Vulnerability and resistance in the spatial heterogeneity of soil microbial communities under resource additions

Kelly Gravuer[a,1], Anu Eskelinen[b,c,d], Joy B. Winbourne[a,e], and Susan P. Harrison[f,1]

[a]Graduate Group in Ecology, University of California, Davis, CA 95616; [b]Department of Physiological Diversity, Helmholtz Center for Environmental Research, 04318 Leipzig, Germany; [c]Department of Physiological Diversity, German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, 04103 Leipzig, Germany; [d]Department of Ecology and Genetics, University of Oulu, FI-90014 Oulu, Finland; [e]Department of Earth & Environment, Boston University, Boston, MA 02215; and [f]Department of Environmental Science and Policy, University of California, Davis, CA 95616

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Spatial heterogeneity in composition and function enables ecosystems to supply diverse services. For soil microbes and the ecosystem functions they catalyze, whether such heterogeneity can be maintained in the face of altered resource inputs is uncertain. In a 50-ha northern California grassland with a mosaic of plant communities generated by different soil types, we tested how spatial variability in microbial composition and function changed in response to nutrient and water addition. Fungal composition lost some of its spatial variability in response to nutrient addition, driven by decreases in mutualistic fungi and increases in antagonistic fungi that were strongest on the least fertile soils, where mutualists were initially most frequent and antagonists initially least frequent. Bacterial and archaeal community composition showed little change in their spatial variability with resource addition. Microbial functions related to nitrogen cycling showed increased spatial variability under nutrient, and sometimes water, additions, driven in part by accelerated nitrification on the initially more-fertile soils. Under anthropogenic changes such as eutrophication and altered rainfall, these findings illustrate the potential for significant changes in ecosystem-level spatial heterogeneity of microbial functions and communities.

grasslands | homogenization | climate change | precipitation | eutrophication

As humans intensify their demands upon ecosystems, it is increasingly recognized that not all desired outcomes can be maximized at any single location (1). In turn, maintaining spatial heterogeneity of ecosystem structure and function has been recognized as a powerful way to maintain diverse functions at the ecosystem scale; for example, grassland ecosystems that are mosaics of fertile and infertile soils can support both plant productivity and native species diversity (2). Predicting how global change could alter spatial heterogeneity in ecosystem processes is therefore critical (3). Understanding the responses of soil microbes to these environmental changes—such as changes in nutrient and water availability—will be central to this effort because soil microbes drive most terrestrial biogeochemical cycles. Soil microbial composition and function are directly influenced by nutrient and water supply, as well as indirectly shaped by the effects of these resources on plant community composition and productivity (4–7). Evidence from plant communities (8–12) suggests that altered water and nutrient inputs have the potential to either reduce or increase spatial heterogeneity in soil microbes and the ecosystem processes they mediate.

Our experimental site was a grassland consisting of several different soil types supporting strikingly different levels of plant productivity and native plant species diversity. Previous measurements of plant communities at this site showed that water addition by itself had little effect on plant biomass on the least fertile soils, thereby contributing to strong differences in plant biomass between soil types; however, nutrient and nutrient + water additions caused spatial homogenization of plant biomass and species composition (13, 14). At this site, we asked whether soil microbial composition and function were likewise reshaped by resource enhancement. We hypothesized that soil microbial communities might be expected to respond to environmental changes similarly to plant community composition (15, 16) and biomass (17) because ecologically significant plant traits can both mediate plant environmental change responses (14, 18, 19) and influence soil microbial composition (7, 20). On the other hand, microbial and plant responses might diverge due to differences such as greater dormancy (21, 22) and responsiveness to smaller precipitation events (23) in microbes than in plants. Moreover, changes in microbial function may or may not parallel those in microbial composition (24, 25).

We investigated how spatial patterns of soil microbial composition and function would be reshaped by fertilization (including major macronutrients and micronutrients), watering (adding harvested rainwater late in the growing season), and the combination of these treatments across an ecosystem containing three distinct soil types with corresponding distinct plant community types (hereafter referred to as harsh serpentine, lush serpentine, and

Significance

Ecosystems can provide diverse functions through their spatial heterogeneity, for example, by supporting high native biodiversity on infertile soils and high productivity on fertile soils. Soil microbial communities may reflect and support this heterogeneity. Anthropogenic perturbations such as nutrient deposition and altered rainfall could alter spatial diversity of soil microbes and the functions they perform. In a 50-ha California grassland, we found that nutrient addition homogenized fungal composition by eroding distinctive mutualist-rich communities found on infertile soils. In contrast, microbial functions maintained or increased their spatial variability under nutrient and water additions, with some functions accelerating more on fertile soils where they were already faster. Our results underscore the need to consider spatial ecosystem variability in studies of global change.

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Data deposition: Sequences have been deposited in the Sequence Read Archive (project PRJNA369163), and functional measurements have been deposited in the Knowledge Network for Biocomplexity (KNB) Data Repository (DOI: 10.5063/F1ST7N62).

[1]To whom correspondence may be addressed. Email: kelly.gravuer@gmail.com or spharrison@ucdavis.edu.

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nonserpentine soils). Specifically, we asked how plot dispersion—the multivariate distances between individual plots and the treatment centroids—differed among the four treatment groups: control (C), fertilized unwatered (F), watered unfertilized (W), and fertilized watered (FW). We calculated these multivariate plot distances using measures of microbial composition and then again using measures of microbial function. In these analyses, we focused on four specific treatment comparisons: fertilization in the presence (FW vs. W) and absence (F vs. C) of watering and watering in the presence (FW vs. F) and absence (W vs. C) of fertilization. To assess bacterial, archaeal, and fungal composition, we used high-throughput DNA sequencing of taxonomic marker genes. To assess microbial function, we measured several indicators of microbially mediated carbon and nutrient cycling rates, including potential activities of extracellular enzymes and two key nitrogen (N) cycling functions (net N mineralization and nitrification rates). All measurements were taken near the end of the plant growing season, between peak plant biomass and the end of the watering treatment. To explore the degree to which effects on function may be present throughout the growing season, we also measured N cycling at several earlier time points (fall, winter, and early spring) and combined these measurements into a fall through early spring N cycling response.

Results

Fungal Composition. For fungal taxonomic composition, fertilization reduced dispersion (as measured by Jaccard similarity) (Fig. 1*A* and *SI Appendix*, Fig. S1*A*). This effect was statistically significant for both the watered and unwatered plots (i.e., fungal taxonomic composition was significantly less variable among FW plots than among W plots and was significantly less variable among F plots than among C plots) (Tables 1 and 2).

Fertilization also reduced dispersion for fungal guild composition (as measured by Euclidean distance among guild relative frequencies); this effect was statistically significant in the unwatered plot group (fungal guild composition was significantly less variable among F plots than among C plots) and of marginal significance in the watered plot group (fungal guild composition was marginally less variable among FW plots than among W plots) (Tables 1 and 2). In addition to these tests of dispersion, we also tested whether treatments significantly shifted the locations of plots in multivariate space (i.e., whether treatments had an effect on fungal composition overall) using PERMANOVA. Because our hypotheses focused on plot dispersion rather than location, we present these PERMANOVA results for all microbial outcomes in *SI Appendix*, Table S1, rather than discussing them here.

Among fungal guilds, fertilization caused a decrease in symbiotroph (mutualist) relative frequency and an increase in pathotroph (antagonist) relative frequency experiment-wide (effect of fertilization in linear mixed effects model was $F = 42.0$, $P < 0.0001$ for symbiotroph relative frequency and $F = 25.4$, $P < 0.0001$ for pathotroph relative frequency). Overall, symbiotrophs comprised $36.3 \pm 1.0\%$ of OTUs in unfertilized plots vs. $29.0 \pm 0.9\%$ in fertilized plots, and pathotrophs comprised $7.6 \pm 0.3\%$ of OTUs in unfertilized plots vs. $9.1 \pm 0.2\%$ in fertilized plots. However, these changes did not manifest equally across plots; matched plots in which unfertilized plots had the highest relative frequencies of symbiotrophs, such as those on the harsh serpentine soil, showed the largest decreases in symbiotroph relative frequency between

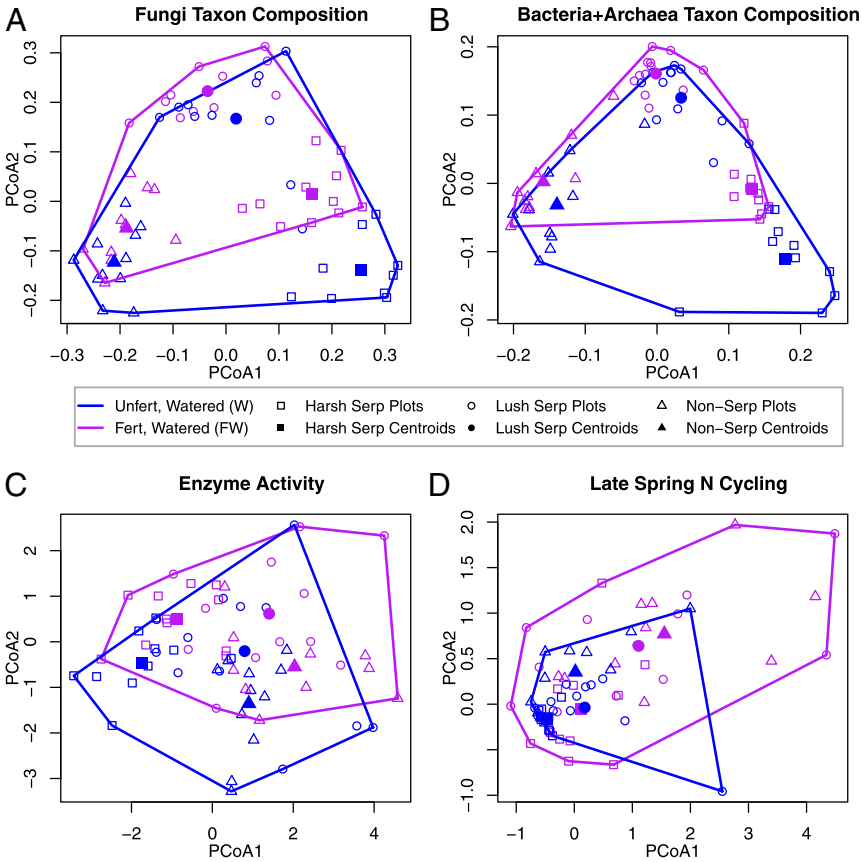

Fig. 1. (*A* and *B*) Reduced variability in microbial composition in response to fertilization of watered plots. (*C*) Variability in enzyme function remained consistent in fertilized vs. unfertilized watered plots. (*D*) Increased variability in late spring N cycling function in response to fertilization of watered plots. These analyses combine effects among and within soils.

Table 1. Effects of treatments on variability (dispersion) of microbial composition and function among plots, analyzed using PERMDISP

				Contrast *P* values for focal comparisons			
	df	F	p (perm)	F vs. C	FW vs. W	W vs. C	FW vs. F
Composition							
Bacteria and archaea presence/absence	3, 125	1.74	0.208	0.774	0.040	0.147	0.351
Bacteria and archaea estimated traits	3, 125	0.16	0.931	0.908	0.547	0.972	0.641
Fungi presence/absence	3, 125	4.02	**0.016**	0.037	0.026	0.431	0.416
Fungi guilds	3, 125	3.01	**0.045**	0.043	0.073	0.471	0.490
Function							
Enzyme potential activity	3, 128	3.65	**0.019**	0.002	0.855	0.011	0.678
Fall through early spring N cycling	3, 128	26.52	**0.0001**	0.0001	0.0001	0.129	0.035
Late spring N cycling	3, 128	14.96	**0.0001**	0.0001	0.011	0.0001	0.929

For contrast *P* values, dark orange indicates contrasts for which the addition of a treatment (treatment combination on left side of the "vs.") reduced variability among plots (compared to the treatment on the right side), with lighter orange indicating a marginally significant reduction. Green indicates contrasts for which the addition of a treatment (treatment combination on left side of the "vs.") increased variability among plots (compared to the treatment on the right side). Boldface values indicate permutation-based *P* values < 0.05 for the overall PERMDISP analysis of each outcome. Treatment codes: C, control; F, fertilized; FW, fertilized + watered; W, watered.

matched unfertilized and fertilized plots (Pearson correlation, $r = -0.75$, $P < 0.0001$), while matched plots in which unfertilized plots had the lowest relative frequencies of pathotrophs, again largely on the harsh serpentine, showed the largest increases in pathotroph relative frequency between matched unfertilized and fertilized plots (Pearson correlation, $r = -0.83$, $P < 0.0001$; Fig. 2 *A* and *B*). These changes led to more homogeneous fungal guild compositions among fertilized plots than among unfertilized plots (Fig. 2*A*).

Bacterial and Archaeal Composition. Fertilization had weak effects on dispersion of bacterial and archaeal taxonomic composition (as measured by Jaccard similarity), reducing dispersion significantly for watered plots (bacterial and archaeal taxonomic composition was significantly less variable among FW plots than among W plots) but having no significant effect on dispersion for unwatered plots (variation in bacterial and archaeal taxonomic composition was statistically similar among F plots vs. among C plots) (Fig. 1*B*, Tables 1 and 2, and *SI Appendix*, Fig. S1*B*). Dispersion of bacterial and archaeal trait-based composition (as measured by Euclidean distance) was not significantly affected by fertilization in either the watered or unwatered plots (variation in bacterial and archaeal trait-based composition was statistically similar among FW plots vs. among W plots and among F plots vs. among C plots) (Tables 1 and 2). In contrast to the significant changes with fertilization in dispersion for some microbial composition metrics, no significant changes in dispersion were observed in response to watering for either the unfertilized (W vs. C) or fertilized (FW vs. F) plots, regardless of the microbial group (fungi or bacteria and archaea) or composition metric tested (Tables 1 and 2).

Microbial Function. Fertilization generally increased dispersion based on microbial functions as measured by Euclidean distance (Tables 1 and 2). Specifically, for N cycling, this effect was statistically significant for both watered and unwatered plots (i.e., FW plots were significantly more variable than were W plots, and F plots were significantly more variable than were C plots) (Tables 1 and 2, Fig. 1*D*, and *SI Appendix*, Fig. S1*D*) and appeared to be driven by increased variability for both N mineralization and nitrification (*SI Appendix*, Table S2). For enzyme functions, the effect of fertilization was significant only for unwatered plots (i.e., F plots were significantly more variable than were C plots) (Tables 1 and 2 and *SI Appendix*, Figs. S1*C* and S2) and was driven primarily by increased variability for enzymes involved in the N cycle (total proteolytic capacity and NAG) (*SI Appendix*, Table S2).

Watering increased dispersion for enzymes and late spring N cycling, based on microbial functions (as measured by Euclidean distance), but only in unfertilized plots (Tables 1 and 2). Increases in net nitrification rate with watering were largest in matched plots where fertility (organic matter, total N, extractable K, and SO_4-S) in the unfertilized plot was highest (*SI Appendix*, Fig. S3), but watering effects on other functions did not show similar relationships with soil properties. In fertilized plots, watering had no significant

Table 2. Effects of treatments on variability (dispersion) of microbial composition and function among plots, expressed as mean distance of each plot to the group centroid ± 1 standard error

	C	F	FW	W
Composition				
Bacteria and archaea presence/absence	41.37 ± 0.55	41.63 ± 0.67	40.74 ± 0.58	42.70 ± 0.68
Bacteria and archaea estimated traits	1.11 ± 0.14	1.13 ± 0.12	1.22 ± 0.13	1.12 ± 0.11
Fungi presence/absence	56.81 ± 0.67	54.71 ± 0.65	55.41 ± 0.49	57.71 ± 0.82
Fungi guilds	0.091 ± 0.007	0.072 ± 0.006	0.078 ± 0.006	0.100 ± 0.010
Function				
Enzyme potential activity	1.91 ± 0.10	2.48 ± 0.16	2.39 ± 0.14	2.43 ± 0.17
Fall through early spring N cycling	1.05 ± 0.10	2.94 ± 0.33	2.06 ± 0.18	0.83 ± 0.06
Late spring N cycling	0.19 ± 0.03	1.31 ± 0.22	1.34 ± 0.16	0.67 ± 0.11

Treatment codes: C, control; F, fertilized; FW, fertilized + watered; W, watered.

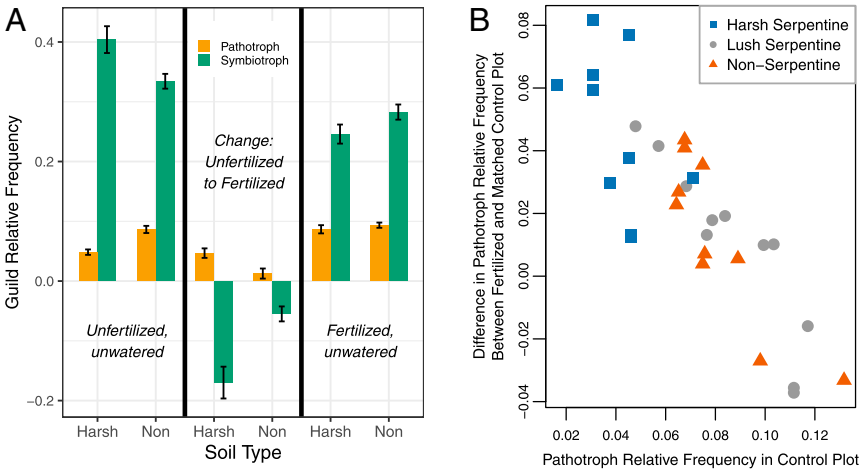

Fig. 2. Reduced variability of fungal guild relative frequencies in response to fertilization of unwatered plots. (*A*) Pathotroph and symbiotroph relative frequencies in (*Left*) unfertilized and (*Right*) fertilized unwatered plots, with (*Center*) the difference in relative frequency of each guild in fertilized (F) vs. unfertilized (C) unwatered plots. Only the harsh serpentine and nonserpentine soils are shown for simplicity; the lush serpentine showed patterns similar to the nonserpentine. Error bars represent 1 SE. (*B*) Pathotroph relative frequency in C plot vs. the difference between pathotroph relative frequency in F plot and pathotroph relative frequency in matched C plot. While on average, pathotroph relative frequencies increased with fertilization, the difference between pathotroph relative frequencies in fertilized vs. matched control plots was larger for matched plots in which the control plot started out with a lower pathotroph relative frequency. Many of the plots experiencing the largest changes were on the harsh serpentine.

effect on dispersion for enzymes and late spring N cycling functions (Tables 1 and 2).

Watering decreased dispersion for fall through early spring N cycling, based on microbial functions (as measured by Euclidean distance), but only in fertilized plots (Tables 1 and 2). In unfertilized plots, watering had no significant effect on dispersion for fall through early spring N cycling (Tables 1 and 2).

Soil Chemical Properties. Prior to treatment application in 2010, dispersion for soil chemical properties (based on Euclidean distances among plots) did not differ among treatments for any of our four focal comparisons (*SI Appendix*, Table S6). In late spring 2013, dispersion (based on Euclidean distances among the same soil chemical properties) was significantly greater in fertilized than in unfertilized unwatered plots (F vs. C) but remained statistically similar in fertilized and unfertilized watered plots (FW vs. W) (*SI Appendix*, Table S6). Late spring 2013 dispersion was significantly smaller in watered than in unwatered fertilized plots (FW vs. F) but remained statistically similar in watered and unwatered unfertilized plots (W vs. C) (*SI Appendix*, Table S6).

Discussion

Microbial composition and function are among the ecological properties considered most essential to understanding how ecological systems can continue to provide services in the face of rapid environmental change. The resource-enhancing changes we applied, especially fertilization, caused initially resource-poor communities to show decreased relative frequencies of mutualistic fungi and increased relative frequencies of antagonistic fungi, thus becoming more similar in fungal composition to the resource-rich soil communities. Conversely, we found resistance to change in the spatial heterogeneity of bacterial and archaeal community composition and of some microbial functions. Other microbial functions showed enhanced spatial heterogeneity as some nitrogen cycling functions sped up most in resource-rich soils where they were initially faster.

The homogenization of fungal composition under fertilization paralleled changes in the plant community, for which the initially less-fertile sites showed greater compositional changes and became more similar to plant communities on initially more-fertile sites (13, 14). Fertilization decreased the relative frequency of symbiotrophic (mutualist) fungi and increased the relative frequency of pathotrophic (antagonist) fungi experiment-wide. However, its largest impact—as judged by relative frequency differences between matched fertilized and unfertilized plots—was where symbiotrophs were initially most frequent and pathotrophs were initially least frequent, which were the least fertile sites. Symbiotrophs are well known to be more abundant in nutrient-poor environments (26, 27) and to decrease under fertilization (15, 28). Pathotrophs may increase under fertilization in response to decreases in symbiotrophs, which protect roots from fungal pathogens (29). Alternatively or additionally, pathotrophs may benefit when fertilization promotes plants with a resource-acquisitive trait syndrome that includes less-defended root tissues (30, 31). In our study, the takeover of harsh serpentine soils by fast-growing exotic plant species that were already prevalent on more fertile soils (14) may have contributed to the observed fungal homogenization.

Conversely, bacterial and archaeal composition was relatively resistant to nutrient addition, possibly because these microbes respond primarily to soil properties such as pH, Ca:Mg, and cation exchange capacity that were not greatly altered by fertilization in this experiment (5, 7, 32) (*SI Appendix*, Tables S4 and S5). Spatial variability of bacterial and archaeal taxonomic composition showed a modest reduction under nutrient addition in watered plots, but this effect was not seen in the abundance-weighted, trait-based measures of bacterial and archaeal composition, nor in unwatered plots. The ability of fungal hyphae to bridge air-filled soil pores, while bacteria and archaea are more dependent on water films to access resources, may contribute to the greater responsiveness of fungi than bacteria and archaea to fertilization without water addition (33). Also, fungi appear to be more strongly associated with plant composition than bacteria and archaea (15, 34–37), possibly because more fungi are plant mutualists or antagonists.

Water addition alone had relatively little effect on the spatial variability of fungal or bacterial and archaeal composition. Other studies have also found that microbes respond to watering in broadly similar ways across different soils in Californian grasslands (38, 39). Given the high capacity of microbes to cope with rainfall variability through dormancy (22), many of the taxa that increased in watered plots would likely also increase during

natural rainfall events, decreasing the ecological novelty of the watering treatment from the microbial perspective.

For some microbial functions related to nutrient cycling, spatial variability increased, as resource additions caused greater increases at fertile sites where levels of these functions were already higher. Both watering in unfertilized plots and fertilization showed this heterogeneity-enhancing effect. Potential contributing factors include the generally lower carbon content and water-holding capacity of less fertile soils (*SI Appendix*, Table S5), which may have limited the responsiveness of nutrient-associated processes to resource addition (40, 41). The granular fertilizer may have contributed to increased functional variability, with some samples containing more nutrient hot spots than others. The major exception was that watering decreased spatial variability for fall through early spring N cycling, particularly net N mineralization rate (*SI Appendix*, Table S2). This effect occurred only in fertilized plots, where watering depleted dissolved organic C, ammonium, nitrate, and (to a lesser extent) P (*SI Appendix*, Table S3, S5) and thereby homogenized these soil properties (*SI Appendix*, Table S6). The seasonal differences in treatment effects highlight the importance of understanding the temporal effects of pulsed environmental changes on ecosystem-level microbial functional patterns (42). Broadly, some aspects of our results for microbial function echo findings from plant communities that water addition by itself has little effect in highly nutrient-limited ecosystems (14), possibly because of microbial resource colimitation.

Our study underlines the value of spatial ecosystem heterogeneity and its potential vulnerability under resource-enhancing changes. At the same time, our results underscore the complexity of soil microbial responses because we found spatial homogenization, resistance, and enhanced spatial heterogeneity in fungal composition, bacterial and archaeal composition, and nutrient cycling functions, respectively. Our findings indicate that global change models of microbially mediated processes (43–46) will require continued ground-truthing across spatially heterogeneous ecosystems.

Materials and Methods

Field Experiment. The experiment was conducted at the University of California McLaughlin Reserve in California (N38°52′, W122°26′), which has a Mediterranean climate with mean temperatures of 7 °C in January and 25 °C in July and rainfall averaging 69.6 cm per year (47). The 1,000 m × 500 m site contains three distinct soil and grassland types (14, 48). The first two are underlain by ultramafic bedrock, which has low Ca:Mg and high levels of some heavy metals (serpentine soils). One serpentine soil type (harsh serpentine) is found on rock outcrops and is shallow, coarse-textured, and low in organic matter and nutrients. The other serpentine soil type (lush serpentine) is found on slopes and valley bottoms and is deeper and finer-textured and has higher organic matter and nutrients, although still low Ca:Mg. The third soil type (nonserpentine) is derived from sedimentary bedrock and is also deeper than the harsh serpentine, with loamy texture and higher levels of organic matter and nutrients than the harsh serpentine. The harsh serpentine has lower plant productivity (138.2 ± 19.4 g/m^2) than the other two soils (613.1 ± 39.5 and 613.9 ± 57.4 g/m^2 for lush serpentine and nonserpentine, respectively) (14). The relative cover of native (vs. exotic) grass and forb species is highest in the harsh serpentine (73.5 ± 4.8%), intermediate in the lush serpentine (26.8 ± 5.6%), and lowest in the nonserpentine (2.9 ± 0.8%) (14). In our study system, the harsh and lush serpentine soil sites were well interspersed with each other; however, it was not possible to completely intersperse the nonserpentine sites as they were separated by a geologic boundary (*SI Appendix*, Fig. S4).

We imposed environmental manipulations beginning in 2010. A watering (precipitation addition) treatment simulated a lengthening of the rainy season, as predicted by some climate change forecasts (49). Using harvested rainwater applied by minisprinklers in the centers of plots, it simulated one additional moderate storm (adding 2.5 cm of water over a 12-h period at night) per week for 8 wk. The total precipitation increase per year was ∼18% over mean annual rainfall (14). A fertilization treatment broadcast a slow-release granular NPK (10-10-10) fertilizer with micronutrients (Lilly Miller Ultra Green; Lilly Miller Brands) (*SI Appendix*, Table S7) in three equal applications (November, early February, and late March) each year, for a total of 10 g/m^2 of N, P, and K annually. Fertilization was carried out multiple times over a prolonged period when it was still raining to provide as temporally and spatially homogeneous nutrient supply rates as possible during the growing season. On each of the three soil types, we applied the full factorial treatment combination to 10 to 12 replicate 3 m × 3 m plots, of which only the center 2 m × 2 m area was sampled to avoid edge effects. In other words, 10 to 12 plots on each soil type received each of the following treatment combinations: fertilized, unwatered (F); watered, unfertilized (W); fertilized and watered (FW); and control (unfertilized, unwatered) (C), for a total of 132 plots (additional experimental details in refs. 13 and 14). See *SI Appendix*, Fig. S4, for a map of all plots, including soil type and treatment applied. We inferred treatment effects using randomized treatment assignment rather than pretreatment measurements, an approach that is supported by the similar levels of pretreatment variability in relevant soil properties across treatment groups (*SI Appendix*, Table S6).

Soil Sampling. We sampled soils in spring 2013 at the end of a below-average water year (51 cm). We collected soils for extracellular enzyme assays on 26 April to 3 May at near-peak plant biomass, comparable to what has been done in other California grassland studies (50–52). We collected soils for microbial community composition and net N mineralization and nitrification assays on 28 to 29 May 2013, which was timed to capture effects of the entire precipitation addition. While these sampling times were each chosen for their own reasons (explained above), the difference of a few weeks between them could limit their comparability. We also sampled soils for net N mineralization and nitrification on 6 to 7 November 2012 (1 wk after the first major rainfall), 4 to 5 February 2013 (when plants were in vegetative form), and 16 to 17 Mar 2013 (when plants were beginning to bolt). Each sampling occurred before fertilizer application.

After removing leaf litter, we collected three composited soil cores (7 cm diameter) per plot to a depth of 7.5 cm, the deepest that could be reliably collected in the harsh serpentine. At the late spring sampling, a subsample for microbial composition was placed in a sterile tube, transported on ice, and stored at −20 °C until DNA extraction. The soil corer was washed with 70% ethanol between sample plots. Remaining soil was also kept on ice and then immediately processed (enzymes) or stored at 4 °C for <48 h until processing (net N mineralization, nitrification, and soil moisture). Soil moisture was measured by comparing mass before and after drying at 105 °C to constant mass; moisture measurements were later corrected to account for the mass of fragments >2 mm in each sample. We sieved remaining soil through 2-mm mesh sieve, air-dried it, and sent the <2 mm fraction to the A&L Western Laboratory for chemical analyses as described in ref. 53.

Function Measurements.

Potential activities of extracellular soil enzymes. We assayed potential activities of five extracellular enzymes and one enzyme group (proteases) on soils sieved through 2-mm mesh sieve (*SI Appendix*, Table S8). Three enzymes were assayed fluorometrically using 4-methylumbelliferone linked substrates (β-1,4-glucosidase [BG], β-*N*-acetylglucosaminidase [NAG], and acid phosphatase [AP]), and two enzymes were assayed colorimetrically using L-dihydroxyphenylalanine (L-DOPA) (polyphenol oxidase [PPO] and peroxidase [PER]). For all assays except the proteases, we followed Saiya-Cork et al. (54), adjusting buffer pH to approximate soil pH (6.8) and incubating fluorometric assays for 2 h and colorimetric assays for 4 h. Activities were calculated following German et al. (55). We measured total proteolytic capacity (i.e., combined activity of the protease enzyme group) under both native substrate conditions (proteins present in the field soil sample) and saturated substrate conditions (a saturating amount of an added protein, casein). Extracts were prepared following Brzostek and Finzi (56), using a pH 6.8 buffer and 4 h incubation. Amino acids, from which proteolytic rates were calculated, were quantified following Darrouzet-Nardi et al. (57).

N cycling: Net N mineralization and net nitrification. We determined initial quantities of ammonium (NH_4^+) and nitrate (NO_3^-) in field samples via extraction with 0.5 M K_2SO_4; 10 g soil and 50 mL K_2SO_4 were shaken for 1 h at 175 rpm before filtering the extract through Whatman #1 filter paper preleached with 0.125 M K_2SO_4. We measured net N mineralization and net nitrification using an aerobic laboratory incubation (58). This method was chosen to capture both the direct influence of field soil moisture and the indirect influence of the plant community via its effects on active soil organic matter quality in the measure of microbial activity potential (59, 60). However, because these N cycling measurements were from relatively short-term laboratory assays of field-collected soils, they may diverge from field N transformations. At the same time as initial extractions, 10 g field moist soil were placed in a specimen cup, incubated in the dark at 22 °C for 7 d, and then extracted with 0.5 M K_2SO_4 as above. Extracts were stored at −20 °C until colorimetric analysis for NH_4^+ and NO_3^- (61, 62). Net N mineralization was calculated as the difference between postincubation and initial NH_4^+ + NO_3^-,

and net nitrification was calculated as the difference between postincubation and initial NO_3^-.

Microbial Composition: DNA Extraction, Sequencing, and Processing. We extracted DNA using a MoBio PowerLyzer kit (MoBio Laboratories) and sent it to Argonne National Laboratory for amplification and sequencing. For bacteria and archaea, the V4 region of the 16S rRNA gene was amplified following Earth Microbiome Project protocols (http://www.earthmicrobiome.org/emp-standard-protocols/16s), with 515F/806R primers from Caporaso et al. (63). For fungi, the ITS1 region was amplified using modified ITS1F and ITS3 primers (64). Paired-end 250 bp amplicon sequencing was performed in two MiSeq runs, one for bacteria and archaea and one for fungi.

For bacterial and archaeal sequences, raw forward and reverse reads were demultiplexed using QIIME 1.9.1 (65). OTUs were then derived using the UPARSE pipeline in USEARCH 8.1 (66). All reads that had more than one barcode difference during the demultiplexing process were removed. Remaining merged reads were then quality filtered using a maximum error threshold of 0.5 and a minimum length of 100. Filtered sequences were dereplicated and clustered into OTUs at 97% similarity, removing singletons and chimeras. The 6.8% of OTUs that could not be mapped to greengenes (version 13_8) (67) with an identity of >75% were discarded (15). The full set of merged reads was then mapped to this OTU set to make the raw OTU table. Taxonomy was assigned to OTUs using the RDP classifier (68) in QIIME 1.9.1. The table was then filtered to remove all OTUs identified as chloroplasts or mitochondria. Next, the OTU table was rarefied to 26,690 sequences per sample in QIIME 1.9.1, retaining 129 of the 132 plots and yielding 18,023 OTUs.

Fungal sequence processing followed Glassman et al. (69) and Nguyen et al. (70). Forward reads were terminated at the ITS3 priming site and reverse reads were terminated at the ITS1f priming site using cutadapt (71). Trimmomatic (72) was used to remove remaining low-quality bases from the ends of reads. Trimmed reads were clustered into OTUs using the UPARSE pipeline in USEARCH 8.1 (66). Forward and reverse reads were merged and quality filtered using a maximum error threshold of 0.25 and a minimum length of 75. Filtered sequences were dereplicated and clustered into OTUs at 97% similarity, removing singletons in the process. Chimeras were removed using uchime_ref with the UNITE database (73). Potentially nonfungal sequences were filtered out by removing 1) OTUs that did not map to UNITE at >60% identity (using usearch_global) and 2) sequences with length/query length <0.85 in a local blast against UNITE, using BLAST+, v 2.3 (74). Taxonomy was assigned using the top local BLAST hit. Merged reads were mapped to the remaining OTUs to make the raw OTU table, and this table was then rarefied to 30,280 sequences per sample in QIIME 1.9.1. The final table retained 129 of the 132 plots (excluding a different 3 plots than were excluded for the bacteria and archaea table) and contained 3,339 OTUs. As read abundance is an uncertain indicator of true relative abundance for fungal ITS sequences (75), the fungal OTU table was converted to a presence–absence representation for analysis, and the bacterial and archaeal OTU was similarly converted to presence–absence for consistency.

Ecological Measures of Microbial Composition. For bacteria and archaea, we used phylogenetic techniques to estimate two traits that are both strongly phylogenetically conserved and ecologically meaningful: rRNA gene copy number and genome size (76, 77). As detailed in Gravuer and Eskelinen (78), we estimated community-weighted mean values for each of these traits in each plot. For fungi, we assigned trophic modes to OTUs using the FUNGuild database (79) in January 2019. Trophic modes (Pathotroph, Saprotroph, or Symbiotroph; hereafter referred to as "guilds") could be assigned to ∼63% of OTUs. We calculated the relative frequency of each guild in each plot (no. of OTUs of guild/total no. of OTUs in plot) to investigate how variability across plots for guild composition changed in response to treatments.

Statistical Analysis. We used the PERMOVA+ add-in for PRIMER version 7 (80) for PERMDISP and PERMANOVA analyses and used R version 3.6.0 (81) for supporting analyses and figure drawing. We used PERMDISP to investigate our central question of how plot dispersion—the multivariate distances between individual plots and the treatment centroids—differed among the four treatment groups: control (C), fertilized unwatered (F), watered unfertilized (W), and fertilized watered (FW). We performed pairwise tests within the PERMDISP analysis, focusing on four specific treatment comparisons: fertilization in the presence (FW vs. W) and absence (F vs. C) of watering and watering in the presence (FW vs. F) and absence (W vs. C) of fertilization. We focused on four treatment groups, rather than on the main effects of fertilization and watering overall, due to the prevalence of watering by fertilization interactions in PERMANOVAs (*SI Appendix*, Table S1).

We performed the PERMDISP analysis for seven outcome metrics, four for microbial composition (taxonomic composition of fungi and bacteria and archaea as well as the two ecological measures of microbial composition: fungal guilds and bacterial and archaeal estimated traits) and three for microbial function (enzyme potential activity, fall through early spring N cycling, and late spring N cycling; see below for seasonal N cycling rationale). For the bacterial and archaeal taxonomic composition and fungal taxonomic composition metrics, measured as presence/absence, we used Jaccard's similarity as the resemblance measure (82). For the fungal guild composition metric, measured as guild relative frequencies (number of distinct OTUs from each guild in plot/total number of OTUs in plot), we used Euclidean distances as the resemblance measure (83).

Data for the fourth composition metric (bacterial and archaeal estimated traits), as well as for all three function metrics, consisted of several continuous numeric variables each with different units of measurement. Specifically, the bacterial and archaeal traits metric consisted of two estimated trait values (rRNA gene copy number and genome size), while the enzymes metric consisted of seven enzyme activity measurements (*SI Appendix*, Table S8, for list, with two measurements for the proteases: activity with native substrate and activity with saturated substrate [casein]). For the N cycling measurements (net N mineralization rate and net nitrification rate), preliminary analyses (e.g., PERMANOVA including season as a fixed effect) showed that treatment effects on N cycling in late spring (when the precipitation treatment directly affected soil moisture; *SI Appendix*, Table S5) differed from treatment effects on N cycling at the earlier time points we had measured (when soil moisture was similar across treatments; *SI Appendix*, Table S3), whereas the earlier time points did not differ significantly from each other (*SI Appendix*, Table S9). Therefore, we created two N cycling metrics: late spring (consisting of the May 2013 net N mineralization rate and net nitrification rate measurements) and fall through early spring (consisting of the net N mineralization rate and net nitrification rate measurements from November 2012, February 2013, and March 2013). For all four of these metrics (separately), we centered and scaled all variables and used Euclidean distance, which can accommodate negative values, as the resemblance measure.

To run the PERMDISP analyses, we used distances to centroids with *P* values based on permutation of least squares residuals (after centering all groups onto a common location) with 9,999 permutations, based on the performance of these options in ref. 84 and examination of our data to confirm that results did not appear to be driven by outliers (which would have recommended use of spatial medians). The permutation procedure did not require assuming that distances to group centroids would be normally distributed. We used PERMANOVAs mainly to inform the strategy for the PERMDISP analyses as explained above and to provide a more complete picture of how the treatments affected each metric (*SI Appendix*, Table S1). However, PERMANOVA results should be interpreted with caution given the heterogeneous dispersions identified by PERMDISP for many of the metrics (80). To explore relationships between treatments and relative frequency of specific fungal guilds, we used the lme4 package in R (85) to build linear mixed effects models with precipitation treatment, nutrient treatment, soil type, and all interactions as fixed effects and irrigation line as a random effect to reflect random error by line. To further investigate these relationships, we matched treated and untreated plots based on geographic proximity, although geologic constraints prevented a complete blocked design.

To test whether there were pretreatment soil chemistry differences among plot groups that could influence dispersion of microbial composition and function, we used PERMDISP to analyze dispersion calculated (using Euclidean distances) from a suite of soil chemical properties measured just prior to treatment application (April 2010). We also analyzed dispersion of the same suite of soil properties for the late spring (2013) soil samples. Soil properties were organic matter (via loss-on-ignition), pH, water-holding capacity, NH_4^+-N, NO_3^--N, Olsen-extractable P, extractable K, S, cation exchange capacity, and Ca:Mg ratio.

Data Availability. Sequences have been deposited in the Sequence Read Archive (project PRJNA369163), and functional measurements have been deposited in the Knowledge Network for Biocomplexity (KNB) Data Repository (DOI: 10.5063/F1ST7N62).

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