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The Effects of Soil Bacterial Community Structure on Decomposition in a Tropical Rain Forest

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Abstract

Soil microorganisms are key drivers of terrestrial biogeochemical cycles, yet it is still unclear how variations in soil microbial community composition influence many ecosystem processes. We investigated how shifts in bacterial community composition and diversity resulting from differences in carbon (C) availability affect organic matter decomposition by conducting an in situ litter manipulation experiment in a tropical rain forest in Costa Rica. We 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 variations 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 and quantity. Our results show that although 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, our results suggest that soil bacterial communities may be either functionally dissimilar or equivalent during decomposition depending on the nature of the organic matter being decomposed.

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Introduction

Microorganisms have been aptly described as the ''engines that drive biogeochemical processes'' (Falkowski and others 2008), yet important questions about the potential effects of changes in microbial community composition on ecosystem function remain (Groffman and Bohlen 1999; Tiedje and others 1999; Nannipieri and others 2003; O'Donnell and others 2005; Condron and others 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 and others 1995; Gholz and others 2000; Cornwell and others 2008). Although the combined importance of litter chemical composition (Gholz and others 2000; Cornwell and others 2008; Grandy and Neff 2008), nutrient availability (Melillo and others 1982; Taylor and others 1989), and climate (Meentemeyer 1978; Aerts 1997; Gholz and others 2000) on decomposition have been clearly established, the effects of variations in microbial community composition have been largely unexplored (Bardgett and others 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 and others 1994; Tiedje and others 1999). Yet, soil microbial communities are incredibly diverse (Fierer and others 2007b), and many ''macroecological'' studies provide evidence that community composition can influence ecosystem processes (Hooper and others 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 variations 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 and others 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 (for example, Schimel 1995; Schimel and others 2005), but there is evidence suggesting that soil microbial community structure has the potential to influence more basic ecosystem processes like decomposition (Waldrop and others 2000; Carney and Matson 2005; Strickland and others 2009; Keiser and others 2011).

Recently, links between the availability of decomposable organic matter and the relative abundance of bacterial subphyla and phyla have been shown (Smit and others 2001; Fierer and others 2007a; Nemergut and others 2010) supporting the notion that higher bacterial taxa can be ecologically distinct (Philippot and others 2010). For example, Fierer and others (2007a) showed that soil carbon (C) availability was positively correlated with the relative abundance of Bacteroidetes and Betaproteobacteria. These two taxa were generally described as copiotrophic (or r-selected) bacteria, whereas the relative abundance of Acidobacteria—described as a generally oligotrophic, or K-selected group—was inversely related to C availability (Fierer and others 2007a). This ecological classification scheme provides a testable and tractable framework for assessing relationships between soil microbial community composition and ecosystem function. Indeed, in an earlier study at the site described here, Cleveland and others (2007) showed that laboratory C additions to soil drove increases in proteobacteria that correlated with an increase in soil CO_2 flux. Similarly, Nemergut and others (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, these studies not only suggest that changes in C availability may alter 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 variations in soil microbial communities are rare because experimentally manipulating microbial community composition in situ is very difficult. As a result, most studies have relied on correlations between community composition and processes to infer structure–function relationships, but 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 and others 2010) despite the fact that they play a dominant role in the global C cycle (Zhao and Running 2010).

The observation made by Nemergut and others (2010), which showed that litter manipulations in a tropical rain forest in Costa Rica drove significant shifts in bacterial 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. We addressed this question using a series of laboratory incubation experiments with soil samples obtained from the same litter manipulation plots described in Nemergut and others (2010). First, we assessed *potential* differences in the ability of distinct soil bacterial 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 and others 2000; Grandy and Neff 2008), we hypothesized that the litter manipulation would alter soil C chemistry, and that this would lead to shifts in bacterial communities and their ability to degrade a wide array of C substrates. Next, we 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 and others 2004), but they are especially important in this wet tropical forest ecosystem (Cleveland and Townsend 2006). Given that the relative abundance of putative copiotrophic bacteria varied positively with C inputs in our study soils (Nemergut and others 2010), we hypothesized that the decomposition rates of DOM would be highest in soil that had received the largest litter inputs. Furthermore, we 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, we assessed the effects of DOM quantity on decomposition rates by adding several known concentrations of DOM to soil samples and assessing relationships among soil type, DOM concentration, and soil respiration rates. Cleveland and others (2010) showed that soil CO_2 fluxes increased with DOM concentration, and Nemergut and others (2010) observed that bacterial communities exposed to similar DOM concentrations had similar compositions. Therefore, we 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 approximately 26°C and mean annual precipitation (MAP) averages more than 5,000 mm y^{-1}, but the site has a distinct dry season (Dec–April) when precipitation averages less than 100 mm $month^{-1}$ and litterfall and standing litter mass are at annual maxima (Cleveland and Townsend 2006). The site is a stratified, closed canopy, highly diverse [100–200 tree species/ha (Kappelle and others 2002)] rain forest that includes many common Neotropical tree species [for example, *Brosimum utile* Kunth Oken. (Moraceae); *Caryocar costaricense* Donn. Sm. (Caryocaraceae); *Hieronyma alchorneoides* Fr. Allem (Phyllanthaceae); *Schizolobium parahybum* Vell. S.F. Blake (Fabaceae); and *Vantanea barbourii* Standl. (Humiriaceae)]. Soil at the site is clay (Wieder and others 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 and others (2006).

Litterfall Manipulation Experimental Design

To test the effects of the quantity of leaf litter inputs on soil microbial community structure and function, we utilized an existing set of in situ litter manipulation plots described by Nemergut and others (2010). In April 2007, we established 30 randomly assigned litter manipulation plots (3 × 3 m). 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 (1×). On average, the 1× and 2× plots received 0.90 ± 0.05 and 1.79 ± 0.11 kg litter $m^{-2}\ y^{-1}$, respectively, over the course of the experiment.

Soil Sampling and Analysis

Soil samples were collected using a hand corer (6 × 10 cm) from each of the 30 litterfall manipulation plots (N = 30 per sampling date), 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 1 week. Soils were sampled in April 2010 for the catabolic response profile analysis, 16S rRNA gene sequencing, and soil chemistry analysis; in Oct 2009 for the native DOM quality incubation experiment; and in Jan 2010 for the native DOM quantity experiment. Previous 16S rRNA gene data showed no significant seasonal differences in bacterial community composition at our site, and differences in bacterial community composition among litter input treatments were consistent across sampling dates (Nemergut and others 2010). Relationships between variables were only assessed for measurements taken on the same samples or subsamples (that is, same collection dates).

We 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, New Jersey, USA). Soil microbial biomass C in fresh soil samples was determined using the chloroform fumigation-extraction method (Brookes and others 1985). In brief, fumigated (5 days) 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, Maryland, USA). We 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 and others 1987). Finally, we assessed compound-specific soil C content on soil subsamples from the catabolic potential assay (see below) using pyrolysis–gas chromatography/mass spectrometry (GCMS) following a method similar to Wickings and others (2011). Soil samples were finely ground and pulse-pyrolyzed using a Pyroprobe 5150 (CDS Analytical Inc., Oxford, Pennsylvania, USA) at 600°C. The pyrolysis products were separated using a gas chromatograph (Trace GC Ultra, Thermo Scientific, Waltham, Massachusetts, USA) fitted with a fused silica capillary column (60 m, 0.25 mm ID), delivered to a mass spectrometer (Polaris Q, Thermo Scientific, Waltham, Massachusetts, 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 soil bacterial community composition between litterfall treatments, we subsampled a set of composited soil samples (by treatment) collected in April 2010 and used in the catabolic potential assay (see below). In brief, DNA was extracted and the 27–338 region of 16S rRNA gene was sequenced using bar-coded pyrosequencing following protocols from Nemergut and others (2010). We used a modified PCR amplification 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, Maryland, 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°C followed by 25 cycles at 94°C for 45 s, 50°C for 30 s, 72°C for 90 s, and a final elongation step at 70°C for 10 min. After sequencing, we conducted all downstream sequence analyses before statistical analysis using

the QIIME pipeline (Caporaso and others 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. We 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. We assessed alpha-diversity in the communities using three metrics: the observed number of OTUs in a sample, the Shannon index (Hill and others 2003), and the phylogenetic diversity index (Faith 1992). We calculated phylogenetic distances between communities using the weighted UniFrac distance metric (Lozupone and Knight 2005).

Catabolic Potential Assay

We assessed soil microbial community metabolic capabilities using catabolic 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, we 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). In brief, 2 g subsamples of each of the 15 soil composites were placed in 60-ml vials fitted with septa (25 vials per composited sample). Next, 2 ml C aliquots (900 mM) of 24 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 lysine), one amino sugar (glucosamine), two proteins (bovine serum albumin 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 before additions.

Twenty-four hours 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, Nevada, 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. We excluded two substrate responses (cellulose and oleic acid) from consideration because they were undetectable in more than one-third of all samples. In addition, we used normalized CO_2 fluxes from each substrate to calculate Simpson's index of diversity (1D) for each sample (Magurran 2004).

Native DOM Incubation Experiment: The Effects of DOM Quality

We further examined the potential effects of litter-driven changes in microbial composition on decomposition dynamics using two laboratory incubation experiments. First, we investigated differences in the ability of the microbial communities to degrade two types of DOM leached from two common tree species from the study site: *Schizolobium parahyba* and *Manilkara staminodella*. Wieder and others (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, Maryland, USA) total organic C analyzer.

After collecting leachate, a set of fresh soil samples (25 g each; $N = 10$ per treatment) were placed in glass Mason jars fitted with lids containing septa and adjusted to 50% water holding capacity (WHC) with deionized water. Two milliliters of each DOM type were added to samples, and the respiration rate at 320 min was calculated by evacuating the headspace and analyzing CO_2 concentrations using a gas chromatograph (Shimadzu, Columbia, Maryland, USA). CO_2 fluxes were calculated as a rate of CO_2 respired per dry weight equivalent of soil.

Native DOM Incubation Experiment: The Effects of DOM Quantity

We 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 air-dried mixed litter collected from litter traps at the site was leached in 700 ml of deionized water for 1 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, and CO_2 concentrations in the incubation vessels were assessed using gas chromatography. Respiration rates at 330 min 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 non-parametric MANOVA (McArdle and Anderson 2001) using the Adonis function (Oksanen and others 2010) 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, we 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, we 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 and others 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, we 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, we 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), we 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. To analyze soil CO_2 responses to additions of varied DOM concentrations, we conducted a 2-way ANOVA with litter input treatment as a fixed factor and DOC concentration as a random factor. CO_2 flux data were log (ln) transformed to meet the assumptions of normality and the heterogeneity of variances, and pair-wise comparisons were made using Tukey HSD tests.

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 different litter treatments varied in their ability to degrade the range of C compounds used in the CRP incubation experiment ($P = 0.02$;

Figure 1). Although all substrate additions resulted in a increases in CO_2 production rates, the proportional decomposition response (that is, 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; Table 1; Appendix A in Supplementary Material).

To assess possible drivers of the observed differences, we explored relationships between a number of soil properties and CRPs. Consistent with previous observations from this litter manipulation experiment (Nemergut and others 2010), the manipulation drove differences in soil nutrient pools, as we observed significantly greater proportions of total soil C and N and greater microbial biomass C in 2× than in 0× plots in the Oct 2009 samples (Table 2). However, the litter manipulation did not result in broad-scale changes in soil C chemistry. We identified 239 distinct pyrolysis products (Appendix B in Supplementary Material), 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.

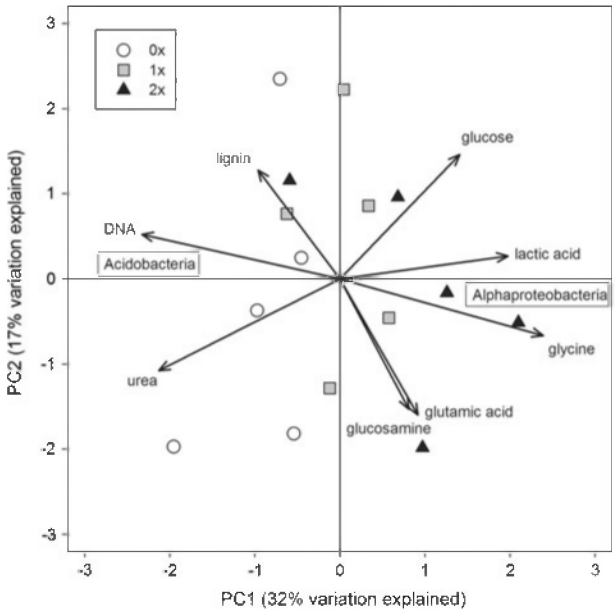

Figure 1. PCoA plot of catabolic response profiles (CRPs) for samples from the litter manipulation plots. Points further apart had more dissimilar CRPs. Vectors represent relationships between CRPs and the relative response to individual C substrates and point in the direction of CRPs with stronger relative 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.

Table 1. Vector Correlation Coefficients (r) and P Values

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.

The 16S rRNA gene sequences showed that the in situ litter manipulations drove significant differences in soil bacterial community composition ($P = 0.001$; Figure 2), and these differences included shifts in specific taxa. For example, Acidobacteria (35%) and Alphaproteobacteria (19%) were the most abundant higher-level taxa across treatments, the relative abundances of these taxa significantly differed among treatments, and variation in total soil C significantly explained variation in bacterial community composition ($P < 0.05$). Specifically, the relative abundance of Acidobacteria and Alphaproteobacteria were significantly lower and higher, respectively, in the 2× plots than in either the 0× or 1× plots ($P < 0.05$), and this trend was also observed in many individual acidobacterial and alphaproteobacterial OTUs. Of the 33 acidobacterial OTUs observed in at least two-thirds of the samples, 13 were relatively less abundant with greater litter inputs (that is, 2× < 1× < 0×) whereas 3 were relatively more abundant with greater litter inputs (that is, 2× > 1× > 0×). For Alphaproteobacteria, of the 21 OTUs found in at least two-thirds of the samples, six showed increases in relative abundance with greater litter inputs, whereas none showed declines in relative abundances with greater litter inputs (Appendix C in Supplementary Material). In addition, the 2× plots contained a significantly higher number of OTUs than the 0× plots, but there were no

Table 2. Soil Properties

Treatment	Soil C (%)		Soil N (%)		Microbial biomass C (μg/g)		
	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]
1×	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]

Different superscript letters indicate significant differences between treatments (α = 0.05). Values represent mean ± 1 SD.
[1]N = 10.
[2]N = 5.

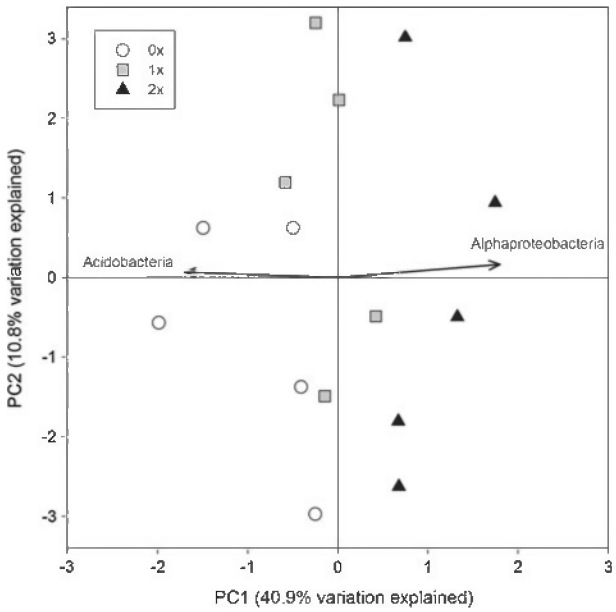

Figure 2. Principal coordinate analysis plot of bacterial community composition for samples from the litter treatment plots. Points further apart had more dissimilar bacterial communities based on weighted UniFrac distances. *Arrows* point in the direction of samples with greater relative abundances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

significant differences in the Shannon or phylogenetic diversity measurements between soil samples from different litter treatments (Table 3). Despite the significant relationship with total soil C content, multivariate analysis showed that variations in soil C chemistry among the different treatments did not significantly explain variations in bacterial community composition.

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 variations in CRPs ($P < 0.05$), but the relationship between CRPs and bacterial community composition was the strongest (Table 4). In addition, the MRM analysis indicated that including the other soil properties (that is, 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, we found significant relationships with CRPs for both Acidobacteria ($\rho = 0.27$; $P = 0.013$) and Alphaproteobacteria

Table 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]
1×	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 (α = 0.05). Measurements were based on 620 sequences per sample, and values represent mean ± 1 SD.

Table 4. Spearman's Rank Correlations (ρ) and P Values Between Soil Catabolic Response Profiles (CRPs) and Explanatory Variables

Characteristics	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.

(ρ = 0.39; P = 0.003; Figure 1) relative abundances.

We 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 A in Supplementary Material). 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 3).

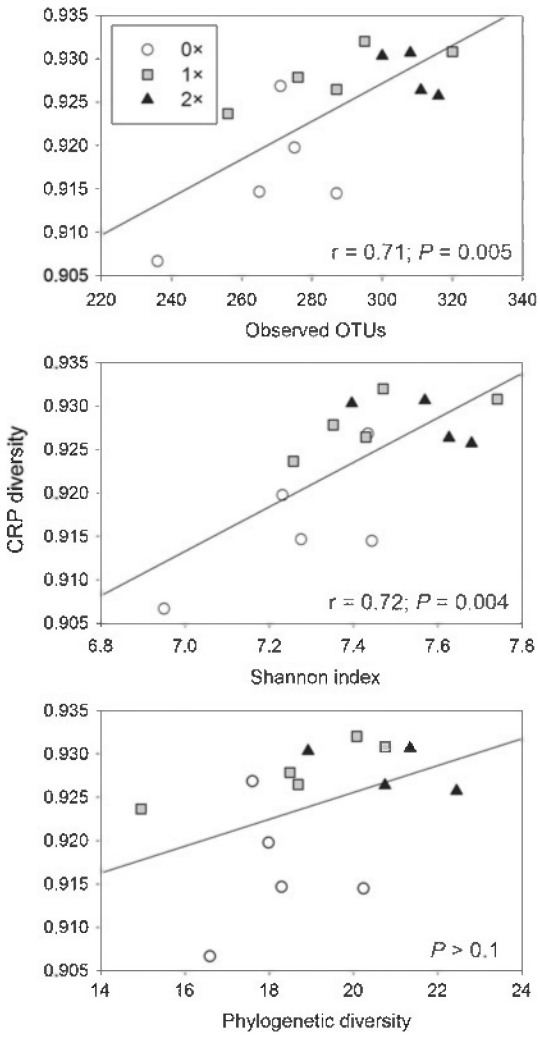

Figure 3. Relationships between the catabolic response profile (CRP) diversity and three measures of bacterial community diversity among all litter input treatments.

Soil Functional Responses to C Substrate Additions: Native DOM Quality

Following the DOM additions, soils receiving greater litter inputs had higher respiration rates across both DOM types (that is, 2× > 1× > 0×; Appendix D in Supplementary Material; $P < 0.05$ in all cases). Respiration rates were 27% lower in the 0× and 70% higher in the 2× soil samples relative to the 1× soils. Soil samples also significantly varied in their response to DOM type ($P < 0.05$). Among all samples, high quality DOM leached from *S. parahyba* elicited a 17% 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 fluxes for different DOM types as there was no statistical interaction between the litter input treatment and the DOM type. Furthermore, after accounting for variations in soil C, soil N, and microbial biomass C (using ANCOVA), litter input treatment did not significantly contribute to variations in CO_2 fluxes, and all other variables significantly explained 89% of the variation in the CO_2 fluxes (P = 0.001).

Soil Functional Responses to C Substrate Additions: Native DOM Quantity

To assess whether shifts in bacterial community composition caused soils receiving greater litter inputs to decompose higher concentrations of DOM more quickly, we calculated whether the CO_2 fluxes among treatments were different among the various DOM concentrations (that is, we explored a possible statistical interaction between litter input treatment and DOM concentration). Results from the ANOVA showed that there was no significant interaction between litter input treatment and DOM concentration. Yet, soil samples from different litter input treatments had significantly different CO_2 fluxes in response to the range of DOM concentrations added in the native DOM quantity incubation experiment ($P < 0.001$) as initial soil respiration rates increased with litter input (that is, 0× < 1× < 2×; $P < 0.05$ in all cases). In addition, CO_2 fluxes increased significantly with DOC concentration ($P < 0.001$; Figure 4).

Discussion

Strickland and others (2009) proposed two competing hypotheses to describe the possible effects of divergent microbial communities on ecosystem processes. The first—functional equivalence—

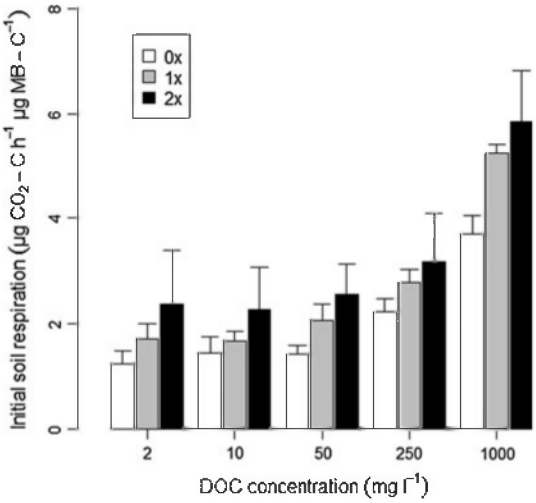

Figure 4. Initial respiration rates of soils normalized by microbial biomass C 330 min after the addition of DOC at several concentrations. *Error bars* mean ± 1 SD.

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 and others (2009) showed that decomposition rates varied depending on the soil inoculum used, and suggested that this was evidence of functional dissimilarity.

Here, we investigated how litter-driven changes in bacterial community composition contribute to differences in organic matter decomposition. Our analyses confirmed that the bacterial communities differed between treatments in ways similar to those previously described (Figure 2; Nemergut and others 2010). We took advantage of these community composition differences to assess whether the soil microbial communities differed in their ability to decompose a range of C compounds. Overall, our results supported our initial hypothesis—soil microbial communities from the 0×, 1×, and 2× plots differed in their ability to decompose varied C compounds (Figure 1). Moreover, among all samples, a substantial amount of the variation in CRPs could be explained by specific variations in bacterial community composition (ρ = 0.46), 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 significantly related to differences in CRPs (ρ = 0.27 and 0.39, respectively).

These findings suggest that variations in community composition, and in particular, the relative abundance of the bacterial taxa Acidobacteria and Alphaproteobacteria, are important in explaining the observed variations in decomposition rates of C substrates. Previously, Acidobacteria have generally been classified as oligotrophic and certain proteobacterial taxa as copiotrophic (Fierer and others 2007a; Nemergut and others 2010). However, it is important to note that not all members of these broad phylogenetic groups fit these categories, and that the functional ecology of the organisms within each group likely varies considerably. Thus, while our results are consistent with a previous study suggesting that in general, Acidobacteria and Alphaproteobacteria fit this ecological classification scheme, further research is needed to determine whether oligotrophic or copiotrophic bacteria as a whole have distinct ecological functions. In addition, our results are consistent with a previous work showing that variations in litterfall C inputs drove predictable shifts in bacterial community composition, and our data also suggest that changes in community composition correspond to changes in the overall ability of the resulting communities to decompose added C substrates. These results support the functional dissimilarity hypothesis, and are consistent with other studies that have shown variations in decomposition rates across different soil microbial communities taken from a single ecosystem (Carney and Matson 2005; Brant and others 2006).

There are several possible explanations for the observed changes in microbial community function between treatments, but our 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 3; Nemergut and others 2010). Thus, we predicted that variations in the delivery of labile C would also alter soil C chemistry (for example, Kiem and others 2000), which could in turn alter community-specific responses to any specific compound array. However, we did not observe differences in the overall soil C chemistry among litter input treatments that are typically associated with variations in soil decomposer communities (Grandy and others 2009; Wickings and others 2011), and soil C chemistry did not explain soil bacterial community structure differences. These findings suggest that soil C quantity was more important to bacterial 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 and others 2002; Bell and others 2005; Waldrop and others 2006; Langenheder and Prosser 2008). At first glance, such variation in diversity does not seem to exist in our samples: bacterial alpha-diversity among soils did not vary significantly across soils receiving different litter inputs in two of the three metrics we used (Table 3). Thus, our results are more consistent with others showing no detectable links between soil C and total bacterial diversity across a wide variety of ecosystems (Lauber and others 2009). The treatments did not seem to drive changes in Shannon or phylogenetic diversity among all samples combined, yet there were strong positive correlations between bacterial diversity and catabolic diversity for two of the three diversity metrics (Figure 3). Therefore, our results provide some additional evidence that catabolic diversity may vary with bacterial diversity in soil. We also observed a positive relationship between catabolic diversity and litter inputs, which is consistent with Degens and others (2000), who showed that catabolic evenness declined with lower soil organic C content. Thus, evidence from this experiment and others suggests that increased soil C might drive increased bacterial diversity that, in turn, leads to an increase in catabolic diversity.

The CRPs effectively illustrate the *potential* effects of varying community composition on the decomposition of individual C substrates, but it is important to note that our native DOM experiments seem to present an entirely different picture of the connections between bacterial community structure and decomposition. We 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. We hypothesized that soil from the 2× plots (with a higher proportion of putative copiotrophic bacteria) would decompose DOM more rapidly than soil from the litter removal plots (with a higher proportion of putative oligotrophic bacteria). Although we observed differences in the amount of respired CO_2 produced following DOM additions, we saw no evidence to suggest that these differences were driven by shifts in bacterial community composition. For example, when manipulating DOM quality, differences in CO_2 fluxes could be explained by variations 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 (89%) of the variation in the CO_2 fluxes during the incubation. Thus, our 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 labile C (Rousk and others 2011; Paterson and others 2011) or SOM (Kemmitt and others 2008).

Similarly, neither of our other two hypotheses regarding the functional dissimilarity in DOM decomposition rates was supported by the incubation data. First, we hypothesized that differences in decomposition rates would vary between bacterial communities based on the biodegradability of the added DOM (Wieder and others 2008). However, we saw no evidence for this in our native DOM quality manipulation experiment: The *S. parahyba* DOM decomposed more quickly than *M. staminodella* DOM, but bacterial community composition did not explain the overall patterns. Next, given the known links between DOM concentration and soil respiration rates in this site (Cleveland and others 2010), we predicted that 2× communities would decompose high concentrations of DOM more rapidly than the 0× communities. Thus, we evaluated whether the difference in CO_2 fluxes between treatments varied across experimental concentrations and found that there was no significant statistical interaction between litter input treatment and DOM concentration (Figure 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. Rather, the observed differences in CO_2 flux rates with increasing DOM concentration observed previously (Cleveland and others 2010) are not driven by variations in microbial community composition per se, but other biogeochemical factors (that is, soil C, soil N, and microbial biomass). Although earlier work has demonstrated links between microbial community structure and ecosystem function (for example, Carney and Matson 2005; Strickland and others 2009; Keiser and others 2011; Paterson and others 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 and others 2003). This is the essence of the functional equivalence hypothesis discussed above (Strickland and others 2009). However, there is growing support for the idea that functional dissimilarity among microbial communities may drive variations even in organic matter decomposition (for example, Condron and others 2010). Results from our native DOM experiments supported the functional equivalence hypothesis: differences in native DOM decomposition rates could not be attributed to differences in microbial community composition. Although similar results have been reported in the literature (for example, Rousk and others 2011), Strickland and others (2009) concluded that decomposer microbial communities were functionally dissimilar. This inconsistency may reflect the fact that Strickland and others (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, our experiment may have more effectively mimicked the type of variations that biotic and/or environmental changes might drive within a single ecosystem. Our results suggest that even when such changes are large (for example, a doubling or removal of litter), resultant shifts in the microbial community may not have significant direct effects on the mineralization of DOM pools.

Nonetheless, our experiments did show inconsistent effects of microbial community composition on decomposition: The results of our DOM experiments support the functional equivalence hypothesis, but the CRP analysis suggests that variations in community composition could drive variations in decomposition. Our 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. The inconsistent results may also be, in part, explained by methodological artifacts. For example, we assessed differences in bacterial community composition among the litter input treatments and related observed differences to variations in decomposition rates of the entire microbial community, which would include organisms in other domains (for example, fungi and Archaea). Thus, although we assessed relationships between decomposition rates and bacterial community composition, other non-bacterial organisms are undoubtedly affecting decomposition rates, especially given that fungi are known to strongly influence decomposition rates in forest ecosystems (Joergensen and Wichern 2008). Additional research may reveal that knowledge of fungal and/or archeal community composition aids in the ability to predict ecosystem process rates.

Next, all of our assays were short-term incubations (that is, $\leq$24 h), but the CRP analysis included a longer incubation period (that is, 24 h) and greater C concentrations than the DOM addition assays (to detect community responses to more refractory C substrates). Thus, it is also possible that the different assays targeted different subsets of microbial communities with varied physiologies. For example, the short-term incubations may have been more heavily biased by bacterial responses. Moreover, the relatively longer CRP analysis could have preferentially assessed the function of better K-selected microbial taxa whereas the DOM addition assays may have preferentially assessed better r-selected taxa, or similarly, the greater C concentrations in the CRP analysis could have solicited the growth of certain microbial taxa over those in the DOM addition assays. Thus, the CRP analysis could have identified differences in microbial function based on a different subset of the microbial community from the DOM addition assays, and certain taxa could have been responsible for functional differences in the CRP analysis that were not strongly probed in the DOM addition assays. Therefore, it is possible that subsets of certain physiologically defined microbial groups may be functionally dissimilar from one another while subsets of other groups are not.

Although methodological issues may have contributed to the inconsistent responses between the CRP and DOM addition assays, there is another possible explanation. Schimel and others (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. Our contrasting results could reflect the fact that DOM decomposition is an aggregate process, whereas the decomposition of pure substrates in the CRP experiment reflects variations 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 reflects 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 although variations 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.

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