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Plot-scale manipulations of organic matter inputs to soils correlate with shifts in microbial community composition in a lowland tropical rain forest

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ABSTRACT

Little is known about the organisms responsible for decomposition in terrestrial ecosystems, or how variations in their relative abundance may influence soil carbon (C) cycling. Here, we altered organic matter *in situ* by manipulating both litter and throughfall inputs to tropical rain forest soils, and then used qPCR and error-corrected bar-coded pyrosequencing to investigate how the resulting changes in soil chemical properties affected microbial community structure. The plot-scale manipulations drove significant changes in microbial community composition: Acidobacteria were present in greater relative abundance in litter removal plots than in double-litter plots, while Alphaproteobacteria were found in higher relative abundance in double-litter and throughfall reduction plots than in control or litter removal plots. In addition, the bacterial:archaeal ratio was higher in double-litter than no-litter plots. The relative abundances of Actinobacteria, Alphaproteobacteria and Gammaproteobacteria were positively correlated with microbial biomass C and nitrogen (N), and soil N and C pools, while acidobacterial relative abundance was negatively correlated with these same factors. Bacterial:archaeal ratios were positively correlated with soil moisture, total soil C and N, extractable ammonium pools, and soil C:N ratios. Additionally, bacterial:archaeal ratios were positively related to the relative abundance of Actinobacteria, Gammaproteobacteria, and Actinobacteria, and negatively correlated to the relative abundance of Nitrospira and Acidobacteria. Together, our results support the copiotrophic/oligotrophic model of soil heterotrophic microbes suggested by Fierer et al. (2007).

1. Introduction

Organic matter decomposition is a fundamental ecosystem process and a primary control over terrestrial carbon (C) storage (Schlesinger, 1997). Multiple studies have shown that both climate and litter quality exert strong controls over decomposition rates (e.g., Melillo et al., 1982; Kirschbaum, 1995; Gholz et al., 2000; Parton et al., 2007). However, soil microorganisms are the primary agents of terrestrial decomposition; microbes decompose organic matter to obtain energy and build biomass, and in so doing, they release CO_2 to the atmosphere (Sanderman and Amundson, 2005; Horwath, 2007). Thus, the *potential* importance of microbial community composition in organic matter decomposition is obvious: microbes are the engines that drive the process (Killham and Prosser, 2007). Yet, no widely used model of decomposition includes explicit parameters describing microbial community composition (Potter et al., 1993; Parton et al., 1994; Thornton et al., 2002).

In part, this lack of consideration of microbes may arise from the notion that biogeochemical processes common to abundant and phylogenetically diverse fractions of the microbial community, such as heterotrophic respiration, may not directly relate to community composition (Schimel, 1995). Indeed, standard ecosystem models treat the microbial community as a "black box", one in which members of the community act as passive catalysts of decomposition (see references in Schimel and Gulledge, 1998). The implicit assumption in this approach is that all members of the decomposer community respond to changes in chemical and abiotic conditions in a similar fashion, and/or that the functional redundancy of decomposing organisms renders shifts in specific taxa unimportant to determining ecosystem-level rates.

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Alternatively, Fierer et al. (2007) proposed an ecological classification scheme for soil bacteria that suggested that different phylogenetic groups may represent different functional groups, and that the relative abundance of functional groups varies with C availability. Specifically, some phyla may be copiotrophic (e.g., Proteobacteria) and relatively more abundant under high-C conditions, while others may be considered oligotrophic (e.g., Acidobacteria). This model is consistent with recent work demonstrating that some taxa exhibit "ecological coherence" at deep levels within the bacterial phylogenetic tree (Philippot et al., 2009). In addition, an assumption of this model is that different taxa process organic matter at different rates even under similar abiotic conditions. Thus, shifts in organic matter resources could drive changes in microbial community composition that, in turn, are important to understanding subsequent patterns in decomposition rates.

To date, support for phylogenetically-based ecological classification schemes is mixed. For example, Smit et al. (2001) assembled microbial community data from multiple soils and showed that higher C soils have larger Proteobacteria:Acidobacteria ratios. Likewise, quantitative PCR (qPCR) analyses on a broad range of soil types demonstrated a decline in acidobacterial relative abundance with decreases in soil C (Fierer et al., 2007). Additions of labile C to soil microcosms have revealed shifts in microbial community structure including a higher relative abundance of Proteobacteria using clone library approaches (Cleveland et al., 2007), qPCR (Fierer et al., 2007) and stable isotope probing (Padmanabhan et al., 2003). However, Strickland et al. (2009) and Kemmitt et al. (2008) observed no relationship between microbial community structure (as assayed by bacterial:fungal ratios and phospholipid fatty acids, respectively) and C mineralization processes in soil. Thus, the copiotrophic/oligotrophic model may depend on the soil type, the experimental approach used, and the analysis techniques employed.

In this study, our overall objective was to examine the relationship between organic matter inputs and soil microbial community structure using a plot-scale field manipulation in a lowland tropical rain forest. Previous data from short-term laboratory soil incubations at this site revealed that litter-leached dissolved organic matter (DOM) amendments drove rapid increases in soil respiration. These increases in CO_2 fluxes corresponded with shifts in microbial community composition that were consistent with the copiotrophic/oligotrophic classification model, including an decrease in the proteobacterial:acidobacterial ratio (Cleveland et al., 2007). Additionally, field measurements have revealed significant seasonal variation in soil respiration in this forest, driven by strong interactions between litterfall and precipitation. Specifically, high litterfall inputs in the dry season fuel large early wet season fluxes of DOM and high rates of soil respiration (Cleveland and Townsend, 2006; Cleveland et al., 2010), suggesting that both litter and precipitation are important controls over organic matter inputs to this system. Together, these data suggest that variations in microbial community composition could be – at least in part – driving the observed variations in soil respiration that correlate with changes in C inputs. However, the extent to which small-scale shifts in microbial community structure and function observed in the laboratory (e.g., Cleveland et al., 2007) relate to the changes documented in the natural environment (Cleveland and Townsend, 2006; Cleveland et al., 2010) is unknown (Schimel and Gulledge, 1998).

Here, we altered both the amount and concentration of organic matter inputs to soils by manipulating both litter and throughfall in a series of experimental plots in a tropical rain forest site in southwestern Costa Rica. In this ecosystem, litter additions and removals increase or decrease, respectively, overall organic matter inputs, while throughfall reductions increased the concentration of litter-leached DOM (Cleveland et al., 2010). We analyzed a number of soil chemical properties across these experimental plots and explored how variations in soil moisture, pH and a variety of indicators of soil C and nitrogen (N) cycling correlate with microbial community composition.

2. Methods

2.1. Site description, manipulations and sampling

The field site is a diverse, lowland tropical rain forest on the Osa Peninsula in southwestern Costa Rica. In April 2007 we initiated a manipulation in which litter inputs were removed at monthly intervals from ten randomly assigned 3 m × 3 m "litter removal" plots and added to ten "double-litter" plots. Over two years we removed $900 \pm 50\ g\ m^{-2}\ y^{-1}$ of fine leaf litter from litter removal plots and estimated that double-litter plots received total annual litter inputs of $1179 \pm 110\ g\ m^{-2}$. In September 2007 we began a throughfall manipulation: "throughfall reduction" plots were constructed using partial throughfall sheds ~1 m above the soil surface on 2.2 m × 2.2 m aluminum frames. On these frames we mounted 5 cm diameter PVC pipe cut longitudinally; pipes were arranged to reduce throughfall to the surface soil by 50% (for more details on the throughfall manipulation see Cleveland et al., 2010). Ten randomly selected plots received no manipulation and served as experimental controls for both litter and throughfall plots.

During the rainy season (April 2008–Jan 2009) the site received 3740 mm of precipitation, with heaviest precipitation between August and October. Over this period we used a set of zero tension lysimeters to quantify DOM inputs to surface soils (Cleveland et al., 2010). Under the canopy, throughfall in control plots totaled 3555 ± 336 mm while throughfall reduction plots received only 1583 ± 212 mm. Notably, the throughfall reduction did not significantly affect soil moisture or total DOC inputs, but approximately doubled the concentration of DOC delivered to surface soils (Cleveland et al., 2010). As expected, total DOC inputs in the litter manipulations were ~40% and ~130% of inputs to control plots in the no-litter and double-litter plots, respectively (Wieder et al., unpublished data).

For the present study, soil samples were collected in three replicate plots from each experimental manipulation and control plots in June and October of 2008. During each sampling event, soil samples for the soil chemical analyses (0–10 cm) were collected using a bulb corer. Soil samples for microbial community analyses (0–5 cm) were collected with an ethanol-cleaned trowel from three replicate plots per treatment generating a total of 24 samples. All samples were transported on ice to the laboratory at the University of Colorado and soils for molecular work were immediately placed at −80 °C until analyses were performed.

2.2. Soil chemical properties

Within 72 h of sample collection, soil samples were sieved to 4 mm and processed for soil chemical analyses. We extracted inorganic N using a 2 M KCl solution (18 h extraction). Ammonium (NH_4^+) in extracts was analyzed colorimetrically on an Alpkem autoanalyzer (OI Analytical, College Station, TX, USA). We extracted microbial biomass C and N using the chloroform fumigation technique with a 0.5 M K_2SO_4 solution (Brookes et al., 1985). Total organic C and N in extracts were analyzed using a high temperature combustion total CN analyzer (Shimadzu TOCvcpn, Kyoto, Japan). Microbial C and N were calculated as the difference between organic C and total N in chloroform fumigated and unfumigated samples with a 0.45 correction factor for extraction efficiency

(Vance et al., 1987). We measured gravimetric soil moisture by drying soil sub-samples at 100 °C for seven days. Oven-dried soils were ground to a fine powder (<0.25 mm) and analyzed for total C and N using high temperature combustion in a Carlo Erba combustion–reduction elemental analyzer (CE Elantech, Lakewood, NJ, USA). Finally, we measured soil pH on air-dried samples in a 2:1 (mass:mass) water slurry.

2.3. Quantitative PCR

Quantitative PCR (qPCR) was used to estimate the relative abundance of bacterial and archaeal 16S rRNA genes in the different soil samples. DNA was extracted from the soils using the PowerSoil DNA extraction kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's instructions. We used the bacterial primers EUB338f and EUB518r as described by Fierer et al. (2005) and the archaeal primers Arch967f (Stahl and Amann, 1991) and Arch1060rMod (GGCCATGCACCWSCTCT) adapted from Moissl et al. (2003). Reactions were assembled using the Agilent Brilliant II SYBR Green qPCR Master Mix (Agilent Technologies, Santa Clara, CA, USA) and each contained ~20 ng of genomic DNA. All reactions were performed in triplicate and a standard curve was run on each plate. Amplifications were performed using a Stratagene MX3005P qPCR machine (Agilent Technologies) and data were analyzed using the manufacturer's MXPro software. To test our samples for the presence of inhibitors, we examined the Ct values (threshold amplification detection values) for a dilution series of our soil genomic DNA extracts. No inhibition was observed ($R^2 = 0.98$).

2.4. Pyrosequencing

Error-corrected bar-coded pyrosequencing was performed as described by Fierer et al. (2008). Briefly, soil genomic DNA was PCR amplified using the primers 5′-GCCTTGCCAGCCCGCTCAGTCAGAGTTTGATCCTGGCTCAG-3′ (containing the bacterial primer 27F) and 5′GCCTCCCTCGCGCCATCAGNNNNNNNNNNNNCATGCTGCCTCCCGTAGGAGT-3′ (containing the bacterial primer 338R and a unique barcode, denoted NNNNNNNNNNNN). Reaction mixtures consisted of 30 μM of each primer, 3 μL of soil genomic DNA, and 22.5 μL of Platinum PCR SuperMix (Invitrogen, Carlsbad, CA, USA); thermocycling conditions followed Fierer et al. (2008). PCRs were performed in triplicate, pooled and cleaned using the UltraClean-htp PCR Clean-up kit (Mo Bio Laboratories) according to the manufacturer's instructions. Equal amounts of amplicons from each soil were used to create a composite sample. Sequencing was completed at the Environmental Genomics Core Facility at the University of South Carolina on a Roche FLX 454 pyrosequencing machine. Data were processed as described by Fierer et al. (2008) and Hamady et al. (2008). We obtained an average of 1384 sequences per soil (range of 1087–2030).

2.5. Data analysis

Archaeal:bacterial ratios, the relative abundances of all taxa and all soil chemical properties were tested for normality and transformed as appropriate prior to statistical tests. Specifically, soil C, soil N, C:N ratios, NH_4^+ pools, pH, bacterial:archaeal ratios and archaeal 16S rRNA gene relative abundance were log transformed; the square root was taken of the relative abundances of Gammaproteobacteria, Nitrospira, Actinobacteria and Acidobacteria. Repeated measures ANOVAs followed by a Tukey's HSD post-hoc test were performed to examine significant differences in the soil chemical properties and in the relative abundance of bacterial taxa between treatment types.

To test the relationship between the field manipulations and bacterial community structure, we performed a repeated measures PERMANOVA (Anderson, 2001; McArdle and Anderson, 2001) in PRIMER v6 (Clarke and Warwick, 2001) using both a Bray–Curtis OTU-based distance metric (Bray and Curtis, 1957) and the UniFrac distance metric (Lozupone and Knight, 2005). To examine the relationship between environmental variables and the overall community structure, Mantel-like tests (RELATE function, Clarke and Warwick, 2001) were performed in PRIMER. Spearman's rank correlation coefficients and significance values were calculated in R (R Development Core Team, 2010). One bacterial:archaeal ratio value was greater than two standard deviations above the mean and was discarded prior to correlation tests.

3. Results

Plot-scale throughfall and litter manipulations drove statistically significant changes in several soil chemical properties (Table 1, ANOVA, $P < 0.05$). The throughfall reduction soils contained significantly more C and N than the no-litter plots. Microbial biomass C and N pools and soil C:N ratios were lower in the no-litter treatment than in the double-litter treatment, while soil C and N concentrations in the double-litter treatment were significantly higher than those in the control and no-litter treatments. Soil NH_4^+ concentrations also varied significantly across treatments: double-litter plots contained significantly higher pools than the control plots. Gravimetric soil moisture was lower in the no-litter plots than in all other treatments. Soil pH showed no significant trend across treatments and no parameters displayed significant differences between sampling dates (repeated measures ANOVA, $P > 0.05$).

Field manipulations also correlated with significant differences in the soil microbial community structure. qPCR-based analysis of the relative abundance of bacterial and archaeal 16S rRNA genes showed that the bacterial:archaeal ratio was significantly higher in the double-litter plots than in the no-litter treatments (Table 1, ANOVA, $P < 0.05$). Pyrosequencing-based analyses of bacterial community composition using both OTU-based (Bray and Curtis, 1957) and the phylogenetically-based UniFrac (Lozupone and

Table 1
Soil characteristics for the different treatments. Shown are the averages of six replicates; values in parentheses are standard deviations. Significantly different (ANOVA, $P < 0.05$, Tukey's HSD post-hoc analysis) groups are denoted with different letters. No seasonally significant patterns in these soil parameters were observed. Bacterial:archaeal = ratio of bacterial 16S rRNA genes/archaeal 16S rRNA genes.

	Soil moisture (%)	Microbial biomass C (μg C/g soil)	Microbial biomass N (μg N/g soil)	Soil C (%)	Soil N (%)	Soil C:N	Soil NH_4^+ (μg/g)	Soil pH	Bacterial:archaeal
Treatment									
Control	46.7 (1.6) A	1244.4 (377.8) AB	266.7 (44.4) AB	5.58 (0.89) ACD	0.47 (0.03) A	11.9 (1.79) AB	9.09 (5.41) A	5.21 (0.44) A	31.3 (33.1) AB
No litter	41.6 (1.2) B	733.3 (222.2) A	200.0 (66.7) A	4.23 (1.15) C	0.37 (0.07) B	11.4 (1.49) A	9.56 (4.34) AB	5.01 (0.13) A	27.7 (30.0) A
Throughfall reduction	48.1 (3.1) A	1244.4 (422.2) AB	266.7 (44.4) AB	6.00 (1.36) BD	0.49 (0.06) AC	12.1 (1.61) AB	12.01 (6.31) AB	5.26 (0.31) A	95.2 (82.2) AB
Double litter	50.9 (3.8) A	1422.2 (200.0) B	311.1 (44.4) B	8.31 (1.48) B	0.59 (0.07) C	14.1 (1.30) B	20.20 (7.21) B	5.25 (0.24) A	138.9 (64.7) B

Knight, 2005) distance metrics also revealed that soils exposed to different treatments harbored significantly different communities (PERMANOVA, $P < 0.001$). Repeated measures ANOVA and PERMANOVA revealed no effect of sampling date on the bacterial to archaeal ratios or the bacterial community composition ($P > 0.05$).

To further examine if changes in specific bacterial taxa drove the observed relationships between community structure and treatment, we performed an ANOVA comparing differences in the relative abundance of all bacterial groups that represented at least 5% (averaged across all replicates) of the total bacterial community (Fig. 1). As described above, we saw no significant effect of time on community structure, thus samples collected in June and October from the same treatment types were treated as replicates in the analyses. The relative abundance of Acidobacteria was significantly higher in the no-litter plots than in the throughfall reductions and the double-litter plots. By contrast, the relative abundance of Alphaproteobacteria was significantly higher in the double-litter and throughfall reduction plots than in the control and no-litter plots. The Acidobacteria:Proteobacteria ratio, an indicator of soil nutrient status (Smit et al., 2001) followed a pattern identical to acidobacterial relative abundance, and was higher in the no-litter plots than in the throughfall reductions and the double-litter soils (ANOVA, $P < 0.01$). Relative abundances of other major taxa were not significantly different between treatments.

Next, we examined the relationships between specific environmental variables (listed in Table 1) and soil microbial community structure irrespective of treatment type. Bacterial:archaeal ratios were positively correlated with soil moisture, soil C, N and NH_4^+ concentrations, and C:N ratios (Table 2, Spearman's rank correlations, $P < 0.05$). These patterns appeared to be driven by the abundance of archaeal 16S rRNA genes relative to the total soil genomic DNA pool. For example, bacterial 16S rRNA gene relative abundance showed no pattern with any of the variables examined while archaeal 16S rRNA gene relative abundance was negatively correlated with all five variables (Table 2). A more detailed analysis of the relationship between archaeal 16S rRNA gene relative abundance and the environmental parameters revealed that all relationships displayed logarithmic-shaped functions (Fig. 2).

The overall bacterial community composition was weakly but significantly correlated with many of the environmental parameters examined (Table 3). For the phylogenetically-based UniFrac metric, total soil N showed the highest correlation with community structure (Rho = 0.35, Table 3). Although we did not see treatment-level differences in soil pH (Table 1), pH showed the strongest correlation with bacterial community structure for the OTU-based metric (Rho = 0.35, Table 3). We also examined the relationships between environmental variables and the relative abundance of different bacterial taxa (Table 4). In general, correlations between soil chemical characteristics and the abundances of specific taxa were stronger than relationships between these parameters and the community structure as a whole. Specifically, the relative abundances of Actinobacteria, Alphaproteobacteria, and Gammaproteobacteria were positively correlated with microbial biomass C and N, soil N and soil C pools, while acidobacterial relative abundance was negatively correlated with microbial biomass C and N, soil moisture, soil C and N pools and pH.

A Mantel-like test revealed a significant relationship between bacterial:archaeal ratios and bacterial community composition (Rho 0.329 and 0.247 for OTU-based metrics and UniFrac-based metrics, respectively; $P < 0.01$). Thus, we also investigated the relationship between the relative abundance of specific bacterial phyla and the bacterial:archaeal ratio. The relative abundances of Actinobacteria, Gammaproteobacteria and Alphaproteobacteria were correlated with higher bacterial:archaeal ratios while Nitrospira and Acidobacteria were correlated with lower bacterial:archaeal ratios (Table 5). Again, this appeared to be driven by changes in the relative abundance of Archaea as bacterial 16S rRNA

Table 2
Shown are the significant ($P < 0.05$) Spearman's rank correlation coefficients as determined in R (R Development Core Team, 2010) for relationships between soil chemical parameters and bacterial and archaeal 16S rRNA genes abundances (as determined by qPCR). Bacterial:archaeal = ratio of bacterial 16S rRNA genes/archaeal 16S rRNA genes. NS = non significant ($P > 0.05$).

	Bacterial:archaeal	Bacterial 16S rRNA genes/μg total DNA	Archaeal 16S rRNA genes/μg total DNA
Soil variable			
Moisture	0.64	NS	−0.55
C	0.89	NS	−0.78
N	0.78	NS	−0.64
NH_4^+	0.78	NS	−0.74
C:N	0.86	NS	−0.81

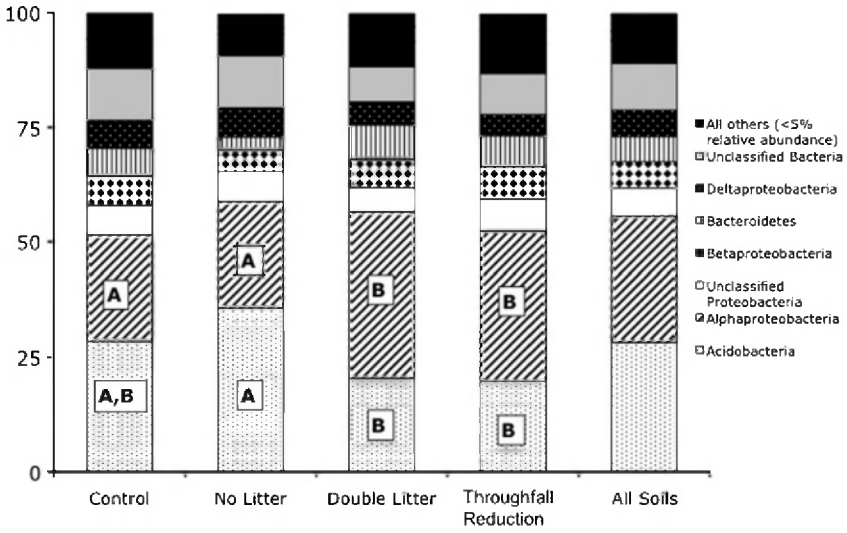

Fig. 1. Relative abundances of dominant (>5% total abundance) bacterial taxa in treatments (n = 6 for each treatment) and in all soils combined. Different letters indicate treatments that are significantly different from one another with regard to the relative abundance of Acidobacteria and Alphaproteobacteria (ANOVA, $P < 0.05$). No other dominant groups were found to be significantly different between treatments.

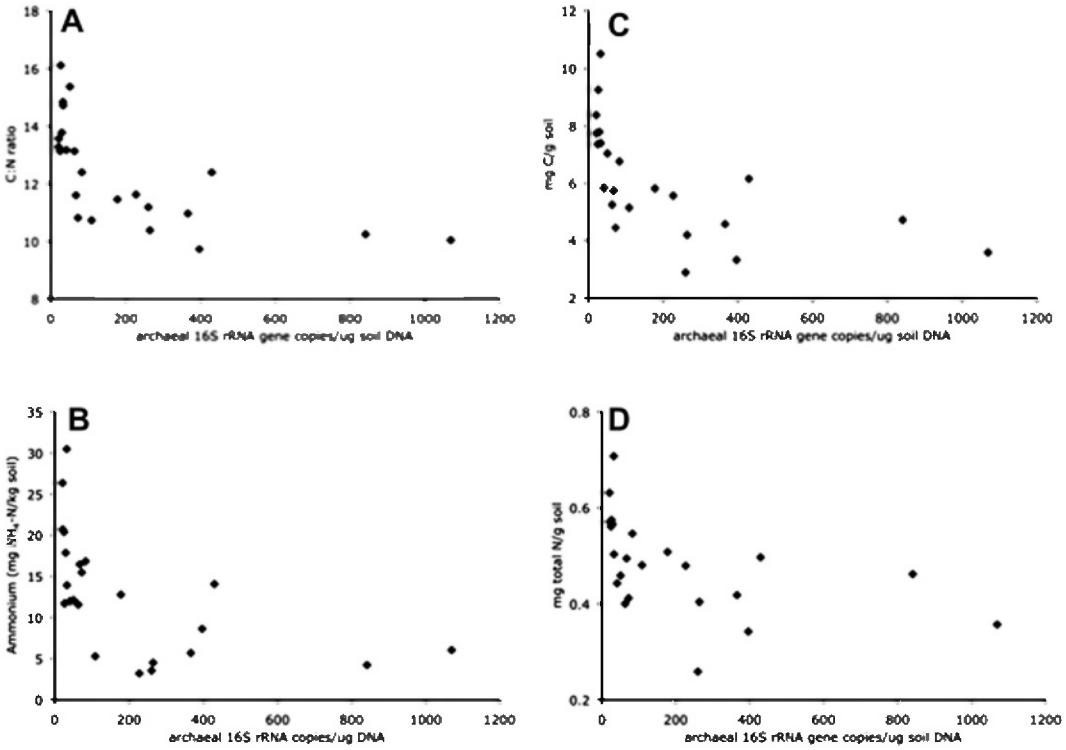

Fig. 2. Relationship between archaeal 16S rRNA gene relative abundance and soil (A) C:N ratios, (B) NH_4^+ pools, (C) C pools, and (D) N pools.

gene relative abundance did not significantly correlate with any taxonomic group (Table 5).

To determine if multiple environmental variables or taxa could predict the relative abundance of soil microbes better than single variables, we performed multiple linear regressions with backwards elimination (Hobbie et al., 2006) on the data shown in Tables 2, 4, and 5. We found that soil C:N ratios and NH_4^+ pools together explained a significantly greater amount of the variation in archaeal 16S rRNA gene relative abundance than any environmental variable alone (Fig. 3A, $R^2 = 0.72$, F-statistic = 29.23, $P < 0.001$). Likewise, the relative abundance of the Gammaproteobacteria and the Nitrospira were better predictors of archaeal 16S rRNA gene abundance than any bacterial taxon considered in isolation (Fig. 3B, $R^2 = 0.68$, F-statistic = 24.19, $P < 0.001$). Finally, total C and pH explained 65% of the variation in acidobacterial relative abundance (Fig. 4, $R^2 = 0.65$, F-statistic = 19.06, $P < 0.001$).

4. Discussion

On average, the microbial community composition in these soils contained high proportions of Alphaproteobacteria and Acidobacteria (Fig. 1), phyla that are abundant in many other soils (Janssen, 2006; Lauber et al., 2009). Actinobacteria, which are also typically numerically significant soil organisms (Janssen, 2006; Lauber et al., 2009), comprised an average of only 3.0% of the communities that we examined. This may be due to the low pH of our soils (Table 1), as actinobacterial relative abundance has been shown to positively correlate with soil pH (Lauber et al., 2009). Taken as a whole, our data demonstrate that multiple aspects of soil C and N cycling in this environment vary in concert with the relative abundance of specific phylogenetic groups of microorganisms including the Archaea, Acidobacteria, Actinobacteria, and the Alpha- and Gammaproteobacteria. Our data also support the copiotroph/oligotroph functional classification model proposed by Fierer et al. (2007). In addition, we found that archaeal:bacterial ratios, as well as the overall bacterial community composition varied by treatment.

As expected, the addition or removal of litter changed a wide range of soil chemical properties; with the exception of NH_4^+ pools and soil pH, all variables were significantly different between the no-litter and the double-litter plots (Table 1). Interestingly, in throughfall reduction plots the soil chemical characteristics we analyzed were statistically indistinguishable from the double-litter plots. Other work on these plots (Cleveland et al., 2010; Wieder et al., unpublished data) shows that both double litter and throughfall reduction manipulations significantly increase the concentration of DOM inputs to surface soils to a similar extent, driving increases in both CO_2 and N_2O fluxes in both treatment types (relative to controls). In general, of the four plot types considered here, litter removal plots appear to be the most resource-poor, while the double-litter and throughfall reduction plots are similarly resource-rich (Table 1).

Those soil chemical differences corresponded with shifts in bacterial community composition between treatments. The no-litter plots harbored a greater relative abundance of Acidobacteria and relatively fewer Alphaproteobacteria as well as

Table 3
Shown are significant ($P < 0.05$) Spearman's rank correlation coefficients for the overall bacterial community structure and various soil chemical parameters using the RELATE test in PRIMER v6 (Clarke and Warwick, 2001). The best correlate of the microbial community structure is indicated in bold for each metric. NS = non significant ($P > 0.05$).

	UniFrac	OTU (Bray–Curtis)
Soil variable		
Microbial biomass C	0.21	NS
Moisture	0.24	NS
Microbial biomass N	0.18	NS
C	0.24	0.22
N	**0.35**	0.19
C:N	NS	0.21
pH	0.28	**0.35**

Table 4
Shown are significant ($P < 0.05$) Spearman's rank correlation coefficients for comparisons of the relative abundance of specific bacterial taxa and soil chemical variables. Soil C:N ratios did not yield significant relationships with the relative abundance of any bacterial taxa. Taxa that did not show a correlation coefficient greater than ±0.60 are not shown. NS = non significant ($P > 0.05$). Spearman's rank correlation coefficients were determined in R (R Development Core Team, 2010).

	Microbial biomass C	Soil moisture	Microbial biomass N	Soil N	Soil C	Soil pH
Taxon (av. relative abundance, range)						
Gammaproteobacteria (2.1%, 0.2–4.2%)	0.70	0.70	0.54	0.73	0.76	NS
Alphaproteobacteria (28.8%, 16.4–42.6%)	0.52	0.52	0.54	0.72	0.68	NS
Actinobacteria (3.0%, 1.2–6.0%)	0.59	NS	0.65	0.52	0.53	NS
Acidobacteria (26.0%, 14.4–42.6%)	−0.60	−0.69	−0.41	−0.73	−0.66	−0.66

higher Acidobacteria:Proteobacteria ratios than the double-litter plots (Fig. 1), a pattern that was recently demonstrated in a field-scale manipulation of C availability using vegetation removal (Thomson et al., 2010). As they did for soil chemical properties, the double-litter and throughfall reduction plots grouped together in terms of the relative abundance of Acidobacteria and Alphaproteobacteria and Acidobacteria:Proteobacteria ratios. The relatively lower abundance of Acidobacteria in the two most resource-rich plots, as well as the highest acidobacterial abundances in the no-litter plots, supports the copiotrophic/oligotrophic model of community effects on decomposition (Fierer et al., 2007).

When we analyzed the relationships between soil chemical parameters and community structure, we found that moisture negatively correlated with the relative abundance of Acidobacteria (Table 4). A recent field manipulation of soil moisture revealed that Acidobacteria are relatively more abundant in dry soils than wet soils (Castro et al., 2010). Consistent with that observation, an analysis of three acidobacterial genomes revealed the potential for these organisms to produce molecules that modulate desiccation resistance (Ward et al., 2009), which may offer Acidobacteria a competitive advantage in drier environments.

We also showed that microbial biomass C and N, soil C, total N, and pH all negatively correlated with acidobacterial relative abundance. As mentioned above, other studies have noted a negative relationship between soil organic matter pools and acidobacterial abundance (Smit et al., 2001; Fierer et al., 2007). Indeed, in a previous experiment using soils from this site, we observed that the relative abundance of Acidobacteria decreased in response to labile C additions in the laboratory (Cleveland et al., 2007). Interestingly, a pyrosequencing-based global-scale analysis of 88 different soils did not reveal a negative relationship between soil C and acidobacterial relative abundance, but instead revealed that soil pH was the best predictor of acidobacterial relative abundance (Jones et al., 2009). Our results demonstrate that soil pH and C together explained more of the variation in acidobacterial relative abundance in these soils than any variable considered in isolation (Fig. 4).

The relative abundance of Alphaproteobacteria was higher in the throughfall reduction and double-litter plots (Fig. 1), a pattern that has been noted in other C-rich soils (Smit et al., 2001; Thomson et al., 2010). Interestingly, 85% of these organisms were related to the Rhizobiales (an N-fixing order of soil bacteria), suggesting that changes in N fixation may be an important consequence of the observed shifts in community structure. Although we did not measure N fixation directly in this study, previous results indicate that rates are substantially higher in the double-litter plots than the control soils (W. Wieder, unpublished data). Together, these data provide a mechanistic explanation for the observed increase in N pools in these treatments (Table 1), and the strong correlation between soil N and Alphaproteobacteria (Table 4). For example, increases in DOC concentrations in both the throughfall reduction and double-litter plots (Cleveland et al., 2010; Wieder et al., unpublished data) would be predicted to increase microbial N demand due to microbial stoichiometric homeostasis (Cleveland and Liptzin, 2007), selecting for a greater relative abundance of organisms that have the ability to fix N (e.g., Rhizobiales).

In a previous laboratory study, experimental DOM additions elicited an increase in the relative abundance of Gammaproteobacteria that correlated with significant increases in soil respiration: 24 h after C additions, Gammaproteobacteria had increased from undetectable levels to comprise over 25% of the community (Cleveland et al., 2007). In our field experiment, the relative abundance of Gammaproteobacteria was also positively correlated with multiple measures of soil organic matter dynamics, including soil C, N, and microbial biomass (Table 4). Additionally, Gammaproteobacteria were significantly more abundant in the throughfall reduction and the double-litter plots than in the control and no-litter treatments (ANOVA, $P < 0.05$), but they nonetheless comprised less than 5% of the bacterial community in all soils. Finally, we did not see a significant relationship between betaproteobacterial relative abundance and soil organic matter as was observed by Fierer et al. (2007). However, Fierer et al. (2007) used a qPCR-based approach to examine changes in community composition in response to C additions, which may not be sufficiently sensitive to distinguish between Gamma- and Betaproteobacteria because of their close phylogenetic relationship.

Bacterial:archaeal ratios varied by a factor of 70 across all soils and treatments analyzed. It is difficult to examine the relative contributions of changes in bacterial vs. archaeal relative abundance to the observed shifts in bacterial:archaeal ratios because of potential differences in PCR inhibitors between samples (Fierer et al., 2005). However, the fraction of archaeal 16S rRNA genes to total soil DNA was variable across samples, while bacterial relative abundance appeared fairly stable (data not shown). As very few soil archaea have been studied in pure culture, the functional

Table 5
Shown are significant ($P < 0.05$) Spearman's rank correlation coefficients for comparisons of the relative abundance of specific bacterial taxa and the bacterial to archaeal ratio as well as the relative abundance of archaea and bacteria as determined by qPCR. NS = non significant ($P > 0.05$). Bacterial:archaeal = ratio of bacterial 16S rRNA genes/archaeal 16S rRNA genes.

	Taxon (av. relative abundance, range)				
	Gammaproteobacteria (2.1%, 0.2–4.2%)	Alphaproteobacteria (28.8%, 16.4–42.6%)	Nitrospira (1.6%, 0–8.0%)	Actinobacteria (3.0%, 1.2–6.0%)	Acidobacteria (26.0%, 14.4–42.6%)
Bacterial:archaeal	0.81	0.70	−0.52	0.62	−0.66
Bacterial 16S rRNA genes/μg total DNA	NS	NS	NS	NS	NS
Archaeal 16S rRNA genes/μg total DNA	−0.78	−0.63	NS	−0.59	0.58

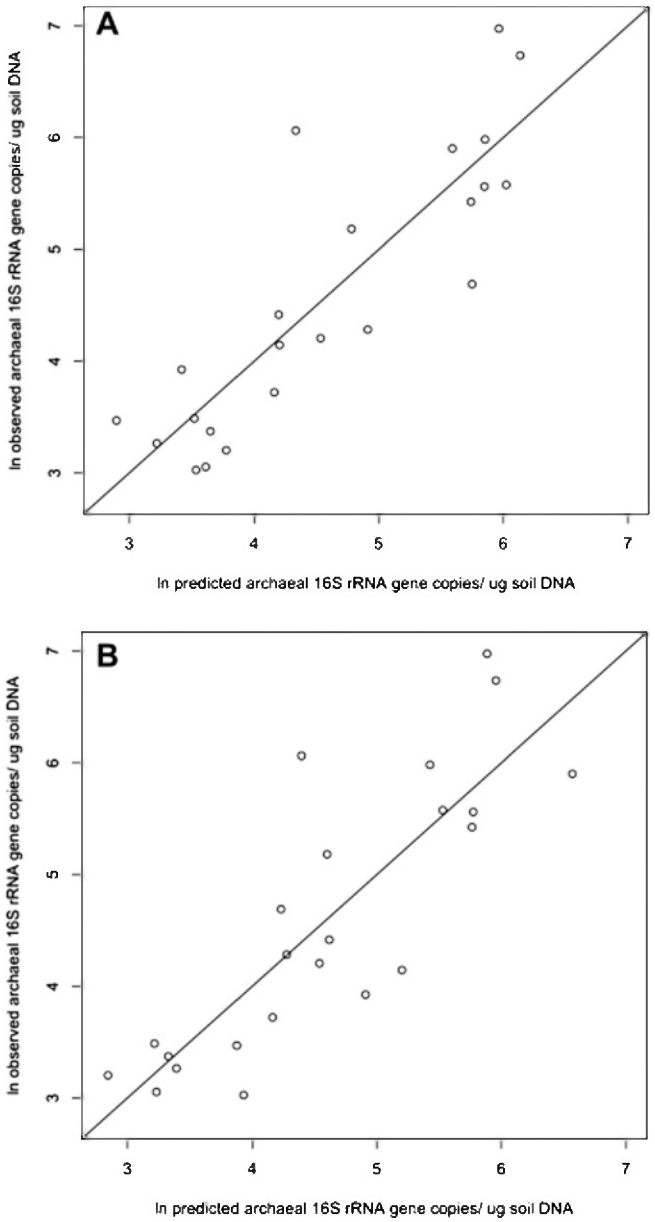

Fig. 3. Multiple linear regression models describing the relative abundance of archaeal 16S rRNA genes based on (A) soil NH_4^+ pools and C:N ratios (ammonium: t-value = −2.74, $P = 0.01$; C:N: t-value = −3.88, $P < 0.001$; ammonium and carbon:nitrogen together: $R^2 = 0.72$, F-statistic = 29.23, $P < 0.001$), and (B) the relative abundance of Nitrospira and Gammaproteobacteria (Nitrospira: t-value = 2.49, P value = 0.02; Gammaproteobacteria: t-value = −4.97, P value < 0.001; Nitrospira and Gammaproteobacteria together: $R^2 = 0.68$, F-statistic = 24.19, $P = 4.6 \times 10^{-6}$).

implications of the correlations observed in this work (Tables 2 and 5) are unknown.

In summary, plot-scale manipulations of organic matter inputs to soils elicited significant shifts in both soil chemical properties and microbial community composition. Litter removal caused declines in soil C pools and soil C:N ratios when compared to litter additions, and the lower total C pools correlated with increases in the relative abundance of both Acidobacteria and Archaea. In the more organic matter-rich plots, higher soil C pools and C:N ratios correlated with increases in the relative abundance of Alpha- and Gammaproteobacteria. The Alphaproteobacteria in the high-C plots were closely related to heterotrophic N-fixers, whose activity may lead to an increase in NH_4^+ pools. These data support the role of the

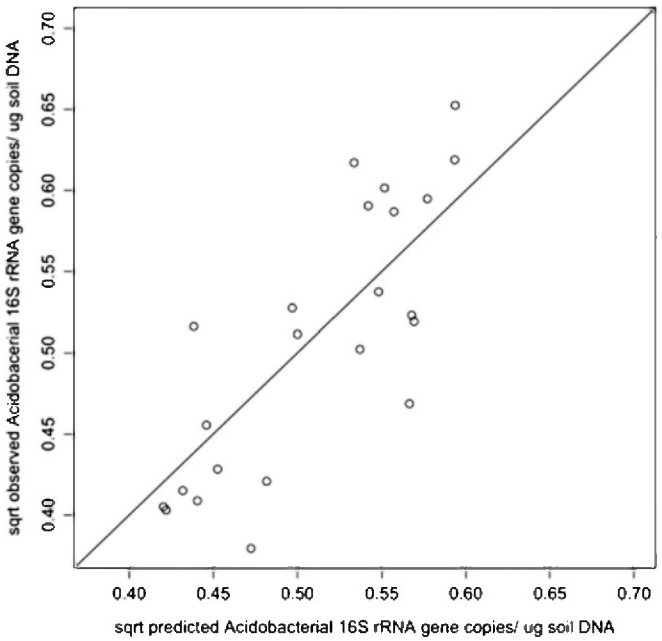

Fig. 4. Multiple linear regression model describing the relative abundance of acidobacterial 16S rRNA genes based on soil pH and soil C (soil C: t-value = −3.61, $P < 0.01$; pH: t-value = −3.68, $P < 0.01$; pH and soil N together: $R^2 = 0.65$, F-statistic = 19.06, $P < 0.001$).

environment in structuring microbial community composition (Martiny et al., 2006). Our results also suggest that rates of phylogenetically widely distributed biogeochemical processes such as heterotrophic decomposition may depend on the relative abundance of specific taxa, and champion the oligotrophic/copiotrophic model proposed by Fierer et al. (2007). Finally, given the similarity in some of the patterns of relative abundance for the different taxa examined (e.g., Actinobacteria, Alphaproteobacteria and Gammaproteobacteria vs. Acidobacteria and Archaea, Tables 2 and 4), and the significant correlations observed between the relative abundance of different taxa (Table 5, Fig. 3B) our results support a recent metaanalysis that demonstrates that diverse suites of microbial lineages tend to co-occur across different environments (Chaffron et al., 2010).

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