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# Changes in microbial community characteristics and soil organic matter with nitrogen additions in two tropical forests

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*Abstract*. Microbial communities and their associated enzyme activities affect the amount and chemical quality of carbon (C) in soils. Increasing nitrogen (N) deposition, particularly in N-rich tropical forests, is likely to change the composition and behavior of microbial communities and feed back on ecosystem structure and function. This study presents a novel assessment of mechanistic links between microbial responses to N deposition and shifts in soil organic matter (SOM) quality and quantity. We used phospholipid fatty acid (PLFA) analysis and microbial enzyme assays in soils to assess microbial community responses to long-term N additions in two distinct tropical rain forests. We used soil density fractionation and $^{13}C$ nuclear magnetic resonance (NMR) spectroscopy to measure related changes in SOM pool sizes and chemical quality. Microbial biomass increased in response to N fertilization in both tropical forests and corresponded to declines in pools of low-density SOM. The chemical quality of this soil C pool reflected ecosystem-specific changes in microbial community composition. In the lower-elevation forest, there was an increase in gram-negative bacteria PLFA biomass, and there were significant losses of labile C chemical groups (O-alkyls). In contrast, the upper-elevation tropical forest had an increase in fungal PLFAs with N additions and declines in C groups associated with increased soil C storage (alkyls). The dynamics of microbial enzymatic activities with N addition provided a functional link between changes in microbial community structure and SOM chemistry. Ecosystem-specific changes in microbial community composition are likely to have far-reaching effects on soil carbon storage and cycling. This study indicates that microbial communities in N-rich tropical forests can be sensitive to added N, but we can expect significant variability in how ecosystem structure and function respond to N deposition among tropical forest types.

*Key words:* *$^{13}C$ NMR; extracellular enzymes; fertilization; Luquillo Experimental Forest, Puerto Rico; nitrogen deposition; nuclear magnetic resonance; nutrient availability; phospholipid fatty acid analysis; PLFA.*

## Introduction

Microbial communities play key roles in carbon (C) and nutrient cycling in ecosystems. Global change drivers such as nitrogen (N) deposition are likely to feed back on ecosystem C and nutrient cycling through the effects on microbial community composition and activity. Nitrogen deposition is increasing rapidly in tropical regions (Galloway et al. 2004), where forests on highly weathered soils tend to be rich in N relative to temperate forests. In these ecosystems, plant growth is typically not N-limited, and large N losses may indicate cycling in excess of plant demand (Vitousek and Farrington 1997, Martinelli et al. 1999, Mirmanto et al. 1999, Harrington et al. 2001). These tropical forests are also characterized by labile soil C pools with rapid turnover times relative to temperate sites (Trumbore 1993). The quantity and chemical quality of this soil C pool is closely coupled with microbial activity (Baldock et al. 1992), yet little is known about how N deposition in tropical regions may alter this relationship.

Long-term N addition to temperate ecosystems can decrease microbial biomass and fungal : bacterial ratios, probably because of N saturation and/or soil acidification in these N-poor ecosystems (Soderstrom et al. 1983, Smolander et al. 1994, Lee and Jose 2003, DeForest et al. 2004, Frey et al. 2004, Wallenstein et al. 2006, Demoling et al. 2008). Decomposition responded both positively and neutrally to increased N in a range of tropical forests (Hobbie and Vitousek 2000, Allison and Vitousek 2004, Waldrop and Firestone 2004, Cusack et al. 2009*a*), suggesting that specific microbial decomposers are sensitive to N availability in N-rich ecosystems.

Soil enzyme activities can provide useful insights into the mechanisms of microbial sensitivity to added N. Soil enzymes can be divided into two broad groups: (1)

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hydrolytic enzymes responsible for the acquisition of C, N, and phosphorus (P) to support primary metabolism; and (2) oxidative enzymes, produced primarily by fungi, which degrade poor-quality, chemically complex compounds like lignin in cometabolic acquisition of nutrients (Sinsabaugh and Moorhead 1994, Sylvia et al. 2004). These groups of enzymes may respond differently to N addition. Where available N is low, N fertilization should shift enzyme production toward increased C or P acquisition, while decreasing the production of oxidative enzymes, which generally scavenge N occluded in plant structural materials (Sinsabaugh and Moorhead 1994). Several temperate-zone studies have observed these expected trends in enzyme responses to N fertilization (Keyser et al. 1978, Kirk and Farrell 1987, Carreiro et al. 2000), although there has been some variability in responses among fungal groups and litter types (Fog 1988, Saiya-Cork et al. 2002, Waldrop et al. 2004). Such changes in decomposer activity provide a key mechanism by which N deposition may alter soil C cycling in tropical forests. It is important to note that enzyme activity provides a functional measure, which can vary independently of microbial community structure (Waldrop et al. 2004).

We used an ongoing N-fertilization experiment in two distinct, N-rich, tropical forests to assess microbial responses to increased N availability, and related changes in the quantity and quality of rapidly cycling soil C. The pool of rapidly cycling C, often operationally defined as low-density soil organic matter (SOM), is central to nutrient cycling and microbial maintenance and is generally most responsive to disturbance (Trumbore 1993, Swanston et al. 2005, Marin-Spiotta et al. 2009). Prior work at these sites revealed a significant decline in rapidly cycling soil C pools with N fertilization for both forest types, with no change in aboveground inputs of C to soils (Cusack et al., *in press*).

We hypothesized that N fertilization in humid tropical forests would increase microbial biomass because of N limitation to the decomposition process (Fig. 1). We also hypothesized that the observed declines in low-density soil C fractions would be characterized by preferential losses of the most labile C compounds, resulting from increased hydrolytic enzyme activities and decreased oxidative enzyme activities (Fig. 1A). We predicted that changes in enzyme activities and soil C chemistry would feed back on microbial substrate utilization. Finally, we explored whether changes in soil enzyme activities with N additions were strictly functional, or if they corresponded to changes in microbial community characteristics (Fig. 1A, B). We chose two tropical forests along an elevation gradient that differed in climate and background N pools. We expected to see a larger microbial response in the upper-elevation forest, where background soil N pools are smaller than in the lower-elevation forest, typical of other tropical forest elevational gradients (Vitousek and Sanford 1986, Cusack et al. 2009*b*).

## Methods

### *Study site*

This study was conducted using soils from the Luquillo Experimental Forest (LEF), a National Science Foundation-sponsored Long Term Ecological Research (LTER) site in Puerto Rico (18.3° N, −65.8° W). Background rates of wet N deposition are relatively low in Puerto Rico (~2.1 kg $N \cdot ha^{-1} \cdot yr^{-1}$), but have more than doubled in the last two decades (National Atmospheric Deposition Program/National Trends Network [NADP/NTN] 2009). The lower-elevation site at 260 m above sea level (a.s.l.) is a wet tropical rain forest (Bruijnzeel 2001) with 3537 mm mean annual precipitation (MAP; Garcia-Montino et al. 1996). The upper-elevation site at 640 m a.s.l. is a lower montane forest characterized by abundant epiphytes and cloud influence (Bruijnzeel 2001) with 4300 mm MAP (McDowell and Asbury 1994). The long-term mean daily temperature is 23°C in the lower-elevation forest, and 21°C in the upper-elevation forests, with little temporal variability within sites in monthly rainfall and mean daily temperature (Brown et al. 1983). Soils in both forests are primarily deep, clay-rich, highly weathered Ultisols (Beinroth 1982). Exchangeable soil nutrients, tree species, and canopy structure vary significantly between the two forests (Weaver 1991, McGroddy and Silver 2000; Appendix A; see Plate 1).

Nitrogen-addition plots in each forest type were established in 2000 at sites described by McDowell et al. (1992). Three 20 × 20 m fertilized plots were paired with control plots of the same size, with ≥10-m buffers between plots. Starting in January 2002, 50 kg $N \cdot ha^{-1} \cdot yr^{-1}$ were added using a hand-held broadcaster, applied in two annual doses of $NH_4NO_3$. Soils for this experiment were collected between 2006 and 2008. Soil samples were taken from the 0–10 cm depth from each plot. This depth was chosen because it had the largest and most consistent response of soil C pool sizes to N additions at these sites (Cusack et al., *in press*). Here we present an overview of our approach. Details of field and laboratory protocols can be found in Appendix A.

### *Soil carbon pools and $^{13}C$ nuclear magnetic resonance spectroscopy*

$^{13}C$ nuclear magnetic resonance (NMR) spectroscopy was combined with density fractionation of soils to measure changes in the chemical composition of rapidly cycling soil C pools. Here two light (i.e., low-density) soil fractions were used to represent the rapidly cycling soil organic matter (SOM) pool (Christensen 1992, Swanston et al. 2004), including (1) a free light fraction (free LF), which closely resembles recent litter inputs and tends to have the fastest turnover in soils (Marin-Spiotta et al. 2008); and (2) an occluded light fraction (occluded LF), which also resembles litter inputs but is physically protected inside of soil aggregates and tends to be older than the free LF (Trumbore 1993). These

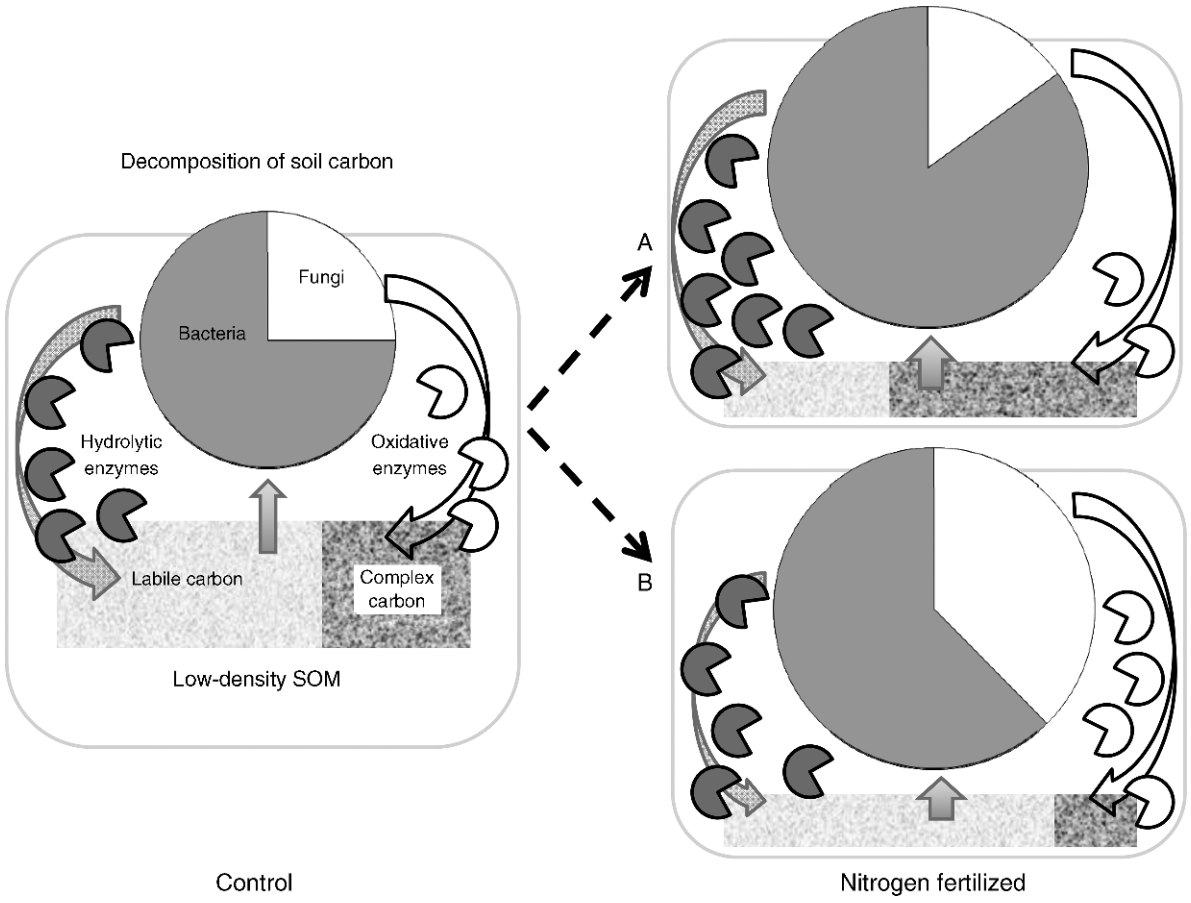


FIG. 1. Two potential effects of nitrogen additions on soil ecology are shown for two forest types in the Luquillo Experimental Forest, Puerto Rico. Both scenarios show overall increases in microbial biomass (circles) and enzyme activities (wedges), with corresponding declines in low-density soil organic matter (SOM) pools. Arrows show microbial enzymes decomposing SOM and feedbacks to the microbial community. (A) The hypothesized increase in hydrolytic enzyme activities (gray wedges) with increased bacterial biomass (gray) and declines in labile carbon compounds (light texture) in SOM. (B) An alternative shift with relative increases in fungal biomass (white), oxidative enzyme activities (white wedges), and subsequent declines in complex carbon compounds (dark texture) from SOM.

low-density soil C fractions were used because they showed strong declines with N fertilization at these sites (Cusack et al., *in press*) and thus were most likely to vary with microbial activity and community composition.

We used $^{13}C$ NMR to compare the C chemistry between fertilized and control plots, and to assess broader differences among the two soil C fractions and source materials (live and dead fine root [<2 cm diameter] tissue, and litterfall). The light C fractions, litterfall, and fine root samples were analyzed using solid-state, variable amplitude cross-polarization and magic-angle spinning (VACP MAS) $^{13}C$ NMR. Peak areas were integrated under the following seven chemical shift regions (and the C compounds they represent): 0–45 (alkyl), 45–65 (N-alkyl/methoxyl), 65–95 (O-alkyl), 95–110 (di-O-alkyl), 110–145 (aromatic), 145–165 (phenolic), and 165–220 ppm (amide/carboxyl) (Baldock et al. 2004). Alkyl C represents lipids and other aliphatics, whereas O-alkyl represents more labile carbohydrates such as cellulose (Baldock et al. 1992). The alkyl : O-alkyl can be used as an index of microbial processing, with higher ratios indicating losses of more labile C relative to poorer-quality C compounds.

### *Microbial biomass and phospholipid fatty acid analysis*

Microbial biomass was initially measured using the chloroform fumigation method (Vance et al. 1987) in August 2006. Phospholipids fatty acid (PLFA) analysis was used for soils collected in August 2007 to measure viable microbial biomass, expressed microbial community characteristics, and changes in fungal vs. bacterial abundance (Frostegard et al. 1991, Frostegard and Baath 1996). Because different plant compounds vary in their isotopic signature, we used the $\delta^{13}C$ of microbial lipids as an indication of shifting microbial substrate utilization (Ehleringer et al. 2000, Waldrop and Firestone 2004). For PLFA extractions, we followed the general methods of White and Ringelberg (1998).

Phospholipid fatty acid (FA) abundance was calculated as nmol FA/g dry soil, and the sum of all PLFA was used as a measure of viable microbial biomass (Federle 1986). The relative abundance of each PLFA (molar percent) was calculated as an indication of community composition. Indicator PLFA were grouped, including iso (i) and ante-iso (a) branched fatty acids (gram-positive bacteria), methyl (Me) branched fatty acids (actinomycetes), monounsaturated and cyclopropyl (cy) lipids (gram-negative bacteria), and 16:1ω5, 18:2ω6,9, and 18:1ω9 (fungal groups) (Federle 1986, O'Leary and Wilkinson 1988, Frostegard and Baath 1996). The 16-C can represent arbuscular mycorrhizal fungi (AMF) (Nordby et al. 1981, Olsson et al. 1995), whereas 18-C PLFAs are general fungal markers (but are negligible in AMF) (Frostegard and Baath 1996, Larsen et al. 1998). The ratio of fungi : bacteria was calculated as the ratio of (18:2ω6,9 + 18:1ω9) : (gram-positive + gram-negative bacteria) (Frostegard and Baath 1996), and the 16:0 phospholipid was used as a general marker for the microbial community (Ziegler et al. 2005).

### *Microbial enzyme analyses*

Soils were collected and analyzed for enzyme activities in August 2007 and March and June 2008. There were no statistically significant interactions between individual enzyme rates and sampling date, so we took time-averaged rates as representative of each plot for comparisons. The general methods in Sinsabaugh et al. (2003) were followed for soil microbial enzyme assays, with modifications as noted in Appendix A. Hydrolytic enzymes were grouped into C, N, and P acquisition enzymes. We calculated total enzyme activities (per g of dry soil) and specific enzyme activities (per nmol of microbial lipid biomass).

### *Statistical analyses*

We used principal components analysis (PCA) to compare general characteristics of the soil C fractions and source materials, using all $^{13}C$ NMR regions, pooling data by component ($n = 12$ for soil fractions and litter, $n = 4$ for live and dead fine roots). The effects of forest type and fertilization on soil C chemistry were tested for litter, free LF, and occluded LF using analysis of variance (ANOVA). Relationships among soil fraction C chemistry, microbial community guilds, and enzyme activities were evaluated using analysis of covariance (ANCOVA), including pH, soil C and N concentrations, and forest type as factors. A fertilization effect was calculated for response variables as the percent change between paired fertilized and control plots for microbial biomass and enzyme activities, or as the difference between paired plots for microbial composition (molar percent) and $\delta^{13}C$, which are already in proportional units. A fertilization effect $>0$ shows a positive change with fertilization between paired plots. Where significant effects were found, we ran post hoc means separation tests using Fisher's least significant difference. Analyses were performed using 7.0.2 JMP software (SAS 2007). For ANCOVA and effect size tests, data were averaged by plot ($n = 3$; all values are reported as mean ± SE). Statistical significance was determined as $P < 0.05$ unless otherwise noted.

## Results

### *$^{13}C$ nuclear magnetic resonance and soil organic matter chemistry*

The free and occluded light soil fractions had significantly different chemical profiles compared to litter and live fine roots, while dead fine roots were similar to the free light fraction (LF) (Fig. 2A). Principal components axis 1, which explained 51% of the variability, separated litter and live fine roots from the light soil C fractions and dead fine roots. This axis was most strongly correlated with O-alkyl ($R^2 = 0.71$), aromatic ($R^2 = 0.82$), and carboxylic C groups ($R^2 = 0.78$, $n = 46$; in each case $P < 0.05$). Axis 2, which explained 27% of the variability, separated litter from other organic matter. This axis was correlated with alkyl C ($R^2 = 0.49$, $n = 46$; $P < 0.05$). A series of $^{13}C$ nuclear magnetic resonance (NMR) spectra from litter to free LF to occluded LF showed a typical decomposition sequence, as did a comparison of dead vs. live fine roots, with relative declines in O-alkyl C vs. alkyl C (Appendices B and C). There was a significant positive correlation between the alkyl : O-alkyl of litter and of free LF when pooling data from the two forests ($R^2 = 0.50$, $n = 12$, $P < 0.05$). Comparing the two forest types, the upper-elevation forest had significantly higher alkyl : O-alkyl for litterfall and the free LF than the lower-elevation forest. Aromatic and phenolic content were also higher in upper-elevation litter (Appendix B).

Light fraction soil organic matter (SOM) chemistry responded differently to added N in the two tropical forests, with losses of more labile C constituents in the lower-elevation forest, and losses of aliphatic C compounds in the upper-elevation forest. Differences between control and fertilized plots were strongest for the free LF in both forests, with significant shifts in the proportion of alkyls vs. O-alkyls/di-O-alkyls (Fig. 2B). In the lower-elevation forest, N additions led to significant declines in the proportion of O-alkyls (–6.9% ± 2.9%; $P = 0.07$) and declines in di-O-alkyls (–12.0% ± 0.8%; $P < 0.05$) in the free LF, representing relative losses of carbohydrates and cellulose from SOM. In contrast, the upper-elevation forest had an increase in O-alkyls (+18.1% ± 6.6%; $P < 0.05$) and di-O-alkyls (+13.7% ± 1.6%; $P < 0.05$) in the free LF, whereas alkyl C declined (–16.5% ± 5.9%; $P = 0.07$), leading to a significant decline in alkyl : O-alkyl, and representing a relative increase in labile C groups and losses of hydrophobic C (aliphatics, waxes, and cutin) in SOM. The occluded LF had fewer significant chemical changes with fertilization, but tended to follow the same patterns as the free LF (Appendix B). The only fertilization effect on litterfall C chemistry was an

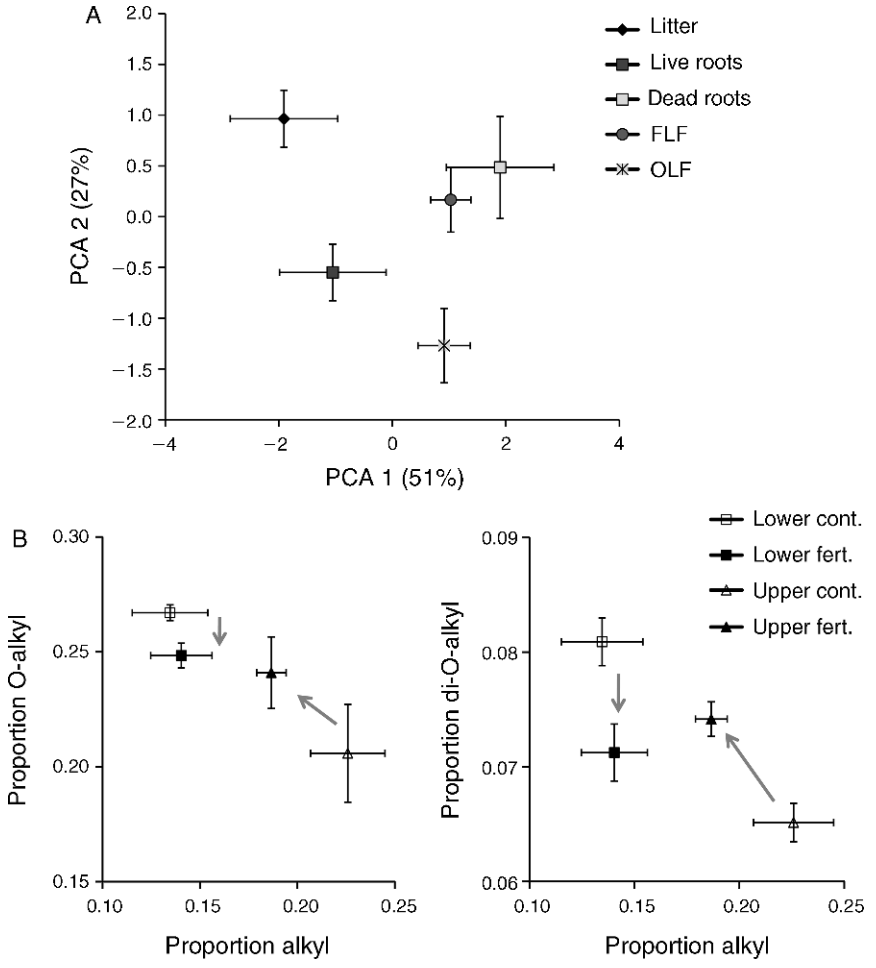


FIG. 2. (A) Results from principal components analysis (PCA) pooling $^{13}C$ nuclear magnetic resonance chemical data from two tropical forests for soil organic matter (SOM) source materials (leaves and live fine roots) and microbially processed soil C pools, including free light fraction (FLF), occluded light fraction (OLF), and dead roots; $n = 12$ for litter, FLF, and OLF; $n = 4$ for roots. (B) Effects of nitrogen fertilization on SOM chemistry as changes in poor-quality carbon (alkyl) relative to labile C compounds (O-alkyl and di-O-alkyl C) in the FLF from 0 to 10 cm, for lower-elevation forest control sites (Lower cont.), lower-elevation forest fertilized sites (Lower fert.), upper-elevation forest control sites (Upper cont.), and upper-elevation forest fertilized sites (Upper fert.). Axes show the proportion of total soil C observed for each chemical shift region. Gray arrows show the direction of change with N fertilization for each forest type. Error bars indicate ±SE.

increase in the proportion of O-alkyls in fertilized plots in the upper-elevation forest (+6.7% ± 1.3%; $P < 0.05$).

### *Microbial community structure*

The strongest fertilization effect of N addition on microbial biomass was in the upper-elevation forest, which had a 36% ± 23% increase in total lipids ($P = 0.1$). Chloroform fumigation showed a trend toward greater microbial biomass C with N fertilization in both forests. Background microbial phospholipid biomass was larger in the lower- vs. the upper-elevation forest, which had significantly higher fungal and bacterial lipids (Appendix D). Microbial phospholipid fatty acid (PLFA) composition in the two tropical forests responded differently to fertilization. In the lower-elevation forest, there was significantly higher bacterial abundance (molar percent; $P < 0.05$) relative to other groups and a trend of higher gram-negative bacterial biomass ($P = 0.07$; Fig. 3) in fertilized vs. control plots, driven by a large increase in the biomass of *cy*19:0 (Appendix D). In the upper-elevation forest, N fertilized soils had significantly higher biomass and molar percentage of fungal lipids 16:1ω5 and 18:2ω6,9 vs. controls (Fig. 3; Appendices D and E). These responses to N fertilization tended to shift fungi : bacteria ratios in different directions in the two forest types (Appendix D). Changes in the microbial community were correlated with changes in free LF soil C chemistry in both forests. In the lower-elevation forest there was a significant negative correlation between gram-negative biomass and di-O-alkyl C in the free LF ($R^2 = 0.71$, $n = 6$; $P < 0.05$). In the upper-elevation forest there was a significant negative correlation between the relative abundance of

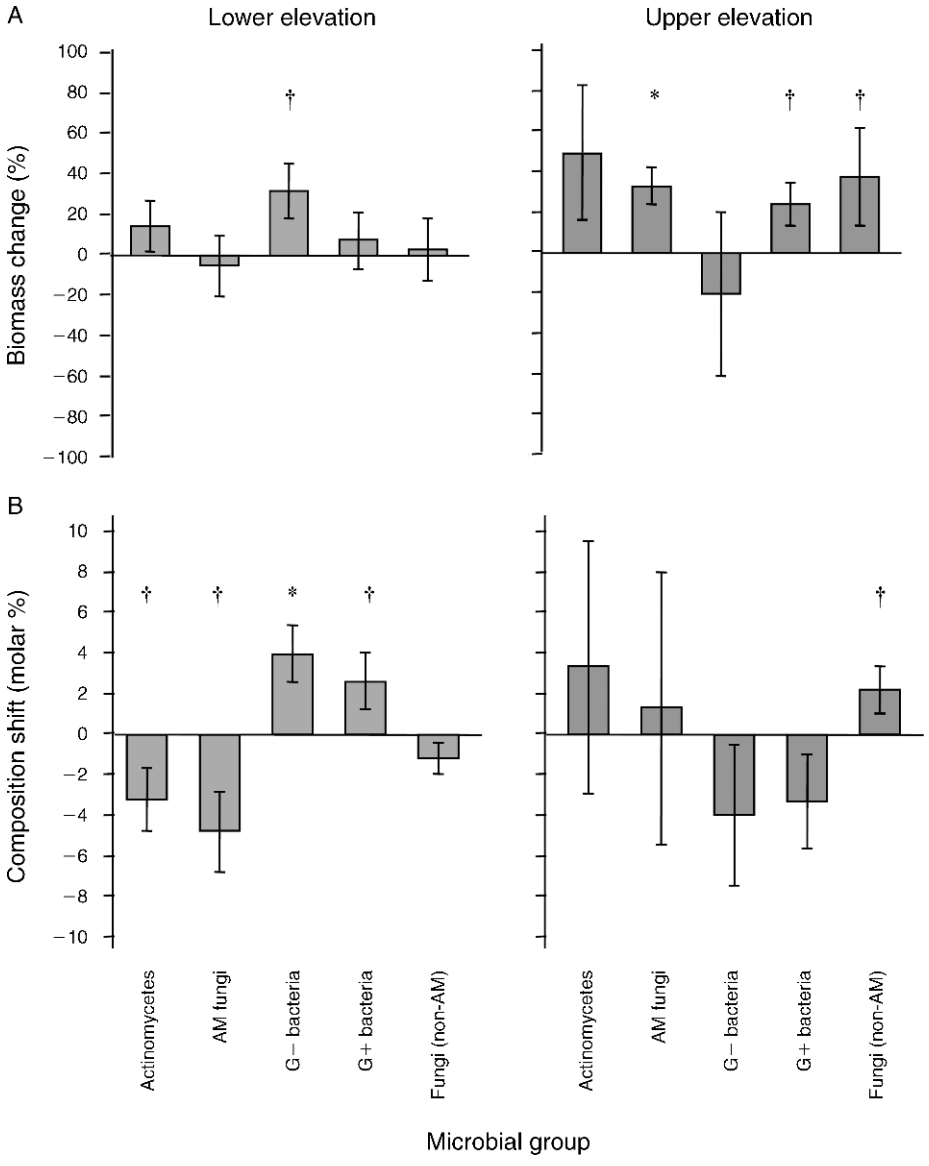


FIG. 3. (A) The effect of N fertilization on percentage change in microbial phospholipid fatty acid (FA) biomass, as measured in nmol FA/g soil. (B) Change in relative abundance of microbes for microbial groups in two tropical forest types (lower and upper elevation). Key to abbreviations: AM, arbuscular mycorrhizal; G+, gram-positive; G–, gram-negative. Error bars indicate ±SE.
* $P < 0.05$, using paired Student's $t$ tests for differences from zero; † $P < 0.1$.

fungi and alkyl C in the free LF ($R^2 = 0.72$, $n = 6$; $P < 0.05$).

### *Microbial enzyme activities*

Oxidative enzyme activities showed the strongest response to N fertilization among enzyme groups. Total oxidative enzyme activities were highly variable among plots within each forest type (Appendix F), reflecting landscape-scale variability. However, when compared between paired plots, fertilization significantly suppressed peroxidase and phenol oxidase activities in the lower-elevation forest, but increased phenol oxidase activity in the upper-elevation forest (Fig. 4A). Responses in hydrolytic enzymes were more varied. In the lower-elevation forest there were significant increases in total cellobiohydrolase (+33% ± 5%) and xylosidase (+8% ± 1%) activities with fertilization (Fig. 4A). In the upper-elevation forest, there was a trend of decreased phosphatase activity with fertilization ($P = 0.1$). In general, the lower-elevation forest had significantly higher total hydrolytic enzyme activities, while the upper-elevation forest had higher total oxidative activities (Appendix E).

Within each forest, total enzyme activities were most strongly correlated with microbial community characteristics, whereas trends across the two forests were

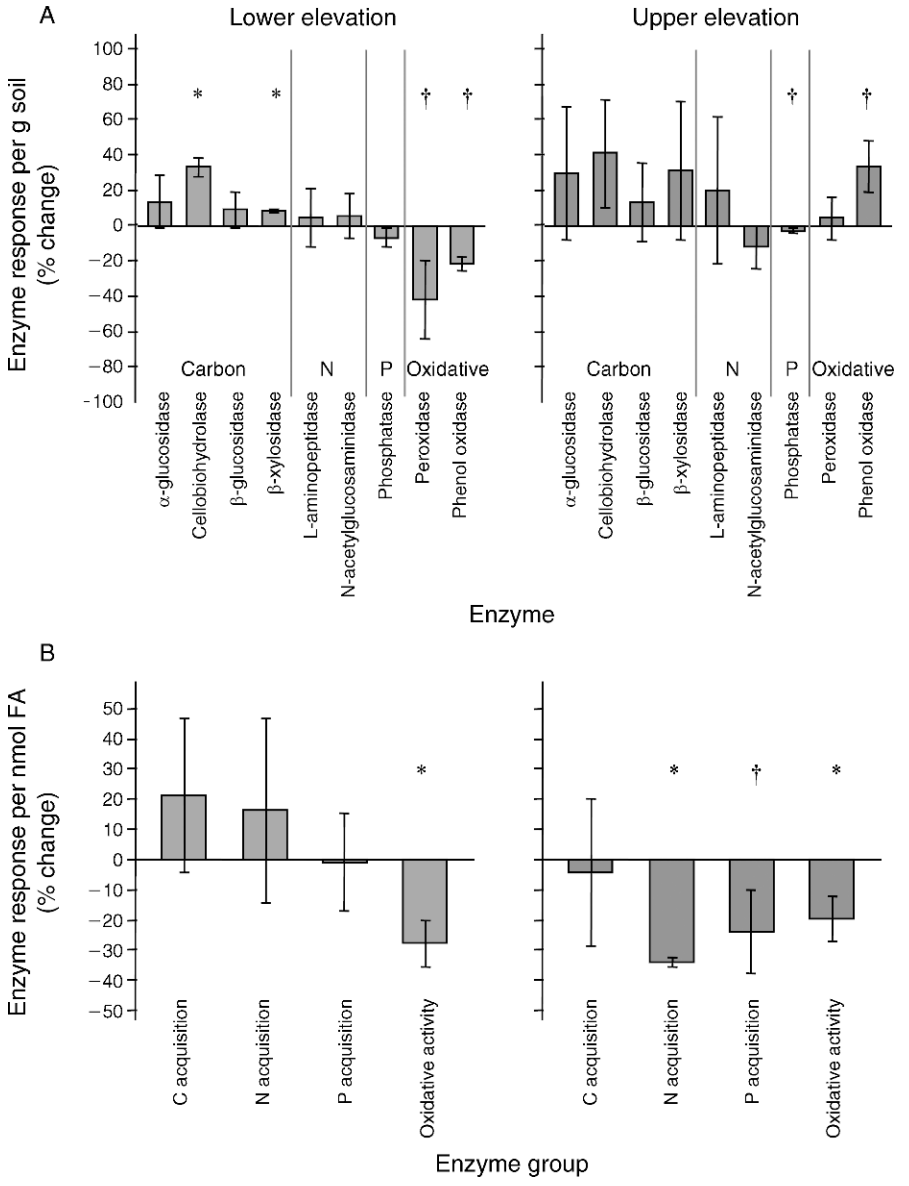


FIG. 4. (A) The percentage change in total enzyme activity per gram of soil between paired fertilized and control plots for microbial enzymes; hydrolytic enzymes are divided by the nutrient acquired (C, N, or P). (B) The percentage change in specific enzyme activity per nmol of microbial fatty acid (FA), grouping enzymes by acquisition activity of C, N, and P, or oxidative activity ($n = 3$). Error bars indicate ±SE.

* $P < 0.05$, using paired Student's $t$ tests for differences from zero; † $P < 0.1$.

correlated with ecosystem-level changes in litterfall chemistry. Within each forest, biomass of the 16:1ω5 fungal PLFA was the strongest predictor of oxidative enzyme activity ($R^2 = 0.39$ in the lower-elevation forest, $R^2 = 0.73$ in the upper-elevation forest, $n = 6$ for each, both positive relationships). Pooling data across forests, litter alkyls were significantly negatively correlated with total C and N acquisition activities ($R^2 = 0.39$ and 0.31, respectively, $n = 12$ each; $P < 0.05$), and litter O-alkyls were negatively correlated with total oxidative activity ($R^2 = 0.34$, $n = 12$; $P < 0.05$).

While total enzyme activities followed changes in the biomass of microbial groups within forest types, specific enzyme activities (i.e., per nmol of microbial lipid) showed a similar trend in the two sites. Fertilized plots had significantly lower specific oxidative enzyme activities in both forests (Fig. 4B) and significantly lower specific N and P acquisition in the upper-elevation forest.

### *Microbial phospholipid fatty acid $\delta^{13}C$*

A significant positive fertilization effect on phospholipid $\delta^{13}C$ in the lower-elevation forest suggested a shift

PLATE 1. The elevation gradient in the Luquillo Experimental Forest, Puerto Rico. Sites for this study represent two points along this gradient: a wet subtropical rainforest (260 m above sea level [a.s.l.]) and a lower montane forest (640 m a.s.l.). Climate, soil nutrients, tree species, canopy structure, and cloud influence vary significantly along the elevational gradient. Photo credit: D. F. Cusack.

in substrate utilization, with increases of 3.5‰ ± 0.8‰ for AMF ($P < 0.05$), 3.7‰ ± 1.4‰ for gram-negative bacteria ($P = 0.08$), 5.4‰ ± 2‰ for gram-positive bacteria ($P < 0.05$), and 2.8‰ ± 0.7‰ for the general 16:0 lipid ($P < 0.05$; Appendix E). Total oxidative enzyme activity was the strongest predictor of the $\delta^{13}C$ of microbial PLFA, with negative correlations in both forests (for the 16:0 general PLFA, $R^2 = 0.84$ and 0.40 in the lower- and upper-elevation forests, respectively, $n = 6$; $P < 0.05$). This corresponded to the decline in total oxidative enzyme activities and enrichment of $^{13}C$ in PLFAs with fertilization in the lower-elevation forest. Phospholipids in the lower-elevation forest tended to be more depleted in $\delta^{13}C$ than in the upper-elevation forest. The $\delta^{13}C$ of forest floor litter was −29.3‰ ± 0.7‰ in the lower-elevation forest and −28.2‰ ± 0.2‰ in the upper-elevation forest (not significant).

## DISCUSSION

We observed significant shifts in microbial community structure and function with N addition in both of the tropical forests studied. However, the nature of the response was different for the two forests, corresponding to divergent shifts in soil organic matter (SOM) chemistry. In the lower-elevation forest, bacterial decomposers increased in importance and were associated with greater hydrolytic enzyme activities and a decrease in the most labile C compounds in SOM (Fig. 1A). The upper-elevation forest followed the trend hypothesized in Fig. 1B, with an increase in fungal importance and oxidative enzyme activities and a decrease in poor-quality C compounds. Interest in the relationship between microbial community structure and ecosystem processes has been growing rapidly (Lewis et al. 2010, Malchair et al. 2010, Peralta et al. 2010), yet few studies explore linkages between microbial community structure and the dynamics of soil C chemistry under elevated N. In one of the few studies on this topic, N fertilization in an alpine ecosystem increased bacterial abundance for some groups and suppressed decomposer fungal abundance, with detectable increases in complex C compounds in SOM (Nemergut et al. 2008). Similarly, N fertilization in northern United States hardwood and pine forests also suppressed fungal biomass, oxidative enzyme activity, and the processing of some C compounds (Frey et al. 2004). Both of these studies found results similar to ours for the lower-elevation forest and agree with our hypothesis. In the upper-elevation Puerto Rican forest, fungal abundance and oxidative enzyme activities increased, and this increase was strongly correlated with losses of aliphatic C (alkyls, found in leaf waxes and cutin), which was surprising for two reasons. First, we expected fungi to respond negatively to added N because of their cometabolic decomposition strategy. Second, oxidative enzyme activities are typically linked to losses of aromatic C (found in lignin), as observed in northern United States hardwood forests (Gallo et al. 2005). Losses of (hydrophobic) aliphatic C compounds may be especially significant in wet tropical forests, where these

compounds have been related to increased soil C storage (Ostertag et al. 2008). These results indicate that there is likely to be significant variability in microbial community structural and functional responses to N deposition among tropical forest types, with potential effects on soil C cycling.

Functional shifts in decomposer activity have not always been accompanied by clear changes in the microbial community at other sites (Gallo et al. 2004, Waldrop and Firestone 2004). In this study, the increase in hydrolytic enzyme activities varied with bacterial abundance and microbial biomass C in the lower-elevation forest, as might be expected in an ecosystem characterized by rapid turnover of organic matter with a high proportion of labile C constituents. More surprisingly, oxidative enzyme activities were strongly correlated to fungal lipid 16:1ω5 in the upper-elevation forest. This marker has most commonly been associated with arbuscular mycorrhizal fungi (AMF) (Nordby et al. 1981, Olsson et al. 1995, Balser et al. 2005), though not always (Olsson and Johnson 2005). Although AMF likely decompose simple N-containing organic compounds in some ecosystems (Tu et al. 2006, Talbot et al. 2008, Whiteside et al. 2009), it is unlikely that they are directly responsible for the production of oxidative enzymes, which are typically produced by saprotrophic fungi (Fog 1988). If this lipid is indeed a marker for AMF in these forests, as in some Hawaiian forests (Balser et al. 2005), then the correlation with oxidative enzymes may result from a positive interaction between AMF and other fungal groups (Starnaud et al. 1995). In general, our data indicate that N deposition may lead both to functional shifts in soil microbial activity and to deeper structural changes in the microbial community.

Notably, the different responses of oxidative enzyme activities to N additions that we observed did not vary with leaf litter chemistry in the same way as northern hardwood systems. There, forest types with poorer litter quality (high lignin and low N) showed the greatest declines in oxidative activity with N addition (Waldrop et al. 2004). On the contrary, we saw increases in oxidative enzymes in the upper-elevation forest, which had litter with higher alkyl : O-alkyl and higher leaf litter lignin concentrations in the dominant tree species (*Cyrilla racemiflors* L., 22.1% lignin) than in the lower-elevation forest (*Dacryodes excelsa* Vahl, 16.6% lignin) (Sullivan et al. 1999). The oxidative enzyme response observed here appears to be more related to local microbial community responses to increased N than to differences in litter quality between sites.

The net changes in microbial biomass and SOM pool sizes were similar at the two sites, in contrast to the patterns in microbial communities and SOM chemistry. In both Puerto Rican forests, aboveground inputs of C to soils did not change with N fertilization (Cusack et al., *in press*), so the decline in the low-density soil C fractions is most likely related to the increases in microbial biomass decomposer activity. Interestingly, the declines in low-density SOM correspond to increases in both dissolved organic C and mineral-associated C at both of these sites (Cusack et al., *in press*), suggesting that increased decomposer activity may have accelerated movement of C into other soil pools. There have been few fertilization studies conducted in tropical forests, and effects on microbial biomass have varied, with declines (Mo et al. 2008), no response (Davidson et al. 2004), and increases (Li et al. 2006). These studies indicate that microbial communities are likely to be sensitive to N deposition in N-rich tropical forests, but it appears that local factors are likely to control directional trends.

The response of specific oxidative enzyme activities to N additions was also similar in the two forests, hinting at some commonality in the physiological response of microbes to N additions. Because the microbial biomass changed with N treatment, whereas soil mass did not, specific enzyme activities showed somewhat different trends than total activities, which we take to be more representative of microbial physiology (i.e., enzymes per unit microbe), whereas total enzyme activities convey net ecosystem function. The reduced specific oxidative enzyme activities in both forests suggest a reduced need for cometabolic N acquisition (Sinsabaugh et al. 2002). Similarly, the decline in the specific activity of N-acquiring enzymes may indicate alleviation of N limitation to decomposition in the upper-elevation forest, where background total soil N pools are smaller than in the lower-elevation forest (Cusack et al. 2009*b*). The relative decline in oxidative enzyme activities with N addition observed in both forests is similar to results from a laboratory incubation with these soils (Cusack et al. 2010). It is possible that the increase in fungal abundance with N addition in the upper-elevation forest was not directly related to N availability, but rather to some indirect effect, such as increased mobilization of base cations, which was observed in N-fertilized plots at these sites (Macy 2004). These changes in specific enzyme activity, together with the increases in microbial groups at each site and declines in the rapidly cycling SOM pools, suggest that added N generally alleviated nutrient limitation to microbial decomposers in both of these tropical forests.

The negative relationship between oxidative enzyme activities and microbial $\delta^{13}C$ suggests a change in microbial substrate utilization. That is, oxidative enzyme activities increase microbial incorporation of C that is relatively depleted in $^{13}C$. This might be expected, since lignin and lipids are naturally 3–6‰ lighter than bulk plant material (Ehleringer et al. 2000). Although lignin and lipids are considered poor C sources for microbial metabolism, with low incorporation into microbial biomass (Sylvia et al. 2004), our results suggest that some of the C released by oxidative enzyme activity is used by microbes. Whether these shifts in substrate utilization with N addition, together with changes in SOM chemistry, will result in further long-

term feedbacks on microbial community structure is still an open question.

### *Conclusion*

Microbial biomass increased with N fertilization in two N-rich tropical forest types, corresponding to smaller pools of rapidly cycling SOM. However, shifts in the chemistry of this SOM pool varied between the two forests, apparently driven by site-specific changes in microbial community characteristics and enzyme activities. Increased bacterial abundance with N addition in a lower-elevation forest corresponded to losses of the most labile C compounds from SOM, whereas increased fungal abundance with N addition in an upper-elevation forest corresponded to losses of hydrophobic aliphatic compounds from SOM. The changes in SOM quantity and chemical quality observed here are likely to have subsequent effects on soil C storage via changes in the chemistry of dissolved organic C and C stabilization. Our results indicate that ecosystem function is sensitive to N deposition in N-rich tropical forests via changes in microbial community characteristics and activity. This study also indicates that C cycling is changing in response to N deposition in tropical forests, driven by complex interactions among microbial community composition, enzymatic capability, and soil C chemistry.

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### Literature Cited

Allison, S. D., and P. M. Vitousek. 2004. Extracellular enzyme activities and carbon chemistry as drivers of tropical plant litter decomposition. Biotropica 36:285–296.

Baldock, J. A., C. A. Masiello, Y. Gelinas, and J. I. Hedges. 2004. Cycling and composition of organic matter in terrestrial and marine ecosystems. Marine Chemistry 92:39–64.

Baldock, J. A., J. M. Oades, A. G. Waters, X. Peng, A. M. Vassallo, and M. A. Wilson. 1992. Aspects of the chemical structure of soil organic materials as revealed by solid-state $^{13}C$ NMR spectroscopy. Biogeochemistry 16:1–42.

Balser, T. C., K. K. Treseder, and M. Ekenler. 2005. Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence. Soil Biology and Biochemistry 37:601–604.

Beinroth, F. H. 1982. Some highly weathered soils of Puerto-Rico. 1. Morphology, formation and classification. Geoderma 27:1–73.

Brown, S., A. E. Lugo, S. Silander, and L. Liegel. 1983. Research history and opportunities in the Luquillo Experimental Forest. Institute of Tropical Forestry, USFS, New Orleans, Louisiana, USA.

Bruijnzeel, L. A. 2001. Hydrology of tropical montane cloud forests: a reassessment. Land Use and Water Resources Research 1:1–18.

Carreiro, M. M., R. L. Sinsabaugh, D. A. Repert, and D. F. Parkhurst. 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81:2359–2365.

Christensen, B. T. 1992. Physical fractionation of soil and organic matter in primary particle size and density separates. Advances in Soil Sciences 20:1–90.

Cusack, D. F., W. W. Chou, W. H. Liu, M. E. Harmon, and W. L. Silver, and LIDET-Team. 2009*a*. Controls on long-term root and leaf litter decomposition in Neotropical forests. Global Change Biology 15:1339–1355.

Cusack, D. F., W. Silver, and W. H. McDowell. 2009*b*. Biological nitrogen fixation in two tropical forests: ecosystem-level patterns and effects of nitrogen fertilization. Ecosystems 12:1299–1315.

Cusack, D. F., W. L. Silver, M. S. Torn, and W. H. McDowell. *In press*. Effects of nitrogen additions on above- and belowground carbon dynamics in two tropical forests. Biogeochemistry.

Cusack, D. F., M. S. Torn, W. H. McDowell, and W. L. Silver. 2010. The response of heterotrophic activity and carbon cycling to nitrogen additions and warming in two tropical soils. Global Change Biology 16:2555–2572.

Davidson, E. A., C. J. R. de Carvalho, I. C. G. Vieira, R. D. Figueiredo, P. Moutinho, F. Y. Ishida, M. T. P. dos Santos, J. B. Guerrero, K. Kalif, and R. T. Saba. 2004. Nitrogen and phosphorus limitation of biomass growth in a tropical secondary forest. Ecological Applications 14:S150–S163.

DeForest, J. L., D. R. Zak, K. S. Pregitzer, and A. J. Burton. 2004. Atmospheric nitrate deposition, microbial community composition, and enzyme activity in northern hardwood forests. Soil Science Society of America Journal 68:132–138.

Demoling, F., L. O. Nilsson, and E. Baath. 2008. Bacterial and fungal response to nitrogen fertilization in three coniferous forest soils. Soil Biology and Biochemistry 40:370–379.

Ehleringer, J. R., N. Buchmann, and L. B. Flanagan. 2000. Carbon isotope ratios in belowground carbon cycle processes. Ecological Applications 10:412–422.

Federle, T. W. 1986. Microbial distribution in soil: new techniques. Pages 493–498 *in* F. Megusar and M. Gantar, editors. Microbial ecology. Society for Microbiology, Ljubljana, Slovenia.

Fog, K. 1988. The effect of added nitrogen on the rate of decomposition of organic-matter. Biological Reviews of the Cambridge Philosophical Society 63:433–462.

Frey, S. D., M. Knorr, J. L. Parrent, and R. T. Simpson. 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. Forest Ecology and Management 196:159–171.

Frostegard, A., and E. Baath. 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22:59–65.

Frostegard, A., A. Tunlid, and E. Baath. 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. Journal of Microbiological Methods 14: 151–163.

Gallo, M., R. Amonette, C. Lauber, R. L. Sinsabaugh, and D. R. Zak. 2004. Microbial community structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils. Microbial Ecology 48:218–229.

Gallo, M. E., C. L. Lauber, S. E. Cabaniss, M. P. Waldrop, R. L. Sinsabaugh, and D. R. Zak. 2005. Soil organic matter and litter chemistry response to experimental nitrogen

deposition in northern temperate deciduous forest ecosystems. Global Change Biology 11:1514–1521.

Galloway, J. N., et al. 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70:153–226.

Garcia-Montino, A. R., G. S. Warner, F. N. Scatena, and D. L. Civco. 1996. Rainfall, runoff and elevation relationships in the Luquillo Mountains of Puerto Rico. Caribbean Journal of Science 32:413–424.

Harrington, R. A., J. H. Fownes, and P. M. Vitousek. 2001. Production and resource use efficiencies in N- and P-limited tropical forests: a comparison of responses to long-term fertilization. Ecosystems 4:646–657.

Hobbie, S. E., and P. M. Vitousek. 2000. Nutrient limitation of decomposition in Hawaiian forests. Ecology 81:1867–1877.

Keyser, P., T. K. Kirk, and J. G. Zeikus. 1978. Ligninolytic enzyme-system of *Phanerochaete chrysosporium* synthesized in absence of lignin in response to nitrogen starvation. Journal of Bacteriology 135:790–797.

Kirk, T. K., and R. L. Farrell. 1987. Enzymatic combustion: the microbial degradation of lignin. Annual Review of Microbiology 41:465–505.

Larsen, J., P. A. Olsson, and I. Jakobsen. 1998. The use of fatty acid signatures to study mycelial interactions between the arbuscular mycorrhizal fungus *Glomus intraradices* and the saprotrophic fungus *Fusarium culmorum* in root-free soil. Mycological Research 102:1491–1496.

Lee, K. H., and S. Jose. 2003. Soil respiration, fine root production, and microbial biomass in cottonwood and loblolly pine plantations along a nitrogen fertilization gradient. Forest Ecology and Management 185:263–273.

Lewis, D. E., J. R. White, D. Wafula, R. Athar, T. Dickerson, H. N. Williams, and A. Chauhan. 2010. Soil functional diversity analysis of a bauxite-mined restoration chronosequence. Microbial Ecology 59:710–723.

Li, Y. Q., M. Xu, and X. M. Zou. 2006. Effects of nutrient additions on ecosystem carbon cycle in a Puerto Rican tropical wet forest. Global Change Biology 12:284–293.

Macy, J. 2004. Initial effects of nitrogen additions in two rain forest ecosystems of Puerto Rico. Thesis. Department of Natural Resources, University of New Hampshire, Durham, New Hampshire, USA.

Malchair, S., H. J. De Boeck, C. Lemmens, R. Ceulemans, R. Merckx, I. Nijs, and M. Carnol. 2010. Diversity-function relationship of ammonia-oxidizing bacteria in soils among functional groups of grassland species under climate warming. Applied Soil Ecology 44:15–23.

Marin-Spiotta, E., W. Silver, C. Swanston, and R. Ostertag. 2009. Soil organic matter dynamics during 80 years of reforestation of tropical pastures. Global Change Biology 15: 1584–1597.

Marin-Spiotta, E., C. W. Swanston, M. S. Torn, W. L. Silver, and S. D. Burton. 2008. Chemical and mineral control of soil carbon turnover in abandoned tropical pastures. Geoderma 143:49–62.

Martinelli, L. A., M. C. Piccolo, A. R. Townsend, P. M. Vitousek, E. Cuevas, W. McDowell, G. P. Robertson, O. C. Santos, and K. Treseder. 1999. Nitrogen stable isotopic composition of leaves and soil: tropical versus temperate forests. Biogeochemistry 46:45–65.

McDowell, W., and C. Asbury. 1994. Export of carbon, nitrogen, and major ions from three tropical montane watersheds. Limnology and Oceanography 39:111–125.

McGroddy, M., and W. L. Silver. 2000. Variations in belowground carbon storage and soil $CO_2$ flux rates along a wet tropical climate gradient. Biotropica 32:614–624.

Mirmanto, E., J. Proctor, J. Green, and L. Nagy. and Suriantata. 1999. Effects of nitrogen and phosphorus fertilization in a lowland evergreen rainforest. Philosophical Transactions of the Royal Society B 354:1825–1829.

Mo, J., W. Zhang, W. Zhu, P. Gundersen, Y. Fang, D. Li, and H. Wang. 2008. Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. Global Change Biology 14:403–412.

National Atmospheric Deposition Program/National Trends Network. 2009. NADP/NTN Monitoring Location PR20, Annual Data Summaries. ⟨http://nadp.sws.uiuc.edu/sites/siteinfo.asp?net=NTN&id=PR20⟩

Nemergut, D. R., A. R. Townsend, S. R. Sattin, K. R. Freeman, N. Fierer, J. C. Neff, W. D. Bowman, C. W. Schadt, M. N. Weintraub, and S. K. Schmidt. 2008. The effects of chronic nitrogen fertilization on alpine tundra soil microbial communities: implications for carbon and nitrogen cycling. Environmental Microbiology 10:3093–3105.

Nordby, H. E., S. Nemec, and S. Nagy. 1981. Fatty acids and sterols associated with citrus root mycorrhizae. Journal of Agricultural and Food Chemistry 29:396–401.

O'Leary, W. M., and S. G. Wilkinson. 1988. Gram-positive bacteria. Pages 117–201 *in* C. Ratledge and S. G. Wilkinson, editors. Microbial Lipids. Academic Press, London, UK.

Olsson, P. A., E. Baath, I. Jakobsen, and B. Soderstrom. 1995. The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. Mycological Research 99:623–629.

Olsson, P. A., and N. C. Johnson. 2005. Tracking carbon from the atmosphere to the rhizosphere. Ecology Letters 8:1264–1270.

Ostertag, R., E. Marin-Spiotta, W. L. Silver, and J. Schulten. 2008. Litterfall and decomposition in relation to soil carbon pools along a secondary forest chronosequence in Puerto Rico. Ecosystems 11:701–714.

Peralta, A. L., J. W. Matthews, and A. D. Kent. 2010. Microbial community structure and denitrification in a wetland mitigation bank. Applied and Environmental Microbiology 76:4207–4215.

Saiya-Cork, K. R., R. L. Sinsabaugh, and D. R. Zak. 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. Soil Biology and Biochemistry 34:1309–1315.

SAS. 2007. JMP 7.0.2. SAS Institute, Cary, North Carolina, USA.

Sinsabaugh, R. L., M. M. Carreiro, and D. A. Repert. 2002. Allocation of extracellular enzymatic activity in relation to litter composition, nitrogen deposition, and mass loss. Biogeochemistry 60:1–24.

Sinsabaugh, R. L., and D. L. Moorhead. 1994. Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. Soil Biology and Biochemistry 26:1305–1311.

Sinsabaugh, R. L., K. Saiya-Corka, T. Long, M. P. Osgood, D. A. Neher, D. R. Zak, and R. J. Norby. 2003. Soil microbial activity in a Liquidambar plantation unresponsive to $CO_2$-driven increases in primary production. Applied Soil Ecology 24:263–271.

Smolander, A., A. Kurka, V. Kitunen, and E. Malkonen. 1994. Microbial biomass C and N, and respiratory activity in soil of repeatedly limed and N-fertilized and P-fertilized Norway spruce stands. Soil Biology and Biochemistry 26:957–962.

Soderstrom, B., E. Baath, and B. Lundgren. 1983. Decrease in soil microbial activity and biomasses owing to nitrogen amendments. Canadian Journal of Microbiology 29:1500–1506.

Starnaud, M., C. Hamel, B. Vimard, M. Caron, and J. A. Fortin. 1995. Altered growth of *Fusarium oxysporum* f.sp. *chrysanthemi* in an in-vitro dual culture system with the vesicular arbuscular mycorrhizal fungus *Glomus intraradices* growing on *Daucus carota* transformed roots. Mycorrhiza 5: 431–438.

Sullivan, N. H., W. B. Bowden, and W. H. McDowell. 1999. Short-term disappearance of foliar litter in three species before and after a hurricane. Biotropica 31:382–393.

Swanston, C., P. S. Homann, B. A. Caldwell, D. D. Myrold, L. Ganio, and P. Sollins. 2004. Long-term effects of elevated

nitrogen on forest soil organic matter stability. Biogeochemistry 70:227–250.
Swanston, C. W., M. S. Torn, P. J. Hanson, J. R. Southon, C. T. Garten, E. M. Hanlon, and L. Ganio. 2005. Initial characterization of processes of soil carbon stabilization using forest stand-level radiocarbon enrichment. Geoderma 128:52–62.
Sylvia, D., J. Fuhrmann, P. Hartel, and D. Zuberer, editors. 2004. Principles and applications of soil microbiology. Second edition. Prentice Hall, Upper Saddle River, New Jersey, USA.
Talbot, J. M., S. D. Allison, and K. K. Treseder. 2008. Decomposers in disguise: mycorrhizal fungi as regulators of soil carbon dynamics in ecosystems under global change. Functional Ecology 22:955–963.
Trumbore, S. E. 1993. Comparison of carbon dynamics in tropical and temperate soils using radiocarbon measurements. Global Biogeochemical Cycles 7:275–290.
Tu, C., F. L. Booker, D. M. Watson, X. Chen, T. W. Rufty, W. Shi, and S. J. Hu. 2006. Mycorrhizal mediation of plant nitrogen acquisition and residue decomposition: impact of mineral nitrogen inputs. Global Change Biology 12:793–803.
Vance, E. D., P. C. Brookes, and D. S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19:703–707.
Vitousek, P. M., and H. Farrington. 1997. Nutrient limitation and soil development: experimental test of a biogeochemical theory. Biogeochemistry 37:63–75.
Vitousek, P. M., and R. L. Sanford. 1986. Nutrient cycling in a moist tropical forest. Annual Review of Ecology and Systematics 17:137–167.
Waldrop, M. P., and M. K. Firestone. 2004. Altered utilization patterns of young and old soil carbon by microorganisms caused by temperature shifts and nitrogen additions. Biogeochemistry 67:235–248.
Waldrop, M. P., D. R. Zak, and R. L. Sinsabaugh. 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biology and Biochemistry 36:1443–1451.
Wallenstein, M. D., S. McNulty, I. J. Fernandez, J. Boggs, and W. H. Schlesinger. 2006. Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. Forest Ecology and Management 222:459–468.
Weaver, P. L. 1991. Environmental gradients affect forest composition in the Luquillo Mountains of Puerto Rico. Interciencia 16:142–151.
White, D. C., and D. B. Ringelberg. 1998. Signature lipid biomarker analysis. Pages 255–272 *in* R. S. Burlage, R. Atlas, D. Stahl, G. Geesey, and G. Sayler, editors. Techniques in microbial ecology. Oxford University Press, New York, New York, USA.
Whiteside, M. D., K. K. Treseder, and P. R. Atsatt. 2009. The brighter side of soils: quantum dots track organic nitrogen through fungi and plants. Ecology 90:100–108.
Ziegler, S. E., P. M. White, D. C. Wolf, and G. J. Thoma. 2005. Tracking the fate and recycling of $^{13}$C-labeled glucose in soil. Soil Science 170:767–778.

## APPENDIX A

Study site and methodological information (*Ecological Archives* E092-053-A1).

## APPENDIX B

$^{13}$C nuclear magnetic resonance chemical regions for organic materials from two tropical forests (*Ecological Archives* E092-053-A2).

## APPENDIX C

$^{13}$C nuclear magnetic resonance spectra for a decomposition sequence (*Ecological Archives* E092-053-A3).

## APPENDIX D

Abundance of indicator phospholipid fatty acids and microbial biomass carbon for two tropical forests (*Ecological Archives* E092-053-A4).

## APPENDIX E

Composition and isotopic signatures of microbial phospholipid fatty acids (*Ecological Archives* E092-053-A5).

## APPENDIX F

Soil enzyme activities with nitrogen additions in two tropical forests (*Ecological Archives* E092-053-A6).