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ORIGINAL ARTICLE

Changes in assembly processes in soil bacterial communities following a wildfire disturbance

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Although recent work has shown that both deterministic and stochastic processes are important in structuring microbial communities, the factors that affect the relative contributions of niche and neutral processes are poorly understood. The macrobiological literature indicates that ecological disturbances can influence assembly processes. Thus, we sampled bacterial communities at 4 and 16 weeks following a wildfire and used null deviation analysis to examine the role that time since disturbance has in community assembly. Fire dramatically altered bacterial community structure and diversity as well as soil chemistry for both time-points. Community structure shifted between 4 and 16 weeks for both burned and unburned communities. Community assembly in burned sites 4 weeks after fire was significantly more stochastic than in unburned sites. After 16 weeks, however, burned communities were significantly less stochastic than unburned communities. Thus, we propose a three-phase model featuring shifts in the relative importance of niche and neutral processes as a function of time since disturbance. Because neutral processes are characterized by a decoupling between environmental parameters and community structure, we hypothesize that a better understanding of community assembly may be important in determining where and when detailed studies of community composition are valuable for predicting ecosystem function.

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Introduction

Over the last few decades, phylogenetic approaches have revealed that microbes exhibit biogeographic patterns in diversity and distribution (Martiny *et al.*, 2006; Hanson *et al.*, 2012) which often mirror those observed for macro-organisms (Langenheder and Prosser, 2008; Langenheder *et al.*, 2010). For example, ample evidence suggests that different ecosystems host distinct types of microbes (Lozupone and Knight, 2007; Nemergut *et al.*, 2011), and that these community differences likely reflect selection (*sensu* Vellend, 2010) acting on trait differences between suites of organisms. Indeed, evidence supports the role of environmental parameters, including pH, salinity and the abundance and quality of carbon (C) in structuring microbial communities (Fierer and Jackson, 2006; Lozupone and Knight, 2007; Logue and Lindström, 2010; Nemergut *et al.*, 2010). Community assembly processes driven by environmental parameters acting on traits are often called 'niche-based' (the term we use here) but they have also been referred to as 'habitat filters' and 'deterministic processes' in the literature.

However, recent work suggests that 'historical filters' or stochastic processes also affect microbial biogeography (Martiny *et al.*, 2006). In particular, there is increasing evidence that dispersal limitations may have a more important role in structuring microbial communities than previously thought (Telford and Vandvik, 2006; Peay *et al.*, 2010; Chytrý *et al.*, 2012). Indeed, the neutral theory of biodiversity (Bell, 2001; Hubbell, 2001) in which dispersal is a key determinant of community

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structure, has been shown to explain a significant portion of bacterial community variation in a variety of systems ranging from tree hole aquatic habitats to wastewater treatment facilities (Sloan *et al.*, 2006; Woodcock *et al.*, 2007; Ofiteru *et al.*, 2010; Caruso *et al.*, 2011; Langenheder and Székely, 2011). Although the relative contribution of niche and neutral processes in determining microbial community structure may vary across systems, evidence is mounting that both can be important (Östman *et al.*, 2009; Ofiteru *et al.*, 2010).

Results suggesting that both dispersal and selection can influence microbial community assembly raise an important question: *what regulates the relative role of niche vs neutral processes in structuring microbial communities*? Work from macrobial systems suggests that a suite of factors including ecosystem productivity, metacommunity (defined here as a set of communities linked by dispersing and interacting taxa) diversity, and dispersal rates are important in the relative balance of these assembly processes (Chase, 2003). Additionally, empirical studies have demonstrated that disturbance can cause an increase in the importance of niche-based processes in structuring communities (Chase, 2007; Jiang and Patel, 2008). Yet, other research suggests that disturbance may promote neutral processes (Didham *et al.*, 2005; Didham and Norton, 2006). Although the specifics of community assembly in response to disturbance may vary with the type and intensity of disturbance, as well as the ecosystem examined, disturbance events frequently kill or severely impact many members of a community. This can 'reset' assembly processes and may create temporal gradients that provide excellent opportunities for examining general rules about community assembly.

Here, we examined bacterial community assembly processes in response to a wildfire. Fires are ecologically important disturbances (Bond *et al.*, 2005) and their effects on plant and animal communities as well as soil biogeochemistry have been widely studied (Certini, 2005; Wang and Kemball, 2005; Ferrenberg *et al.*, 2006). Recent work has also shown that fire induces microbial community shifts characterized by an increase in the relative abundance of Firmicutes and/or β-proteobacteria and an increase in the ratio of bacteria to fungi (Yeager *et al.*, 2005; Smith *et al.*, 2008; Waldrop and Harden, 2008; Bárcenas-Moreno *et al.*, 2011). However, the relative role of niche *vs* neutral assembly processes in driving these community shifts is unknown. Fire-based disturbance can lead to major shifts in a variety of environmental parameters that are likely to have large direct and indirect effects on the soil microbial community through niche-based processes. For example, fires typically result in an ephemeral pulse of ammonium (NH_4^+), creation of a reactive charcoal layer, and subsequent changes in pH (Peitikäinen et al., 2000; DeLuca and Sala, 2006; Wardle *et al.*, 1997, 1998). On the other hand, because fire causes large reductions in the standing soil microbial biomass (Hart *et al.*, 2005; Wang *et al.*, 2012), dispersal, which can be largely stochastic, may lead to an increase in the relative importance of neutral processes in early community assembly.

In this study, we used pyrosequencing of bacterial 16S rRNA genes and null deviation analysis (Chase and Myers, 2011) to examine changes in bacterial community assembly processes following a wildfire that killed all vegetation and consumed the surface litter layer of a conifer forest. We sampled the bacterial community and chemistry of soils from a burned site and from an adjacent unburned forest stand at 4 and 16 weeks after the fire. On the basis of previous studies, we expected that burned soils would demonstrate an increase in soil pH and ammonium pools, a decrease in soil organic matter and alpha (local) and gamma (regional) diversity, and shifts in bacterial community composition. Given the importance of dispersal in early recovery processes, we hypothesized that the burned communities at 4 weeks would show a greater relative importance of neutral processes than unburned communities. By 16 weeks, we hypothesized that niche-based processes, driven by the major shifts in soil chemistry, would become more important for microbial community assembly in the burned sites. Our data yielded insights into microbial community assembly following disturbance, which may be important for a better understanding of the relationships between assembly processes, microbial community structure and ecosystem function.

Materials and methods

Study site and sample collection

A total of 100 samples, 25 each from burned and unburned soils, collected at both 4 (October 2010) and 16 weeks postfire (January 2011) were analyzed in this study. Soils were sampled roughly 1 m from the base of living (unburned) and dead (burned) tree trunks near the southeastern edge of the Fourmile Fire (40.039N, 105.391W), that was ignited on 6 September 2010 on the eastern slope of the Colorado Front Range, Boulder County, CO, USA. Forests were dominated by ponderosa pines (*Pinus ponderosa scopulorum*) and Douglas firs (*Pseudotsugae menziesii glauca)* on similar northeastern aspects, between 2100–2285 m asl. The climate, fire history and soils of these forests were described by Schoennagel *et al.*, (2011) and Veblen *et al.*, (2000). Unburned and burned sites were ~300 m apart with each site roughly 150 m from the dividing fire-line between burned and unburned forest. Trees in both treatments were located within a 650 m^2 plot with a minimum of 3 m and a maximum of 25 m separating individual trees; soils were collected from under the

same trees on both dates. Samples were a composite of three 130.5 cm^3 cores from the top 5 cm of mineral soil with litter and visible organic material excluded, and were transported on ice, sieved through 2 mm mesh, and stored at −80 °C for DNA extraction or at +4 °C for biogeochemical analyses.

DNA extraction and pyrosequencing of partial 16 S rRNA genes

DNA was isolated using the MO BIO Power Soil DNA Extraction kit (MO BIO Laboratories, Carlsbad, CA, USA), and was processed as described in Nemergut *et al.*, (2010) and Knelman *et al.*, (2012). A fragment of the 16S rRNA gene encoding the V1-V2 region was amplified using modified primers of 27F and 338R adapted for Titanium chemistry (454 Life Sciences, Bradford, CT, USA). PCR reactions were performed in triplicate with 10 µl of sterile H_2O, 10 µl of 5 PRIME hot master mix (5 PRIME, Gaithersburg, MD, USA), 2 µl (5 µM) of the reverse primer, 1 µl (10 µM) of the forward primer and 2 µl of the sample DNA. Samples were denatured for 3 min at 94 °C followed by 25 cycles at 94 °C for 45 s, 50 °C for 30 s, 72 °C for 90 s and a final elongation step at 70 °C for 10 min. Three replicate PCR products were quantified, pooled and cleaned using MO BIO UltraClean-htp PCR Clean-up kits and 16S rRNA gene amplicons were sent to the Environmental Genomics Core Facility (Engencore) at University of South Carolina for 454 Life Sciences GS FLX Titanium pyrosequencing.

Sequence Analysis

Pyrosequencing data were screened using the QIIME (version 1.2.1) toolkit (Caporaso *et al.*, 2010) with the following parameters: quality score >25, sequence length >200 and <400, maximum homopolymer of 6, 0 maximum ambiguous bases and 0 mismatched bases in the primer. OTUs were denoised using Denoiser (Reeder and Knight, 2010) and were picked at the 97% identity level using UCLUST (Edgar, 2010) in QIIME. OTUs were randomly subsampled in QIIME so each library contained 1142 sequences (the fewest in a single sample). Quality data were not obtained from five samples which were excluded from analyses. The taxonomic identity of OTUs was assigned using RDP in QIIME, and QIIME was used to generate a weighted UniFrac distance matrix (Lozupone and Knight, 2005; Lozupone *et al.*, 2006) and a Bray–Curtis distance matrix (Bray and Curtis, 1957). QIIME was also used to generate α and γ diversity metrics (OTU richness (unique OTUs), Shannon diversity, phylogenetic diversity, Pielou's evenness and dominance (probability of randomly sampling two individuals of the same OTU, Caparaso *et al.*, 2010)). All sequencing data have been deposited in the MG-RAST database (http://metagenomics.anl.gov/).

Soil analysis and microbial biomass

Soil moisture, pH and total C and nitrogen (N) were measured on samples collected on both dates. Soil moisture was determined with the gravimetric method after drying soils at 60 °C for 48 h. Soil pH was measured using a 1:5 ratio of soil to de-ionized H_2O. Total C and N were determined by grinding and combustion in an elemental analyzer as described by (Knelman *et al.*, 2012). NH_4^+, dissolved organic N (DON), dissolved organic C (DOC), and microbial biomass were measured for only the 16 week samples by adding 40 ml of 0.5 M K_2SO_4 to 10 g of soil, shaking the mix for 1 h, and filtering through Whatman no.1 paper (Whatman Incorporated, Florham Park, NJ, USA). NH_4^+ concentrations were determined using the sodium salicylate method and absorbance at 650 nm on a microplate reader (Mulvaney, 1996). DOC and DON were determined using a TIC/TOC analyzer. For DOC, biomass C = EC/kEC, where EC = extractable C from soil, and kEC (extractable C from microbial biomass) was estimated at 0.45 (Beck *et al.*, 1997). DON was determined by Kjeldahl Digestion of 20 ml of extract; N = EN/kEN where kEN was estimated at 0.54 (Brookes *et al.*, 1985). Microbial C and N pools were calculated as the difference between DOC and DON from non-fumigated and 5-day chloroform fumigated soils (Brookes *et al.*, 1985; Beck *et al.*, 1997).

Data Analysis

Burned and unburned soil chemistry, α diversity measures, and weighted UniFrac matrices were compared with SAS-JMP 9.0.0 (JMP 2011) using one-way analysis of variance followed by Tukey's HSD means comparisons (Kruskal–Wallace tests followed by Steel-Dwass means comparisons when test assumptions were not met). To avoid violating assumptions of sample independence, sample OTU dissimilarities (β-diversity, calculated as mean group Bray–Curtis dissimilarity) were compared using ADONIS followed by the permutation method of 'betadisper' in the Vegan package for the R platform (Oksanen *et al.*, 2011; Team RDC 2011). Because the hypotheses tested here focus on the difference between burned and unburned soils and less on differences between geographically collocated samples, and because samples were removed from the site, repeated measures analyses or paired tests were not used. Variables that were measured only in January (NH_4^+, DOC/DON, microbial biomass) were compared via *t*-tests or Mann–Whitney U-tests. When effective, log or $\log_{10}$ transformations were applied to meet test assumptions. Our figures and tables contain back-transformed values with statistical comparisons based on transformed data as noted.

PERMANOVA and non-metric multidimensional scaling were completed on the Bray–Curtis distance matrix in PC-ORD (McCune and Mefford, 2011) and used to compare community composition in burned

and unburned samples. Mantel tests for correlations between environmental factors and the soil bacterial community of burned and unburned soils at 4 and 16 weeks were calculated in PC-ORD. Edaphic data are available in the MIMARKS database (Yilmaz *et al.*, 2011).

We used the null deviation approach (Chase and Myers, 2011) to examine bacterial community assembly. This technique uses a null model to create stochastically assembled communities from the regional species pool to determine the degree to which observed β diversity patterns deviate from stochastic assembly. The null deviation approach disentangles variation in community compositional dissimilarity across sites from variation due to changes in α (local) and γ (regional) diversity (Chase and Myers, 2011). This approach assesses changes in β diversity that result from the relative influence of niche and neutral processes and not from changes in α diversity. We measured the null deviation as the relative difference of the observed β diversity from the null-model β diversity, $(\beta_{obs}-\beta_{null})/\beta_{null}$, where β diversity was measured as Sorenson-Czekanowski dissimilarity. For each sample, the expected β diversity under the null model was calculated from 10 000 stochastically assembled communities. Gamma diversity for each null model was calculated from each within-treatment (burned *vs* unburned at 4 and 16 weeks) species pool. As this analysis requires presence-absence data and does not weight species by their abundance (unlike the non-metric multidimensional scaling and Mantel tests described above) it is sensitive to noise from rare species. Therefore, taxa with very low abundances (<1% of sequences per community) were removed from pyrosequencing data before analyses (Ofiteru *et al.*, 2010). To test for treatment differences in the null deviation we conducted permutation tests by first randomly permuting treatment labels, then resimulating null models and recalculating null deviations for each of 5000 permutations.

Results

Bacterial Community Diversity and Structure

After rarefaction to an equal sequencing depth, we found a total of 4760 unique OTUs across all samples (Supplementary Figures S1 and S2a). Unburned soils contained 2596 OTUs at 4 weeks and 2627 OTUs at 16 weeks postfire. Burned soils had 1889 OTUs at 4 weeks and 1656 at 16 weeks—28 and 37% lower γ diversity than was observed in unburned soils, respectively. Unburned soil had 850 and 1002 unique (that is, not found in samples from any other treatment/date) OTUs and burned soil had 357 and 273 unique OTUs at 4 and 16 weeks, respectively. A total of 698 'generalist' OTUs were found in both burned and unburned soils on both dates.

Burning significantly reduced α diversity for both dates (Supplementary Figure S2 b–f) regardless of the diversity metric (richness, Shannon, phylogenetic diversity, evenness or dominance) applied. Alpha diversity within burned or unburned soils did not change significantly between sampling dates. Specifically, burned soils had an average of 31 and 50% lower OTU richness than unburned soil at 4 and 16 weeks, respectively (Supplementary Figure S2b; $F_{3,84}=37.49$, $P<0.0001$). The Shannon diversity index of burned soil (four weeks = 6.12, 16 weeks = 5.46) was significantly lower than that of unburned soil (4 weeks = 7.68, 16 weeks = 7.92) (Supplementary Figure S2c, $F_{3,84}=26.69$, $P<0.0001$). Finally, burning reduced phylogenetic diversity by 28% at 4 weeks (49.61 *vs* 68.76) and 42% at 16 weeks (41.99 *vs* 72.65) (Supplementary Figure S2d; $F_{3,84}=33.98$, $P<0.0001$). OTU evenness was lower in burned soil than in unburned at 4 (0.74 *vs* 0.87) and 16 weeks (0.69 *vs* 0.89) (Supplementary Figure S2e; $F_{3,84}=22.23$, $P<0.0001$), while dominance was higher in burned soils at 4 (0.10 *vs* 0.02) and 16 weeks (0.09 *vs* 0.01) (Supplementary Figure S2f; $F_{3,84}=10.17$, $P<0.0001$). Average β diversity, in this case mean pairwise Bray-Curtis dissimilarity and UniFrac distance, was also altered by burning (Supplementary Figures S2g and h). Fire caused a significant increase in Bray-Curtis dissimilarity at 4 and 16 weeks (PERMANOVA, $P=0.0002$), and a significant increase in UniFrac for both dates (PERMANOVA, $P=0.0002$).

Burned and unburned soils harbored significantly different bacterial communities 4 and 16 weeks postfire (PERMANOVA, $P<0.001$) and within both treatments between dates (PERMANOVA, $P<0.001$). Non-metric multidimensional scaling clustered samples by treatment and date, with burned soils displaying a greater spread between samples than for unburned communities (Figure 1), consistent with the observed increases in β diversity.

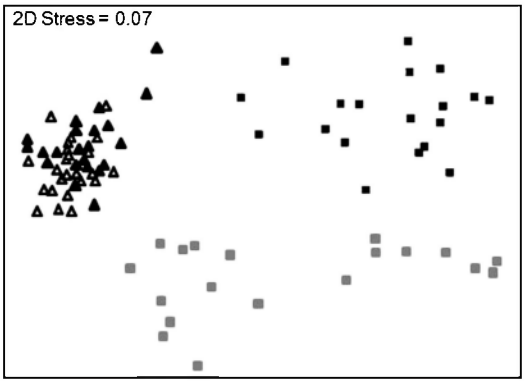

Figure 1 Non-metric multidimensional scaling ordination based on Bray-Curtis distances showing the change in bacterial community composition and increase in β diversity in burned soil bacterial communities (4 week communities = gray squares; 16 week communities = black squares) compared with unburned soil bacterial communities (4 week = open triangles; 16 week = solid triangles) 4 and 16 weeks after the fire.

The relative abundances of seven dominant bacterial phyla (or subphyla in the case of Proteobacteria) differed significantly between burned and unburned soils at 4 (Supplementary Figure S3; $\chi^2_6 = 58.07$, $P < 0.0001$) and 16 weeks ($\chi^2_6 = 75.72$, $P < 0.0001$); and within the burned samples between dates ($\chi^2_6 = 28.73$, $P < 0.0001$, Supplementary Figure S3). Proportional phyla and subphyla abundances in unburned soils were not different between dates ($\chi^2_6 = 1.29$, $P > 0.95$). *Firmicutes* were in low relative abundance in unburned soils for both dates, but dominated burned soils. *Betaproteobacteria* were in low relative abundance in the 4 week burned samples, but increased at 16 weeks to become the second most abundant taxon.

Soil chemistry

Burning reduced soil C and N ($F_{3,96} = 36.61$, $P < 0.0001$), with 65% less C and 48% less N in burned than unburned samples from 4 weeks, and 69% less C and 60% less N in the burned soil from 16 weeks (Table 1). Burning reduced microbial biomass (microbial C; measured only at 16 weeks) by 77% compared with unburned samples (Supplementary Table S1). The C:N ratio of burned soils was 39% lower than that of unburned soils at 4 weeks, and 24% lower at 16 weeks ($F_{3,96} = 107.94$, $P < 0.0001$; Table 1). Burning increased pH for both sample dates, while pH showed an overall decrease between 4 and 16 weeks regardless of treatment ($F_{3,97} = 20.5$, $P < 0.0001$, Table 1). Soil moisture was lower in burned soils at 4 weeks ($F_{3,93} = 10.38$, $P < 0.0001$), with no difference between treatments at 16 weeks (Table 1). Burned soils had greater than a 12-fold increase in mean NH_4^+ concentration (only measured at 16 weeks), averaging $3.12\,\mu g^{-1}g$ in unburned soils and $38.87\,\mu g\,g^{-1}$ in burned soils ($U = 623$, $Z = -6.01$, $P < 0.0001$, Table 1). Burning did not significantly change DOC, but increased DON (both measured only at 16 weeks) by 60% over unburned soil ($t_{24,24} = -6.0$, $P < 0.0001$, Table 1).

Community Assembly Processes

The null deviation approach (Chase and Myers, 2011) created stochastically assembled communities from the regional species pool to determine the degree to which observed β diversity patterns deviate from stochastic assembly. A null deviation close to zero suggests that neutral processes are more important in structuring the community, whereas larger positive or negative null deviations suggest that niche-based processes are more important. After 4 weeks, burned communities deviated significantly less from the stochastic assembly model than unburned communities (permutation test, $P = 0.02$; Figure 2). After 16 weeks, however, burned communities (relative null deviation $= -0.17$) deviated significantly more from the stochastic assembly model than unburned communities ($P = 0.001$; Figure 2). Importantly, the unburned sites showed a moderate but consistent deviation from the stochastic assembly model (relative null deviation $= -0.12$; Figure 2), with no significant changes in the null deviation value between the 4 and 16 week samples ($P = 0.36$). Burned sites, by contrast, were significantly more stochastic at 4 weeks than at 16 weeks ($P < 0.001$).

Community structure and soil environmental characteristics

Our analysis revealed that the relationship between environmental characteristics and soil microbial community structure varied with sampling date and disturbance. We found no significant correlations between environmental characteristics and community structure for the 4 week samples (Table 2). For the 16 week burned samples we observed significant correlations between community composition and pH. By contrast, we observed significant correlations between soil C:N and community composition for the 16 week unburned soils. Tests of correlations between the bacterial community and environmental factors yielded similar results regardless of whether weighted or unweighted community metrics were considered (data not shown).

Discussion

A severe wildfire provided an opportunity to examine the relative roles of niche *vs* neutral assembly processes in recently disturbed soil

Table 1 Comparison of unburned and burned soil properties at 4 and 16 weeks after a stand-replacing wildfire

Sample	*Treatment*	*%Moisture*	*pH*	*%C*	*%N*	*C:N*	NH_4^+	*DOC*	*DON*
4 weeks	Unburned	21.03 (2.07)[a]	7.30 (0.12)[b]	5.75 (0.59)[a]	0.23 (0.02)[a]	26.07 (0.71)[a]			
	Burned	9.70 (1.05)[b]	8.00 (0.25)[a]	2.03 (0.16)[b]	0.12 (0.01)[b]	16.00 (0.39)[c]			
16 weeks	Unburned	7.91 (1.32)[b]	6.92 (0.09)[c]	7.96 (1.04)[a]	0.30 (0.04)[a]	26.07 (0.45)[a]	3.12 (0.37)	0.34 (0.06)	0.02 (0.003)
	Burned	8.18 (0.85)[b]	7.34 (0.12)[b]	2.44 (0.39)[b]	0.12 (0.02)[b]	20.02 (0.39)[b]	38.87 (3.48)	0.28 (0.04)	0.06 (0.005)
	P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	ns	<0.0001

Abbreviations: DOC, dissolved organic C; DON, dissolved organic N; ns, not significant.Untransformed means (± 1 s.e.), *P* from analysis of variance with transformed values for variables measure in both sample dates; *P* from *t*-test or Mann–Whitney U for variables measured for one sample date. Means followed by different letters represent significant differences from Tukey's HSD comparisons ($P < 0.05$).

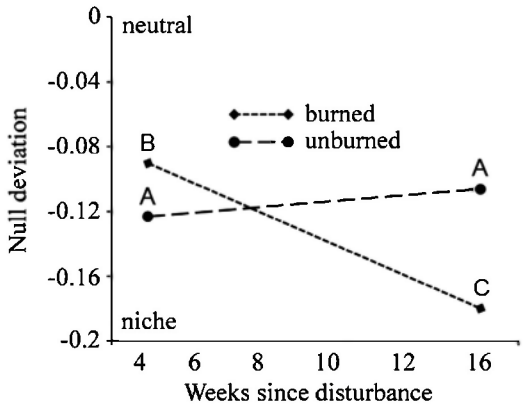

Figure 2 Plot showing the null deviation (Chase and Myers, 2011) of burned and unburned communities 4 and 16 weeks after the fire. A null deviation close to zero suggests that neutral processes are more important in structuring the community, whereas larger positive or negative null deviations suggest that niche-based processes are more important. Different letters indicate significant differences between sample dates based on permutation tests ($P<0.05$).

bacterial communities. As expected, we found that burning caused substantial changes in soil bacterial diversity (Supplementary Figure S2), community structure (Figure 1, Supplementary Figures S1 and S3) and soil chemistry (Table 1). We also found that bacterial secondary succession proceeded very rapidly in the postdisturbance landscape, as communities from 4 and 16 weeks postburn were significantly different, not only in terms of the OTUs present, but also with respect to the phyla/subphyla proportional abundances (Figure 1, Supplementary Figures S1 and S3). Seasonal effects are known to influence soil microbial community abundances and activities (Monson *et al.*, 2006; Schmidt *et al.*, 2007) and, consistent with these observations, we observed a small but significant difference in the bacterial community structure (Figure 1) of unburned samples from 4 (fall) and 16 (winter) weeks. As well, pH and soil moisture were different between the two sampling timepoints (Table 1). However, the magnitude of the differences in burned *vs* unburned soil community structure, along with significant reductions in γ and α diversity in both sample dates suggest that fire effects (direct or indirect) on microbial community assembly and secondary succession are much stronger than seasonal shifts over this time period.

A null deviation value close to zero suggests that community assembly is highly stochastic and neutral processes are more important in structuring the community. Larger positive or negative null deviations suggest that niche-based processes are more important, and environmental filters, for example, could have strong influences on community assembly. Regardless of disturbance or sampling time, null processes were important in structuring soil microbial communities (Figure 2).

Table 2 Bacterial community structure association with environmental factors in unburned and burned soils at 4 and 16 weeks after fire

Treatment group	*Soil factor*	*Mantel r*	$P \leqslant 0.05$
4 weeks unburned	All factors combined	0.117	0.175
	C:N ratio	−0.071	0.300
	%N	−0.020	0.531
	%C	−0.020	0.552
	pH	−0.138	0.213
	H_2O	0.129	0.155
4 weeks burned	All factors combined	0.116	0.130
	C:N ratio	−0.025	0.459
	%N	−0.052	0.349
	%C	−0.033	0.431
	pH	−0.098	0.218
	H_2O	0.125	0.125
16 weeks unburned	All factors combined	0.267	0.059
	C:N ratio	0.320	0.007*
	%N	0.121	0.162
	%C	0.119	0.144
	pH	0.083	0.226
	H_2O	0.246	0.069
	NH_4^+	−0.015	0.496
	DON	−0.086	0.306
	DOC	−0.078	0.337
16 weeks burned	All factors combined	0.208	0.109
	C:N ratio	−0.048	0.380
	%N	0.027	0.295
	%C	−0.003	0.649
	pH	0.303	0.013*
	H_2O	−0.082	0.335
	NH_4^+	0.214	0.101
	DON	0.202	0.071
	DOC	0.099	0.210

Abbreviations: DOC, dissolved organic C; DON, dissolved organic N.Mantel tests were completed with a Bray–Curtis distance matrix for OTU counts and a Euclidean distance matrix for soil factors. OTUs with $\geqslant$10 sequences per sample were included. Significance for each test was determined from 5000 randomized Monte Carlo runs.
*indicates significant relationships ($P<0.05$).

However, our analysis also revealed that fire caused a quantifiable change in assembly processes (that is, the relative importance of niche *vs* neutral processes) that shifted with time since disturbance. As with the observed changes in diversity and community structure, these shifts were evident over very short time frames: communities in the soils 4 weeks postburn were shaped by neutral processes (smaller null deviations) significantly more so than unburned communities, while burned communities at 16 weeks were shaped by niche processes (larger null deviations) more than unburned communities.

Interestingly, we also observed an increase in β diversity among postburn communities (Figure 1, Supplementary Figure S2e,f). Such increases in β diversity have been interpreted as support for neutral processes in community assembly (Kraft *et al.*, 2007; Chase and Myers, 2011). However, our null deviation analysis supports that the observed increases in β diversity are due to both increases (4 weeks) and decreases (16 weeks) in neutral processes in burned soils relative to unburned sites (Figure 2). The initial increase in β diversity may

thus reflect the stochastic nature of dispersal, and the fact that 'seed' microbes from air and precipitation vary in both space and time. As succession proceeds, however, our data suggest that this increase in β diversity reflects an increase in niche-based processes. Other work has demonstrated that fire tends to increase biogeochemical heterogeneity, likely due to variation in the severity of the burn across the landscape (for example, Turner et al., 2007; Hamman *et al.*, 2008). Thus, these increases in environmental variation may be reflected, at least to some degree, in the changes in community composition observed in the burned soils, combined with an increase in niche-based processes in shaping communities. These shifts in community assembly may also be reflected in the trait differences of the dominant taxa. For instance, spore-forming Firmicutes —which may be more easily dispersed— are most abundant in the 4 week postburn soils. As well, Betaproteobacteria, which commonly dominate early successional landscapes (Nemergut *et al.*, 2007; Sattin *et al.*, 2009) are more abundant at 16 weeks, when the decrease in soil organic matter (Table 1) may select for more oligotrophic taxa.

We performed Mantel tests to examine correlations between environmental parameters and community structure. We measured a suite of standard soil chemical parameters (Table 1) but did not observe any significant relationships between these variables and community structure, (regardless of whether comparisons were completed with abundance based analyses or unweighted analyses), within treatments for the 4 week samples (Table 2). For the 16 week samples, β diversity in the burned samples was correlated with pH while unburned communities were correlated with soil C:N. Correlation coefficients for these relationships were roughly similar, and some have interpreted these as evidence for the amount of variation in community structure that is explained by niche-based processes. However, as noted by (Anderson *et al.*, 2011), extreme caution should be taken in interpreting these relationships in terms of assembly mechanisms because of the potential for unmeasured environmental variation as well as the possibility of spatial structure in environmental parameters. Thus, these analyses provide hypotheses about the potential sources of local variation in bacterial community structure, but are not inconsistent with our null deviation analyses.

These observed differences in community assembly over very short time scales may reconcile the fact that different researchers have found support for increases in both niche (Chase, 2007; Jiang and Patel, 2008) and neutral (Didham *et al.*, 2005; Didham and Norton, 2006; Leibold and McPeek, 2006) processes following disturbance. Indeed, our results suggest dynamic shifts in community assembly processes in postdisturbance landscapes, leading us to propose a conceptual model describing

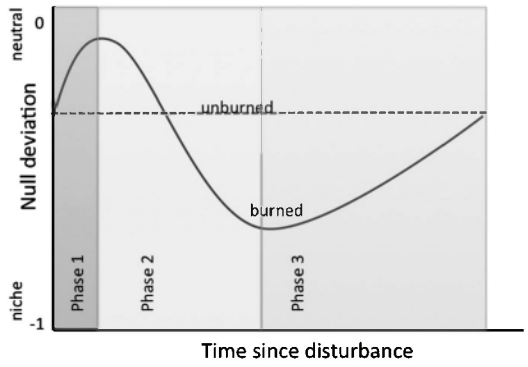

Figure 3 The three hypothesized phases of community assembly following disturbance. Phase 1 is characterized by more neutral assembly processes; Phase 2 is more niche-based and Phase 3 is increasingly more neutral.

these changes. Specifically, we hypothesize that time since disturbance features at least three distinct phases in community assembly (Figure 3). Phase 1 immediately follows disturbance, and is characterized by a brief increase in the relative role of neutral processes in community assembly, perhaps because stochastic dispersal processes are strongly affecting community structure. This is supported by other work that suggests that ecological equivalence may be more likely immediately following a severe disturbance event or at the onset of primary succession when immigrants face less competition (Leibold and McPeek, 2006). During phase 2, organisms begin to grow and divide, and niche-based processes in the postdisturbance landscape act as strong filters on microbial community composition. This can be characterized by increases in β diversity if the disturbance was heterogeneous at the landscape level, or decreases if it was more homogenous. Similar results have been observed in other experimental systems as niche-based processes were shown to be important following drought as well as density-independent mortality disturbance events (Chase, 2007; Jiang and Patel, 2008). Finally, over longer time scales (phase 3), the environment becomes less harsh and neutral processes again become more important in shaping community structure. To some degree this supports other work that suggests that neutral processes may dominate community assembly within successional stages while niche processes may dominate during transition periods between successional stages (Denslow, 1980; Ellner and Fussmann, 2003; Cadotte, 2007).

An important caveat of our data is that we lack an understanding of community assembly from a functional level. It is possible that examining assembly processes using metagenomics or metatranscriptomics would reveal different patterns in the relative importance of niche *vs* neutral processes. For example, Burke *et al.* (2011) recently showed that microbial community succession on marine algae displayed functional convergence but

lacked taxonomic coherence. This suggests a high degree of functional redundancy in microbial communities, which may decouple structure and function. If these same processes are at work in the postdisturbance landscape that we examined, analysis of the 16S rRNA gene data may suggest that communities are assembled by a larger predominance of neutral processes than is actually the case. Future studies should use both SSU rRNA and metagenomic approaches to examine community assembly processes, as trait-based approaches may yield deeper understandings of the mechanisms driving assembly.

As well, such trait-based approaches may be important for guiding approaches for how and where to sample microbial communities to understand ecosystem processes. We now possess the tools to reveal high-resolution details about temporal and structural changes in microbial community structure. As microbial community structure drives function, some argue that there is value in knowing 'who does what' to understand and predict ecosystem processes (Zak *et al.*, 2003; Monson *et al.*, 2006; Van Der Heijden *et al.*, 2008). However, as mentioned above, many studies reveal that environmental factors are important determinants of microbial community structure (Fierer and Jackson, 2006; Lozupone and Knight, 2007; Logue and Lindstrom, 2010; Nemergut *et al.*, 2010). Also, these same parameters are vital in regulating ecosystem processes (Bonan and Shugart 1989; Paul and Clark, 1996), raising the question: how much added value is provided by detailed investigations of microbial structure data? Indeed, a better understanding of microbial community assembly processes, and where and when they may change in response to disturbances, could be fundamental to understanding links between structure and function. We hypothesize that if communities are largely structured by neutral processes, then while environmental factors will still affect ecosystem processes in these communities by influencing the physiologies of individual microorganisms, soil communities should exhibit less of a direct link between edaphic factors and processes. In other words, the degree to which niche *vs* neutral processes guide microbial community assembly will affect the strength of the relationship between environmental factors and ecosystem processes.

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