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Testing the functional significance of microbial community composition

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Abstract. A critical assumption underlying terrestrial ecosystem models is that soil microbial communities, when placed in a common environment, will function in an identical manner regardless of the composition of that community. Given high species diversity in microbial communities and the ability of microbes to adapt rapidly to new conditions, this assumption of functional redundancy seems plausible. We test the assumption by comparing litter decomposition rates in experimental microcosms inoculated with distinct microbial communities. We find that rates of carbon dioxide production from litter decomposition were dependent upon the microbial inoculum, with differences in the microbial community alone accounting for substantial (~20%) variation in total carbon mineralized. Communities that shared a common history with a given foliar litter exhibited higher decomposition rates when compared to communities foreign to that habitat. Our results suggest that the implicit assumption in ecosystem models (i.e., microbial communities in the same environment are functionally equivalent) is incorrect. To predict accurately how biogeochemical processes will respond to global change may require consideration of the community composition and/or adaptation of microbial communities to past resource environments.

Key words: bacteria; carbon mineralization; community composition; decomposition; functional diversity; functional equivalence; functional redundancy; fungi; leaf litter; microorganisms.

Introduction

Many of the ecosystem processes critical to the functioning of ecosystems, such as decomposition, are carried out in whole or in part by microorganisms. Ecosystem models designed to explain and predict how the rates of these ecosystem processes change in response to changing environmental conditions, such as temperature and moisture, typically treat the microbial community as a single, homogenously functioning entity (Parton et al. 1983). Yet it is increasingly being recognized that microbial community composition plays a role in determining ecosystem process rates (Hendrix et al. 1986, Schimel and Gulledge 1998, Reed and Martiny 2007). However, even in those models (Hunt and Wall 2002) that explicitly consider microbial community composition (e.g., bacterial vs. fungal dominance), the assumption is still that the environment ultimately controls ecosystem process rates. That is, changes in microbial community composition exert proximate control on process rates but the contemporary environment ultimately structures the community. This hypothesis, which we refer to as "functional equivalence," has recently been challenged (Reed and Martiny 2007) given the finding that historical contingencies, and not just the contemporary environment, structure microbial communities both at local and regional scales (Martiny et al. 2006, Ramette and Tiedje 2007). The counter-hypothesis, which we refer to as "functional dissimilarity," is significant because it proposes that the responses of ecosystem processes to global changes are not ultimately fixed by environmental conditions (Balser and Firestone 2005). On the contrary, it proposes that microbial community composition (i.e., the whole community genotype) may, in combination with the environment, ultimately (not just proximally) determine ecosystem process rates.

The hypothesis of functional equivalence does appear intuitive. For example, given that there are many thousands of species of microorganisms in a given soil (Fierer et al. 2007) and that microbes can rapidly adapt to new environments (Goddard and Bradford 2003), one might assume that this equates with a high degree of functional redundancy (Andrén and Balandreau 1999, Behan-Pelletier and Newton 1999, Wall and Virginia 1999). Any spatial or temporal change in community composition would then produce a negligible change in ecosystem processes regulated by microbes (Bell et al. 2005, Franklin and Mills 2006, Wertz et al. 2006, Cardinale et al. 2007, Jiang 2007, Verity et al. 2007). There is an expectation that the greatest functional redundancy will be observed for biogeochemical processes performed by a broad array of soil microorgan-

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isms, such as the mineralization of carbon compounds during litter decomposition, rather than for processes such as nitrogen fixation (Schimel 1995, Bell et al. 2005) that are performed only by specific microbial groups. Work by Balser and Firestone (2005) tends to support this divide. They observed functional differences for microbial processes such as nitrogen mineralization but not for carbon mineralization. Indeed, equivalence in organic carbon degradation has been suggested by others among both local and regional heterotrophic microbial communities in both aquatic (Langenheder et al. 2005, 2006) and terrestrial habitats (Balser and Firestone 2005, Ayres et al. 2006, Wertz et al. 2006).

There is evidence from both plant and animal communities that changes in community composition impact ecosystem process rates (Tilman et al. 1996, 2001, Naeem and Wright 2003, Taylor et al. 2006, Cardinale et al. 2007). Similarly, evidence is now accumulating that microbial community composition influences ecosystem process rates, albeit the identification of causation, as opposed to correlation, is not always conclusive (Schimel and Gulledge 1998, Behan-Pelletier and Newton 1999, Wall and Virginia 1999, Reed and Martiny 2007). To identify causation both common garden and reciprocal transplant approaches are required, where both ecosystem processes and community composition are monitored across time in different environments (Reed and Martiny 2007).

Here we used a long-term (300-day) experimental litter–soil system that combines both a common garden and a reciprocal transplant approach to test between the following competing hypotheses for litter mineralization: (1) different soil microbial communities are functionally equivalent vs. (2) different soil microbial communities are functionally dissimilar. The hypothesis of "functional equivalence" is based on the expectation that the high diversity and/or adaptive ability of soil microbes confer functional redundancy across different microbial communities (i.e., only the environment impacts function). The competing hypothesis of "functional dissimilarity" is based on the expectation that historical contingencies play a role in shaping the functioning of microbial communities. To test between the hypotheses, we collected soil inocula from three locations in the continental United States where we might expect the microbial community composition to differ either because of geographic distance (a surrogate for history) and/or contemporary habitat differences (Martiny et al. 2006). Litters from the dominant plant species at each of the three locations were also collected, sterilized, and then milled to generate three, distinct, common garden environments. We inoculated each litter in a factorial design with each of the three soils. If different inocula yielded distinct rates of litter decomposition then the hypothesis of functional dissimilarity was supported, and if rates were similar then this supported the hypothesis of functional equivalence. We posed a sub-hypothesis under the hypothesis of functional dissimilarity that stated that soil inocula that shared a common history with a litter (i.e., soils collected from the same location as the litter) would mineralize that litter more rapidly than soil inocula without a shared history (the "home field advantage" hypothesis [Gholz et al. 2000]). Our findings support the hypotheses of functional dissimilarity and home field advantage, suggesting that differences in microbial community composition may contribute, as ultimate and not just proximal controls, to spatial and temporal variation in ecosystem carbon dynamics.

Methods

Microcosm design

Litter and soil were collected from three sites within the continental United States (see Plate 1). Grass litter (*Hordeum murinum*) was collected from Sedgwick Reserve, California (34°42′ N, 120°03′ W), pine (*Pinus taeda*) from Duke Forest, North Carolina (35°58′ N, 79°05′ W), and rhododendron (*Rhododendron maximum*) from the Coweeta Long Term Ecological Research site, North Carolina (35°00′ N, 83°30′ W). Pine and rhododendron were collected as recent litterfall and grass litter as standing-dead material. Litter was oven-dried (65°C) and then milled (2 mm). Litter was sterilized by autoclaving (121°C, 20 min) twice in succession and again 24 h later. Mineral soil (0–5 cm) was collected together with the litter. Soils were passed through a 2-mm sieve, homogenized, and then stored at 5°C until use as an inoculum. We refer to each soil inoculum as rhododendron, pine, or grass inoculum and each litter type as rhododendron, pine, or grass litter.

To 1 g of litter we added 0.5 g dry mass equivalent of soil inoculum and mixed them by vortexing in a 50-mL plastic centrifuge tube. The mixture was adjusted to and maintained at 50% water-holding capacity, which is favorable for microbial activity. Tubes were incubated at 20°C and 100% humidity during the 300-d experiment. Our design was a 3×3 combinatorial setup (i.e., all litters crossed with all soil inocula) plus additional "no-litter" soils. In all, 24 replicates per treatment were constructed, permitting destructive harvest of replicates on incubation days 25, 99, and 300 for chemical and microbial analyses (see *Clone library construction and sequencing*). The soil inoculum accounted for an average of $<5\%$ of the total CO_2 flux across all treatment combinations, and soil carbon (much of which is not likely to be readily bioavailable) accounted for $<5\%$ of total carbon in each microcosm (see Appendix A).

Determination of carbon mineralization rates and litter chemistry

Carbon mineralization rates were determined across 300 days using a static incubation procedure as described in Fierer et al. (2003). Carbon mineralization rates were determined on eight replicate samples per treatment combination at 22 time points during the course of the incubation, and carbon dioxide production

rates were corrected for the contribution of the corresponding soil inoculum by subtracting the carbon mineralization rates of the "no-litter" soils. Since samples were harvested on days 25, 99, and 300 in order to assess the composition of the microbial community (see *Clone library construction and sequencing* below), we decided that those harvested microcosms should correspond to the same eight replicates per treatment measured up to a given harvest date. Specifically, those microcosms harvested on days 25, 99, or 300 were the same microcosms on which we measured carbon mineralization rates during days 2–25, 26–99, or 100–300 of the incubation, respectively. This resulted in three incubation periods corresponding to days 2–25, 26–99, and 100–300. Since a treatment within each of these incubation periods was composed of different replicates, we measured all microcosms regardless of their specified incubation period on days 25 and 99 in order to insure that each set of replicates had similar carbon mineralization rates. It should be noted that microcosms from all three incubation periods (i.e., three sets of eight replicates) were measured on day 25, but only microcosms from the last two incubation periods (i.e., two sets of eight replicates) were measured on day 99, since the first set of microcosms had already been destructively harvested. No significant difference in mineralization rates was detected for samples from differing incubation periods measured on the same day ($F_{2,357} = 0.12$; $P = 0.89$). Since no differences in mineralization rates were detected between samples measured on the same day but differing in incubation period we felt confident in using the entire data set to calculate cumulative carbon mineralized across the entire 300-d period of this experiment (See *Statistical analyses* below).

Total carbon, total nitrogen, and C:N ratios for each inocula and litter were determined using an NA1500 CHN analyzer (Carlo Erba Strumentazione, Milan, Italy).

Clone library construction and sequencing

For each of the nine unique litter–soil inoculum combinations, DNA was extracted from samples at three time points (days 25, 99, and 300 of the incubation periods) using the PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, California, USA), after grinding 1 g of each sample with a mortar and pestle in liquid nitrogen. We monitored microbial community composition at each of these three time points in order to determine whether microbial community composition ever converged, an approach suggested by Reed and Martiny (2007). The DNA was extracted from three replicate samples per litter–soil inoculum combination at each time point, and these were pooled prior to polymerase chain reaction (PCR) amplification. This yielded a total of 27 unique DNA samples from which the clone libraries were constructed.

The DNA was also extracted and clone libraries were constructed from an additional 12 samples (two of the grass litter–grass soil inoculum treatments and two of the rhododendron litter–rhododendron soil inoculum treatments at each of the three time points). This was done to assess variability in microbial community composition within a given treatment at a given time point. In all cases, the replicate samples (the clone libraries constructed from the same litter–soil inoculum–time point combination) were essentially identical with respect to microbial community composition, and therefore we present only the results from the 27 clone libraries generated by the different treatments (nine litter type/soil inoculum combinations at three time points).

Clone libraries were constructed by amplifying the DNA samples with the universal primer pair 515f (5′-GTGCCAGCMGCCGCGGTAA-3′) and 1391r (5′-GACGGGCGGTGWGTRCA-3′). This primer pair amplifies small subunit rRNA genes from all three domains (Archaea, Bacteria, and Eukarya) (Baker et al. 2003, Cullen and MacFarlane 2005, Martiny et al. 2006) yielding a PCR product that is 850–1100 base pairs (bp) in length. Although this primer set may not necessarily amplify all taxonomic groups equally, any amplification bias should be consistent across all of the samples. Each 50-μL PCR reaction contained 1× PCR buffer, 2.5 mmol/L $MgCl_2$, 0.2 mmol/L of each dNTP, 0.2 μmol/L of each primer, 0.5 units of *Taq* DNA polymerase (Invitrogen, Carlsbad, California, USA), and 2 μL of template DNA. Each DNA sample was amplified with three replicate PCR amplifications (30 cycles each, annealing temperature of 52°C) with the amplicons from each sample pooled prior to cloning. Amplicons were cleaned using the Promega WizardSV Gel Extraction and PCR Clean Up kit (Promega, Madison, Wisconsin, USA).

Amplicons were cloned using TOPO TA for Sequencing kit (Invitrogen) following the manufacturer's instructions. Plates were grown overnight at 37°C before plasmid amplification and sequencing. A total of 32 clones were sequenced per sample at Agencourt Bioscience (Beverly, Massachusetts, USA). Despite the small size of these libraries, they were sufficiently large to allow us to compare quantitatively community structure at a general level of phylogenetic resolution (see *Sequence analyses*).

Sequence analyses

Sequences were binned into major taxonomic groups (i.e., metazoan, bacteria, and fungi) using the BLAST algorithm (Altschul et al. 1997) against the complete European Ribosomal RNA database (*available online*).[5] Sequences with expect (E) values greater than 1×10^{-100} to nearest neighbors were not included in the analyses. Of the clones sequenced, ~3% of the sequences were not

[5] ⟨http://www.psb.ugent.be/rRNA/index.html⟩

TABLE 1. Taxonomic description of the microbial communities of each litter type–soil inoculum combination at each of the three time points.

			All groups					
Litter type	Soil inoculum	Incubation day	Bacteria (%)	Fungi (%)	Proteobacteria (%)	Acidobacteria (%)	Actinobacteria (%)	CFB (%)
Rhododendron	grass	25	62.1	37.9	24.1	13.8	6.9	17.2
	pine	25	51.6	48.4	25.8	9.7	3.2	9.7
	rhododendron	25	36.7	63.3	23.3	9.4	3.0	0.0
	grass	99	58.6	41.4	37.9	13.8	0.0	3.4
	pine	99	40.0	60.0	6.7	20.0	6.7	6.7
	rhododendron	99	47.3	52.7	31.6	11.4	2.2	1.1
	grass	300	46.4	53.6	28.6	7.1	3.6	3.6
	pine	300	76.7	23.3	23.3	30.0	0.0	23.3
	rhododendron	300	82.7	17.3	30.0	36.6	3.6	4.4
Pine	grass	25	72.4	27.6	55.2	17.2	0.0	0.0
	pine	25	78.3	21.7	56.5	21.7	0.0	0.0
	rhododendron	25	71.4	28.6	46.4	25.0	0.0	0.0
	grass	99	85.2	14.8	37.0	40.7	3.7	0.0
	pine	99	70.4	29.6	40.7	14.8	0.0	14.8
	rhododendron	99	87.1	12.9	51.6	35.5	0.0	0.0
	grass	300	68.4	31.6	57.9	5.3	0.0	0.0
	pine	300	75.0	25.0	41.7	8.3	0.0	12.5
	rhododendron	300	93.3	6.7	50.0	40.0	0.0	0.0
Grass	grass	25	87.1	12.9	32.7	4.0	3.8	41.7
	pine	25	89.7	10.3	55.2	6.9	3.4	17.2
	rhododendron	25	86.2	13.8	65.5	3.4	13.8	0.0
	grass	99	92.7	7.3	21.6	1.1	4.8	47.2
	pine	99	96.8	3.2	35.5	0.0	12.9	35.5
	rhododendron	99	87.5	12.5	68.8	6.3	12.5	0.0
	grass	300	93.0	7.0	22.5	1.2	1.1	25.3
	pine	300	96.8	3.2	22.6	0.0	3.2	67.7
	rhododendron	300	100.0	0.0	76.7	6.7	3.3	3.3

Notes: Taxonomic description was determined by the 30–32 clones sequenced per library. Firmicutes refers to the "*Bacillus–Clostridium*" group, and CFB refers to the "*Cytophaga–Flavobacterium–Bacteriodes*" group. The "other bacteria" category consists primarily of sequences identified as either Planctomycetes or Verrucomicrobia. The "other fungi" category is evenly divided between sequences identified as Basidiomycota or Zygomycota.

included in the analyses as they were either of low quality, chimeric, or were neither bacterial nor fungal. The taxonomic classification of the fungal sequences was determined by BLAST search against the European Ribosomal RNA database. The bacterial sequences were aligned against the Greengenes database (DeSantis et al. 2006*b*) using the NAST aligner (DeSantis et al. 2006*a*) and classified into taxonomic groups with the Greengenes "classify" utility (*available online*).[6] Only those sequences sharing >90% similarity to reference sequences over a 600-bp region were classified. Taxonomic characteristics of the microbial communities are provided in Table 1. Sequences were deposited in GenBank under the accession numbers EU729748–EU730583 and EU725929–EU726201 for bacteria and fungi, respectively.

The bacterial and fungal sequences were combined together and aligned against bacterial and fungal guide sequences downloaded from the Silva ssu RNA database (*available online*).[7] Sequences were aligned using MUSCLE (Edgar 2004) and a neighbor-joining tree containing all bacterial and fungal sequences from the constructed libraries. In addition, an outgroup (*Haloferax volcanii*) was constructed in MEGA (Tamura et al. 2007).

We used the weighted UniFrac algorithm (Lozupone et al. 2006) to determine whether soil inocula affected the composition of the microbial communities on each litter type. UniFrac provides an overall estimate of the phylogenetic distance between each pair of communities by examining the fraction of the total branch length within a single phylogenetic tree that is unique to either of the two communities (as opposed to being shared by both; Fierer et al. 2007).

Statistical analyses

Statistical analyses of mineralization data were performed using S-Plus 7.0 (Insightful, Seattle, Washington, USA). To examine treatment effects on mineralization rates across time, a linear mixed-effects model was used in which time, inoculum source, and litter type (all discrete variables) were treated as fixed effects and permitted to interact. Microcosm identity was included as a random effect to account for the repeated sampling across time of microcosms. We analyzed the cumulative carbon mineralization rates using ANOVA with litter type, inoculum source, and block as discrete variables; inoculum source and litter type were permitted to

[6] ⟨http://greengenes.lbl.gov/cgi-bin/nph-classify.cgi⟩
[7] ⟨http://silva.mpi-bremen.de/⟩

TABLE 1. Extended.

Firmicutes (%)	Other bacteria (%)	Ascomycota (%)	Other fungi (%)
0.0	0.0	37.9	0.0
0.0	3.2	38.7	9.7
0.0	1.0	45.2	18.1
0.0	3.4	34.5	6.9
0.0	0.0	46.7	13.3
0.0	1.0	44.5	8.2
0.0	3.6	50.0	3.6
0.0	0.0	20.0	3.3
0.0	8.0	11.6	5.7
0.0	0.0	27.6	0.0
0.0	0.0	21.7	0.0
0.0	0.0	21.4	7.1
3.7	0.0	14.8	0.0
0.0	0.0	29.6	0.0
0.0	0.0	12.9	0.0
0.0	5.3	31.6	0.0
0.0	12.5	25.0	0.0
0.0	3.3	6.7	0.0
1.4	3.6	8.9	4.0
3.4	3.4	6.9	3.4
3.4	0.0	13.8	0.0
3.5	14.6	7.3	0.0
9.7	3.2	0.0	3.2
0.0	0.0	12.5	0.0
32.8	10.1	7.0	0.0
0.0	3.2	3.2	0.0
0.0	10.0	0.0	0.0

interact. Cumulative carbon mineralization rates were determined by integrating values under the curve of the 300-d sampling period. Since replicates were destructively harvested for molecular analyses, to generate cumulative values we blocked replicates within a treatment by mineralization rates and generated the cumulative values from samples within a block. This approach enabled us to determine whether litters crossed with their home (as opposed to foreign) soil inoculum resulted in the highest, cumulative carbon mineralization, permitting us to explore our "home field advantage" hypothesis. Pairwise comparisons of means were explored using the Tukey method to assess differences between inocula within the same litter type. For statistical significance we assumed an α level of 0.05 and, when reported as such, data were $\log_{10}$-transformed to conform to assumptions of homoscedasticity (verified using model checking).

RESULTS

Carbon mineralization

Across the course of the incubation we noted that in most cases, and regardless of the inoculum–litter combination, that carbon mineralization dynamics followed an approximately similar three-stage pattern. This consisted of an initial peak in mineralization rates during the first 25 d, a secondary peak lasting from about day 30 to as much as day 150, and finally a decline phase characterized by a gradual decrease in mineralization rates over time (Fig. 1).

When examining each litter in turn, for rhododendron litter we found a significant two-way interaction between inoculum and time on carbon mineralization rates for each incubation period ($P < 0.0001$ in all cases; Table 2, Fig. 1A). The interactions, across all incubation periods (i.e., days 2–25, 26–99, and 100–300), showed that mineralization dynamics were different between at least two of the inocula and that these differences between the inocula were dependent upon time. For pine litter a significant two-way interaction between inoculum and time was found for the first two incubation periods ($P < 0.0001$ in both cases; Table 2, Fig. 1B). In the third incubation period (days 126–300), the interaction was not significant but the main effects of inoculum ($P < 0.0001$) and time ($P < 0.0001$) were (thus, relative differences between inocula were time independent). For grass litter we found a significant interaction between inoculum and time on carbon mineralization rates for incubation periods one, two, and three ($P < 0.05$, $P < 0.0001$, and $P < 0.001$, respectively; Table 2, Fig. 1C).

To examine further the dynamics we saw in the time course data, we looked at cumulative mineralization across the 300-d experiment (Fig. 2). This examination showed a significant two-way interaction term of inoculum and litter on cumulative carbon mineralization rates ($P < 0.001$; Table 3). We investigated this result by looking at the inoculum effect for each litter type individually to test our sub-hypothesis of "home field advantage."

For each litter type we found a significant effect of inoculum on cumulative carbon mineralization ($P < 0.001$ in all cases). Post hoc analyses on rhododendron litter showed that all three inocula differed, with the rhododendron inoculum yielding a higher cumulative value than either the pine or grass inoculum (Fig. 2A). For pine litter, all three inocula again differed except in this instance the pine inoculum yielded a higher cumulative value than either of the other two inocula (Fig. 2B). For grass litter, both grass and rhododendron inocula yielded a higher cumulative value than did the pine inoculum (Fig. 2C).

Microbial community

For the UniFrac analyses we examined each litter type separately (building a separate tree for each litter type), combining the sequences for each soil inoculum from the three time points. By doing this, we could examine the overall influence of the inoculum source on microbial community composition in each litter type across the entire incubation period. We found that, as expected, litter type influenced microbial community composition (data not shown). However, within each litter type, we also found a significant influence ($P < 0.01$) of soil inoculum on microbial community composition (Fig. 3), as determined by both the UniFrac significance test and

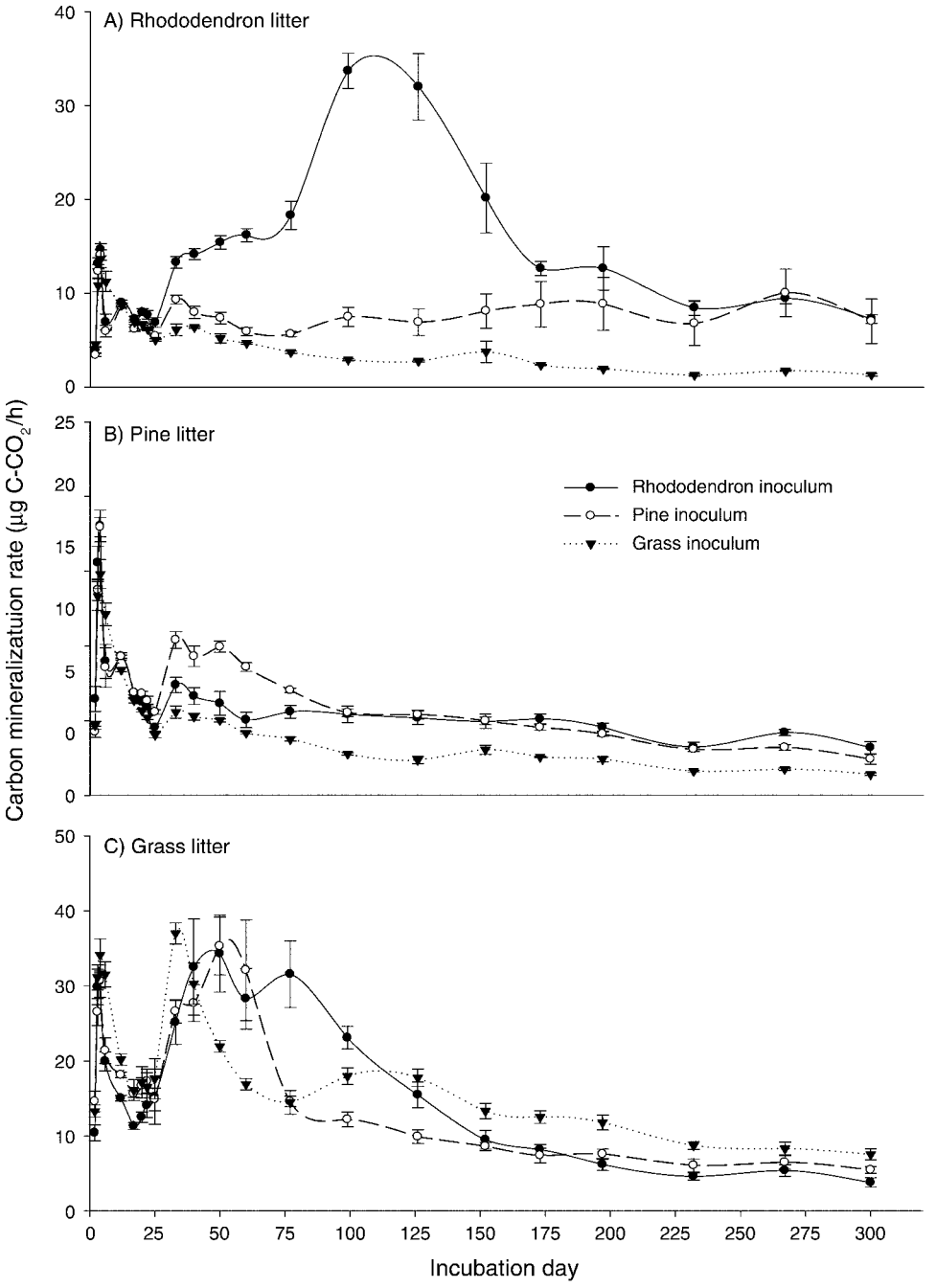

FIG. 1. Time course of carbon mineralization (mean ± SE, $n = 8$ replicates) for each litter type and soil inoculum combination. In all cases across all time periods there was a significant inoculum × time interaction on CO_2 production rates ($P < 0.05$), except in the case of pine litter between incubation days 126 and 300, for which there were significant main effects of inoculum source and time only ($P < 0.0001$ in both cases; see Table 2). (A) Mineralization dynamics for rhododendron litter. In this instance rhododendron inoculum is native to this litter type. (B) Mineralization dynamics for pine litter; pine inoculum is native to this litter type. (C) Mineralization dynamics for grass litter; grass inoculum is native to this litter type.

the phylogenetic (P) test (Martin 2002). In most instances, the inoculum effects on the microbial communities were apparent at even the coarsest levels of taxonomic resolution (e.g., shifts in bacterial : fungal ratios or shifts in the dominance of particular bacterial phyla; Table 1). Notably, the resulting microbial community composition from the different inocula was most dissimilar in the grass litter microcosms and most similar in the rhododendron litter microcosms. That is, the more chemically complex the litter habitat, the more

TABLE 2. Linear mixed-effects model results for the effect of inoculum and time on carbon mineralization rates.

Litter type	Incubation period (days)	Inoculum effect			Inoculum × time effect		
		df	*F*	*P*	df	*F*	*P*
Rhododendron	2–25	2, 21	6.12	<0.01	16, 168	5.08	<0.0001
	26–99	2, 21	285.33	<0.0001	10, 105	18.73	<0.0001
	100–300	2, 21	51.85	<0.0001	12, 126	6.34	<0.0001
Pine	2–25	2, 21	3.2	0.061	16, 168	3.65	<0.0001
	26–99	2, 21	59.16	<0.0001	10, 105	4.03	<0.0001
	100–300	2, 21	93.63	<0.0001	12, 126	NS	NS
Grass	2–25	2, 21	6.61	<0.01	16, 168	1.85	<0.05
	26–99	2, 21	4.40	<0.05	10, 105	6.22	<0.0001
	100–300	2, 21	13.01	<0.001	12, 126	3.15	<0.001

Notes: Results were analyzed based on litter type and incubation period. *F* and *P* values are reported for significant ($P < 0.05$) model terms; NS, not significant. All data were $\log_{10}$-transformed to correct for heteroscedasticity. The *F* and *P* values are estimated in linear mixed-effects models using, in this case, restricted maximum likelihood (REML) estimates and are not calculated using least-squares methods (Pinheiro and Bates 2000). Hence, means and sums of squares values are not calculated.

similar the resulting community compositions from the different inocula (Fig. 3, Table 1). Furthermore, microbial communities on the same litter habitat never converged compositionally across the course of this experiment (Fig. 3, Table 1).

DISCUSSION

The three-stage pattern observed in mineralization dynamics across time (Fig. 1) is consistent with a recently proposed mathematical model (Moorhead and Sinsabaugh 2006) that incorporates three guilds of microbial decomposers. However, we found that the magnitude and characteristics of each stage within a given litter habitat were strongly determined by the inoculum source (Fig. 1, Table 2). Indeed, the main effect of inoculum explained 20% of the variation in cumulative carbon mineralization across litter types, and its interaction with litter type explained an additional 25% of the variation (Fig. 2, Table 3). When examining the inoculum effect for each litter separately, it explained between 22% and 86% of the variation in cumulative carbon mineralization (Fig. 2, Table 3). The significant inoculum effects and inoculum interactions with time refute the hypothesis that microbial communities are functionally equivalent. These results are contrary to those studies (Balser and Firestone 2005, Langenheder et al. 2005, 2006, Ayres et al. 2006, Wertz et al. 2006) that did not observe differences in carbon mineralization rates between different microbial communities, and these results demonstrate that microbial communities may impact processes that are generally presumed to be carried out equivalently by a wide array of organisms (Schimel 1995). Indeed, the competing hypothesis of functional dissimilarity was supported across the course of the entire 300-day experiment. Given that the composition of the communities in a given litter habitat, from different inocula, were consistently distinct across time (Fig. 3, Table 1), differences in function in the same

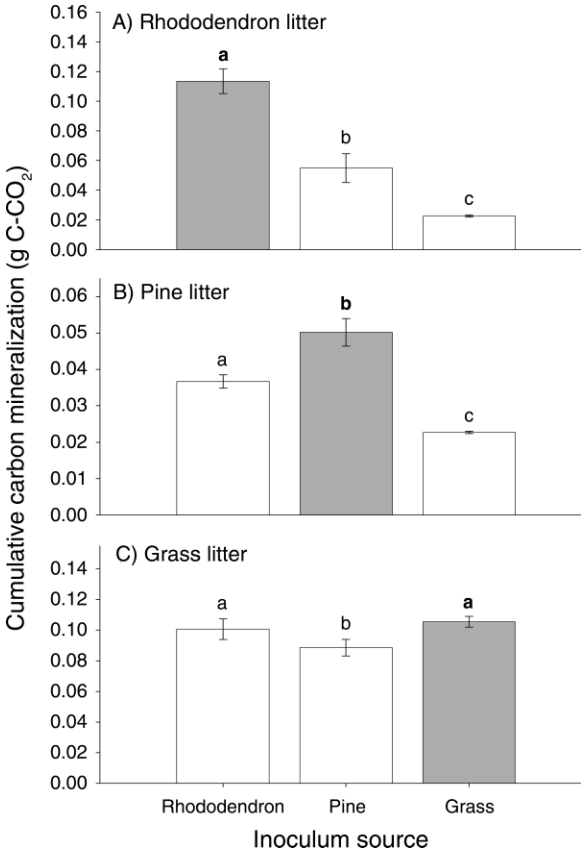

FIG. 2. Cumulative carbon mineralized (mean ± SE, $n = 8$ replicates) for each litter type by inoculum across the 300-day experiment. The inoculum effects for each litter type were highly significant ($P < 0.001$). Letters denote significant differences between treatments within each litter type using a Tukey post hoc pairwise comparison, and letters in boldface denote the home litter × home inoculum combination, as do gray bars. (A) Cumulative carbon mineralization values for rhododendron litter, with rhododendron inoculum being native to this litter type. (B) Cumulative carbon mineralization values for pine litter. (C) Cumulative carbon mineralization values for grass litter.

TABLE 3. ANOVA results with percentage sums of squares explained (%SS) for the effects of inoculum across all litter types and for the effect of inoculum within each litter type on cumulative carbon mineralization.

Source of variation	df	SS	%SS	*F*	*P*
All					
Inoculum	2	5.6	19.6	179.8	<0.001
Litter	2	13.2	46.1	422.2	<0.001
Block	7	1.8	6.2	16.3	<0.001
Inoculum × litter	4	7.1	24.9	114.12	<0.001
Error	56	0.9	3.0		
Rhododendron					
Inoculum	2	10.2	86.2	145.0	<0.001
Block	7	1.1	9.7	4.6	<0.01
Error	14	0.5	4.2		
Pine					
Inoculum	2	2.4	82.5	114.5	<0.001
Block	7	0.4	12.4	4.9	<0.01
Error	14	0.1	5.1		
Grass					
Inoculum	2	0.1	22.3	17.6	<0.001
Block	7	0.4	68.9	15.6	<0.001
Error	14	0.1	8.9		

Note: All data were $\log_{10}$-transformed.

litter habitat may be attributed to the composition of the microbial community (Reed and Martiny 2007).

When the rhododendron and pine inocula were combined with their "home" litter resources, the combinations yielded significantly greater cumulative mineralization than instances in which the inocula were "foreign" to the litter (Fig. 2A, B, Table 3). The grass inoculum yielded cumulative mineralization values that were equivalent to the rhododendron inoculum and higher than the pine inoculum only with grass litter (Fig. 2C, Table 3). These results support the "home field advantage" hypothesis (Gholz et al. 2000, Ayres et al. 2006), suggesting that soil microbial communities display some adaptation (or pre-adaptation) to their past resource environment.

If adaptation to the past resource environment explains the "home field advantage" phenomenon that we observed (Fig. 2), this implies that decomposer communities may be selected for, at least in part, by the specific characteristics of past litter inputs as both Gholz et al. (2000) and Ayres et al. (2006) have suggested. More specifically, litter characteristics may lead to changes in the composition of a given microbial community, as observed in this study (Fig. 3, Table 1). Indeed, we chose three litter resources that presented a gradient in litter chemical complexity and recalcitrance, as indicated by both C:N ratios (Appendix A) and by in situ decomposition rates reported for identical or very similar litter types (Andren et al. 1992, Sanchez 2001, Ball et al. 2008), with rhododendron representing the most, and grass the least, complex and recalcitrant litter. Potentially then, these differences in resource quality could account for the strong home field advantage observed for both the rhododendron and pine inocula on their respective home litters and the weaker home field advantage observed for the grass inoculum on the grass litter (Fig. 2, Table 3). Notably, Langenheder et al. (2005) observed that a microbial community inoculum from a lake with high-quality dissolved organic carbon (DOC) yielded respiration rates lower than inocula from low-quality DOC environments, when exposed to common garden environments with low-quality DOC. Also, soils pre-exposed to a pesticide or herbicide may degrade that compound more rapidly in subsequent applications (Taylor et al. 1996, Laha and Petrova 1997). Together, these results suggest that the ability of microbial communities to decompose carbon compounds may be contingent on past exposure to similar compounds (Taylor et al. 1996, Laha and Petrova 1997) and that the more chemically complex litter carbon is, the more likely community function will be contingent on history and not simply on chemical quality alone.

Our study cannot unambiguously disentangle whether the functional differences between the inocula were the

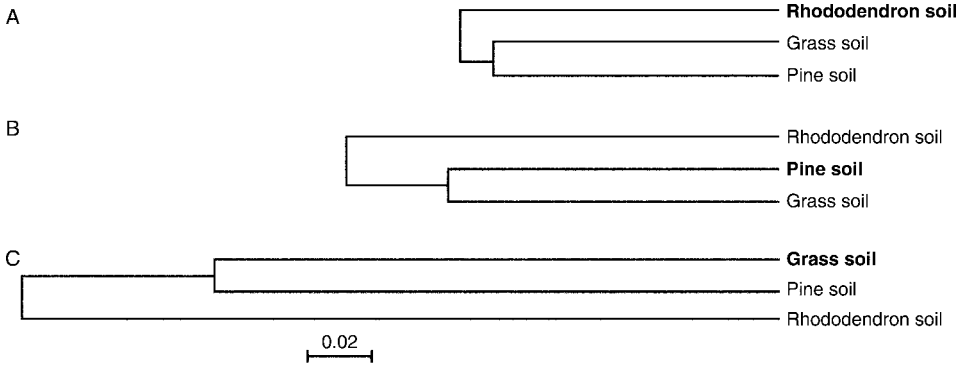

FIG. 3. Relative UniFrac distances (Lozupone et al. 2006) between the decomposer communities developing from the three soil inocula in (A) rhododendron litter, (B) pine litter, and (C) grass litter. The "home" soil inoculum is highlighted in boldface. The sequence data from the three sampling dates are combined for these analyses to illustrate overall differences in the microbial communities. For each litter type, the three inocula always yielded distinct communities ($P < 0.01$), as determined by the UniFrac significance test and the phylogenetic (P) test (see *Results: Microbial community*).

PLATE 1. Sites (U.S.) where litter and soil inocula were collected: (top left) Sedgwick Reserve, California, (lower left) Duke Forest, North Carolina, and (right) Coweeta Long Term Ecological Research site, North Carolina (Photo credits: B. A. Ball).

result of differences in the abundance of different taxa or the degradative capacities of individuals from the same taxa from different inocula. If only the former mechanism applied then communities of more similar composition would function more similarly. We did not observe this relationship; instead, those communities that developed on rhododendron litter were most similar in composition (Fig. 3) and less similar with regards to their function (Figs. 1 and 2). This observation was investigated statistically and the similarity plots are shown in Appendix B. Intriguingly, these data suggest that the manner in which microbial communities respond compositionally to environmental variation may be a poor predictor of their impacts on ecosystem function and communities that are more similar in taxonomic composition may not necessarily be more similar with regards to their functional capabilities. Or to rephrase that statement using the definitions from the biodiversity–ecosystem functioning literature, functional response groups may not correlate with functional effect groups (Naeem and Wright 2003). This may make prediction of the manner in which changing microbial community composition will impact ecosystem functioning highly uncertain. However, at least from the functional response group perspective, the microbes behaved like animal and plant communities. That is, the harsher environmental filter (i.e., rhododendron $>$ pine $>$ grass litter) generated communities of more similar phylogenetic composition (Fig. 3).

Ecosystem models are generally designed without incorporation of the potential effects of variation in microbial community composition or the functional capacities of individuals from the same taxonomic level on ecosystem processes (Zak et al. 2006). Yet, under similar environmental conditions, our results show that the effects of different microbial inocula can account for $>85\%$ of the variation in litter carbon mineralization (Table 3) and significantly alter the temporal dynamics of this process (Fig. 1). Before exploring the consequences of these findings and their possible implications for predicting ecosystem carbon dynamics, we propose two necessary areas of research. First we should resolve

whether the taxonomic composition of microbial communities or the past exposure of communities to specific resource conditions predominately impacts ecosystem function through time. That is, does community composition impact future functioning and/or is functioning a product of the selection environment (Loehle and Pechmann 1988, Goddard and Bradford 2003)? Second, and building on ideas relating to adaptation, future work should resolve whether microbial communities from different selection environments functionally converge when placed in a similar environment. Although our experiment ran for 300 days, to reliably test this possibility we would need an experimental design in which microbial communities sourced from differing environments were exposed to continuous inputs of the same resource. By using such an approach we may be able to determine how rapidly microbial communities adapt to new resource conditions and whether different communities functionally converge. Such studies might then inform us as to how rapid land use conversion may impact microbially mediated biogeochemical processes and would be vital to our understanding of the degree to which ecosystem functions are resistant and resilient to environmental change.

Our findings suggest that soil microbial communities of differing composition are functionally dissimilar. We propose future work to explore further the assumption of functional equivalence for soil microbial communities. If the weight of evidence supports the alternate hypothesis of functional dissimilarity, then the historical factors that shape microbial community functioning must be explored in ecosystem models to predict accurately how the carbon cycle will respond to global change.

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APPENDIX A

Initial percentages of C and N and the C:N ratios of the litters and the soil inocula (*Ecological Archives* E090-031-A1).

APPENDIX B

Bray-Curtis similarity matrices of function (cumulative carbon mineralization) for each litter type and incubation period and cluster diagram of function for each treatment across all incubation periods (*Ecological Archives* E090-031-A2).