

Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition

Cory C. Cleveland · Diana R. Nemergut ·
Steven K. Schmidt · Alan R. Townsend

Received: 16 June 2006 / Accepted: 10 November 2006 / Published online: 13 December 2006
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Abstract Organic matter decomposition and soil CO₂ efflux are both mediated by soil microorganisms, but the potential effects of temporal variations in microbial community composition are not considered in most analytical models of these two important processes. However, inconsistent relationships between rates of heterotrophic soil respiration and abiotic factors, including temperature and moisture, suggest that microbial community composition may be an important regulator of soil organic matter (SOM) decomposition and CO₂ efflux. We performed a short-term (12-h) laboratory incubation experiment using tropical rain forest soil amended with either water (as a control) or dissolved organic matter (DOM) leached from native plant litter, and analyzed the effects of the treatments on soil respiration and microbial community composition. The latter was

determined by constructing clone libraries of small-subunit ribosomal RNA genes (SSU rRNA) extracted from the soil at the end of the incubation experiment. In contrast to the subtle effects of adding water alone, additions of DOM caused a rapid and large increase in soil CO₂ flux. DOM-stimulated CO₂ fluxes also coincided with profound shifts in the abundance of certain members of the soil microbial community. Our results suggest that natural DOM inputs may drive high rates of soil respiration by stimulating an opportunistic subset of the soil bacterial community, particularly members of the Gammaproteobacteria and Firmicutes groups. Our experiment indicates that variations in microbial community composition may influence SOM decomposition and soil respiration rates, and emphasizes the need for in situ studies of how natural variations in microbial community composition regulate soil biogeochemical processes.

C. C. Cleveland (✉) · D. R. Nemergut ·
A. R. Townsend
INSTAAR: An Earth and Environmental Sciences
Institute, University of Colorado, 450 UCB/1560 30th
Street, Boulder, CO 80303, USA
e-mail: Cory.Cleveland@Colorado.Edu

D. R. Nemergut
Environmental Studies Program, University of
Colorado, 397 UCB, Boulder, CO, USA

S. K. Schmidt · A. R. Townsend
Department of Ecology and Evolutionary Biology,
University of Colorado, 334 UCB, Boulder, CO, USA

Keywords Carbon cycle · Decomposition ·
Dissolved organic matter · DOM · Microbial
community composition · Organic matter · Soil
respiration

Introduction

Organic matter decomposition is a fundamental process, regulating rates of net carbon (C)

storage and nutrient cycling in terrestrial ecosystems. Decades of research have informed the development of ecosystem models that describe decomposition and heterotrophic soil respiration rates primarily as functions of abiotic environmental factors including temperature, moisture, and organic matter substrate quantity and quality (Meentemeyer 1978; Swift et al. 1979; Scott-Denton et al. 2003; Davidson and Janssens 2006; Scott-Denton et al. 2006). However, the potential effects of temporal variations in microbial community structure on these processes are seldom explicitly acknowledged (but see Fang et al. 2005). Instead, most conceptual and empirical decomposition models treat the microbial community as a “black box” in which microorganisms act as passive catalysts of decomposition reactions of substrates whose rates vary as functions of temperature and moisture (Raich and Schlesinger 1992; Lloyd and Taylor 1994; Parton et al. 1994).

Simple soil respiration-climate relationships are convenient for models attempting to describe ecosystem CO₂ fluxes, but many studies show contradictory responses of soil respiration to variations in climate (Davidson and Janssens 2006). Soil moisture correlates with soil respiration rates in some sites (e.g., Buchmann 2000; Schwendenman et al. 2003), but only weakly predicts rates of soil respiration in others (Scott-Denton et al. 2003; Cleveland and Townsend 2006; Monson et al. 2006). The inability of climate to adequately explain variation in soil respiration rates suggests that soil CO₂ fluxes are regulated by a more complex set of variables and interactions.

For example, heterotrophic CO₂ fluxes also depend on the availability of soluble, labile C sources, and microbial respiration rates are tightly linked to the chemistry and amount of organic matter entering the soil (Schlesinger and Andrews 2000; Wardle et al. 2004). In addition, three emerging lines of evidence suggest that short- and long-term variations in soil microbial community composition (e.g., Schmidt et al. 2007) could also influence soil respiration rates. First, microbial community structure varies significantly within soil types—both spatially and temporally—with research indicating that within

a single site, rhizosphere microbial community composition and activity vary by plant species and across seasons (Grayston et al. 1998; Bardgett et al. 1999; Wardle et al. 2004; Carney and Matson 2005; Schmidt et al. 2007). Second, manipulative experiments have shown that specialized organisms respond to certain C compounds. Padmanabhan et al. (2003) found that specific and unique soil bacterial assemblages were responsible for the decomposition of individual soil organic compounds, including glucose, naphthalene, phenol, and caffeine. Finally, the types of microorganisms within a site that respond to C inputs vary through time, suggesting that temporal shifts in community structure may directly affect soil respiration rates following C inputs. For example, in a subalpine forest soil, C inputs consistently stimulated members of the Betaproteobacteria, but the specific *types* of soil Betaproteobacteria that responded in summer were genetically and physiologically distinct from those responding in winter (Monson et al. 2006).

These studies and others support proposed links between microbial community composition and ecosystem function (Adams and Wall 2000; Hooper et al. 2000; Wardle et al. 2004). Unfortunately, relatively little is known about the types of microorganisms that decompose native soil organic matter (SOM), or how variations in microbial activity in response to labile C inputs are related to soil microbial community composition. Here, we asked a simple question: in an ecosystem where high rates of soil respiration are positively related to seasonal increases in C availability (Cleveland and Townsend 2006), could seasonal increases in soil respiration be related to variations in microbial community composition? To begin to address this question, we performed a soil incubation experiment using soil collected from a mature, lowland tropical rain forest. Previous research from the study site revealed a strong, positive relationship between labile C availability and soil respiration rates (Cleveland and Townsend 2006), and we hypothesized that dissolved organic matter (DOM)-stimulated increases in soil respiration coincide with rapid, measurable shifts in soil microbial community composition.

Materials And Methods

Study sites and sampling regime

Soil and litter samples were collected from a primary tropical rainforest on the Osa Peninsula in southwestern Costa Rica (see Cleveland et al. 2006 for complete site description). Soils at the site are highly weathered, nutrient poor Ultisols. The site lies within the tropical wet lowland forest bioclimate, with an average annual temperature of 26.5°C (Holdridge et al. 1971). Average annual rainfall exceeds 5,000 mm (Cleveland and Townsend 2006), but like most tropical rainforests, this area experiences a dry season (from January to March on the Osa Peninsula). During the 3-month dry season, more than half of the annual litter falls on the forest floor (>500 g C m⁻²; Cleveland and Townsend 2006), providing a large pool of soluble, highly decomposable C (e.g., Don and Kalbitz 2005) that may fuel high rates of soil respiration during the early rainy season (Cleveland and Townsend 2006). In this experiment, our objective was to simulate a DOM pulse leached from litter layer during a low-intensity rainstorm—an event that occurs almost daily in this site during the early rainy season (Cleveland and Townsend 2006).

We collected 8 × 10 cm surface soil cores from each of ten 25 m² plots in the dry season using a hand corer (see Cleveland et al. 2006 for soil physical and chemical characteristics). Immediately after collection, soil samples were transported on ice in an insulated cooler to the laboratory (within 72 h of collection), and stored at ~4°C until analysis. Soil samples were gently hand-homogenized (to 4 mm) to remove roots and other organic debris. Following homogenization, samples were split into paired experimental samples. All incubation experiments were initiated within 96 h of soil sampling to avoid artifacts incurred during long-term storage.

Soil incubation experiment

To simulate an episodic DOM leaching event, we added soluble organic material leached from freshly fallen litter samples to soil. About 100 g of mixed, air-dried plant litter obtained from

litter traps in the dry season (litter harvested at 2-week intervals throughout the year; Cleveland and Townsend 2006) were extracted in 1 L of de-ionized water for 12 h at 22°C, pre-filtered to 0.45 μm and sterile filtered to 0.22 μm. The C concentration of the resulting leachate (650 mg/L) was measured using a Shimadzu TOC 5050A total organic carbon analyzer (Shimadzu Corporation, Kyoto, Japan). Sterility of the leachate was confirmed using Biolog microtiter plates (Biolog Inc., Hayward, CA, USA); no color development was observed after 24, 48 or 72 h, indicating that the leachate was free of all viable bacteria. Approximately 25 g of field-moist soil (~15 g dry weight) were placed in 1 L glass vessels and pre-incubated at 26°C (±1°C) for 24 h in a Precision 815 low-temperature incubator (Precision Scientific, Winchester, VA, USA).

After pre-incubation and equilibration at 26°C, all vessels were aerated and samples (*N* = 10 replicates per treatment) received ~5 mL doses (i.e., an amount to bring each soil sample to ~50% of water holding capacity) of DOM (~225 μg DOM-C g⁻¹ soil; +DOM). To separate water (soil wet-up) versus C-addition effects on microbial community activity and composition, we also amended a parallel set of samples with ~5 mL of de-ionized water (*N* = 10 replicates; +H₂O). Following treatment additions, glass vessels were immediately sealed with caps equipped with rubber septa for gas sampling, incubated at 26°C in darkness for 12 h, and sampled for CO₂ at 2, 4, 7, 9, and 12 h using glass gas-tight syringes. Sample CO₂ concentrations were determined at each time point using a Shimadzu GC-14 gas chromatograph equipped with a thermal conductivity detector.

DNA extraction and analysis

Our strategy was to sample soil microbial community composition when soil respiration achieved maximum rates. Previous experiments showed that following C additions to these soils, microbial respiration rates were highest after ~12 h of incubation at 26°C (Cleveland et al. 2002). Therefore, after the 12-h headspace CO₂ sampling was complete, ~5 g of soil from each

microcosm were removed to sterile conical tubes and preserved at -80°C until DNA extraction.

Soil DNA was extracted from soil samples using a modification of the protocol described by Moré et al. (1994). Briefly, 0.5 g of soil from each of the $+\text{H}_2\text{O}$ and $+\text{DOM}$ samples were agitated in 1 mL phosphate extraction buffer using a bead beater and centrifuged at $14,000g$ for 10 min. The resulting supernatant was removed to a fresh tube and incubated on ice for 5 min with $200\ \mu\text{L}$ of 7.5 M ammonium acetate, centrifuged at $14,000g$ for 3 min, and the supernatant transferred to a fresh tube. Samples were then extracted with an equal volume of phenol : chloroform : isoamyl alcohol (25:24:1), DNA was precipitated with $200\ \mu\text{L}$ of isopropanol overnight at -20°C and washed with 1 mL of 70% ethanol. Following extraction, the individually extracted DNA samples from each treatment were pooled and purified over Sepharose 4B packed columns (Sigma, St. Louis, MO, USA) as described in Jackson et al. (1997).

PCR, cloning, and sequencing

Approximately 30 ng of DNA were amplified with universal bacterial 16S rDNA primers [8f and 1492r (Lane 1991)]. Each reaction included 400 nM of each primer, $200\ \mu\text{M}$ of each dNTP, 1.25 U of Taq DNA polymerase (Promega, Madison, WI, USA) in Taq DNA polymerase buffer [$2.5\ \text{mM}\ \text{MgCl}_2$ (Promega)]. After an initial denaturation step at 94°C (1 min), PCR was performed using 30 cycles at 94°C for 1 min, $58 \pm 5^{\circ}\text{C}$ for 30 s and 72°C for 2.5 min with a terminal 10 min extension at 72°C . Resulting PCR products were gel purified using QIAquick gel purification columns (Qiagen, Valencia, CA, USA), ligated into the vector TOPO 2.1 (Invitrogen Inc., Carlsbad, CA, USA) and transformed into *Escherichia coli* following the manufacturer's instructions.

Transformants were inoculated into 96-well deep-dish plates containing 1.5 mL Luria broth (1% NaCl, 1% tryptone, 0.5% yeast extract) and $50\ \text{mg}\ \text{mL}^{-1}$ ampicillin. Cultures were agitated at $200g$ for 14 h at 37°C , plasmid DNA was extracted and purified (Sambrook and Russell 2001), and pelleted DNA samples were air-dried

(20 min.) and re-suspended in 50 mL Tris-HCl pH 8.5. Inserted 16S rRNA genes were PCR amplified from the plasmids using the primers M13F and M13R (Invitrogen Inc.), and purified 16S rRNA genes were sequenced with the T7 promoter primer and the M13-9 primer using the BigDye Terminator Cycle Sequencing kit Version 3.0 (PE Biosystems, Foster City, CA, USA) according to the manufacturer's directions. Sequencing products were analyzed at the Iowa State University DNA Sequencing Facility.

Data analysis

All soil respiration data were tested for homoscedasticity (Levene's test for equal variances), normality, and skewedness (SPSS, Chicago, IL, USA). Significant differences in maximum respiration rates following DOM additions in the incubation experiment were tested with a paired-samples *t*-test ($\alpha = 0.05$; Snedecor and Cochran 1989).

DNA sequences were edited in Sequencher 4.1 (Gene Codes Co., Ann Arbor, MI, USA) and subjected to BLAST searches (Altschul et al. 1990). The 16S rRNA gene sequences were also subjected to chimera check in RDP (Cole et al. 2003) and Bellerophon (Huber et al. 2004). These sequences were aligned in Dr. Phil Hugenholtz's 16S rRNA ARB database (<http://rdp8.cme.msu.edu/html/alignments.html>) in ARB (Ludwig et al. 2004). Phylogenetic affiliations were assigned based on both the ARB alignment and nearest BLAST matches. Operational taxonomic unit (OTU) designations were assigned using DOTUR (Schloss and Handelsman 2005) with a distance-based phylogenetic tree constructed in ARB. Lineage-per-time plots were also constructed in DOTUR (Schloss and Handelsman 2005). Detailed phylogenies of the Gammaproteobacteria and the Firmicutes were constructed by aligning close relatives from the ARB database and BLAST matches with our sequences in ARB. Alignments were used to generate a neighbor-joining phylogenetic tree in PAUP (Swofford 2001) using the distance optimality criterion. All trees were subjected to bootstrap analyses (1,000 replicates) using both the parsimony and neighbor-joining optimality criterion.

Results

Soil respiration rates

Dissolved organic matter additions ($3,375 \mu\text{g C}$ as DOM) rapidly stimulated respiration in the soil microcosms (Fig. 1). After the 12-h incubation, soil respiration rates in the +DOM samples were $35.1 \pm 1.7 \mu\text{g CO}_2\text{-C g}^{-1} \text{h}^{-1}$, versus only $9.1 \pm 1.2 \mu\text{g CO}_2\text{-C g}^{-1} \text{h}^{-1}$ in the +H₂O samples (Fig. 1A). Twelve hours after the DOM addition, the +DOM samples had respired $\sim 100\%$ more CO₂ than the control soil samples ($2,981 \pm 245 \mu\text{g CO}_2\text{-C}$ vs. $1,503 \pm 80 \mu\text{g CO}_2\text{-C}$ in +DOM and +H₂O samples, respectively; Fig. 1B). In contrast, the soil wet-up (water only) had little effect on soil respiration rates. Immediately following water additions, soil respiration rates in +H₂O samples increased slightly (Fig. 1A), but rates of

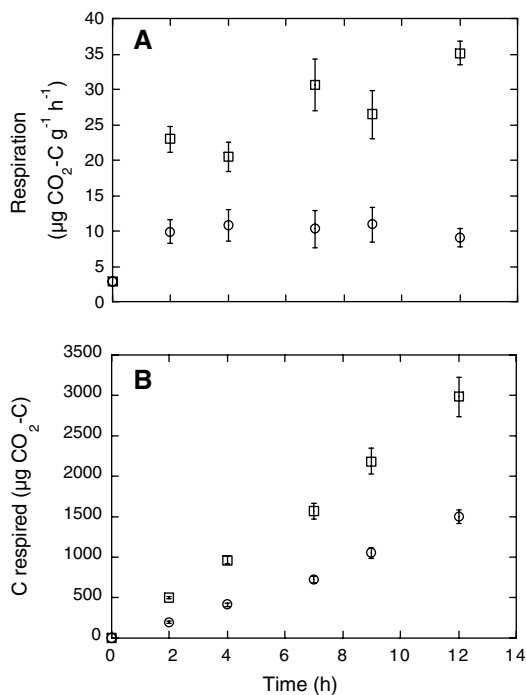


Fig. 1 (A) Soil respiration rates; and (B) total CO₂ respired in soil incubations following water (*open circles*) or dissolved organic matter (DOM; *open squares*) additions ($3,375 \mu\text{g C}$ as DOM) to tropical rain forest soil. Soil respiration rates are expressed on a soil mass (*dry weight*) basis. Values represent the mean of ten replicates per treatment, and *error bars* are ± 1 SE

soil respiration in the +H₂O samples did not differ significantly over the 12-h incubation (Fig. 1A).

Soil microbial community composition

We analyzed a total of 102 sequences from the +H₂O ($N = 46$) and +DOM samples ($N = 56$), and there were some notable similarities in community composition between the two treatments. Overall, broad-scale (i.e., division- and sub-division-level) diversity was similar in both sets of samples, with sequences within nine and ten major bacterial clades represented in the +H₂O and +DOM samples, respectively (Fig. 2). Sequences related to several of these clades were found in both the +H₂O and +DOM soil samples, including the Acidobacteria, Firmicutes, Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Verrucomicrobia, and the Planctomycetes. However, more detailed comparisons of the communities revealed that, in spite of the similarities between the two treatments at broader phylogenetic levels, the types of organisms within the +H₂O and +DOM communities were very different. For example, we compared the actual number of unique sequences ($>3\%$ 16S rRNA gene sequence difference) in the two communities over various genetic distances with the theoretical maximum number of possible unique sequences (i.e., if there was no overlap in the two communities), and the theoretical minimum number of possible unique sequences (i.e., if each sequence present in one community was also present in the other community; Fig. 3). Results indicated that at genetic distances of less than 10%, the +H₂O and +DOM communities were distinct from each other.

Lineage per time (LPT) curves, in which the number of unique microbial phylotypes is plotted as a function of genetic distance, provide an index of the amount of phylogenetic diversity within a community. LPT plots for these communities also revealed profound differences between the +H₂O and +DOM soils. The +H₂O curves showed a gradual decline, suggesting that phylogenetic diversity was relatively high. In contrast, in the +DOM samples, LPT curves showed an initial, relatively steep decline, indicating that many of the individual phylotypes in the +DOM samples

Fig. 2 Relative abundance of 16S rRNA gene phylotypes in (A) +H₂O; and (B) +DOM samples. Colors represent division or sub-division-level clades (Proteobacteria) and the black lines represent unique phylotypes (i.e., >3% different from all other sequences)

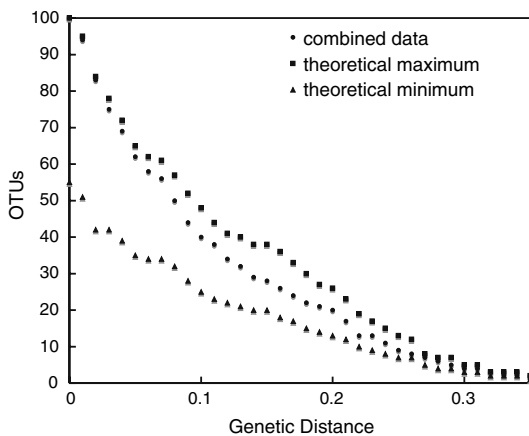
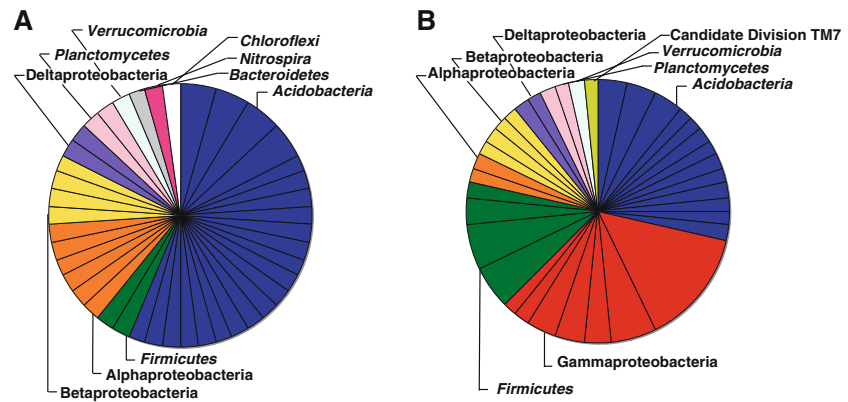


Fig. 3 Estimated number of unique phylotypes in the combined (+DOM and +H₂O) libraries (circles), compared to the theoretical minimum number of phylotypes (i.e., if all sequences in one library were found in the other library; triangles) and the theoretical maximum number of phylotypes (i.e., if none of the sequences present in one library were found in the other library; squares). This plot indicates that although there was some division-level sequence overlap in the +DOM and +H₂O libraries, the sequences in the +H₂O and +DOM libraries were very different

were closely related, and that the depth of phylogenetic diversity in the +DOM samples was lower than in the +H₂O samples (data not shown).

Our sampling and analysis of microbial community composition in the soil samples was not exhaustive, therefore it is difficult to make definitive statements about the abundance of specific bacterial lineages or overall microbial diversity,

but our results do show some clear and important differences. For example, perhaps the most striking difference between +DOM and control soil communities was the increase in abundance of non-Acidobacteria sequences in the +DOM soil. Sequences related to the Acidobacteria clade were numerically dominant in both the +H₂O and +DOM samples, but they represented nearly 60% of the sequences in the control library and only about 30% in the +DOM library (Fig. 2). In addition, in the control samples, non-Acidobacterial sequences included members of nine major clades, and the frequencies of individuals within those groups were fairly equally represented; no single non-Acidobacterial clade represented more than 11% of the sequences, and most individual clades comprised <4% of the sequences. In contrast, in the +DOM samples, non-Acidobacterial sequences were dominated by individuals from the Gammaproteobacteria and Firmicutes groups, which comprised 34 and 16% of the +DOM sequences, respectively. In the +H₂O samples, phylotypes from the Firmicutes group were present in small numbers (~4%), but the sequences were not closely related to the Firmicute sequences in the +H₂O samples (data not shown). Sequences related to the Gammaproteobacteria were not detected in the +H₂O samples.

Discussion

Soil moisture content can limit decomposition and soil respiration rates (e.g., Housman et al.

2006; Potts et al. 2006). However, in this tropical rain forest site, soil moisture (to 10 cm depth) remains relatively constant throughout the year, even during the 3-month dry season when precipitation is <100 mm/month (Cleveland and Townsend 2006). In this experiment, water-only additions did not significantly enhance rates of soil respiration (Fig. 1a). The lack of a strong CO₂ response to the soil wet-up indicates that even though soil samples were collected in the dry season, soil moisture was not limiting to heterotrophic respiration at that time. This result is consistent with previous data from this site showing that soil moisture does not correlate strongly with rates of soil respiration or other soil processes (Cleveland and Townsend 2006).

Although seasonal increases in rainfall do not drive significant variation in soil moisture, they do facilitate the movement of DOM from litter to soil, driving significant dry-to-wet season increases in soil respiration (Cleveland et al. 2006; Cleveland and Townsend 2006). In this experiment, microbial respiration responded rapidly to dissolved organic C additions in the +DOM samples (Fig. 1). Many other studies showing rapid microbial responses to labile C additions have added relatively high concentrations of simple, pure, C-rich compounds that stimulate microbial activity, but that may not necessarily be analogous to native organic material that is present in natural ecosystems. Thus, while the high rates of microbial respiration we observed in response to C additions (in general) are not surprising, the rapid response immediately following additions of relatively dilute, litter-leached native DOM is noteworthy for several reasons.

First, leached DOM is a complex, heterogeneous suite of C compounds that vary in complexity and decomposability (Neff and Asner 2001; Cleveland et al. 2004), yet it still stimulated rapid rates of microbial respiration in our incubation. In addition, previous *in situ* DOM additions elicited similarly high-respiration rates, suggesting that rapid respiratory responses to episodic DOM inputs may be important in regulating ecosystem function in this site (Cleveland et al. 2002). Next, while the concentration of the DOM added here (~650 mg L⁻¹) was higher than typical tropical rain forest throughfall or soil

solution concentrations (Schwendenmann and Veldkamp 2005), it did represent realistic concentrations of DOM through the litter layer (Neff and Asner 2001; Cleveland et al. 2004). Previous research at this site showed that as much as 50% of dry season litter C is susceptible to leaching in the first 60 days of the rainy season (Cleveland et al. 2006). If so, a typical daily 10 mm rainfall event in the early rainy season and subsequent leaching of the dry season litter pool (250 mg C m⁻²) could generate a pulse of DOM of ~200 mg L⁻¹ to the soil surface. The increase in soil respiration that we observed following additions of native, leached DOM suggests that episodic DOM fluxes *in situ* may also rapidly stimulate high rates of soil respiration.

Rapid increases in microbial activity following pulses of labile C inputs are common, but do such increases represent a community-wide response, or are they accomplished via shifts in the abundance of certain microbial taxa? Our results provide compelling evidence that increases in soil respiration following C inputs may be dominated by a subset of soil microbes, with DOM inputs causing rapid shifts in soil bacterial community composition. In the +H₂O samples, as is common in many soils, the Acidobacteria was the numerically dominant group, but the abundances of individuals within other division-level groups were relatively evenly distributed. However, at the peak of soil respiration, microbial community structure in the +DOM soil was markedly different. In particular, C additions appeared to strongly stimulate organisms within the Gammaproteobacteria and Firmicutes clades.

The dominance of organisms within the Gammaproteobacteria and Firmicutes groups, and their relative increase in abundance in response to C additions, suggests that members of these groups quickly responded to the additions of organic material added in the +DOM soil, leading to rapid increases in soil respiration. Detailed phylogenetic analyses of the Gammaproteobacteria and Firmicutes sequences revealed that they are related to the order Enterobacteriales and the genus *Bacillus*, respectively (Figs. 4, 5), groups that include an array of both pathogenic and symbiotic bacteria. Interestingly, members of these orders have been

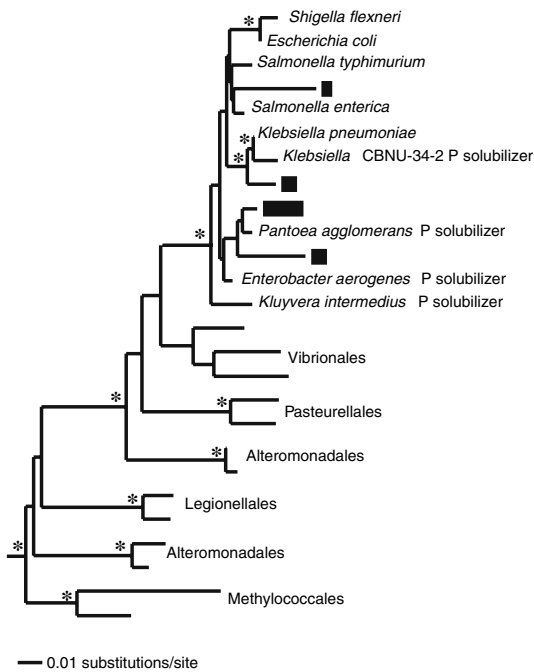
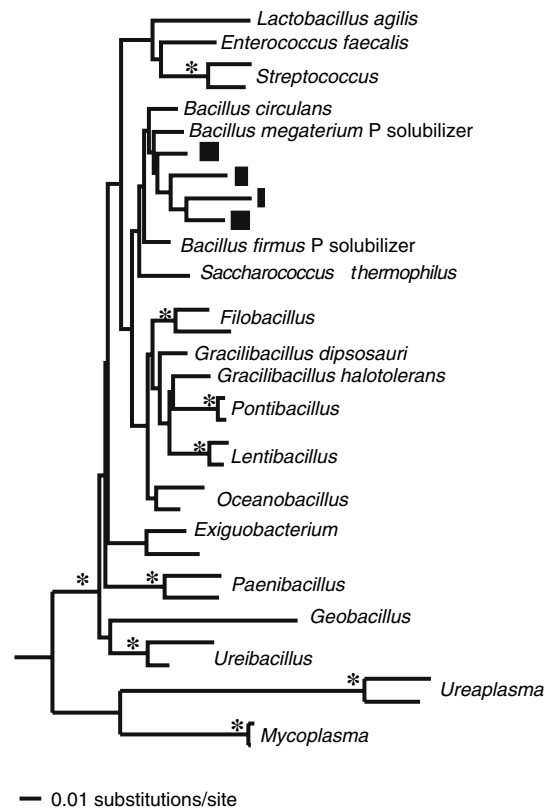


Fig. 4 Neighbor-joining phylogenetic tree showing representative sequences from the clones related to the Enterobacteriales in the +DOM samples. Rectangles represent sequences from this study, and the size of the rectangle symbolizes the number of sequences obtained. Reference taxa were selected based on nearest BLAST matches. Tree is rooted with *Rhodobacter sphaeroides* (X53853), *Bartonella bacilliformis* (X60042) and *Rhizobium hainanensis* (U71078). Asterisk indicates a parsimony and distance bootstrap (1,000 replicates) value of 80 or higher. Accession numbers: *Shigella flexneri* (X80679), *Escherichia coli* (X80724), *Salmonella typhimurium* (X80681), *Salmonella enterica* (AL627282), *Klebsiella pneumoniae* (X87276), *Klebsiella sp.* CBNU-34-2 (AY335553), *Pantoea agglomerans* (AY335552), *Enterobacter aerogenes* (AY335552), *Kluyvera intermedius* (AF297470), Vibrionales [*Vibrio natriegens* (X74714), *Vibrio cholerae* (X74696), *Photobacterium histaminum* (D25308)], Pasteurellales [*Alteromonas macleodii* (X82145), *Chromohalobacter marismortui* (X87221)], Legionellales [*Legionella gormanii* (Z32639), *Legionella pneumophila* (M59157)], Alteromonadales [*Francisella tularensis* (L26086), *Thiomicrospira crunogena* (L40810)], Methylococcales [*Methylococcus capsulatus* (M29023), *Methylococcus sp.* (X72769)]

identified as potential phosphate solubilizers, capable of accessing calcium-, iron- and aluminum-bound phosphate minerals (Rodríguez and Fraga 1999; Chung et al. 2005; Souchie et al. 2006). This is noteworthy given previous work at this site indicating that the decomposition of



soluble C in soil is strongly phosphorus (P) limited (Cleveland et al. 2002; Cleveland and Townsend 2006). While by no means definitive, the presence of relatives of known P-solubilizing organisms in the +DOM samples suggests an interesting hypothesis: that the ability to solubilize P may give the Enterobacteriales and the Bacillus a competitive advantage in these P-poor soils, allowing them to rapidly grow in response to episodic C inputs.

To our knowledge, our data are among the first to show that increases in soil respiration following native DOM additions to soils correspond with a rapid shift in microbial community composition. Yet, rapid shifts in microbial composition in response to changing environmental conditions and/or resources have been observed by microbial ecologists for some time. More than 75 years ago, Winogradsky (1925) observed increases in zymogenous organisms following substrate additions, with a return to more “stable,” slow growing communities following substrate depletion. More

◀ **Fig. 5** Neighbor-joining phylogenetic tree showing representative sequences from the clones related to the genus *Bacillus* in the +DOM samples. Rectangles represent sequences from this study, and the size of the rectangle symbolizes the number of sequences obtained. Reference taxa were selected based on nearest BLAST matches. Tree is rooted with *Trichodesmium* sp. (X70767), *Nostoc punctiforme* (AF027655) and *Gloeotheca membranacea* (X78680). Asterisk indicates a parsimony and distance bootstrap (1,000 replicates) value of 80 or higher. Accession numbers: *Lactobacillus agilis* (M58803), *Enterococcus faecalis* (AE016830), *Streptococcus* [*Streptococcus pneumoniae* (X58312), *Streptococcus macedonicus* (Z94012)], *Bacillus circulans* (D78312), *Bacillus megaterium* (D16273), *Bacillus firmus* (D16268), *Saccharococcus thermophilus* (L09227), *Filobacillus* [*Filobacillus salipiscarius* (AB194046), *Filobacillus milensis* (AJ238042)], *Gracilibacillus dipsosauri* (AB101591), *Gracilibacillus halotolerans* (AF036922), *Pontibacillus* [*Pontibacillus chungwhensis* (AY603362), *Pontibacillus marinus* (AY603977)], *Lentibacillus* [*Lentibacillus kapialis* (AY667493), *Lentibacillus salarius* (AB231905)], *Oceanobacillus* [*Oceanobacillus chironomi* (DQ468656) *Oceanobacillus profundus* (DQ386635)], *Exiguobacterium* [*Exiguobacterium aurantiacum* (X70316), *Exiguobacterium* sp. (X86064)], *Paenibacillus* [*Paenibacillus validus* (M77489), *Paenibacillus azotofixans* (D78318)], *Geobacillus* [*Geobacillus thermoglucosidasius* (AJ781265)], *Ureibacillus* [*Ureibacillus koreensis* (DQ348072), *Ureibacillus rudaensis* (DQ348071)], *Ureaplasma* [*Ureaplasma canigenitalium* (D78648), *Ureaplasma parvum* (L08642)], *Mycoplasma* [*Mycoplasma capricolum* (U26046), *Mycoplasma mycoides* (U26044)]

recently, Fierer et al. (2007) suggested an ecological classification of bacteria that proposed that the abundance of copiotrophic and oligotrophic bacteria in soil is regulated by the abundance of labile C substrates, and that C decomposition rates (which include heterotrophic soil respiration) vary directly with copiotrophic bacterial abundance. For example, in low C environments, oligotrophic groups like the Acidobacteria were most dominant, but in high C soils, copiotrophic groups like the Proteobacteria, and Bacteroidetes increased in relative abundance (Fierer et al. 2007). In our experiment, we observed a similar pattern. DOM additions caused a relative decrease in Acidobacterial sequences, and relative increases in Gammaproteobacteria and Firmicutes groups. Our results support a model in which abundant bacterial phyla may be ecologically classified according to their physiology, and in which the abundance of these ecologically defined groups (i.e., copiotrophic and oligotrophic

groups) may be strongly regulated by C availability (Fierer et al. 2007). However, our results also suggest that microbial community structure and composition may vary rapidly in response to short-term changes in C availability. In other words, while low-soil C availability (on average) may indeed favor oligotrophic organisms, natural and frequent increases in C availability in many ecosystems may promote short-term variations in community composition, and favor the temporary dominance of copiotrophic organisms.

The increased abundance of Gammaproteobacteria and Firmicutes sequences (Fig. 2) following DOM additions suggests that members of these two divisions may be largely responsible for the observed increases in soil respiration in our incubation experiment, contributing to a growing body of research showing that members of the these clades may dominate many important biogeochemical processes in natural ecosystems. The importance of the Gammaproteobacteria, in particular, in soil C cycling has also been shown in other studies. Padamanbhan et al. (2003) showed that phylotypes within the Gammaproteobacteria clade were responsible for the decomposition of pulses of suite of organic substrates including naphthalene, caffeine, and glucose. A molecular characterization of phenol-degrading bacteria also showed that close relatives of both the Gammaproteobacteria and the Firmicutes were important phenolic compound mineralizers in soil (Abd-El-Haleem et al. 2002). These studies, among others, demonstrate that members of the Gammaproteobacteria and Firmicutes divisions are particularly adept at responding to increases in labile soil C, and that in systems where natural pulses of diverse C compounds are common, these groups may be disproportionately important to the decomposition (and hence respiration) of labile C compounds.

Traditionally, ecosystem models have focused on the role of abiotic factors in regulating organic matter decomposition rates. However, such simplifications are often inadequate for explaining observed variations in soil respiration (Davidson and Janssens 2006; Monson et al. 2006; Sacks et al. 2006). Our results provide evidence of strong links between variations in both microbial community composition and soil respiration, and suggest that

a complete understanding of decomposition dynamics and soil respiration (and perhaps other soil biogeochemical processes) in natural ecosystems may require a more thorough understanding of temporal shifts in microbial community composition. While results from a short-term laboratory experiment with limited sample numbers may not accurately describe microbial dynamics in situ, they do provide both justification and a framework for further research investigating links between soil microbial community structure and function in the natural environment. Many forces, including land use change (Bornemann and Triplett 1997; Nüsslein and Tiedje 1999), variations in nutrient fertility (Bardgett et al. 1999), and climate variability (Monson et al. 2006), have been shown to drive either short-term variations or perhaps long-term or permanent changes in microbial community composition. Understanding how natural and disturbance-driven processes affect microbial community dynamics, and how these, in turn, interact with abiotic factors to regulate soil biogeochemical processes, may be important in generating more accurate predictions of the response of terrestrial ecosystems to environmental change.

Acknowledgments We thank H. and M. Michaud of the Drake Bay Wilderness Camp for providing field access and logistical support in Costa Rica, and Francisco Campos and the Organización para Estudios Tropicales (OET) and the Ministerio de Ambiente y Energía (MINAE) in Costa Rica for assisting with research permits and logistics. We are grateful to R. Ley for valuable discussions and insight during the experimental design and data analysis, and Sarah Flood Page for assistance with sequence editing and analysis. We also thank K. Kalbitz and two anonymous reviewers for valuable comments on the manuscript. This work was supported by National Science Foundation (NSF) Grant DEB-0089447 (to A.R.T. and S.K.S.) and by NSF Grant DEB-0515744 (to A.R.T. and C.C.C.)

References

- Abd-El-Haleem D, Moawad H, Zaki EA, Zaki S (2002) Molecular characterization of phenol-degrading bacteria isolated from different Egyptian ecosystems. *Microb Ecol* 43:217–224
- Adams GA, Wall DH (2000) Biodiversity above and below the surface of soils and sediments: linkages and implications for global change. *Bioscience* 50: 1043–1049
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215:403–410
- Bardgett RD, Lovell RD, Hobbs PJ, Jarvis SC (1999) Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. *Soil Biol Biochem* 31:1021–1030
- Borneman J, Triplett EW (1997) Molecular microbial diversity in soils from eastern amazonia: Evidence for unusual microorganisms and microbial population shifts associated with deforestation. *Appl Environ Microbiol* 63:2647–2653
- Buchmann N (2000) Biotic and abiotic factors controlling soil respiration rates in *Picea abies* stands. *Soil Biol Biochem* 32:1625–1635
- Carney KM, Matson PA (2005) Plant communities, soil microorganisms, and soil carbon cycling: Does altering the world belowground matter to ecosystem functioning? *Ecosystems* 8:928–940
- Chung H, Park M, Madhaiyan M, Seshadri S, Song J, Cho H, Sa T (2005) Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. *Soil Biol Biochem* 37:1970–1974
- Cleveland CC, Townsend AR, Schmidt SK (2002) Phosphorus limitation of microbial processes in moist tropical forests: Evidence from short-term laboratory incubations and field experiments. *Ecosystems* 5:680–691
- Cleveland CC, Neff JC, Townsend AR, Hood E (2004) Composition, dynamics and fate of leached dissolved organic matter in terrestrial ecosystems: results from a decomposition experiment. *Ecosystems* 7:275–285
- Cleveland CC, Reed SC, Townsend AR (2006) Nutrient regulation of organic matter decomposition in a tropical rain forest. *Ecology* 87:492–503
- Cleveland CC, Townsend AR (2006) Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere. *Proc Natl Acad Sci USA* 103:10316–10321
- Cole JR, Chai B, Marsh TL, Farris RJ, Wang Q, Kulan SA, Chandra S, McGarrell DM, Schmidt TM, Garrity GM, Tiedje JM (2003) The RibosomalDatabase Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. *Nucleic Acids Res* 31:442–443
- Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* 440:165–173
- Don A, Kalbitz K (2005) Amounts and degradability of dissolved organic carbon from foliar litter at different decomposition stages. *Soil Biol Biochem* 37:2171–2179
- Fang C, Smith P, Smith JU, Moncrief JB (2005) Incorporating microorganisms as decomposers into models to simulate soil organic matter decomposition. *Geoderma* 129:139–146
- Fierer N, Bradford MA, Jackson RB (2007) Towards an ecological classification of soil bacteria. *Ecology* (in press)

- Grayston SJ, Wang S, Campbell CD, Edwards AC (1998) Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biol Biochem* 30:369–378
- Holdridge LR, Grenke WC, Hatheway WH, Liang T, Tosi JA (eds) (1971) *Forest Environments in Tropical Life Zones: a pilot study*. Pergamon, Oxford
- Hooper DU, Bignell DE, Brown VK, Brussaard L, Dangerfield JM, Wall DH, Wardle DL, Coleman DC, Giller KE, Lavelle P, Van der Putten WH, De Ruiter PC, Rusek J, Silver WL, Tiedje JM, Wolters V (2000) Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: Patterns, mechanisms, and feedbacks. *Bioscience* 50:1049–1061
- Housman DC, Naumburg E, Huxman TE, Charlet TN, Nowak RS, Smith SC (2006) Increases in desert shrub productivity under elevated carbon dioxide vary with water availability. *Ecosystems* 9:374–385
- Huber T, Faulkner G, Hugenholz P (2004) Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. *Bioinformatics* 20:2317–2319
- Jackson CR, Harper JP, Willoughby D, Roden EE, Churchill PF (1997) A simple, efficient method for the separation of humic substances and DNA from environmental samples. *Appl Environ Microbiol* 63:4993–4995
- Lane DJ (1991) 16S/23S rRNA Sequencing. In: Stackebrandt E, Goodfellow M (eds) *Nucleic acid techniques in bacterial systematics*. Wiley, Chichester, pp 115–175
- Lloyd J, Taylor JA (1994) On the temperature dependence of soil respiration. *Funct Ecol* 8:315–323
- Ludwig W, Strunk O, Westram R et al (2004) ARB: a software environment for sequence data. *Nucleic Acids Res* 32:1363–1371
- Meentemeyer V (1978) Macroclimate and lignin control of litter decomposition rates. *Ecology* 59:465–472
- Monson RK, Lipson DL, Burns SP, Turnipseed AA, Delany AC, Williams MW, Schmidt SK (2006) Winter forest soil respiration controlled by climate and microbial community composition. *Nature* 439:711–714
- Moré MI, Herrick JB, Silva MC, Ghiorse WC, Madsen EL (1994) Quantitative cell-lysis of indigenous microorganisms and rapid extraction of microbial DNA from sediment. *Appl Environ Microbiol* 60:1572–1580
- Neff JC, Asner GP (2001) Dissolved organic carbon in terrestrial ecosystems: synthesis and a model. *Ecosystems* 4:29–48
- Nüsslein K, Tiedje JM (1999) Soil bacterial community shift correlated with change from forest to pasture vegetation in a tropical soil. *Appl Environ Microbiol* 65:3622–3626
- Padmanabhan P, Padmanabhan S, DeRito C et al (2003) Respiration of ¹³C-labeled substrates added to soil in the field and subsequent 16S rRNA gene analysis of ¹³C-labeled soil DNA. *Appl Environ Microbiol* 69:1614–1622
- Parton WJ, Schimel DS, Cole CV, Ojima DS (1994) A general model for soil organic matter dynamics: sensitivity to litter chemistry, texture and management. In: Bryant RB, Arnold RW (eds) *Quantitative modeling of soil forming processes*. Soil Science Society of America Special Publication 39, Madison, pp 147–167
- Potts DL, Huxman TE, Cable JM, English NB, Ignace DD, Eilts JA, Mason MJ, Weltzin JF, Williams DG (2006) Antecedent moisture and seasonal precipitation influence the response of canopy-scale carbon and water exchange to rainfall pulses in a semi-arid grassland. *New Phytol* 170:849–860
- Raich JW, Schlesinger WH (1992) The global carbon dioxide flux in soil respiration and its relationships to vegetation and climate. *Tellus* 44B:81–99
- Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol Adv* 17:319–339
- Sacks WJ, Schimel DS, Monson RK, Braswell BH (2006) Model-data synthesis of diurnal and seasonal CO₂ fluxes at Niwot Ridge, Colorado. *Global Change Biol* 12:240–259
- Sambrook J, Russell DW (2001) *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
- Schlesinger WH, Andrews JA (2000) Soil respiration and the global carbon cycle. *Biogeochemistry* 48:7–20
- Schloss PD, Handelsman J (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl Environ Microbiol* 71:1501–1506
- Schmidt SK, Costello EK, Nemergut DR, Cleveland CC, Reed SC, Weintraub MN, Meyer AF, Martin AM (2007) Microbial turnover and seasonal succession drive biogeochemical cycles in the alpine. *Ecology* (in press)
- Schwendenmann L, Veldkamp E, Brenes T, O'Brien JJ, Mackensen J (2003) Spatial and temporal variation in soil CO₂ efflux in an old-growth neotropical rain forest, La Selva, Costa Rica. *Biogeochemistry* 64:111–128
- Schwendenmann L, Veldkamp E (2005) The role of dissolved organic carbon, dissolved organic nitrogen, and dissolved inorganic nitrogen in a tropical wet forest ecosystem. *Ecosystems* 8:339–351
- Scott-Denton LE, Sparks KL, Monson RK (2003) Spatial and temporal controls of soil respiration rate in a high-elevation, subalpine forest. *Soil Biol Biochem* 35:525–534
- Scott-Denton LE, Rosenstiel TN, Monson RK (2006) Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. *Global Change Biol* 12:205–216
- Snedecor GW, Cochran WG (1989) *Statistical Methods*. Iowa State University, Ames
- Souchie EL, Saggin-Júnior OJ, Silva EMR et al (2006) Communities of P-solubilizing bacteria, fungi and arbuscular mycorrhizal fungi in grass pasture and secondary forest of Paraty, RJ-Brazil. *An Acad Bras Cienc* 78:183–193

- Swift MJ, Heal OW, Anderson JM (1979) Decomposition in terrestrial ecosystems. University of California, Berkeley
- Swofford DL (2001) Phylogenetic analysis using parsimony (*and other methods), 4th edn. Sinauer Associates, Sunderland, MA
- Wardle DA, Bardget RD, Klironomos JN, Setälä H, Van der Putten WH, Wall DH (2004) Ecological linkages between aboveground and belowground biota. *Science* 304:1629–1633
- Winogradsky SN (1925) Etudes sur la microbiologie du sol. I. Sur la method. *Ann Inst Pasteur* 40:455–520