, changes in litter nutrient stoichiometry could exacerbate

phosphorus (P) limitation. Next, shifts in C inputs may drive changes in soil C chemistry (i.e., Kiem et al. 2000), causing feedbacks on soil C mineralization rates that result from differences in the decomposability of individual C substrates. Additionally, changes in litterfall may drive changes in soil abiotic conditions – like soil moisture (Sayer 2006) and soil O_2 availability, which could affect soil C mineralization rates (Silver et al. 1999, Schuur 2001).

Changing litter inputs could also affect losses of soil CO_2 to the atmosphere both directly (as a function of increasing C substrate) and indirectly (via effects on the decomposition of SOC). For example, based on first-principles, soil CO_2 fluxes would be predicted to vary proportionally with C inputs, and accordingly, litter removal treatments often drive declines in soil CO_2 fluxes (Li et al. 2004, Vasconcelos et al. 2004, Sulzman et al. 2005, Sayer et al. 2007, Schaefer et al. 2009). However, CO_2 fluxes often respond more strongly than expected (i.e., disproportionately) to litterfall augmentation, suggesting that increasing C inputs accelerate decomposition of extant soil C (Fontaine et al. 2004, Sulzman et al. 2005, Carney et al. 2007, Fontaine et al. 2007, Sayer et al. 2007, Schaefer et al. 2009). Taken together, these studies and observations provide a framework for investigating the potential significance of shifts in the quantity of plant-derived soil C inputs on belowground C pools and fluxes.

Unfortunately, the potential effects of increasing soil C inputs on soil C storage, cycling and losses (in general) are still unclear, and only a handful of studies have directly investigated the effects of varying plant C inputs on soil C cycling in tropical forests (Sayer 2006, Sayer et al. 2007). Although there has never been a CO_2 enrichment experiment (akin to the FACE experiments) conducted in a tropical rain forest, some

evidence suggests that NPP is likely to increase in some tropical forests via CO_2 enrichment (Hickler et al. 2008), climate change (Raich et al. 2006), or both. Conversely, others have suggested that NPP could decrease in tropical forests due to rising temperatures and drought (e.g., Clark et al. 2003, Nepstad et al. 2007) or through deforestation (Achard et al. 2002). Despite their dominant role in the global C cycle (Field et al. 1998, Bonan 2008), the consequences of potential changes in litter inputs on soil C cycling in tropical forests are largely unknown.

Thus, my overall objective was to assess the effects of changing C inputs on soil C pools and fluxes in a tropical rain forest. To do so, I utilized a litter manipulation experiment in a lowland tropical forest in Costa Rica, and I addressed several hypotheses. First, I hypothesized that DOM movement from the litter to the soil would vary as a direct function of litter inputs. Next, I hypothesized that shifts in litter inputs would drive proportional changes in soil C concentrations and shifts in soil C chemistry. Third, I hypothesized that greater DOC fluxes and higher soil C content in response to increasing litter inputs would elicit higher CO_2 losses from the ecosystem, and that declining DOC fluxes and soil C content in response to litter removal would elicit lower CO_2 losses from the ecosystem.

Finally, given the potential implications of accelerated tropical soil C losses to the atmosphere and global climate, I also investigated the mechanisms driving variation in soil CO_2 fluxes among the litter manipulation treatments with those controlling seasonal variation in CO_2 fluxes. Since rainfall and litterfall vary seasonally in many tropical forests (including the site studied here; Cleveland and Townsend 2006), I expected several environmental variables such as DOM quantities, microbial activity, O_2

concentrations, and nutrient concentrations to vary in accordance with these seasonal fluctuations. I also took the opportunity to assess a commonly overlooked potential control on seasonal variation in soil decomposition rates, bacterial community composition. Microbial community composition has been hypothesized to be an important contributor to CO_2 flux rates (McGuire and Treseder 2010), yet its influence on temporal variation in CO_2 fluxes is unclear since there are mixed results indicating whether communities actually vary on seasonal time scales (Krave et al. 2002, Carney and Matson 2006, Waldrop and Firestone 2006, Cruz-Martinez et al. 2009), and a previous study at the site observed no significant differences between bacterial community compositions from three sampling dates (Nemergut et al. 2010). However, given that bacterial community composition shifted dramatically with differences in C additions both in the lab and *in situ* at the site (Cleveland et al. 2007, Nemergut et al. 2010), I hypothesized that the soil bacterial community composition would be linked with seasonal fluctuations in DOM and CO_2 fluxes. Overall, I hypothesized that seasonal patterns in rainfall and litterfall would drive variation in DOM inputs, affecting bacterial community compositions and, in turn, regulate CO_2 fluxes.

METHODS

Study site

This 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 receives ~5,000 mm of rainfall per year, and has a mean annual temperature of ~26 °C. The majority of the precipitation falls during the

wet season (roughly April to December), while the dry season typically receives < 100 mm $month^{-1}$ (Cleveland and Townsend 2006). Additionally, litterfall at the site displays strong seasonal patterns with maximum litterfall rates (~90 g C m^{-2} $month^{-1}$) taking place during the dry season (Figure 2-4; Cleveland and Townsend 2006). Soil at the site is an ultisol that formed on the Osa basaltic complex (Berrange and Thorpe 1988).

Experimental design

In April 2007, a litter manipulation experiment was initiated consisting of a set of 3 × 3 m litterfall removal (0×) plots, control (1×) plots, and litterfall addition (2×) plots (N = 10 per treatment) (Wieder et al. 2011). At monthly intervals, litter was 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, ~900 g m^{-2} y^{-1} of litter was removed from the 0× plots and added to the 2× plots.

Litter layer throughfall and DOM fluxes

To quantify DOM delivery from the litter layer to the soil surface, a set of zero-tension lysimeters was constructed using 10 × 50 cm PVC pipe cut longitudinally. Each half was used to create one lysimeter and installed in each plot so that they were flush with the soil surface. Each lysimeter was equipped with a drain valve and a length of rubber tubing that carried leachate into polyethylene collection carboys placed in opaque buckets buried outside the plots. The lysimeters were filled with washed gravel and the surfaces were covered with 0.5 mm mesh to exclude large debris. Throughfall quantity was determined every 3-4 d by weighing the carboys, and subsamples from each carboy were immediately frozen for chemical analysis. In addition, canopy throughfall was

collected and measured using a set of five 314 cm^2 funnels deployed throughout the site that drained to another set of carboys, and throughfall C and nutrient fluxes were calculated as the differences between lysimeter and throughfall values. DOC and dissolved organic nitrogen concentrations in the leachate (DON) were measured in the subsamples using a Shimadzu TOC-VCPN total organic C and total N analyzer (Shimadzu Inc., Columbia, MD, USA).

Soil characterization

Soil C and nitrogen (N) concentrations, gravimetric soil moisture content, microbial biomass C and N concentrations, and fine root biomass were measured in all plots approximately every four months in all plots by collecting 0-10 cm soil samples with hand corers. After sampling, all roots were removed by hand, rinsed with deionized water, transported to the laboratory in coin envelopes, dried at 60 °C for 72 h, and weighed to determine fine root biomass, and soil samples were transported to the laboratory in sealed plastic bags in coolers on ice. In the laboratory, 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). Microbial biomass C and N was determined on fresh soil samples using the chloroform fumigation-extraction method (Brookes et al. 1985) on fresh soil samples (stored at 4 °C for less than 72h). Briefly, for each sample, soil microbial biomass was assessed by measuring the difference in 0.5 M K_2SO_4extractable C between fumigated and unfumigated subsamples. Organic C and N in the extracts was measured using a TOC-VCPN analyzer, and

microbial biomass C and N was 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).

Soil inorganic N and P fluxes were measured using ion-exchange resin capsules (Unibest, Bozeman, MT, USA). The resin capsules were carefully inserted into each plot at a depth of 10 – 15 cm every 2 – 4 months using a small hand trowel, making efforts to minimize disturbance. Quantities of inorganic N (ammonium; NH_4^+ and nitrate; NO_3^-) and P (PO_4^{3-}) 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). N and P fluxes were calculated fluxes by dividing the quantities of nutrients bound to the capsules by the number of days they were exposed to the soil.

Soil O_2 concentrations were measured at weekly intervals in each of the ten plots from April 2008 – March 2009. Briefly, in each plot, the open end of a 5 × 12 cm chamber was inserted ~9 cm into the soil, and the opposite end was sealed but fitted with a stopcock to allow gas sampling. Prior to each sampling event, chambers were allowed to equilibrate for 6 d, and O_2 concentrations were measured by extracting 50 mL of headspace from the chamber and injecting the sample into a chamber surrounding an O_2 probe (YSI 550A, YSI Incorporated, Yellow Springs, OH, USA). Continuous volumetric soil moisture content was also measured in a subset of the control plots (n = 4) at hourly intervals using HOBO sensors, and 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.

C chemistry was assessed using pyrolysis-gas chromatography/mass spectrometry (Wickings et al. 2011). Soil samples were collected from each plot in April 2010 using a hand corer (0 – 10 cm) and transported to the laboratory where they were sieved to 4 mm. Five composites per treatment were created by combining randomly selected pairs. Subsamples from each composite were oven-dried (60 ºC for 48 h), finely ground, pulse-pyrolyzed using a Pyroprobe 5150 (CDS Analytical Inc., Oxford, PA, USA) at 600 °C and delivered to a gas chromatograph (Trace GC Ultra, Thermo Scientific, Waltham, MA, USA) fitted with a fused silica capillary column (60 m, 0.25 mm ID) where individual compounds were separated and passed onto the mass spectrometer (Polaris Q, Thermo Scientific, Waltham, MA, USA). Putative identifications were determined 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).

CO_2 fluxes

Soil CO_2 fluxes were measured in all plots from April 2007 – March 2009. Initially, a set of permanently deployed ~80 cm^2 polyvinylchloride plastic collars were randomly placed in each plot (to 10 cm), and CO_2 fluxes were measured weekly using a vented, closed soil chamber system (LI-6400, LI-COR, Lincoln, Nebraska, USA). Following chamber equilibration, CO_2 concentrations were measured for 3-5 min, and fluxes were calculated using linear regression. Cumulative CO_2 production was calculated by linearly interpolating fluxes between measurements.

Bacterial community analysis

I assessed soil bacterial community composition in five randomly selected control plots sampled in September 2008, January 2009, and March 2009. During each sampling event, 0-5 cm samples were collected aseptically using a trowel that was sterilized with ethanol between samplings, transported on ice to the laboratory and stored at -80 °C until analysis. Communities were assessed using bar-coded pyrosequencing (Leff et al. 2011). Briefly, DNA was extracted from each sample, and the 27-338 region of the 16S rRNA gene was PCR amplified with primers which attached bar-code sequences to each amplified sequence. Next, the PCR products from each sample were combined and sequenced at Engencore (The University of South Carolina, Columbia, SC, USA) using Titanium chemistry (454 Life Sciences, Bradford, Connecticut, USA). I performed the sequence data analysis using the QIIME pipeline (Caporaso et al. 2010) which filters out sequences containing sequencing mistakes, assigns sequences to samples, assigns sequences to discrete operational taxonomic units (OTUs; 97% similarity), and identifies the taxonomy of OTUs. I controlled for differences in the number of sequences obtained per sample by randomly selecting 620 sequences per sample for downstream analysis. Phylogenetic distances between communities were calculated using the weighted UniFrac distance metric (Lozupone and Knight 2005).

Statistical analysis

One-way ANOVAs and Tukey post-hoc tests were used to test for differences among treatments and control plots, and measurements taken at different time points were averaged for each plot. Heterogeneity of variances among treatments was checked using a Levene's test and corrected, if necessary, by *ln* transforming the appropriate data. In one case (inorganic N flux), I was unable to meet this assumption even after trying

several transformations. Therefore, I used a non-parametric test, and a post-hoc test was not conducted for this variable. To test for differences in soil C chemistry and bacterial communities, I used nonparametric MANOVA (McArdle and Anderson 2001) with the corresponding distance matrices.

I compared CO_2 fluxes and soil C concentrations among the litter manipulation treatments and control plots using repeated measures ANOVA and Tukey post-hoc tests with measurement dates as factors. Differences in CO_2 fluxes between treatments and controls were compared for 0× and 2× plots using a t-test. To assess relationships between CO_2 fluxes and average variable measurements across all plots, I used simple linear regression and calculated Pearson product-moment correlations. Simple linear regression and Pearson product-moment correlations were also used to evaluate relationships between seasonal variation in CO_2 fluxes in the control plots and individual variable measurements. In all cases, I checked for non-linear relationships, and transformed data using *ln* transformations as necessary. . In order to model seasonal variation in CO_2 fluxes using multiple explanatory variables, I used both backwards and forwards elimination procedures. I considered the best-fit model between these different procedures as the one with the lowest AIC. With all ANOVA and regression tests, I checked that data met assumptions of independence and normality, and I used a significance threshold of $\alpha = 0.05$ for all tests. All the aforementioned tests were performed in SPSS v. 17 (SPSS, Chicago, Illinois, USA) except for the nonparametric MANOVA, which was implemented using the Adonis function (Oksanen et al. 2011) in R v. 2.9.2 (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

DOM Fluxes

DOC and DON fluxes from the litter layer to the soil surface varied among litter treatments and the control plots from April 2007 – February 2008; 0× plots had significantly lower DOC fluxes than control and 2× plots (44, 103, and 123 mg m^{-2} d^{-1}, respectively; $P < 0.001$) and significantly lower DON fluxes than control and 2× plots (1.07, 2.27, and 2.02 mg m^{-2} d^{-1}, respectively; $P < 0.001$). During the 12 month period beginning in March 2008 (after allowing a full wet season for the treatments to take effect), DOC fluxes were 47% greater in the 2× plots and 58% lower in the 0× plots relative to the controls ($P < 0.02$ in both cases; Table 2-1), yet DON fluxes were largest in the control plots and significantly lower in the 0× plots (Figure 2-1; Table 2-1). DOC and DON concentrations displayed similar patterns as fluxes during the 12 month period beginning in March 2008. DOC concentrations were greater in plots with higher litter inputs, and DON concentrations were greatest in control plots. However, differences in DON concentrations between treatments and the control were not significant (Table 2-1). There were also strong temporal variation in DOC and DON fluxes (Figure 2-1); DOC and DON fluxes in control plots varied seasonally and were positively correlated with rainfall (Appendix 2-2).

Litter input effects on soil C pools and other soil characteristics

Soil C content was not significantly different between treatments at the beginning of the experiment, but the 2× plots had a significantly greater soil C content than the 0× plots after eight months of litterfall manipulation ($P = 0.01$). Additionally, the repeated

measures ANOVA indicated that soil C was significantly different in each of the litter input treatments (i.e., 0× < control < 2×) over the course of the 12 month period beginning in March 2008 ($P < 0.05$; Figure 2-2). Furthermore, there was a significant effect of sampling date on soil C as well as a significant time × treatment interaction ($P < 0.05$). By March 2009 (~23 months after the initiating the litter input treatments), soil C concentrations in the 0× and 2× plots were 26% lower and 31% higher than in the control plots, respectively. Soil C concentrations did not vary significantly in the control plots between the beginning of the experiment and March 2009 ($P < 0.1$; Figure 2-2).

During the 12 month period beginning in March 2008, there were also significant treatment differences and/or differences among the treatments and the control plots for all of the other edaphic characteristics that were measured ($P < 0.05$) except O_2 concentrations ($P > 0.1$; Table 2-1; Figure 2-3). Among all variables, differences in PO_4^{3-} fluxes were greatest between the 0× and 2× plots (171% larger in the 2× plots), but fine root biomass, soil C, microbial biomass C, microbial biomass N, soil N, C:N ratios, and gravimetric moisture also increased in 2× plots relative to 0× plots (91%, 78%, 56%, 46%, 43%, 23%, and 13%, respectively; Table 2-1).

239 distinct pyrolysis products were found when characterizing the soil C chemistry among the treatments and control. However, I did not observe significant treatment-driven differences in the overall soil C chemistry in my experiment.

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. However, 0× plot CO_2 fluxes declined relative to control and 2× plots after only three months of litterfall removal (Figure 2-4). When comparing soil CO_2

fluxes in the litter input treatments over the course of a year (beginning in March 2008), I found that fluxes in the 2× plots were significantly greater than fluxes in the 0× plots ($P < 0.01$). However, the fluxes in the 2× plots were only marginally greater than those from the control plots ($P = 0.06$). Differences in CO_2 fluxes between control and 0× plots were not significant during this period. Additionally, among all plots, there was substantial temporal variation in CO_2 fluxes over the course of the year, with fluxes peaking during the early wet season. There was a significant time × treatment interaction ($P < 0.01$); 2× fluxes tended to be greater compared to other treatments during those months with greater CO_2 emission rates (Figure 2-4).

On average, CO_2 fluxes from the 0× and 2× plots were 16% lower and 26% higher, respectively, than those in the control plots during the 12 month period beginning on March 8, 2008, and fluxes were significantly different between 0× and 2× plots ($P = 0.01$). However, differences in CO_2 fluxes between 2× and control plots also showed substantial temporal variation. In the 2× plots, the largest and most consistent differences in CO_2 fluxes occurred during the early rainy season (late April through late June) and late rainy season/early dry season transition (November - January), whereas, the differences between the 0× and control plots were more consistent across time points (Figure 2-5). I also observed differences in total soil CO_2 produced over this period. For example, the 2× plots lost a significantly greater amount of CO_2 than 0× plots ($P < 0.01$), and 0× and 2× plots produced 15% less and 33% more CO_2 than control plots, respecively—a significant difference ($P < 0.01$). Differences in CO_2 production between 0× and control plots and 2× and control plots were approximately even until the 2009 dry season (January – March 2009; Figure 2-5).

Across the litter input treatments and control, microbial biomass N (r = 0.63), microbial biomass C (r = 0.58), soil C (r = 0.56), and PO_4^{3-} flux (r = 0.53; Figure 2-6) correlated most strongly with CO_2 emissions ($P < 0.05$ in all cases), but several other variables were also significantly correlated (Appendix 2-1). Of the variables that were measured, only DON fluxes and concentrations, gravimetric soil moisture, and O_2 concentrations did not vary significantly with soil CO_2 fluxes ($P > 0.1$; Appendix 2-1).

Temporal variation in edaphic characteristics and bacterial communities

Precipitation, soil moisture, O_2 concentrations, litter input, and microbial biomass C all displayed strong seasonal variation in the control plot soils. Seasonal variation in soil moisture and O_2 concentration were inversely correlated with one another (Figure 2-3; Appendix 2-2), and, as expected, soil tended to have a higher moisture during periods of greater precipitation (i.e., the wet season). Litter inputs tended to increase during the wet season to dry season transition and decrease in the late dry season/early rainy season (Figure 2-4). There were increases in microbial biomass and soil nutrient fluxes during rainy season time points, and there were no visible seasonal trends in fine root biomass (Figure 2-5).

As previously observed by Nemergut et al. (2010) using different sample dates, there were not OTU-level differences in soil bacterial communities between sampling dates in the control plots ($P > 0.1$). Additionally, I did not observe differences in the relative abundance of Acidobacteria or Betaproteobacteria between sampling dates. However, I found that the relative abundance of Alphaproteobacteria was significantly (52%) greater in March 2009 than in September 2008 in the control plots ($P < 0.05$).

When I investigated relationships between the temporal variation in CO_2 fluxes and other variables in control plots during the March 2008 to March 2009 period, I found that O_2 concentration was the strongest predictor of CO_2 fluxes ($r = 0.69$; $P < 0.01$). Correspondingly, soil moisture content was inversely correlated with CO_2 fluxes ($r = -0.60$; $P < 0.01$). Litter input, DOC concentration, and rainfall were weakly but significantly correlated with CO_2 flux ($P < 0.05$), while DOC, DON, and DON concentration were not ($P > 0.1$; Appendix 2-2). Together, O_2 concentration and rainfall produced the best model of CO_2 fluxes I identified and accounted for 53% of the temporal variation in soil respiration. My ability to assess relationships among CO_2 fluxes, fine root biomass, microbial biomass C, soil C, soil N, and nutrient fluxes was limited due to infrequent sampling, but none of these variables showed close relationships with temporal variation in CO_2 fluxes (Figures 5 and 6).

DISCUSSION

As expected, the experimental litter manipulation had strong effects on both DOC fluxes and the concentration of DOC moving from the litter layer to the soil (Figure 2-1; Table 2-1). This finding supports the hypothesis that litter inputs drive changes in DOC fluxes and corroborates previous work suggesting that the quantity of soil litter inputs is an important determinant in the availability of C for internal soil C cycling (Cleveland et al. 2006, Cleveland and Townsend 2006). However, both litter addition and removal treatments tended to drive declines in DON fluxes (but only significantly so in 0× plots). I expected DON fluxes to decrease in the 0× plots due to decreased litter inputs, but the decline in DON fluxes in the 2× plots was unexpected. One possible explanation could

be that increases in litter C drove higher rates of N immobilization in the litter layer (Park et al. 2002). In general, however, the results suggest that DOM fluxes in this ecosystem are sensitive to the magnitude of litter inputs and contrast with studies conducted in temperate ecosystems where litterfall manipulations did not significantly impact DOM concentrations (e.g., Park and Matzner 2003, Nadelhoffer et al. 2004, Lajtha et al. 2005). This discrepancy may be due to the greater precipitation at the study site, which causes high organic matter losses from the litter layer via leaching, and thus, DOM fluxes may be more sensitive to changes in litter inputs in wet ecosystems (like tropical forests) than in mesic or dry ecosystems.

Litter manipulations also had significant effects on soil C pools, with litter addition and litter removal driving significant increases and declines in total soil C, respectively (Figure 2-2; Table 2-1). However, the rate of change is noteworthy; the litter removal plots lost ~26% of the initial SOC pool after only two years of treatment (Figure 2-2). These findings support my hypothesis that litter inputs would cause proportional differences in soil C pools, but contrast with those of Nadelhoffer et al. (2004) who did not observe significant effects of litter addition or removal on 0-10 cm soil C concentrations after five years of manipulation in a temperate forest. This discrepancy could be due to inherent differences between temperate and tropical forest ecosystems such as lower DOM fluxes (i.e., greater C mineralization in the litter layer and/or slower transfer of litter C to soil pools) or greater soil C saturation in temperate forest soils. For example, work in temperate agroecosystems suggests that soil C may be stabilized via physical and chemical mechanisms that are limited by soil edaphic characteristics (e.g., texture; Six et al. 2002, Plante et al. 2006). Thus, if soil C exchange sites are saturated,

soil C pools would not be predicted to increase with increasing litter inputs (Stewart et al. 2007). In my experiment, the roughly linear effect of litter inputs on soil C (i.e., soil C concentrations in the 2× plots increased approximately the same magnitude as soil C concentrations in the 0× plots declined; Figure 2-2) suggests that the soil at this site was not C saturated. This could have been due to their high clay content (> 70%; C. Cleveland *unpublished data*) which may have prohibited C saturation with the size of the additions that were introduced. Additionally, in contrast to previous studies in temperate ecosystems, the large and rapid declines in soil C in the 0× plots suggest that a substantial fraction of soil C in this system is not well stabilized via physical and/or chemical mechanisms and may actually be susceptible to mineralization by heterotrophs. The lack of significant differences in C chemistry supports this notion, as it suggests that soil decomposers did not selectively mineralize more labile C compounds, and thus, C was not likely to have been predominately stabilized via chemical recalcitrance. Nonetheless, the results contrast with a study in a temperate agroecosystem, which found that depletion of plant-derived C inputs resulted in shifts in soil C chemistry (Kiem et al. 2000).

The results also revealed several noteworthy effects of litterfall manipulations on belowground biogeochemical cycling. First, litter removal drove declines in soil moisture while litter additions had no effect—a result found in other litterfall manipulation studies (Sayer 2006). Yet, the declines in soil moisture in the 0× plots did not translate to changes in O_2 concentrations (Table 2-1), suggesting that declines in litterfall in wet tropical forests may not affect belowground processes (including C cycling) via an increase in O_2 availability. I did, however, find that litter removal had a strong effect on soil microbial biomass; microbial biomass C decreased by approximately

25% in the 0× plots relative to the controls (Table 2-1). This finding is consistent with others who have found similar effects of litter removal (Sayer 2006, Feng et al. 2009), and could impact rates of C mineralization and other nutrient transformations. Additionally, the differences in nutrient – particularly P fluxes – in response to the litter manipulation is noteworthy because it suggests that litter inputs may affect belowground processes through changes in nutrient availability and/or stoichiometry that occur in concert with changes in C inputs. For instance, increased P fluxes due to greater litter inputs could have important consequences for tropical forests, many of which are characterized by low soil P availability (Cleveland et al. 2011). Similarly, N fluxes might decrease with increasing litter due to constraints lifted by increased availability of other nutrients such as P, and N could be more strongly retained in the ecosystem (Wieder et al. 2011). Lastly, fine root biomass was affected by litter inputs (i.e., greater biomass in 2× plots than 0× plots), suggesting that altered belowground conditions due to increased litter inputs promoted root growth. This could have taken place through increased nutrient availability or moisture with greater litter inputs. Together, these results demonstrate a potential cascade of shifting conditions in response to changing litter inputs.

Changing litter inputs also had strong effects on soil C losses (i.e., via soil respiration). For example, the results indicated that two-fold increases in litter inputs caused a much greater increase in soil CO_2 fluxes than would be predicted from the declines in CO_2 fluxes in response to litter removal. Although not significantly different from those in either manipulation treatment, control plots tended to have intermediate CO_2 fluxes (Figure 2-4). Previous work from temperate and relatively dry tropical

locations has shown similar results in response to litter additions and removal (Li et al. 2004, Vasconcelos et al. 2004, Sulzman et al. 2005, Sayer et al. 2007, Schaefer et al. 2009). However, declines in soil respiration in response to litter removal were greater in other tropical ecosystem studies (Li et al. 2004, Vasconcelos et al. 2004, Sayer et al. 2007). There are two likely explanations for the result that CO_2 flux increases due to litter additions outpaced the magnitude of CO_2 flux declines due to litter removal. First, previous studies have noted that litter additions cause increases in CO_2 production rates that cannot be explained by litter C additions alone (Sulzman et al. 2005, Sayer et al. 2007, Schaefer et al. 2009), and this has been attributed to priming effects, whereby C in SOM pools is mineralized by microorganisms that may be stimulated by fresh labile C inputs (Kuzyakov et al. 2000). Thus, the results could be attributable to a priming effect. However, another possible explanation for these results is that greater litter inputs drove increases in root biomass, which, along with heterotrophic respiration, caused greater CO_2 fluxes. In fact, fine root biomass explained a significant amount of the variability in CO_2 fluxes in the experimental plots ($r = 0.42$; Appendix 2-1), and fine root biomass increased (although not significantly) by 72% in the 2× plots compared to the controls (Table 2-1). Furthermore, if a priming effect did occur in the 2× plots, it did not lead to a decline in soil C pools, as the increase in soil C concentrations in the 2× plots (relative to the controls) was roughly equal to the decrease in 0× plots at the end of the experiment (Figure 2-2). Thus, increased root biomass seems to be a likely explanation for the disproportionate CO_2 fluxes from the 2× plots.

Interestingly, neither DOM fluxes nor concentrations were the most important driver of CO_2 fluxes across the experimental and control plots. DOC fluxes and

concentrations significantly explained variation in CO_2 fluxes along with many of the other variables that were measured (including fine root biomass), but microbial biomass, soil C, and PO_4^{3-} fluxes were stronger predictors of soil CO_2 emissions (Appendix 2-1). The strong relationship between soil C concentrations and CO_2 fluxes suggests that C substrate availability was, indeed, an important control. CO_2 fluxes may have had a stronger relationship with soil C than DOC inputs (either quantities or concentrations) because soil C is a better indicator (and contains a larger pool) of available C in the experimental plots than C leached through the litter layer. Although litter solubility is an important determinate of litter decomposition in the wet tropics (Wieder et al. 2009), DOC inputs represented only roughly 8% of total litter C inputs over the course of the second year of the experiment. Thus, the data suggest that the majority of litter C enters the soil through mechanisms other than leaching, which are important in contributing to microorganism-available C. Additionally, the results suggest that microbial activity and inorganic P availability were important factors regulating soil respiration rates. These findings are supported by some previous studies which observed significant changes in the microbial biomass due to litter input manipulation (Li et al. 2004, Sayer 2006, Feng et al. 2009), but not all studies observed this trend (Fisk and Fahey 2001, Sayer et al. 2007).

Nutrient availability often constrains litter decomposition rates (Swift et al. 1979), and at this site, P fertilization was shown to stimulate soil heterotrophic respiration rates (Cleveland et al. 2006, Cleveland and Townsend 2006). The strong correlation between soil P fluxes and CO_2 fluxes I observed (Figure 2-6) is consistent with that previous work. However, it is noteworthy that despite the positive correlation between soil P fluxes and soil CO_2 fluxes, inorganic N fluxes were inversely correlated with CO_2 fluxes

(Appendix 2-1). This is consistent with the observation that DOC: DON ratios were much greater in the 2× plots than the other plots, and with previous work suggesting that soil N availability in this ecosystem does not limit soil respiration rates (Cleveland and Townsend 2006). In other words, although DON availability was lower relative to C availability in the 2× plots, low DON transport and availability did not seem to constrain soil respiration rates.

In contrast to the factors influencing CO_2 fluxes across the litterfall manipulation plots, temporal variation in CO_2 fluxes in the control plots were most strongly related to O_2 concentrations. Correspondingly, CO_2 fluxes had an inverse relationship with soil moisture and rainfall, and together, O_2 concentrations and rainfall produced the best model of seasonal variation from the data that were collected. These results are supported by other evidence in tropical forests that O_2 concentrations strongly control decomposition rates (i.e., Schuur 2001). Litter inputs and DOC flux, however, were not significant predictors of temporal variation in CO_2 fluxes (Appendix 2-2). While seasonal trends in O_2 and rainfall are inversely related, the additional explanatory power of rainfall beyond that of O_2 concentration suggests rainfall negatively influences CO_2 fluxes in ways other than decreasing O_2 availability. This could be through DOC dilution, which has been shown to decrease soil CO_2 production (Cleveland et al. 2010), and the importance of DOC concentration is suggested by its significant positive relationship with CO_2 fluxes. These results demonstrate that the factors determining seasonal variation in CO_2 fluxes at the site were fundamentally different from those determining variation among soils receiving different litter inputs where O_2 concentrations and moisture did not relate to CO_2 fluxes, but DOC fluxes did (Appendix 2-1). Additionally,

other factors that appeared to impart controls over CO_2 fluxes across the litterfall manipulation plots, such as microbial biomass, P fluxes, and fine root biomass, did not seem to vary in accordance with temporal variation in CO_2 fluxes (Figure 2-4 and 2-6). Taken together, these results do not support my hypothesis that patterns in rainfall and litterfall drive patterns of soil DOM inputs and respiration. Instead, they suggest a model where seasonal soil respiration is most strongly determined by shifts in rainfall which determines soil moisture, O_2 concentrations, and, more subtly, DOC concentrations.

Microbial community composition has also been proposed as an important control over soil decomposition rates (Allison and Martiny 2008, McGuire and Treseder 2010), yet direct evidence is rare. There are mixed results indicating whether microbial communities influence *in situ* SOM decomposition rates in tropical forests (e.g., Carney and Matson 2005, Leff et al. in revision), but laboratory and field studies at the site indicate that bacterial communities respond strongly to differences in organic matter inputs (Cleveland et al. 2007, Nemergut et al. 2010), and community shifts under elevated CO_2 could be responsible for differences in SOM decomposition rates (Carney et al. 2007). Additionally, soil extracellular enzyme activities likely reflecting differences in microbial activity varied during different sampling dates at the site (Weintraub et al. in review). Thus, I hypothesized that seasonal patterns in soil DOM fluxes at the site would elicit shifts in bacterial community composition and mediate DOM decomposition rates and therefore, CO_2 fluxes. However, I did not detect significant whole-community differences in bacterial community composition at three dates that featured considerably different CO_2 fluxes. These results agree with findings by Nemergut et al. (2010), who also did not observe differences in soil bacterial

communities at the site among three additional dates. These findings suggest that bacterial community composition does not strongly control seasonal variation in CO_2 fluxes at the site. That said, faster growing bacterial groups (i.e., copiotrophs) which likely include many Proteobacteria (e.g., Nemergut et al. 2010) could still have subtle influences on decomposition rates as I detected significant differences in the relative abundance of Alphaproteobacteria between the two dates that had the greatest differences in CO_2 fluxes. The inconsistency between the profound bacterial community composition shifts due to differences in organic matter inputs (Nemergut et al. 2010, Leff et al. in revision) and the more subtle shifts between sampling dates could be due to the fact that DOM inputs did not strongly control seasonal variation in CO_2 fluxes, and bacterial community structure may be more sensitive to resource availability than O_2 concentration. However, further research is needed to test this hypothesis.

Overall, my results suggest that tropical soil C pools may quickly respond to future changes in soil litterfall inputs, and they reveal a key difference between tropical forests (where changes were rapid) and temperate forests (where litter manipulation effects on soil C pools are more subtle). Furthermore, the mechanisms controlling CO_2 fluxes out of the soil may be profoundly different between seasonal variation and variation due to shifts in C inputs. Since roughly 30% of the world's soil C is in the tropics (Jobbagy and Jackson 2000), and soil C represents a C pool more than twice as large as plants (Schlesinger 1997), even small shifts in the size of this pool are likely to have consequences for global C cycling. Thus, these results demonstrate the potential for shifts in forest litterfall inputs to impact C sequestration on a global scale.

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FIGURES

Figure 1-1

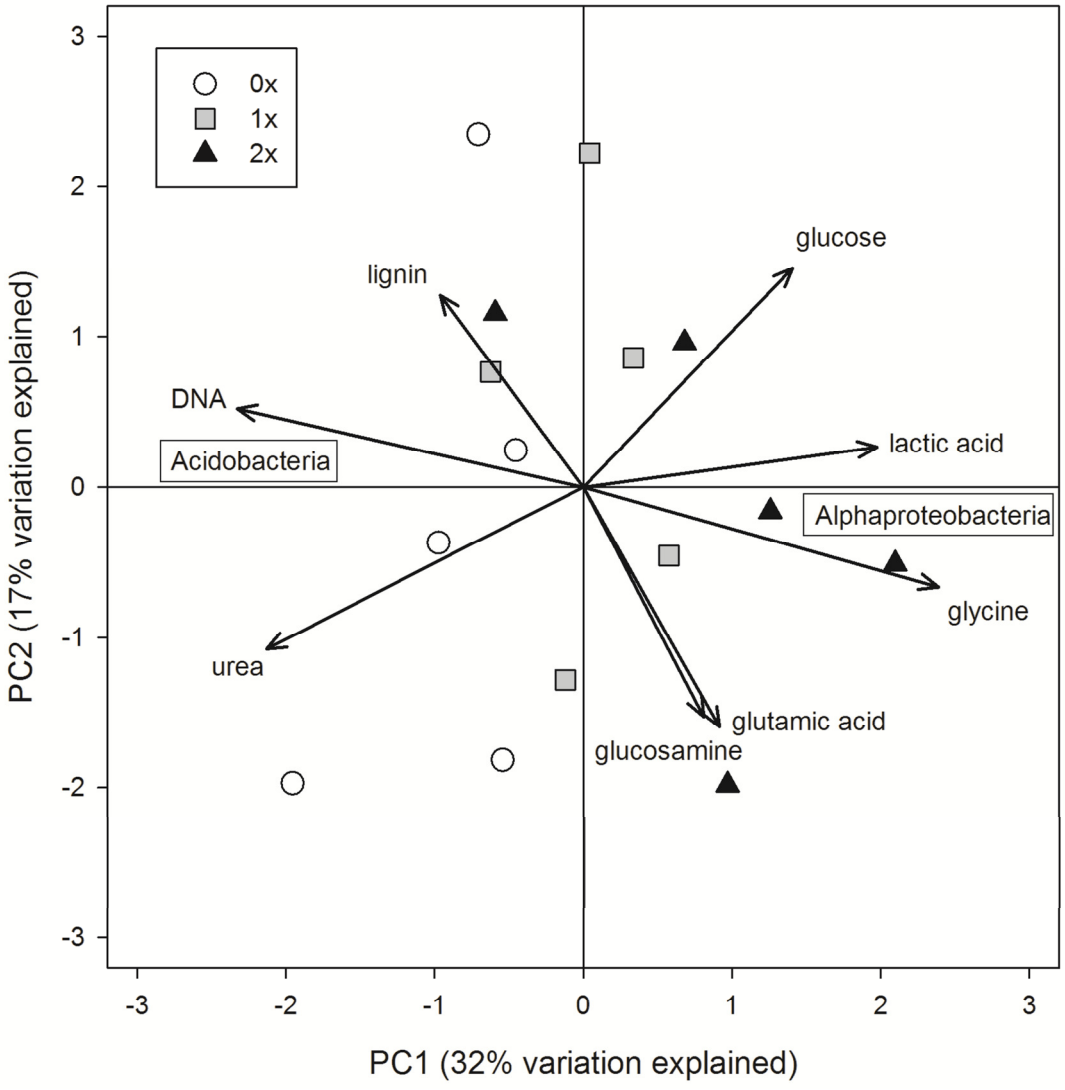

Figure 1-2

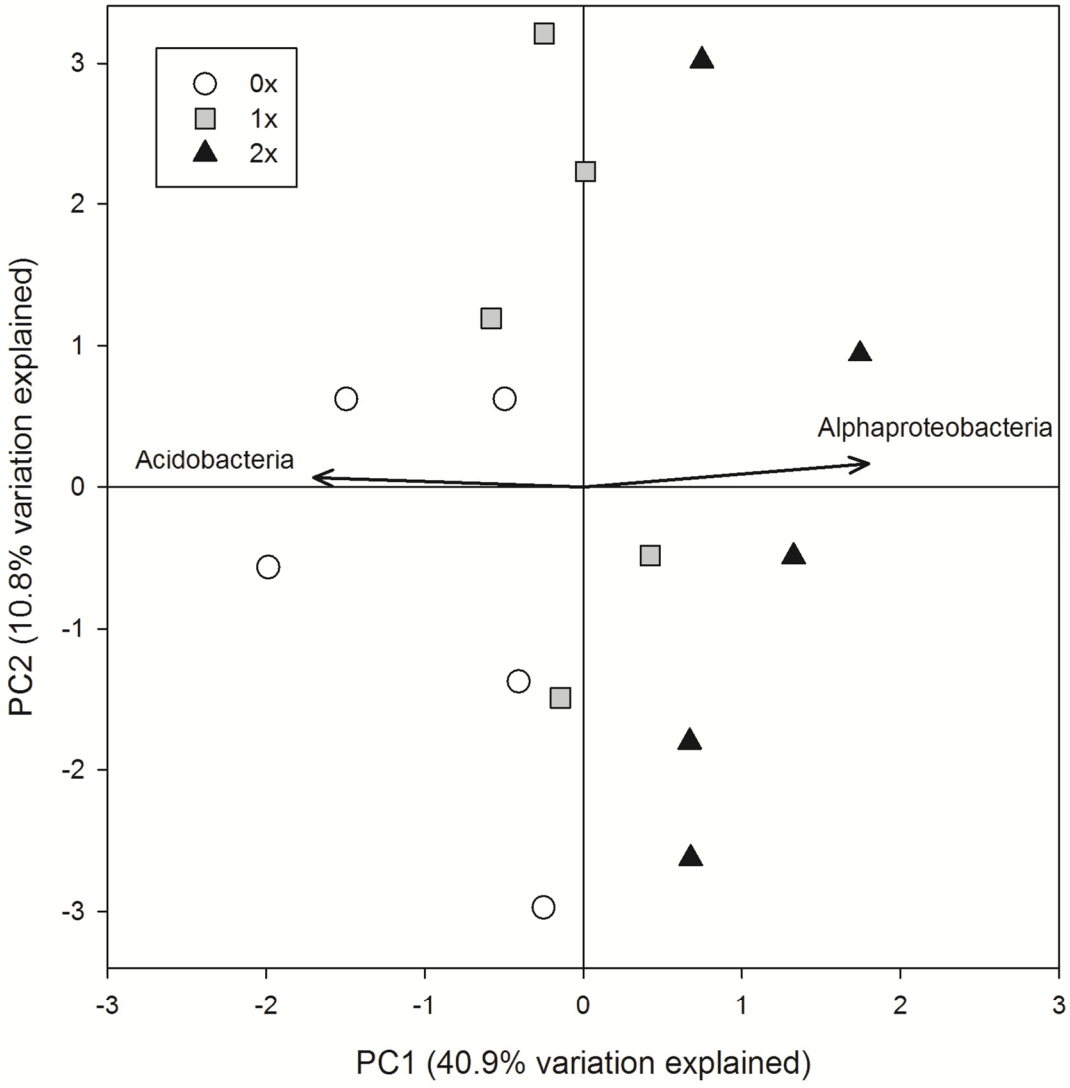
0x
1x
2x
Acidobacteria
Alphaproteobacteria
PC2 (10.8% variation explained)
PC1 (40.9% variation explained)
3
2
1
0
-1
-2
-3
-3
-2
-1
0
1
2
3

Figure 1-3

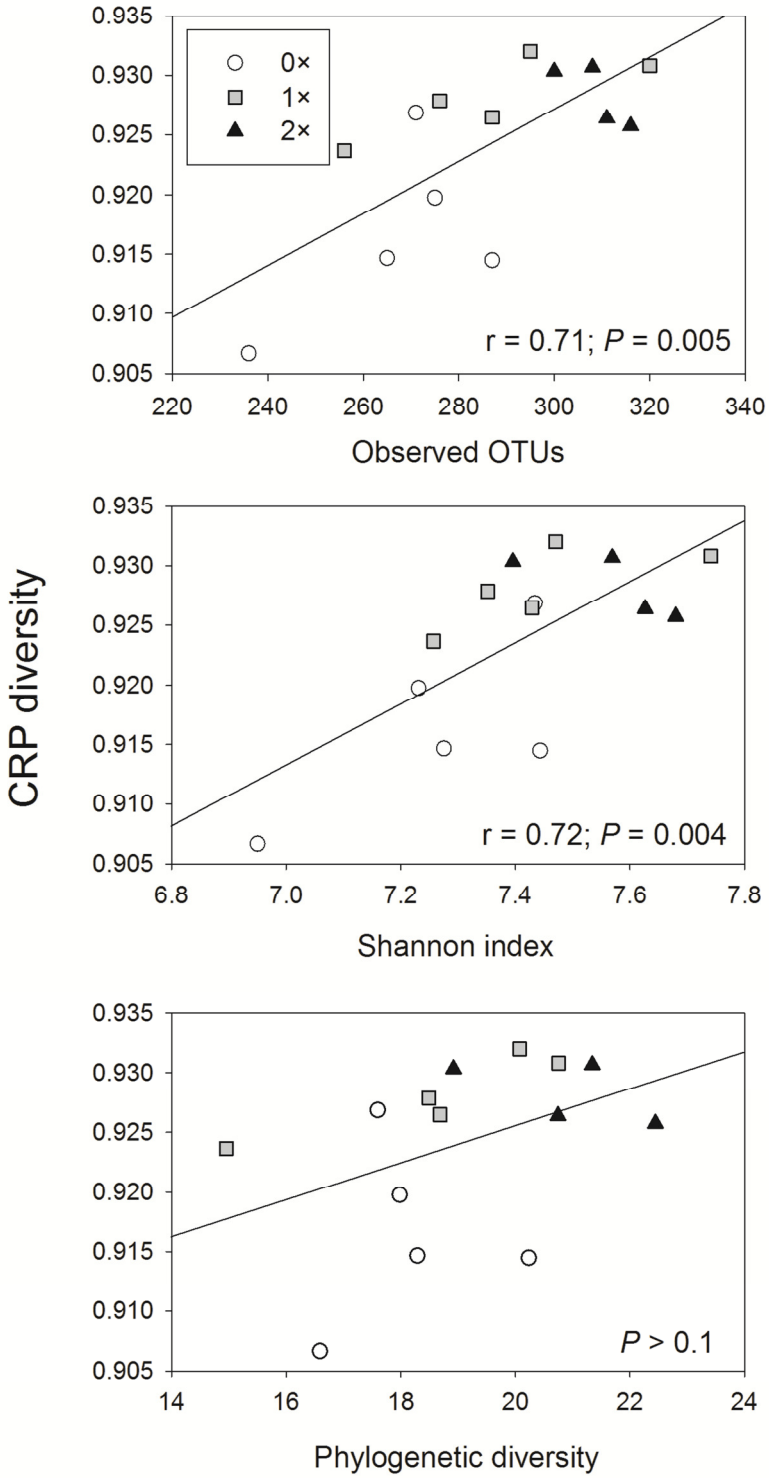
0×
1×
2×
r = 0.71; P = 0.005
Observed OTUs
r = 0.72; P = 0.004
Shannon index
P > 0.1
Phylogenetic diversity
CRP diversity

Figure 1-4

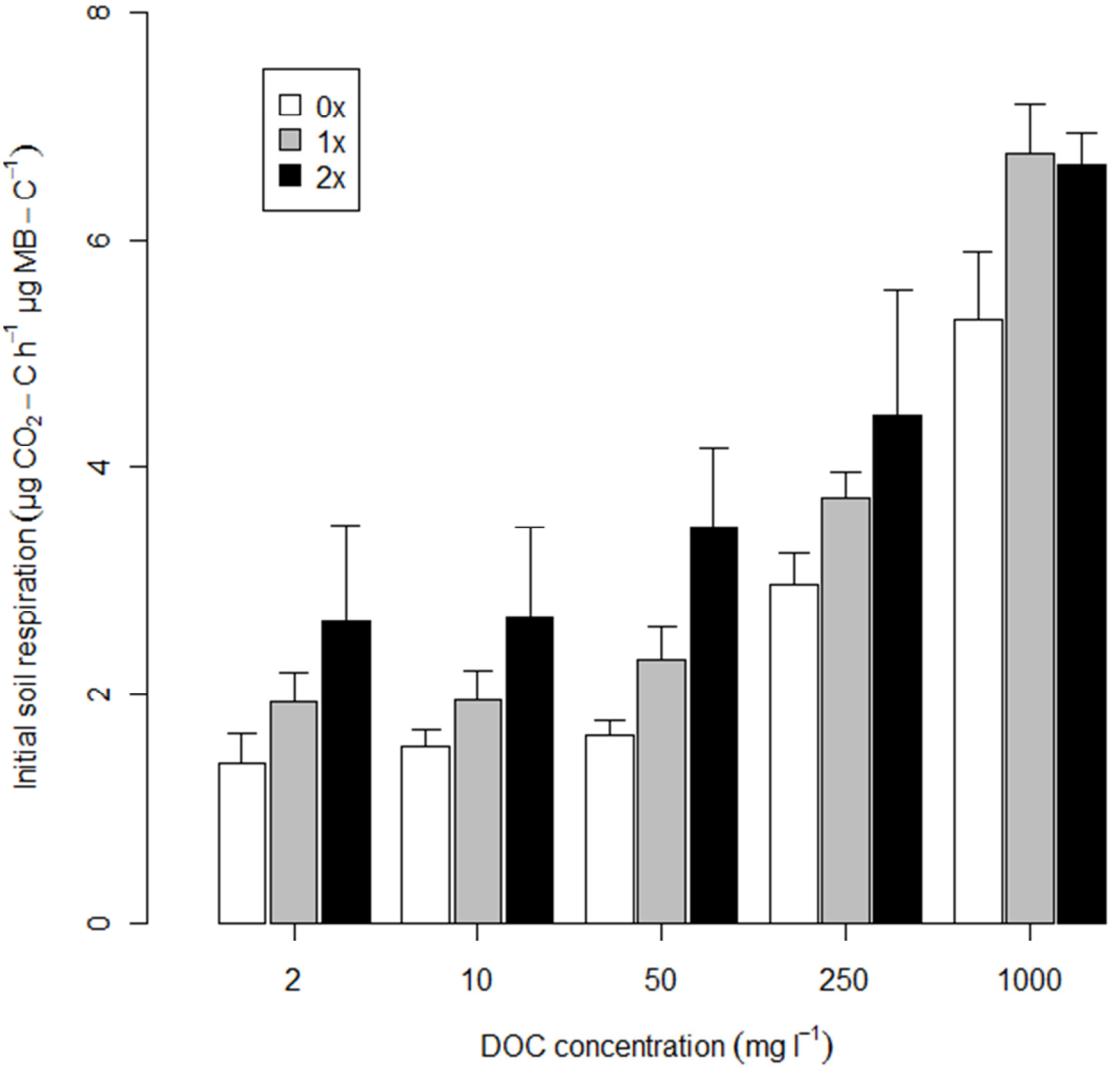
0x
1x
2x
Initial soil respiration (µg CO2 – C h-1 µg MB – C-1)
0
2
4
6
8
2
10
50
250
1000
DOC concentration (mg l-1)

Figure 2-1

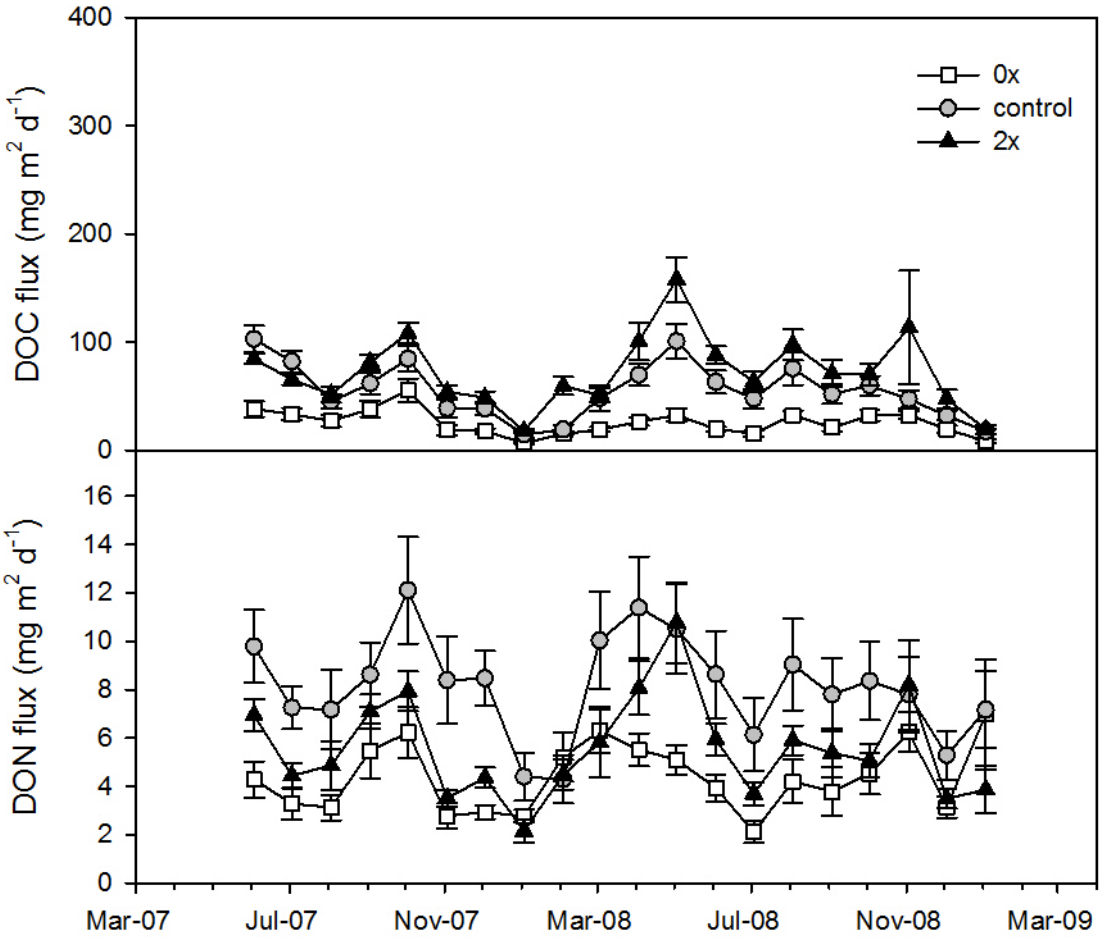
DOC flux (mg m^2 d^{-1})
DON flux (mg m^2 d^{-1})
0x
control
2x
400
300
200
100
0
16
14
12
10
8
6
4
2
0
Mar-07
Jul-07
Nov-07
Mar-08
Jul-08
Nov-08
Mar-09

Figure 2-2

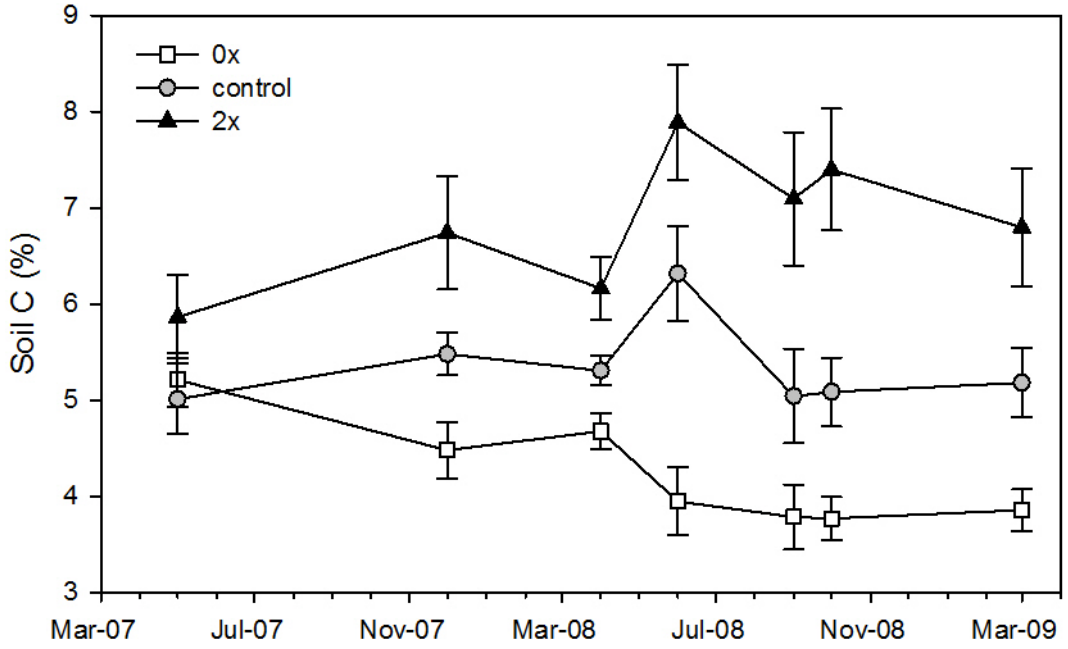
0x
control
2x
Soil C (%)
9
8
7
6
5
4
3
Mar-07
Jul-07
Nov-07
Mar-08
Jul-08
Nov-08
Mar-09

Figure 2-3

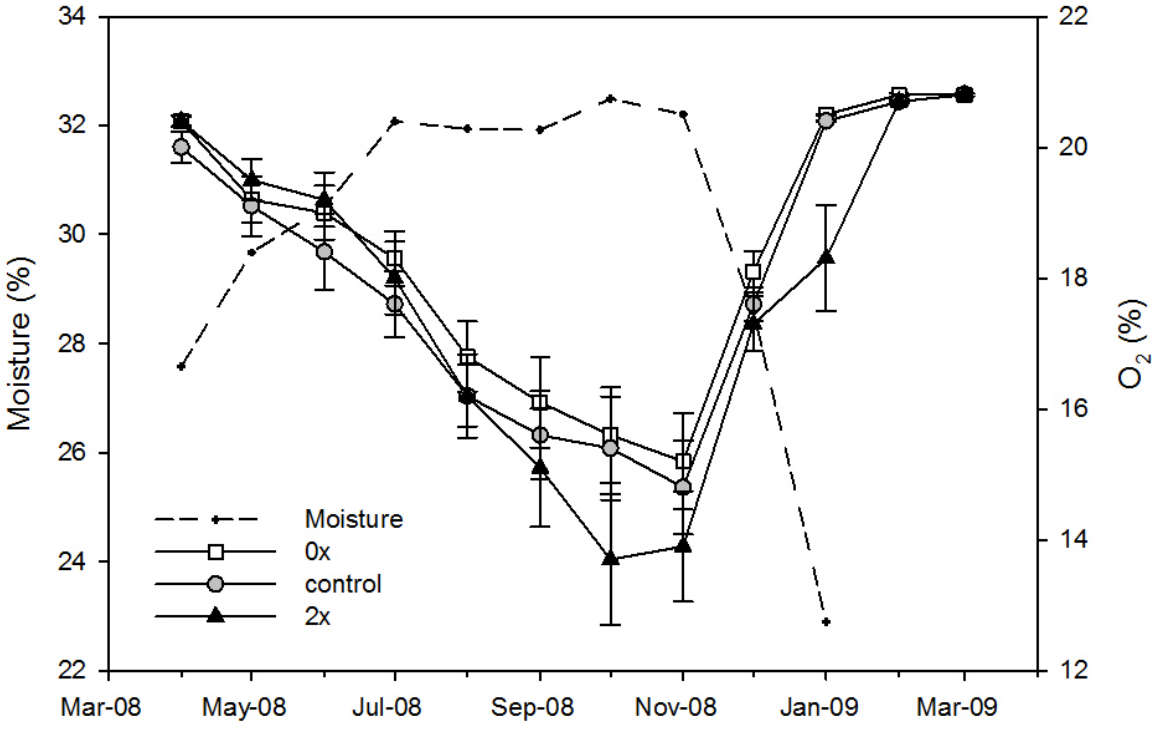
Moisture (%)
O_2 (%)
34
32
30
28
26
24
22
22
20
18
16
14
12
Mar-08
May-08
Jul-08
Sep-08
Nov-08
Jan-09
Mar-09
Moisture
0x
control
2x

Figure 2-4

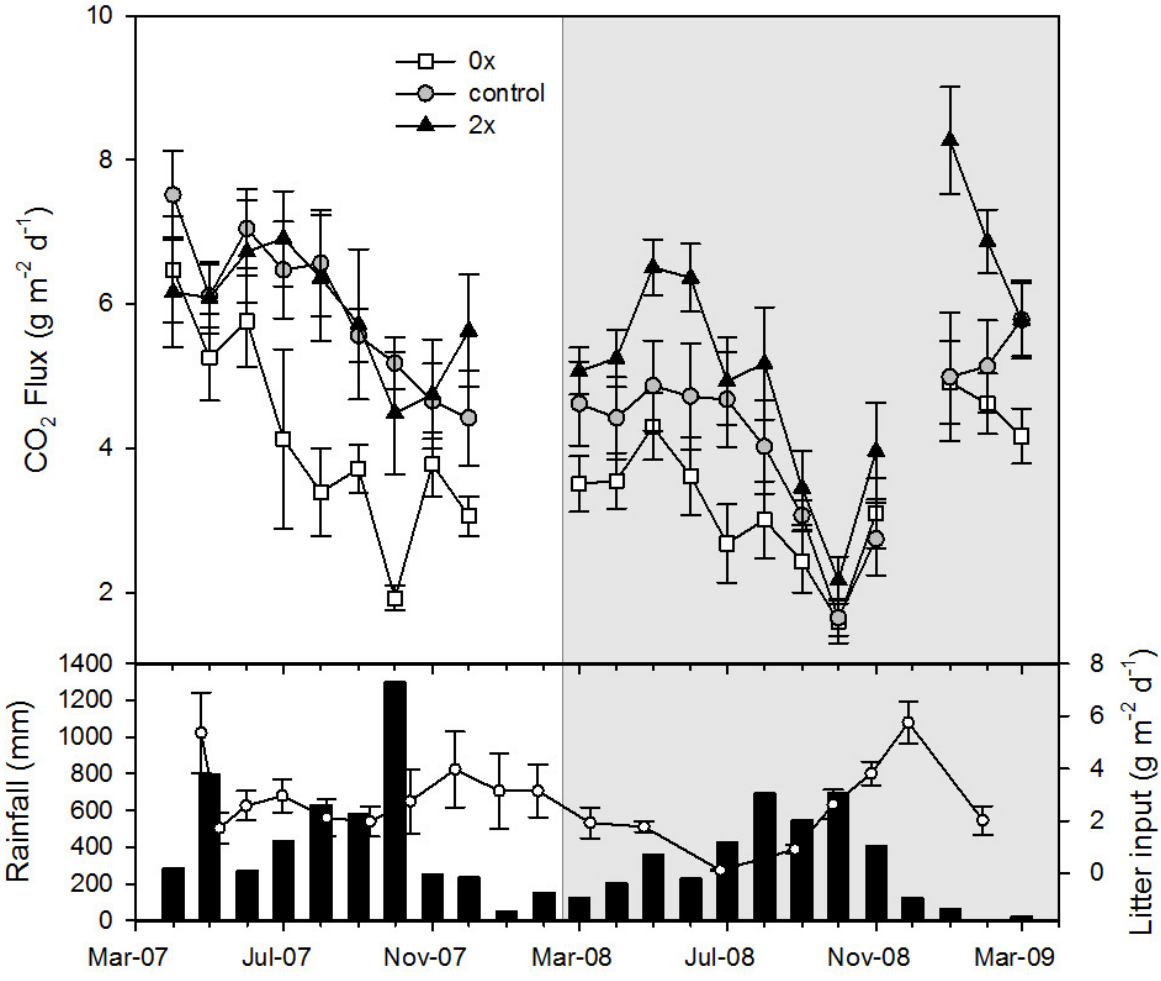
0x
control
2x
CO_2 Flux (g m^{-2} d^{-1})
Rainfall (mm)
Litter input (g m^{-2} d^{-1})
Mar-07
Jul-07
Nov-07
Mar-08
Jul-08
Nov-08
Mar-09

Figure 2-5

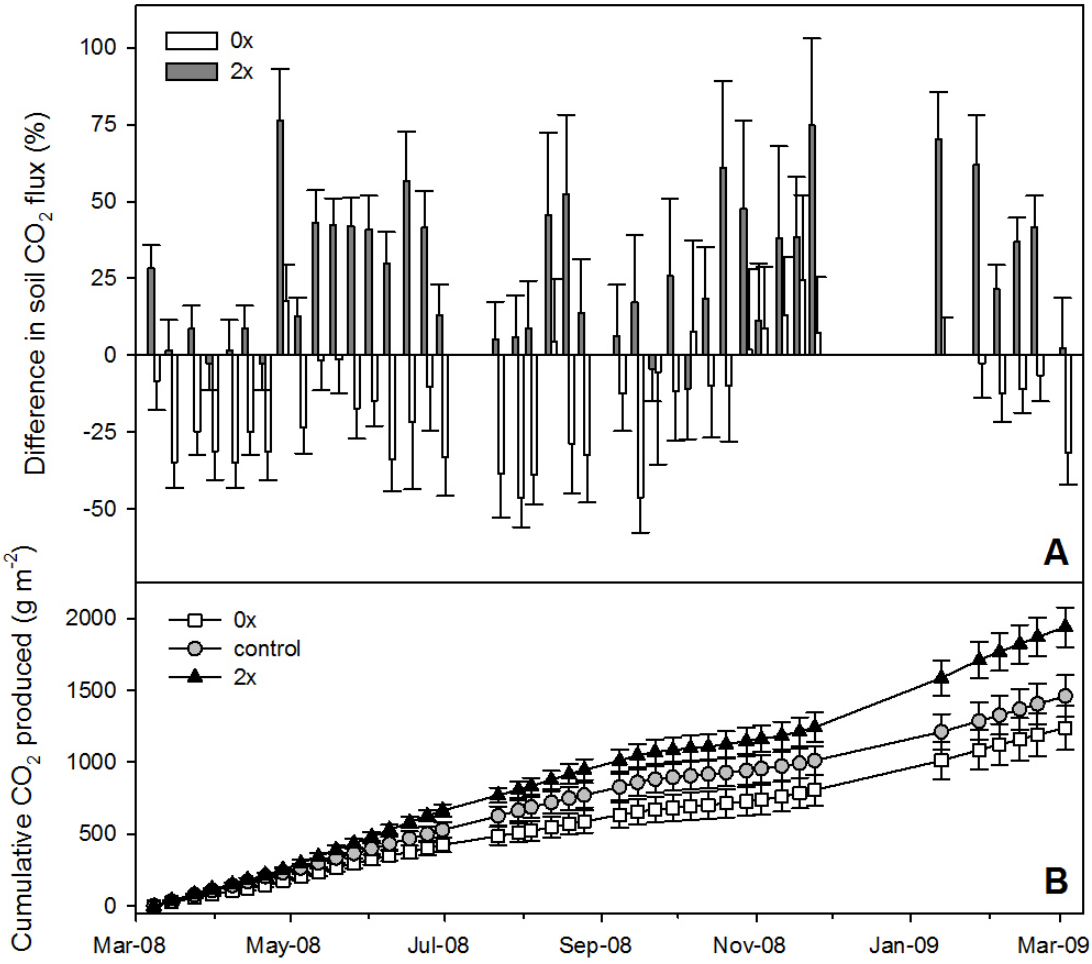
0x
2x
Difference in soil CO_2 flux (%)
100
75
50
25
0
-25
-50
A
0x
control
2x
Cumulative CO_2 produced (g m^{-2})
2000
1500
1000
500
0
B
Mar-08
May-08
Jul-08
Sep-08
Nov-08
Jan-09
Mar-09

Figure 2-6

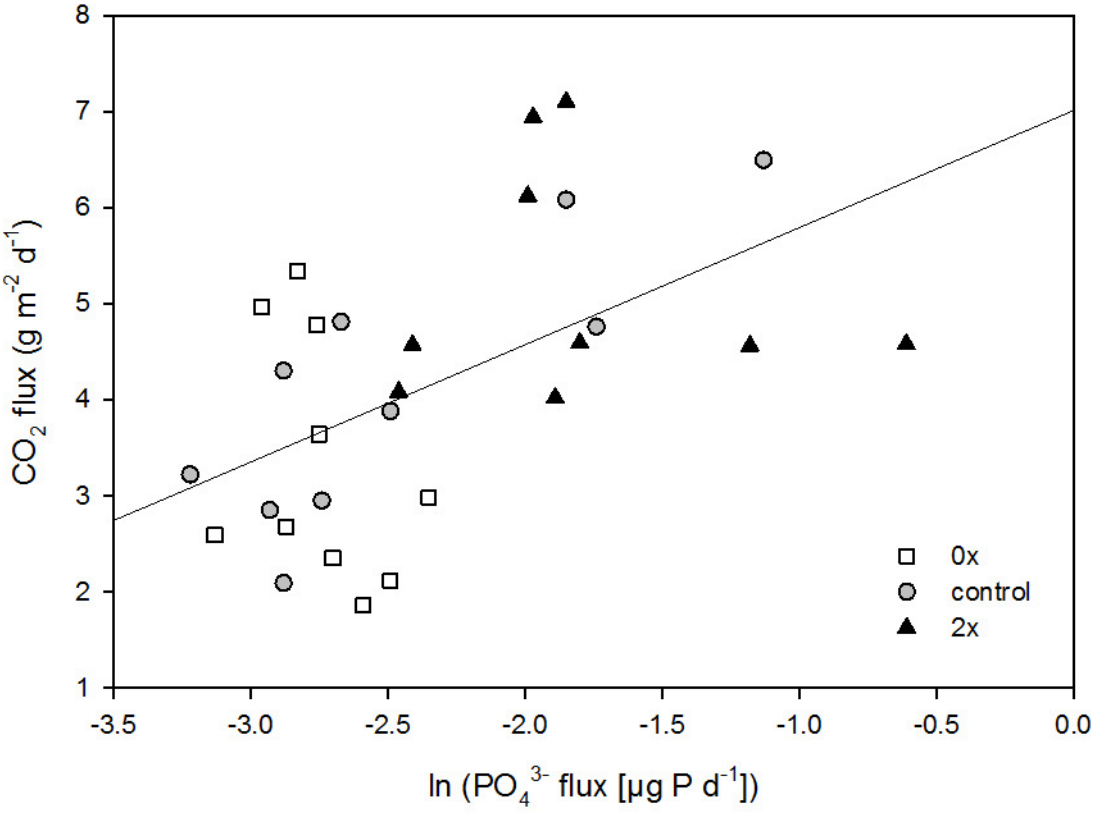
8
7
6
5
4
3
2
1
-3.5
-3.0
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
CO2 flux (g m-2 d-1)
ln (PO4 3- flux [µg P d-1])
0x
control
2x

FIGURE CAPTIONS

Figure 1-1. Principal coordinates analysis plot of catabolic response profiles (CRPs) for samples from the litter manipulation plots. Points further apart had more dissimilar CRPs. Vectors represent relationships between CRPs and the relative response to individual C substrates and point in the direction of CRPs with stronger relative responses to the substrates. Vectors were only plotted for substrates that were significantly correlated with the first two principal coordinates. Acidobacteria and Alphaproteobacteria labels were plotted using vector fitting at locations where points closer to them represented CRPs from samples with greater relative abundances of the indicated bacterial clade.

Figure 1-2. Principal coordinate analysis plot of bacterial community composition for samples from the litter treatment plots. Points further apart had more dissimilar bacterial communities based on weighted UniFrac distances. Arrows point in the direction of samples with greater relative abundances of Alphaproteobacteria and Acidobacteria.

Figure 1-3. Relationships between the catabolic response profile (CRP) diversity and three measures of bacterial community diversity among all litter input treatments.

Figure 1-4. Initial respiration rates of soils normalized by microbial biomass C 1.7 h after the addition of DOC at several concentrations. Error bars represent means ± 1 SD.

Figure 2-1. Dissolved organic carbon (DOC) and nitrogen (DON) fluxes in the litter removal (0×), control, and litter addition (2×) litter treatments over the course of the experiment. Values represent monthly means ± 1 SE.

Figure 2-2. Surface (0-10 cm) total soil C concentration in litter removal (0×), control, and litter addition (2×) plots from April 2008 to March 2009. Values represent means ±1 SE.

Figure 2-3. Volumetric soil moisture in control plots and mean (± 1 SD) soil O_2 concentrations in litter removal (0×), control, and litter addition (2×) plots through time.

Figure 2-4. Monthly average (± 1 SE) CO_2 fluxes from litter removal (0×), control, and litter addition (2×) plots from April 2007 through March 2009. Monthly rainfall and litter inputs (± 1 SD) are shown to illustrate seasonal variation. The gray background highlights the time period approximately a year after the experiment establishment during which I assessed relationships between CO_2 fluxes and potential controls across the litter manipulation plots.

Figure 2-5. A) Mean differences (± 1 SE) between CO_2 fluxes from litter removal (0×) and litter addition (2×) plots and control plots from March 8, 2008 to March 3, 2009 for each sampling date measured. B) Mean cumulative CO_2 production (± 1 SE) for 0×, control, and 2× plots over the same period.

Figure 2-6. The relationship between mean CO_2 and soil inorganic P fluxes across the litter removal (0×), control, and litter addition (2×) soils.

TABLES

Table 1-1. Figure 1-1 vector correlation coefficients

Substrate	r	*P*
DNA	0.903	0.001
Glucosamine	0.656	0.033
Glucose	0.768	0.007
Glutamic acid	0.698	0.021
Glycine	0.940	0.001
Lactic acid	0.756	0.006
Lignin	0.606	0.049
Urea	0.904	0.001

Values are Pearson correlations (**r**) between the proportional responses of selected C substrates used in the catabolic response profile analyses and the first two principal coordinates of the catabolic response profiles.

Table 1-2. Soil properties

	Soil C (%)		Soil N (%)		Microbial biomass C (µg/g)		
Treatment	10/2009[1]	4/2010[2]	10/2009[1]	4/2010[2]	10/2009[1]	1/2010[1]	4/2010[2]
0×	3.80 ± 0.63[a]	4.06 ± 1.01[a]	0.27 ± 0.04[a]	0.26 ± 0.07[a]	1088 ± 188[a]	857 ± 220[a]	1174 ± 324[a]
control	4.81 ± 0.93[a]	5.12 ± 1.76[a]	0.32 ± 0.06[ab]	0.32 ± 0.08[a]	1363 ± 228[ab]	922 ± 189[ab]	1289 ± 301[a]
2×	6.54 ± 1.81[b]	6.37 ± 1.90[a]	0.36 ± 0.10[b]	0.38 ± 0.09[a]	1766 ± 462[b]	1226 ± 401[b]	1592 ± 301[a]

[1]N = 10
[2]N = 5

Different superscript letters indicate significant differences between treatments ($\alpha = 0.05$). Values represent means ± 1 SD.

Table 1-3. Diversity measurements for bacterial communities in soils

Treatment	Unique OTUs	Shannon index	Phylogenetic diversity
0×	266 ± 19^{a}	7.26 ± 0.20^{a}	18.1 ± 1.3^{a}
control	286 ± 23^{ab}	7.45 ± 0.18^{a}	18.5 ± 2.2^{a}
2×	302 ± 15^{b}	7.53 ± 0.13^{a}	20.3 ± 1.7^{a}

Different superscript letters indicate significant differences between treatments ($\alpha = 0.05$). Values represent means ± 1 SD.

Table 1-4. Spearman's rank correlations (ρ) between soil catabolic response profiles (CRPs) and explanatory variables

Characteristic	ρ	*P*
Bacterial community	0.46	< 0.001
pH		NS
Soil moisture		NS
Soil C	0.32	0.019
Soil C chemistry	0.28	0.033
Soil N	0.35	0.007
C:N	0.29	0.015
Microbial biomass C	0.39	0.003

Correlations were calculated using Mantel tests. CRPs were represented by a Bray-Curtis distance matrix, bacterial community compositions were represented by a weighted UniFrac distance matrix, soil C composition with a Bray-Curtis matrix, and all other variables used Euclidean distance matrices. NS = not significant.

Variable	0×	control	2×
DOC Flux (mg m^{-2} d^{-1})	27.0 ± 7.1[a]	64.0 ± 27.5[b]	94.1 ± 33.2[c]
[DOC] (mg L^{-1})	3.4 ± 1.2[a]	7.3 ± 2.3[b]	11.0 ± 2.9[c]
DON Flux (mg m^{-2} d^{-1})	4.7 ± 1.4[a]	8.7 ± 4.7[b]	6.6 ± 2.2[ab]
[DON] (mg L^{-1})	0.9 ± 0.3[a]	1.3 ± 0.7[a]	0.9 ± 0.3[a]
DOC:DON	7.17 ± 2.09[a]	8.15 ± 2.29[a]	14.6 ± 2.92[b]
Soil C (%)	4.00 ± 0.62[a]	5.35 ± 0.78[b]	7.12 ± 1.27[c]
Soil N (%)	0.35 ± 0.05[a]	0.45 ± 0.04[b]	0.50 ± 0.1[b]
Soil 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 ± 1.2[a]	17.7 ± 1.2[a]	17.4 ± 1.4[a]
Microbial biomass C (µg g^{-1})	897 ± 150[a]	1206 ± 167[b]	1397 ± 304[b]
Microbial biomass N (µg g^{-1})	169 ± 34[a]	198 ± 42[ab]	246 ± 55[b]
Inorganic N flux (µg d^{-1})	7.15 ± 3.89	18.51 ± 15.08	3.28 ± 1.77
Phosphate flux (µg d^{-1})	0.07 ± 0.02[a]	0.11 ± 0.09[a]	0.19 ± 0.14[b]
Fine root biomass (kg ha-1)	1446 ± 862[a]	1608 ± 728[ab]	2765 ± 1534[b]

Table 2-1. Soil properties in the litter removal (0×), control, and litter addition (2×) treatments from March 8, 2008 to March 3, 2009. Different superscript letters indicate significantly different mean values between treatments for each variable ($P < 0.05$). Values represent 12 month means ± 1 SD.

APPENDICES

Appendix 1-1

Mean proportional decomposition responses to C compounds added in the catabolic response profile analysis

	Relative proportion by treatment[1]		
Substrate	0x	1x	2x
Amylopectin	0.5 ± 0.5	0.4 ± 0.2	0.1 ± 0.1
Amylose	2.2 ± 1.1	2.1 ± 0.8	2.2 ± 1.0
BSA	1.7 ± 0.7	2.3 ± 0.6	1.5 ± 0.5
Casein	0.5 ± 0.3	0.3 ± 0.4	0.0 ± 0.0
Chitin	1.2 ± 0.6	1.0 ± 0.6	0.4 ± 0.4
Citric acid	13.2 ± 2.0	11.7 ± 2.4	10.9 ± 3.3
DNA	10.4 ± 1.7	9.5 ± 1.7	7.9 ± 1.5
Fructose	6.4 ± 1.4	6.1 ± 1.5	6.3 ± 1.5
Glucosamine	1.8 ± 0.5	1.7 ± 0.8	1.7 ± 0.5
Glucose	5.8 ± 3.0	6.7 ± 1.2	7.0 ± 1.7
Glutamic acid	5.7 ± 1.3	5.1 ± 1.6	5.5 ± 2.2
Glutamine	5.7 ± 2.3	6.0 ± 1.4	7.7 ± 1.8
Glycine	2.9 ± 1.1	4.6 ± 1.1	6.0 ± 1.8
Glycogen	10.6 ± 1.7	8.8 ± 2.1	9.5 ± 2.1
Histidine	3.1 ± 1.0	4.8 ± 1.0	5.3 ± 2.5
Humic acid	0.7 ± 0.6	1.2 ± 0.4	0.6 ± 0.9
Lactic acid	3.1 ± 1.0	3.7 ± 0.3	5.3 ± 2.2
Lignin	4.6 ± 2.1	4.5 ± 1.0	2.9 ± 0.2
Linoleic acid	2.2 ± 0.7	2.4 ± 0.7	2.1 ± 0.7
Lysine	0.3 ± 0.3	0.9 ± 0.2	0.8 ± 1.2
Sucrose	4.3 ± 1.3	5.9 ± 1.7	6.4 ± 0.8
Urea	13.3 ± 3	10.5 ± 2.1	9.9 ± 2.3
Diversity[2]	0.916 ± 0.007[a]	0.928 ± 0.003[b]	0.928 ± 0.003[b]

[1]Values represent means ± 1 SD

[2]Mean Simpson's index; different superscript letters indicate significantly different values ($P < 0.05$)

Appendix 2-1

Variable	CO_2 flux	DOC flux	[DOC]	DON flux	[DON]	DOC:DON	Grav. soil moisture	$[O_2]$	Fine root biomass	Soil C	Soil N	Soil C:N	Micr. biomass C	Micr. biomass N	Inorganic N flux
DOC flux	0.48**														
[DOC]	0.34	0.92***													
DON flux	0.16	0.70***	0.54**												
[DON]	-0.07	0.42*	0.42*	0.80***											
DOC:DON	0.49**	0.63***	0.68***	-0.09	-0.31										
Gravimetric Soil moisture	0.26	0.64***	0.67***	0.23	0.09	0.58***									
$[O_2]$	-0.03	-0.03	-0.08	0.07	-0.03	-0.08	-0.41*								
Fine root biomass	0.42*	0.23	0.26	-0.17	-0.12	0.47**	0.55**	-0.3							
Soil C	0.57**	0.62***	0.64***	0.08	-0.06	0.7***	0.82***	-0.3	0.66***						
Soil N	0.5**	0.51**	0.48**	0.14	0.02	0.49**	0.82***	-0.32	0.71***	0.92***					
Soil C:N	0.45*	0.61***	0.71***	0.03	-0.12	0.81***	0.44*	-0.03	0.32	0.67***	0.37*				
Microbial biomass C	0.58***	0.55**	0.5**	0.13	0.00	0.56**	0.71***	-0.23	0.66***	0.86***	0.9***	0.42*			
Microbial biomass N	0.63***	0.40*	0.36	-0.03	-0.13	0.54**	0.57**	-0.15	0.72***	0.82***	0.88***	0.41*	0.9***		
Inorganic N flux	-0.46*	-0.12	-0.19	0.32	0.35	-0.51**	-0.03	0.02	-0.46*	-0.5**	-0.32	-0.62***	-0.37*	-0.56**	
PO_4^{3-} flux	0.53**	0.45*	0.43*	0.07	-0.12	0.55**	0.51**	-0.24	0.51**	0.69***	0.68***	0.42*	0.59***	0.61***	-0.38*

Pearson correlations between measured variables from March 8, 2008 to March 3, 2009 using data from all litterfall manipulation plots.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Appendix 2-2

Variable	CO_2 Flux	Litter input	DOC	[DOC]	DON	[DON]	Moisture	$[O_2]$
Litter input	**-0.26**							
DOC	0.16	-0.14						
[DOC]	**0.34**	**-0.28**	-0.12					
DON	0.04	-0.00	**0.84**	-0.11				
[DON]	0.08	-0.05	**-0.59**	**0.79**	**-0.36**			
Moisture	**-0.60**	0.04	-0.12	**-0.36**	**-0.30**	-0.23		
$[O_2]$	**0.69**	**-0.55**	-0.06	**0.75**	-0.08	**0.50**	**-0.75**	
Rainfall	**-0.29**	0.07	**0.49**	**-0.28**	**0.39**	**-0.50**	0.24	**-0.40**

Pearson correlations between means of frequently measured variables in control plots at sampling dates from March 8, 2008 to March 3, 2009. Bold values are significant correlations ($P < 0.05$).