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ORIGINAL ARTICLE

Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlying grassland

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Climate change impacts on soil microbial communities could alter the structure of terrestrial ecosystems and biogeochemical cycles of the Earth. We used 16S rRNA gene microarrays to evaluate changes in the composition of grassland soil microbial communities under rainfall amendments simulating alternative climate change scenarios, and to compare these to responses of overlying plants and invertebrates. Following 5 years of rainfall manipulation, soil bacteria and archaea in plots where natural rain was supplemented differed little from ambient controls, despite profound treatment-related changes in the overlying grassland. During the sixth and seventh year, seasonal differences in bacterial and archaeal assemblages emerged among treatments, but only when watering exacerbated or alleviated periods of particularly aberrant conditions in the ambient climate. In contrast to effects on plants and invertebrates, effects on bacteria and archaea did not compound across seasons or years, indicating that soil microbial communities may be more robust than associated aboveground macroorganisms to certain alterations in climate.

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Introduction

Scientists have now amassed a large body of evidence documenting biological responses to recent climate change (Parmesan, 2006; Rosenzweig *et al.*, 2008). This work has focused primarily on macrobiota, whereas climate change impacts on microbial communities remain little understood. Given the fundamental role of microbial communities in biogeochemical cycling, responses to changing climate could have repercussions for ecosystem structure and feedbacks to the climate system (Wardle *et al.*, 2004). With relatively short generation times and rapid growth under favorable conditions, microbial communities could be among the fastest components of an ecosystem to respond to changing environmental conditions (Wolters *et al.*, 2000; Prosser *et al.*, 2007). On the other hand, the high functional and genetic diversity, potentially rapid evolutionary rates and vast dispersal capabilities of microbes may mitigate responses to environmental change (Giller *et al.*, 1997; Finlay and Clarke, 1999; Girvan *et al.*, 2005; Prosser *et al.*, 2007). To date, the enormous diversity of soil microbial communities (Rossello-Mora and Amann, 2001; Torsvik *et al.*, 2002; Gans *et al.*, 2005) has precluded their comprehensive characterization and limited our understanding of climatic effects to broad functional or taxonomic groupings across a community (Fierer *et al.*, 2003; Zak *et al.*, 2003; Waldrop and Firestone, 2006a, b; Rinnan *et al.*, 2007) or specific subsets within a community (Horz *et al.*,

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2004, 2005). Here, we use 16S rRNA gene microarrays to profile the composition of soil bacterial–archaeal communities in detail and to compare the form and timescale of their ecological response to rainfall manipulation with that of overlying plant and invertebrate communities.

Since 2001, 36 plots in a northern California grassland have been subjected to one of three precipitation-addition regimes designed to mimic predictions of alternative climate change models (National Assessment Synthesis Team, 2000; Suttle *et al.*, 2007). Previous work has shown that effects of rainfall amendment on plant and invertebrate communities depend heavily on the timing of the increase in rainfall (Suttle *et al.*, 2007). Increased rainfall during the current winter rainy season has had little discernible effect on aboveground communities. Added spring and summer rainfall that serves to extend the rainy season, on the other hand, has produced dramatic changes in the composition and diversity of grassland plants and invertebrates, in part because seasonal effects have generated strong ecological feedbacks that compound across years (Suttle *et al.*, 2007). Following the fifth year of rainfall manipulation, we initiated work to determine how these different precipitation regimes and associated aboveground changes impact underlying soil bacterial and archaeal communities. Plant and microbial communities are potentially powerful mutual drivers in the response of terrestrial ecosystems to global change (Wardle *et al.*, 2004), though associations between them are not well understood. With a combined aboveground—belowground, field-based approach, we examined the magnitude of response in each to a shared change in environmental conditions.

Materials and methods

Experimental background and sample collection

The field experiment was conducted at the Angelo Coast Range Reserve in Mendocino County, California (39°44′17.7″N, 123°37′48.4″W). Beginning January 2001, rainfall amendments were imposed over replicate 70-m^2 grassland plots in accordance with projections from two leading climate change models (Figures 1a and b) (National Assessment Synthesis Team, 2000). Treatments included increased winter rainfall (January–March), increased spring rainfall (April–June) and a watering control (ambient rainfall). Each water-addition plot receives 14–16 mm of rainfall over ambient every 3 days for 87 days.

After 5 years of precipitation amendment, we designated 12 neighboring plots for combined analysis of aboveground and belowground communities. Across the following 5 years (years 6 and 7 of rainfall amendment), we sampled plant and microbial composition of these plots early in the rainy season (December 10), late in the rainy season (April 1), at peak plant production and diversity (May 31) and during summer drought (July 1). At each time point, we collected four separate soil subsamples per plot. Subsamples consisted of two 2-cm-diameter 15-cm-deep soil cores collected inside predesignated 400-cm^2 quadrants arrayed within each plot (Figure 1c). Immediately before soil collection, we harvested all aboveground plant tissues within these quadrants for measurement of biomass and species composition (see Supplementary materials and methods for details and for information on invertebrate sampling protocols). Soil subsamples were combined to obtain measurements of the plot's soil moisture content, pH and available nitrate and ammonium (see Supplementary materials and methods for protocols).

Bacterial–Archaeal community analysis

We extracted DNA from soils within 24 h of collection from the field. After soil subsamples were manually homogenized to break down soil aggregates, we extracted DNA from 0.25 g (approximate dry weight) of each subsample using the Power Soil DNA kit (MO BIO, Carlsbad, CA, USA) according to the manufacturer's instructions. Extracted DNA was quantified by gel electrophoresis and equal concentrations from each subsample were pooled by plot for further analysis. Amplification of the bacterial and archaeal 16S rRNA gene for microarray analysis and conventional sequencing is described in detail in Supplementary materials and methods.

For microarray analysis, DNA was biotin labeled and hybridized to a high-density 16S rRNA gene microarray. Microarrays consisted of ~500 000

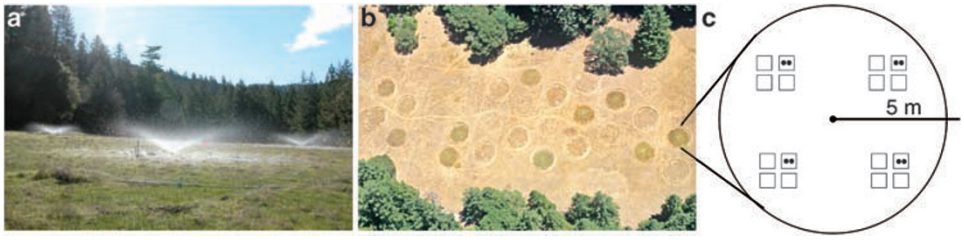

Figure 1 The field experiment. (**a**, **b**) Artificial rainfall is delivered from sprinklers that distribute water evenly over replicate 70 m^2 plots. (**c**) Soils and plants were collected for analysis from four quadrants per plot at each of four time points over the year, beginning early in the rainy season (December 10) and continuing into the summer drought (July 1).

probe sets designed to distinguish among >8700 distinct taxa (detailed information in Supplementary materials and methods and Brodie *et al*. (2006)). PhyloChip washing, staining and scanning were performed as described by Masuda and Church (2002). PhyloChip data were measured at taxon/OTU level (similar to 99% sequence homology) but were summarized to subfamily level (approximately 94% sequence homology) for further analyses to minimize OTU representation-by-subfamily biases. A positive fraction of 0.90 (that is, hybridization of >90% of taxon-specific probes) was our threshold for detection and positive identification of a specific taxon within a sample (Brodie *et al*., 2006, 2007).

Data analysis and array validation

Probe hybridization intensities allow for comparisons of taxonomic membership and the relative abundance of individual taxa among samples. We used nonmetric multidimensional scaling (Kruskal and Wish, 1978; Clarke, 1993) to structure high-dimensional community composition data along simple axes expressing overall compositional similarity and dissimilarity among plots (more information in Supplementary materials and methods). We tested for treatment-based differences in community composition with multi-response permutation procedures (Mielke, 1984; Mielke and Berry, 2001), and followed this multivariate analysis with univariate statistical screening (analysis of variance (ANOVA); $P<0.05$) to determine which taxa most strongly drove these patterns.

We verified the depth of coverage provided by microarrays by constructing and sequencing 16S rRNA gene clone libraries for samples collected from each treatment in December 2005 and May 2006 (from 110 to 284 high-quality clones used for analysis, Supplementary materials and methods and Table S1). Only 4% of organisms detected by cloning were not detected by the microarray (at the aforementioned 0.90 detection threshold) (Supplementary Table S2), supporting the comprehensiveness of microarray coverage for these communities.

Results and discussion

We tested soils in December 2005 (following the onset of the rainy season but before the sixth year of water addition) to evaluate the cumulative impact on the microbial community of the previous 5 years of rainfall amendment and aboveground change. We found that the overall structure of soil bacterial–archaeal communities was statistically indistinguishable among treatments (Figure 2a), even as overlying communities of plants and invertebrates had diverged markedly (Suttle *et al*., 2007; Figure 2b). Across the next 2 years, microbial composition remained statistically indistinguishable among treatments through all but two time points (Figure 2a). Treatment-related differences did emerge in April 2006 and July 2006, but these differences did not persist even to the following sampling date (May 2006 and December 2006, respectively). In fact, compositional differences across sampling dates were more pronounced than differences among treatments at any single sample point (Figure 3). Under the region's Mediterranean-type climate, winter rainy seasons that can deliver more than 200 cm of rainfall in just a few months are followed by prolonged periods of drought in which no rain may fall for 6 months or more. Sampling across this temporal gradient, we found strong seasonal dynamics in the composition of soil bacterial–archaeal communities, particularly in 2005–2006, when sampling dates were distinguished by sharp contrasts in climatic conditions (Supplementary Figure S2).

Treatment-related differences detected in April and July 2006 were absent in April and July 2007 (Figure 2a), suggesting that the 2006 differences were not driven solely by experimental watering, but more likely arose from an interaction between watering and ambient climatic conditions. Both the April and July 2006 sample dates followed periods of aberrant climate at the field site. First, March 2006 was among the wettest Marches on record in northern coastal California (California Climate Tracker, http://www.wrcc.dri.edu/monitor/cal-mon/index.html) (that is, rank seven in a 113-year record). Against this backdrop of unusually high ambient precipitation (Supplementary Figure S2), supplemental watering led to suppressed abundances of many bacterial groups relative to control plots (Figure 4). The strongest effect was in winter-addition plots, where increased moisture stress would result directly from water addition throughout the rainy season, but we also saw decreased abundances of many groups in spring-addition plots. These differences were attributable, perhaps, to the thick moss covering that had accumulated in spring-addition plots (Supplementary Table S3), as mosses reduce moisture loss from the soil relative to bare ground or vascular plant cover (Zimov *et al*., 1995). Following the end of that rainy season, drought conditions began abruptly when an unusually hot period over the first several weeks of May (Supplementary Figure S2) dried surface soils and killed annual plant species that had only recently germinated. Over the 8 years of the study, this loss of spring-germinating plants was observed only in 2006. The hot spell and rapid soil surface drying appear to have accelerated natural seasonal dynamics in the microbial community, whereas the addition of water buffered these changes and drove spring-addition plots apart from the other treatments in both overall composition (Figure 2a) and individual bacterial abundances (Figure 4).

Variation in the relative abundance of specific taxa among treatments (Figure 4 and Supplementary Figure S1) can be difficult to attribute to specific

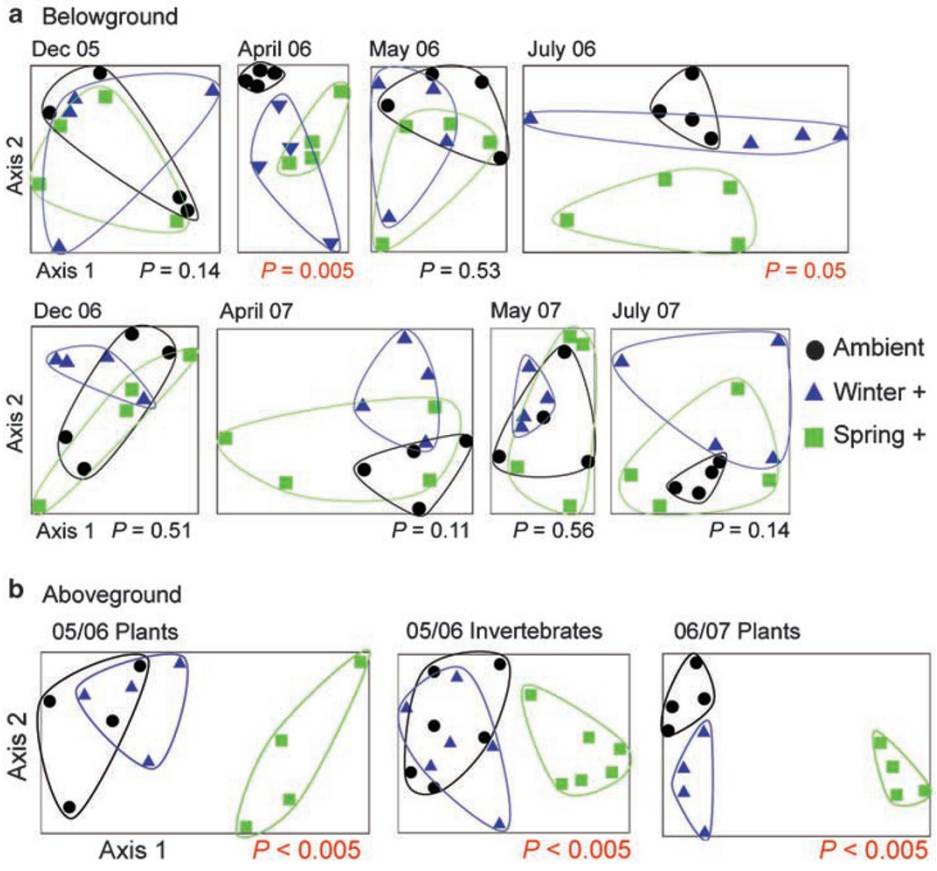

Figure 2 Effects of 5+ years of rainfall amendment on (**a**) belowground and (**b**) aboveground communities. Ordinations illustrate results from nonmetric multidimensional scaling of (**a**) taxon-by-taxon abundance data across all subfamilies of bacteria and archaea detected by microarrays at each time point and (**b**) species-specific production data and family-specific abundance data across all plants and invertebrates, respectively (Supplementary Table S4). Distances among points express relative dissimilarities in overall community composition among plots. *P*-values denote significance levels from statistical testing for differences in community composition among treatments using multi-response permutation procedures.

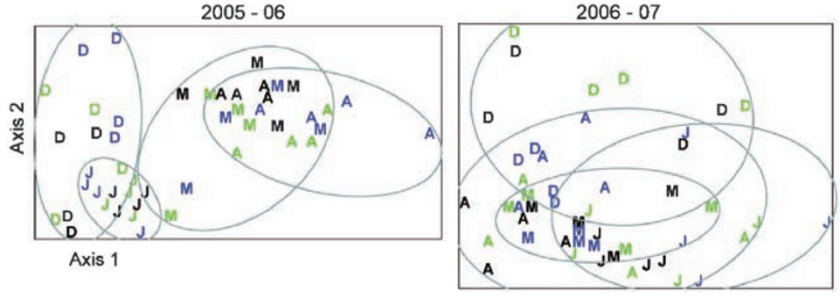

Figure 3 Variation in soil bacterial–archaeal community composition with respect to watering treatment (color) and sampling date (letter). Distances among points express relative dissimilarities in overall community composition among plots and time points. Blue, winter-addition plots; green, spring-addition plots and black, control plots. D, December 10; A, April 1; M, May 31; J, July 1.

environmental drivers, given the complexity of the soil system and gaps in our understanding of the physiology and ecology of most soil microorganisms. Where information exists for specific organisms, however, similar responses by less well-characterized groups may provide clues to their functions and activities in the soil environment. In July 2006, for example, many Bacteroidetes, betaproteobacteria and gammaproteobacteria were favored in spring-addition plots (Figure 3). These groups generally adopt a more r-selected life-history strategy, typified by rapid responses to high resource availabilities (Smit *et al.*, 2001; Fierer *et al.*, 2007). Such conditions are characteristic of spring-addition plots, where soils remain moist, early-senescing plant species are actively decomposing and plant

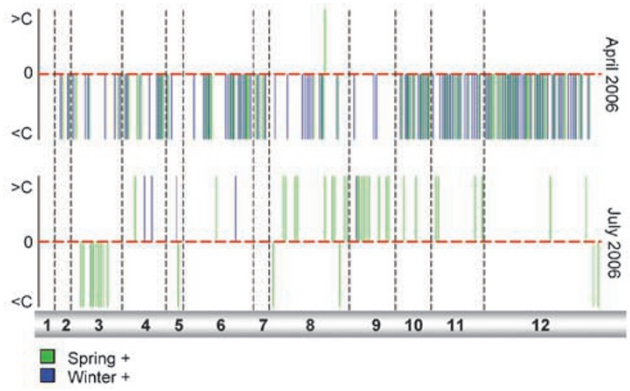

Figure 4 Treatment-based differences in relative abundance of individual subfamilies, as indicated from univariate screening of all subfamily-level data (analysis of variance (ANOVA): unadjusted $P \leqslant 0.05$). Each colored vertical bar represents a subfamily for which abundance in a water-addition treatment (green, spring addition; blue, winter addition) differed statistically from the control. Bars extending upward from the dotted red line to '>C' denote greater abundance in a water-addition treatment than in the control; bars extending downward to '<C' denote lower abundance than in the control. Subfamilies are grouped at broader taxonomic levels as follows: (1) Archaea, the Bacterial phyla; (2) Acidobacteria; (3) Actinobacteria; (4) Bacteroidetes; (5) Cyanobacteria; (6) Firmicutes and (7) Verrucomicrobia, and the Proteobacteria classes (8) Alphaproteobacteria; (9) Betaproteobacteria; (10) Deltaproteobacteria; (11) Gammaproteobacteria and (12) other phyla.

species with later phenologies remain active. In contrast, numerous Actinobacteria were suppressed in spring-addition plots at this sampling date. Most Actinobacteria are strict aerobes, generally adopting a slow-growing, K-selected strategy suited to low resource availability (Fierer *et al.*, 2007) and found in lower abundances in wetter soils (Goodfellow and Williams, 1983; Alekhina *et al.*, 2001).

Conclusions

Global climate change could have important consequences for patterns of species composition and biodiversity across landscapes, and there is great concern over how these will impact ecosystem productivity and function (Loreau, 2001; Tilman *et al.*, 2001; Chapin *et al.*, 2005). Microbial composition and function are sensitive to variability and extremes in soil conditions (Stark and Firestone, 1996; Gulledge and Schimel, 1998; Fierer *et al.*, 2003), and researchers working within global change experiments have found that environmental perturbations can impact both individual bacterial groups (Horz *et al.*, 2004, 2005) and aggregate community-level properties such as biomass and respiration (Zak *et al.*, 2000). We detected changes in microbial abundance and composition in response to climatic amendment, but sampling repeatedly across seasons and years found that these responses were short lived and left little legacy.

Microbial communities in Mediterranean-type grassland soils encounter pronounced fluctuations in soil moisture content across the year. The climatic history of these ecosystems would select for microbial populations that are resilient to highly variable environmental conditions (Waldrop and Firestone, 2006a, b). This may explain why responses to imposed shifts in baseline precipitation regimes were minimal compared to compositional changes observed across the year (Figure 3). When treatment effects did emerge following periods of more extreme conditions, they were short lived against background dynamics. Predicted increased frequency of extreme weather events (National Assessment Synthesis Team, 2000) and changes in baseline conditions to levels outside the range of historical climatic regimes may be necessary to initiate longer-term or compounding changes in the bacterial and archaeal composition of these soils.

Results from this experiment indicate a degree of robustness to climate alteration, in the form of elevated rainfall, by soil microorganisms not seen in overlying macroorganisms. Through 7 years of precipitation amendment, we found much less change in the composition of soil bacterial and archaeal communities than in overlying plants and animals. Most plant and invertebrate taxa in this grassland complete their life cycles within a single year, yet experimental manipulation of the timing of the rainy season generated strong interannual feedbacks that led to dramatic differences in the composition and diversity of aboveground communities 5+ years into the experiment (Figure 2b; Suttle *et al.*, 2007). In contrast, soil bacterial and archaeal communities remained statistically indistinguishable among treatments after 5 years and through most of the 2-year sampling period that followed (Figure 2a). Research has shown that many characteristics of a plant assemblage—composition (Hunt *et al.*, 1988; Bardgett *et al.*, 1999; Smalla *et al.*, 2001; Wieland *et al.*, 2001; Nunan *et al.*, 2003; Ayres *et al.*, 2006), diversity (Gruter *et al.*, 2006) and production (Zak *et al.*, 2003)—can affect the microbial composition of underlying soils. Any snapshot measurement in our experiment might have affirmed this idea for our grassland system as well, but analyzing across seasons we find a soil community characterized by marked seasonal dynamics and longer-term decoupling from aboveground change.

There is speculation that the broad taxonomic distribution of functional traits within microbial communities may confer functional robustness to losses of taxa and changes in composition (Giller *et al.*, 1997; Wolters *et al.*, 2000; Griffiths *et al.*, 2001; Wertz *et al.*, 2007). Intriguingly, this study shows that microbial community composition itself can be robust both to changing climate and to associated changes in plant production and species composition.

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Data deposition

The sequences reported in this paper have been deposited in the GenBank database (accession numbers EF515877 to EF516982).

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Supplementary Information accompanies the paper on The ISME Journal website (http://www.nature.com/ismej)