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Developing climate-smart restoration: Can plant microbiomes be hardened against heat waves?

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Abstract. Heat waves are increasing in frequency and intensity, presenting a challenge for the already difficult practice of ecological restoration. We investigated whether pre-heating locally sourced rhizosphere soil (inoculum) could acclimatize plants to a field-imposed heat wave in a restoration setting. Soil heating in the laboratory caused a marked shift in rhizosphere bacterial community composition, accompanied by an increase in species evenness. Furthermore, pre-heated rhizosphere soil reduced plant height, number of leaves, and shoot mass of the C_4 grass, blue grama (*Bouteloua gracilis*), and it reduced the shoot mass of the C_3 grass, Arizona fescue (*Festuca arizonica*) in the glasshouse. Following transplantation and the application of a field heat wave, pre-heated inoculum did not influence heat wave survival for either plant species. However, there were strong species-level responses to the field heat wave. For instance, heat wave survivorship was over four times higher in blue grama (92%) than in Arizona fescue (22%). These results suggest that the use of C_4 seeds may be preferable for sites exhibiting high heat wave risk. Further research is needed to understand whether inocula are more effective in highly degraded soil in comparison with partially degraded soils.

Key words: *heat waves; grassland; restoration; rhizosphere; arbuscular mycorrhizal fungi; rhizobacteria.*

Introduction

Soil degradation and extreme weather events pose concurrent threats to the stability and productivity of terrestrial ecosystems (Frank et al. 2015). According to climate models, the frequency and intensity of heat waves, extended periods of unusually high heat stress (Robinson 2001), will become more frequent and more severe (Meehl and Tebaldi 2004, Jentsch and Beirkuhnlein 2008, IPCC 2014). Several past examples include the European, North American, and Russian heat waves of 2003, 2006, and 2010, which increased human mortality (Vandentorren et al. 2004, Poumadére et al. 2005), extended forest fires (Tressol et al. 2008) and reduced aboveground productivity (Ciais et al. 2005, Allen et al. 2010). Furthermore, extreme events can exacerbate soil degradation through mass mortality of plant populations (Breshears et al. 2005, Gibbens et al. 2005, Gitlin et al. 2006, Meisner et al. 2013). Currently, one-third of Earth's land area is moderately to severely degraded (FAO 2015) and extreme events will make it increasingly difficult to rehabilitate ecosystems. Recent research suggests that rhizosphere microbiomes confer plant abiotic stress tolerance, but this knowledge has not yet been applied to climate-smart ecological restoration (Mariotte et al. 2017).

The rhizosphere is the region of soil directly influenced by plant roots and contains free-living and symbiotic bacteria, fungi, and eukaryotes. Rhizosphere engineering is an emerging field that aims to increase plant growth and alleviate plant stress either by exploiting whole microbial communities (Carbajo et al. 2011) or individual taxa (Dessaux et al. 2016). Rhizosphere organisms can directly influence plant growth under stressful conditions by synthesizing phytohormones (Aroca et al. 2008, Lim and Kim 2009, Kang et al. 2014), amino acids (Ruiz-Lozano et al. 1995), and enzymes (Lim and Kim 2013), and by transferring soil water to plant roots (Khalvati et al. 2005). Rhizosphere organisms can also benefit plants indirectly, by altering internal (endogenous) production of auxins (Verbon and Liberman 2016), provisioning nitrogen and phosphorus (Hu et al. 2017), and through microbe-to-microbe facilitation (Dupponois and Garbaye 1991). In addition, some taxa can physically improve soil structure; arbuscular mycorrhizal hyphal networks enhance soil aggregate stability and improve infiltration (Kohler et al. 2017), and some bacteria produce biofilms, a sticky polysaccharide matrix that retains water (Seneviratne et al. 2010). Overall, meta-analyses suggest that arbuscular mycorrhizal fungi (AMF) and rhizobacterial inoculants enhance plant growth by 29% and 40% under drought, respectively (Jayne and Quigley 2014, Rubin et al. 2017).

While plant growth promoting rhizobacteria (PGPR) and AMF have had promising results in glasshouse and field trials, there are potentially negative consequences of the commercialization and global transport of inocula, including loss of native soil biodiversity and even the facilitation of invasive species (Schwartz et al. 2006). A diverse soil microbiome has been associated with elevated plant productivity (van der Heijden et al. 1998, Hu et al. 2017) and resistance to abiotic perturbations (Yachi and Loreau 1998, Awasthi et al. 2014). In contrast, the majority of agricultural inoculation studies use only one to three microbial species and typically occur in highly controlled glasshouse conditions, which differs from actual agricultural and restoration

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scenarios in which hundreds to thousands of microbial taxa might be present.

In ecological restoration, it is common to harden plants by exposing them to abiotic stress prior to out-planting. The practice promotes epigenetic or biochemical modifications, such as the production of heat shock proteins, which enhance growth and survival under future abiotic stress (Bruce et al. 2007, Walter et al. 2011, Crisp et al. 2016). A similar stress priming phenomenon may also be mediated through rhizosphere microbial communities: *Brassica rapa* rhizosphere soil that had been exposed to drought promoted plant growth and future drought tolerance in the host plant to a greater extent than drought-naïve soil (Lau and Lennon 2012). Furthermore, local strains of AMF sourced from xeric Mediterranean soils increased lavender biomass and N and K adsorption more under drought conditions compared to non-local strains (Marulanda et al. 2007). This raises the question: Can we feasibly harvest and harden locally sourced soil organisms to facilitate ecological restoration?

We characterized the effects of pre-heating a locally sourced rhizosphere soil community on plant performance both in the glasshouse and under a field-imposed heat wave. We hypothesized that soil heating would shift the microbial community toward species that are more genetically adapted to high temperatures (Bárcenas-Moreno et al. 2009). Second, we hypothesized that the same heated soil would improve plant survival under a heat wave.

Methods

Experiment 1: Laboratory and glasshouse experiment

During February 2015, we collected seeds and rhizosphere soil from two codominant grass species, Arizona fescue (*Festuca arizonica*; C_3) and blue grama (*Bouteloua gracilis*; C_4), within USFS land adjacent to the Arboretum at Flagstaff (Flagstaff, Arizona, USA; elevation 2,180 m, 35°9′41.08″ N, 111°43′53.25″ W). Seeds were collected from ~30 individuals, and rhizosphere soil was collected by shaking soil from the roots of six plants, which were randomly selected from the 30 individuals. Soil was pooled, homogenized, and distributed into two treatments for the glasshouse experiment: (1) kept for one week at room temperature (control) and (2) heated at 45°C for one week inside an incubator (model IGS180, Thermo Scientific, Waltham, Massachusetts, USA).

To determine the microbial community composition of the control and heated inoculum treatments, we extracted DNA from six replicates from the pooled control and heated inoculum using a MoBio PowerSoil DNA Isolation Kit (QIAGEN, Germantown, Maryland, USA). Quantitative PCR (qPCR) was used to measure 16S rRNA gene abundance, a proxy for the total number of bacteria and archaea per gram of soil. Standard curves were generated using 10-fold serial dilutions of 16S rRNA gene amplicons, which were prepared using soil DNA and primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACVSGGGTATCTAAT-3′) (Caporaso et al. 2012) containing Illumina sequence adaptors P5 (5′-AATGATACGGCCACCACCGA) and P7 (5′-CAAGCAGAAGACGGCATACGA), respectively, at the 5′ end of each primer to prevent decreased primer efficiency due to amplicon degradation (Dhanasekeran et al. 2010). The 10 μL qPCRs consisted of 1 μL of template DNA and 9 μL of master mix (0.75 mmol/L of each primer, 0.01 U/μmol/L Phusion HotStart II Polymerase [Thermo Fisher Scientific, Fremont, California, USA], 1× Phusion HF buffer, 1× EvaGreen fluorescent dye [Biotium, Fremont, California, USA], 3.0 mmol/L $MgCl_2$, 6% glycerol, and 200 μmol/L dNTPs). The assay was performed on a Bio-Rad CFX384 Touch real-time PCR detection system (Bio-Rad Laboratories, Hercules, California, USA), using the following program: 95°C for 2 min, followed by 40 cycles of 95°C for 30 s, 64.5°C for 30 s, and 72°C for 1 min. All qPCRs were run in triplicate.

Samples were sequenced on an Illumina MiSeq system (Illumina, San Diego, California, USA). Two PCR steps were used to prepare the samples, as in Berry et al. (2011). Each sample was first amplified using primers 515F and 806R in triplicate 10 μL PCRs containing 1 μmol/L of each primer, 0.01 U/μL Phusion HotStart II Polymerase (Thermo Fisher Scientific, Waltham, Massachusetts, USA), 1X Phusion HF buffer (Thermo Fisher Scientific), 3.0 mmol/L $MgCL_2$, 6% glycerol, and 200 μmol/L dNTPs. PCR conditions were 95°C for 2 min; 15 cycles of 95°C for 30 s, 55°C for 30 s, and 60°C for 4 min. Initial PCR products were pooled, checked on a 1% agarose gel, 10-fold diluted, and used as a template in the subsequent tailing reaction with region-specific primers that included the Illumina flow cell adapter sequences and a 12-nucleotide Golay barcode (15 cycles identical to initial amplification conditions).

DNA sequences were analyzed with the software package Quantitative Insights into Microbial Ecology version 1.7 (QIIME; Caporaso et al. 2010a). Open reference OTU picking was performed at 97% identity using UCLUST (Edgar 2010). The most abundant sequence for each OTU was aligned with PyNAST (Caporaso et al. 2010b) against the Greengenes v13_5 database (DeSantis et al. 2006), and taxonomy was assigned using the Ribosomal Data Project classifier (Wang et al. 2007). Any operational taxonomic units (OTUs) that accounted for <0.05% of the total sequences were discarded (Bokulich et al. 2013). The bacterial libraries were rarified so that sequencing efforts did not affect diversity comparisons. The QIIME L7 (species level) OTU table was used for subsequent diversity and community analyses.

In February 2015, following seed and soil collections, grasses were germinated in trays containing sterilized commercial potting mix for two weeks before transplantation into 164-cm^3 Cone-tainers (Stuewe and Sons, Tangent, Oregon, USA). Before planting, control and heated inoculum were wetted to field capacity. In each Cone-tainer, a single layer (2.5 cm^3) of inoculum was placed between layers of steam-sterilized commercial potting mix consisting of one-third peat moss, one-third perlite, and one-third vermiculite. We transplanted 45 seedlings of each of the two plant species (Arizona fescue and blue grama) and inoculum treatments (heated and control) into the Cone-tainers. In order to simulate the options available to restoration professionals, we also planted 21 seedlings in steam-sterilized commercial potting mix with no inoculum added, for later use in the field experiment (see *Experiment 2: Field experiment*). Every two weeks, we monitored plant height, number of leaves, and chlorophyll content (SPAD 502+, Spectrum Technologies, Aurora, Illinois, USA). Grasses were grown from March 2015 until May 2015 (two months) in the glasshouse, before harvesting a portion of the

grasses to evaluate biomass and arbuscular mycorrhizal fungal (AMF) root colonization.

After two months of growth, we randomly selected 20 grasses from the heated inoculum and control inoculum treatments, separated roots from shoots, and washed roots in reverse osmosis water. Roots were further subsampled for mycorrhizal determination. We dried roots and shoots for two days at 60°C to determine dry root and shoot mass. We weighed the wet root samples and back-calculated the total mass using a wet to dry relationship determined from the dried subsamples. We quantified percent root length colonization by loading roots into Simport tissue cassettes (Simport Scientific, Beloeil, Quebec, Canada), clearing roots in 10% KOH overnight, and rinsing them several times in tap water. To stain roots, we boiled roots for five minutes in a 5% Sheaffer black calligraphers ink (Providence, Rhode Island, USA)/47.5% household acetic acid/47.5% distilled water solution, as detailed in Vierheilig et al. (1998). We de-stained roots for 20 minutes in a tap water solution acidified with a few drops of vinegar. Next, we sliced roots into 1-cm pieces using a razor blade and mounted them on 75 × 25 mm Gold Seal Plain Slides (Thermo Fisher Scientific, Waltham, Massachusetts, USA) using polyvinyl acetate (PVA) and rectangular coverslips. Mycorrhizal fungal structures within the root cortex (vesicles, arbuscules, coils, intraradical hyphae) along with other (non-AM) fungi were quantified using the magnified line intersect method at 200× magnification (McGonigle et al. 1990), and total percent AMF colonization was calculated as the sum of the percent colonization by vesicles, arbuscules, coils, and intraradical hyphae for each sample.

Experiment 2: Field experiment

Field research took place within a disturbed and compacted 10 × 40 m strip of land (a former service road) at the Merriam Powell Research Center at the Arboretum at Flagstaff (Fig. 1). The service road was initially constructed in 1980 and cordoned off from further use in 2014, the year before this experiment was conducted. The road was never seeded. Our study design leveraged the unique access to electrical infrastructure provided by the Arboretum, while simulating a fine-scale restoration program, similar to roadcut revegetation efforts in U.S. national parks (Paschke et al. 2000). Vegetation composition within the road footprint was a mixture of ruderal native and nonnative species including yellow sweet clover (*Melilotus officinalis*), white sweet clover (*Melilotus* alba), bindweed (*Convolvulus arvensis*), squirreltail (*Elymus elymoides*), spreading fleabane (*Erigeron divergens*), wooly cinquefoil (*Potentilla hippiana*), and western wheat grass (*Pascopyrum smithii*). The adjacent undisturbed area was a mixed C_3-C_4 grassland dominated by blue grama, Arizona fescue, pine dropseed (*Blepharoneuron tricholepsis*), and mountain muhly (*Muhlenbergia montana*).

The experimental design consisted of six 1-m^2 plots (three heated, three control) in a randomized split plot block design, with three blocks. Blocks were 5 m apart, and each control plot was 1 m from each heated plot (within each block). During mid-June 2015, after five total months of growth in the glasshouse, we randomly selected and transplanted 21 replicates of each species and inoculum treatment (heated, control, and non-inoculated) among the three heat wave plots and three control plots (there were seven replicates for each species and inoculum treatment combination per plot). Grasses were planted in stratified rows, alternating by inoculum treatment. We watered plants once per week until the heat wave was initiated one month later in July 2015. Around each plot, we constructed a frame using 3/4″ galvanized steel pipe (1 inch = 2.54 cm) and custom pipe fittings (MSC Industrial, Melville, New York, USA; Fig. 1A). We mounted four 1,000 watt ceramic infrared heat lamps (Mor Electric, Comstock Park, Michigan, USA) housed in steel ALEX-F fixtures on the corner of each heated plot (Fig. 1A), as in Kimball (2005). To wire the lamps, we spliced the lamp terminals to 12-gauge (20 amp) weatherproof extension cords and 1,000 watt dimmer switches allowed user control of heat output. To achieve even heating distribution, the optimal height was 1 m and the optimal angle was 45°, similar to Kimball et al. (2008). Unheated plots received the steel pipe frame and aluminum "dummy lamps" constructed to match the dimensions of the lamp fixtures.

To ensure that the experimental treatments were effective, we installed one soil temperature sensor (model MPS-6, METER Environment, Pullman, Washington, USA), one soil moisture sensor (model GS1, METER Environment), and one air temperature sensor (iButton; Maxim Integrated, San Jose, California, USA) within each plot and recorded hourly measurements. In addition, we used a handheld infrared thermometer to measure leaf temperature and surface soil temperature daily, as well as an infrared camera (model FLIR-1, FLIR Systems, Wilsonville, Oregon, USA) to confirm an even heating distribution within each plot. To calculate experimental treatment effects, we averaged all the hourly or daily values per plot and then calculated the means and standard errors for the three plots in each treatment.

Heating lamps were turned on 8 July 2015 and turned off 14 days later on 22 July 2015. We measured height, leaf, number, chlorophyll content, and survivorship on 7 July 2015, the day before the heat wave, and on 5 August 2015, two weeks after the heat lamps were turned off, to account for time-lag effects of extreme climate events on plant productivity (Wang et al. 2003).

Data analysis

Unless otherwise specified, all analyses were performed in R Statistical Software (version 3.2.2; R Core Team 2015). To evaluate initial effects of soil heating on plant growth (Experiment 1), we conducted fixed effects linear models on AMF and non-AMF root colonization, inoculum 16S gene copies/g soil, inoculum bacterial diversity, root mass, shoot mass, and root:shoot ratio. Non-significant results for tests of dispersion (PERMDISP, $P = 0.44$) affirmed the suitability of permutational multivariate analysis of variance (PERMANOVA) for bacterial community analyses (Anderson et al. 2008). We evaluated bacterial community structure at the L7 level (species level) using the untransformed relative abundance file, constructed a Bray Curtis dissimilarity matrix, and created a nonmetric multidimensional scaling (NMDS) ordination (5,000 iterations) followed by PERMANOVA in PRIMER 7+ PERMANOVA (Clarke and Gorley 2015).

To evaluate whether heated inoculum primed plant performance under the field heat wave (Experiment 2), we

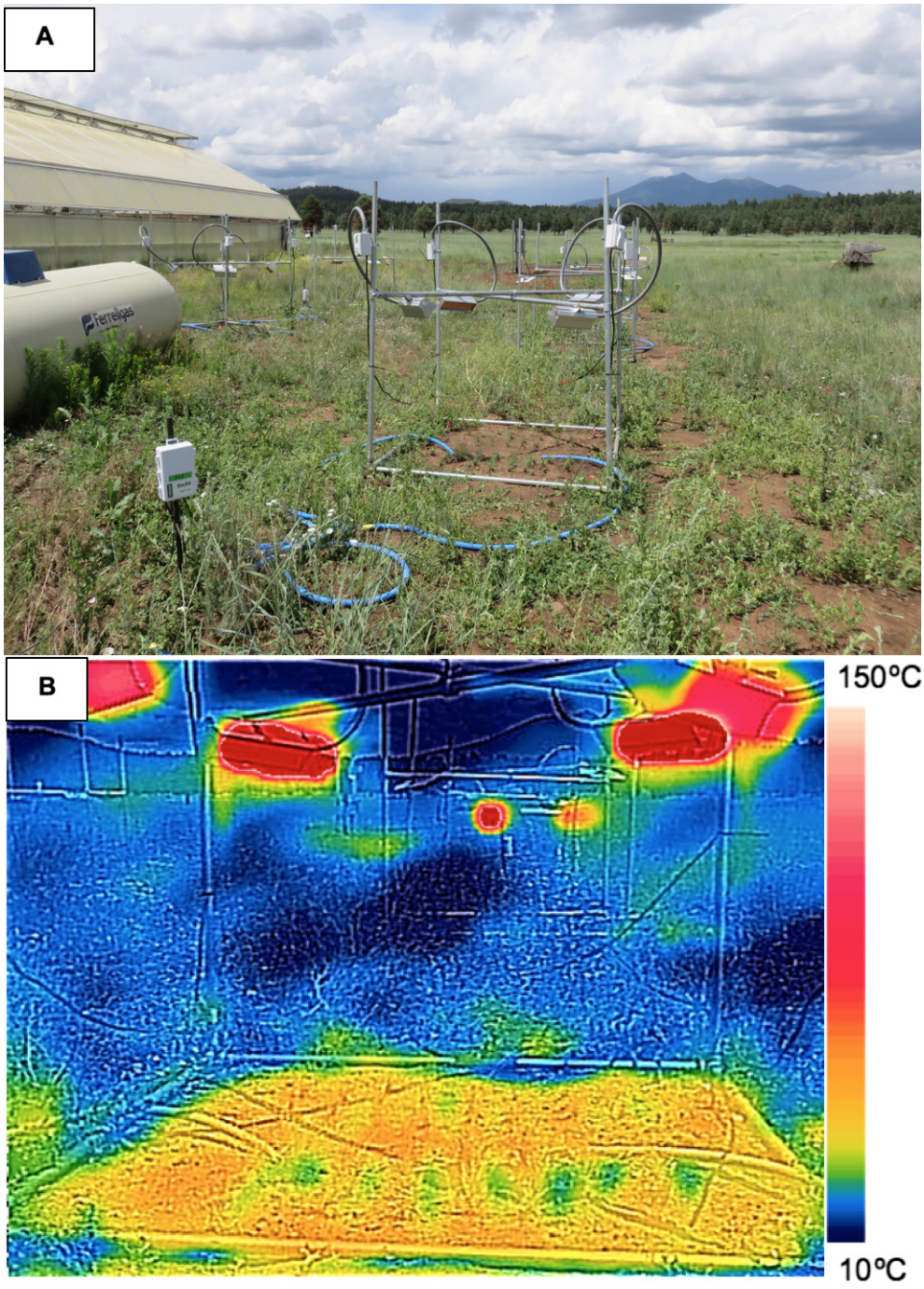

FIG. 1. (A) Experimental design consisting of steel pipe, ceramic lamps and data loggers in the disturbed strip of land (a former road). (B) Infrared image demonstrating an even heating distribution within plots. Cooler blue-green sections are planted grasses.

calculated the percent change in plant performance variables (height, number of leaves, and chlorophyll) using the mean value at the start of the two-week heat wave, x_{start}, and the mean value for each plant variable two weeks after heat wave, x_{end}, for each species, treatment, and inoculum combination using the following formula:

$$\%\Delta = \frac{x_{\text{end}} - x_{\text{start}}}{x_{\text{start}}} \times 100.$$

Due to a highly unbalanced design because of high mortality of Arizona fescue in the heat wave plots, we were unable to use conventional modeling methods to infer the main effects and interactions among plant species, inoculum treatments, and the heat wave on plant response variables (Shaw and Mitchell-Olds 1993). Therefore, we conservatively inferred the effects of heat wave and inoculum treatments using the means and 95% bootstrapped confidence intervals (Mooney and Duval 1993). We individually bootstrapped each subsample of

data (10,000 restarts) using the "boot" package (Canty and Ripley 2015), using the percentile method to construct 95% CIs. Effects of heating and inoculum treatments were inferred when the 95% CI for the percentage change in plant performance in the control plots did not overlap the 95% CI for the percentage change in heated plots.

Survivorship was calculated as a percent change, per plot, of the number of living grasses at the start of the heat wave, to the number of grasses that were still alive two weeks after the heat wave. To evaluate survivorship, which was recorded as 1 or 0 for each plant, we used mixed effects logistic regression in the lme4 package (Bates et al. 2015) and the lmerTest package in R (Kuznetsova et al. 2015). To obtain *P* values for mixed-effects models, we used the Satterwaite approximation (Schaalje et al. 2002). For each species data set, we tested for the main and interactive fixed effects of the heat wave and inoculum treatments, with block as a random effect.

Results

Experiment 1: Laboratory and glasshouse experiment

Soil heating (in the laboratory) reduced soil water content from 20% to 0.03% and from 22% to 0.4% for the blue grama and Arizona fescue rhizosphere soils, respectively, indicating a heat-induced drought. While soil heating did not affect the total number of 16S gene copies (Table 1), it increased species evenness (Shannon's H) of both Arizona fescue and blue grama rhizosphere soils (Table 1). Bacterial community composition differed between the two plant species' rhizospheres and between heated and control soils (Fig. 2). There was an interaction between plant species and heat waves, in which heat waves had a stronger effect on the blue grama rhizosphere bacterial community than the Arizona fescue rhizosphere bacterial community (Fig. 2).

Total root AMF colonization was similar in blue grama and Arizona fescue roots (20%), and soil heating did not alter AMF colonization in either species (Table 1). Similarly, soil heating did not affect the abundance of non-AMF in either species' roots. However, Arizona fescue roots were colonized predominantly by non-AMF (45-47%), whereas blue grama roots were colonized at similar rates by AMF (21%) and non-AMF (22–24%, Table 1).

For blue grama, pre-heated soil inoculum decreased height, number of leaves, and shoot mass, and increased root:shoot ratio in comparison with control inoculum (Table 2). However, Arizona fescue growth was largely

Table 1. Microbial analyses from the glasshouse experiment (Experiment 1).

Inoculum	16S gene copies/g soil (N = 6)	16S diversity (Shannon's H; N = 6)	AMF (%; N = 20)	Non-AMF (%; N = 20)
Arizona fescue				
Control	138,204 (12,077)	4.67 (0.01)	19.63 (3.65)	47.36 (6.43)
Heated	135,965 (19,595)	**4.72 (0.02)****	18.53 (3.29)	45.98 (5.67)
Blue grama				
Control	145,687 (9,194)	4.71 (0.02)	21.32 (3.60)	22.14 (0.02)
Heated	138,285 (13,730)	**4.75 (0.01)****	21.36 (3.17)	23.78 (4.35)

Notes: Values are means with SE in parentheses. Individual main effects models were performed on each response variable. AMF, arbuscular mycorrhizal fungi. N indicates the number of replicates for each inoculum treatment. Significant results from main effects models are shown in bold-face type.** $P < 0.01$.

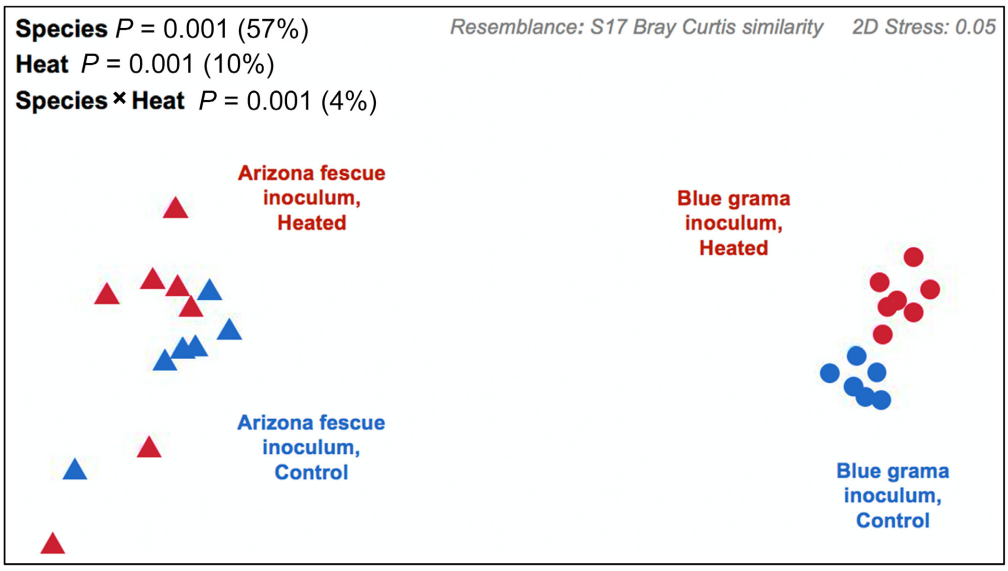

Fig. 2. Nonmetric multidimensional scaling (NMDS) ordination for blue grama and Arizona fescue rhizosphere bacterial communities following 45°C of heating for one week. Taxa were analyzed at the species (L7) level. Values in parentheses represent the percent variation explained by main effects and interactions from the PERMANOVA test.

TABLE 2. Plant performance results from the glasshouse experiment (Experiment 1).

Inoculum	Height (cm; $N = 20$)	Number of leaves ($N = 20$)	Chlorophyll (SPAD units; $N = 20$)	Shoot dry mass (g; $N = 20$)	Root dry mass (g; $N = 20$)	Total dry mass (g; $N = 20$)	Root:shoot ($N = 20$)
Arizona fescue							
Control	14.64 (0.42)	13.85 (0.75)	15.23 (1.26)	0.066 (0.003)	0.142 (0.009)	0.208 (0.008)	2.244 (0.183)
Heated	14.31 (0.42)	14.85 (0.65)	12.77 (1.33)	**0.061 (0.004)****	0.132 (0.009)	0.192 (0.012)	2.248 (0.145)
Blue grama							
Control	20.03 (0.67)	6.5 (0.53)	6.50 (0.53)	0.074 (0.003)	0.072 (0.005)	0.146 (0.007)	0.818 (0.062)
Heated	**19.04* (0.97)**	**5.30* (0.45)**	8.74 (1.20)	**0.057 (0.006)*****	0.072 (0.006)	0.129 (0.009)	**1.309 (0.276)***

Notes: Values are means with SE in parentheses for the final measured values at the end of the five-week growth period. Individual main effects models were performed on each response variable. SPAD units are a relative index of chlorophyll content. *N* indicates the number of plants in each inoculum treatment. Significant results from main effects models are shown in bold-face type. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

unaffected by soil heating, with the exception of shoot mass, which decreased due to soil heating (Table 2).

Experiment 2: Field experiment

We achieved an even distribution of heating within the plots, as confirmed by infrared imaging (Fig. 1B). We increased air temperature by an average of 15.8°C, soil temperature (at 15 cm) by 12.6°C, surface soil temperature by 18.7°C, and leaf temperature by 18.1° and 13.8°C for Arizona fescue and blue grama, respectively (Table 3). The treatment represented a realistic heat wave for this field site: the maximum air temperature recorded by Merriam Powell Research Center instrumentation during summer 2017, when the data stream became available, was 36.5°C, <3°C lower than the mean air temperature recorded by iButtons in the heat wave plots (39.1°C). Volumetric water content was unaffected by the heat wave treatment (Table 3).

The field heat wave produced contrasting effects in the two plant species. For Arizona fescue, the heat wave negatively affected number of leaves and chlorophyll content (Table 4). In contrast, the heat wave did not negatively affect any performance measures for blue grama (Table 5). Heat wave survivorship was also over four times higher in blue grama (92%) than in Arizona fescue (22%, Figs. 3, 4).

Heated inoculum did not improve any plant performance measures under the field heat wave for either plant species, in comparison with the control inoculum treatment (Table 4, Table 5). Furthermore, heated inoculum did not affect heat wave survivorship for either plant species (Figs. 3, 4).

TABLE 3. Treatment effects of the field heat wave, Experiment 2.

Treatment	Air temp. (°C)	Volumetric water content (m^3/m^3)	Soil temp. (°C), 15 cm	Surface soil temp. (°C)	Leaf temp., Arizona fescue (°C)	Leaf temp., blue grama (°C)
Control ($N = 3$)	23.29 (0.02)	0.23 (0.01)	22.50 (4.05)	38.7 (11.33)	31.50 (5.06)	29.60 (3.89)
Heat wave ($N = 3$)	39.08 (1.64)	0.24 (0.04)	35.10 (3.19)	57.37 (13.34)	49.60 (7.03)	43.40 (8.02)
Difference	15.79	0.01	12.60	18.67	18.10	13.80

Notes: Air temperature (temp.), volumetric water content (the mass of water per mass of dry soil), and soil temperature were recorded hourly over the two-week period, and surface soil temperature and leaf temperature were monitored daily. *N* indicates the number of plots within each treatment. We averaged all the hourly or daily values per plot and then calculated the mean values and standard errors (in parentheses) using the three mean plot values.

TABLE 4. Effects of the field heat wave, Experiment 2, on plant performance for Arizona fescue.

	Control plots				Heat wave plots			
Inoculum	N	Height (% change)	Number of leaves (% change)	Chlorophyll content (% change)	*N*	Height (% change)	Number of leaves (% change)	Chlorophyll content (% change)
Control	16	18.84 (−3.18, 40.20)	62.10 (32.50, 91.87)	97.29 (11.29, 207.70)	6	−16.00 (−37.61, 4.75)	**−43.07 (−63.50, −18.59)**	**−82.09 (−97.15, −62.95)**
Heated	14	32.01 (17.96, 46.31)	70.72 (39.16, 104.43)	87.34 (6.28, 188.53)	3	−8.69 (−33.33, 21.05)	**−54.36 (−58.33, −52.11)**	**−69.33 (−80.76, −56.00)**
None	11	28.03 (11.46, 47.81)	84.52 (43.84, 130.18)	31.87 (−39.26, 125.58)	3	3.30 (−16.67, 26.58)	**−63.75 (−90.91, −30.77)**	**−84.60 (−85.80, −83.33)**

Notes: N is the final sample size of living plants, after the heat wave, which was used for calculations of mean percent change in plant variables. Initial sample size was 21 for each species and inoculum treatment. Values are means with 95% bootstrapped CIs in parentheses. Effects of heat waves and inoculum treatments were conservatively inferred when the 95% CIs did not overlap. The heat wave significantly reduced the relative change in number of leaves and chlorophyll content (shown in bold-face type), but not plant height, for all inoculum treatments, and there were no differences in plant variables among inoculum treatments.

TABLE 5. Effects of the field heat wave, Experiment 2, on plant performance for blue grama.

	Control plots				heat wave plots			
	N	% Δ height	% Δ Leaf num.	% Δ Chlor. content	N	% Δ height	% Δ Leaf num.	% Δ Chlor. content
Control Inoculum	21	44.54 (23.81, 80.71)	55.68 (32.96, 89.06)	86.44 (29.19, 169.18)	20	57.63 (34.90, 82.52)	**143.49 (102.09, 183.54)**	271.71 (25.21, 760.18)
Heated inoculum	18	73.21 (45.73, 109.58)	85.84 (59.13, 125.71)	167.00 (51.72, 340.26)	19	69.56 (36.93, 128.75)	164.58 (115.95, 241.89)	113.15 (22.52, 261.47)
No inoculum	20	40.69 (19.12, 67.85)	82.62 (51.67, 119.88)	105.29 (−0.01, 292.40)	18	40.22 (13.44, 66.28)	86.94 (50.87, 139.24)	54.90 (6.45, 111.70

Notes: *N* is the final sample size of living plants, after the heat wave, which was used for calculations of mean percent change in plant variables. Initial sample size was 21 for each species and inoculum treatment. Values are means with 95% bootstrapped CIs in parentheses. Effects of heat waves and differences among individual inoculum treatments were conservatively inferred when the 95% CIs did not overlap. The heat wave increased the relative change in number of leaves for the control inoculum treatment (shown in bold-face type), but there were no differences in plant variables among inoculum treatments.

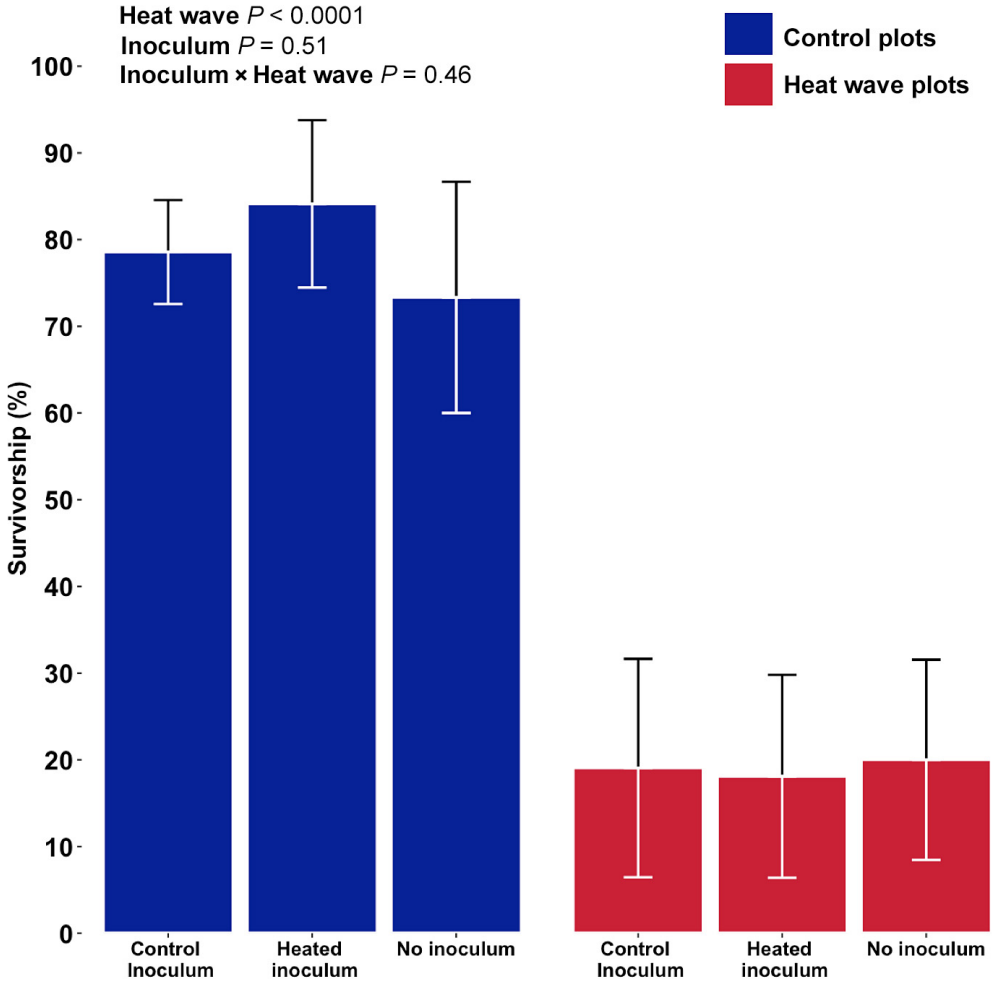

FIG. 3. Effect of field heat wave and inoculum treatments on survivorship for Arizona fescue. Graph depicts percent survival per plot (mean ± SE). The heat wave significantly reduced survivorship of Arizona fescue, and inoculum treatments did not alter survivorship.

DISCUSSION

Extreme events, such as prolonged periods of hot, dry conditions will likely play a large role in steering vegetation trajectories due to their effects on population-level plant mortality (Smith 2011, Meisner et al. 2013). Furthermore, the role of the microbial community under these extreme weather events cannot be ignored, because microbes may respond more rapidly to environmental stressors than plants can due to their fast turnover time (Lau and Lennon 2012). We observed a shift in bacterial community composition after one week of sustained soil heating, which was accompanied by an increase in species evenness. Furthermore, heated inoculum decreased blue grama growth in the glasshouse,

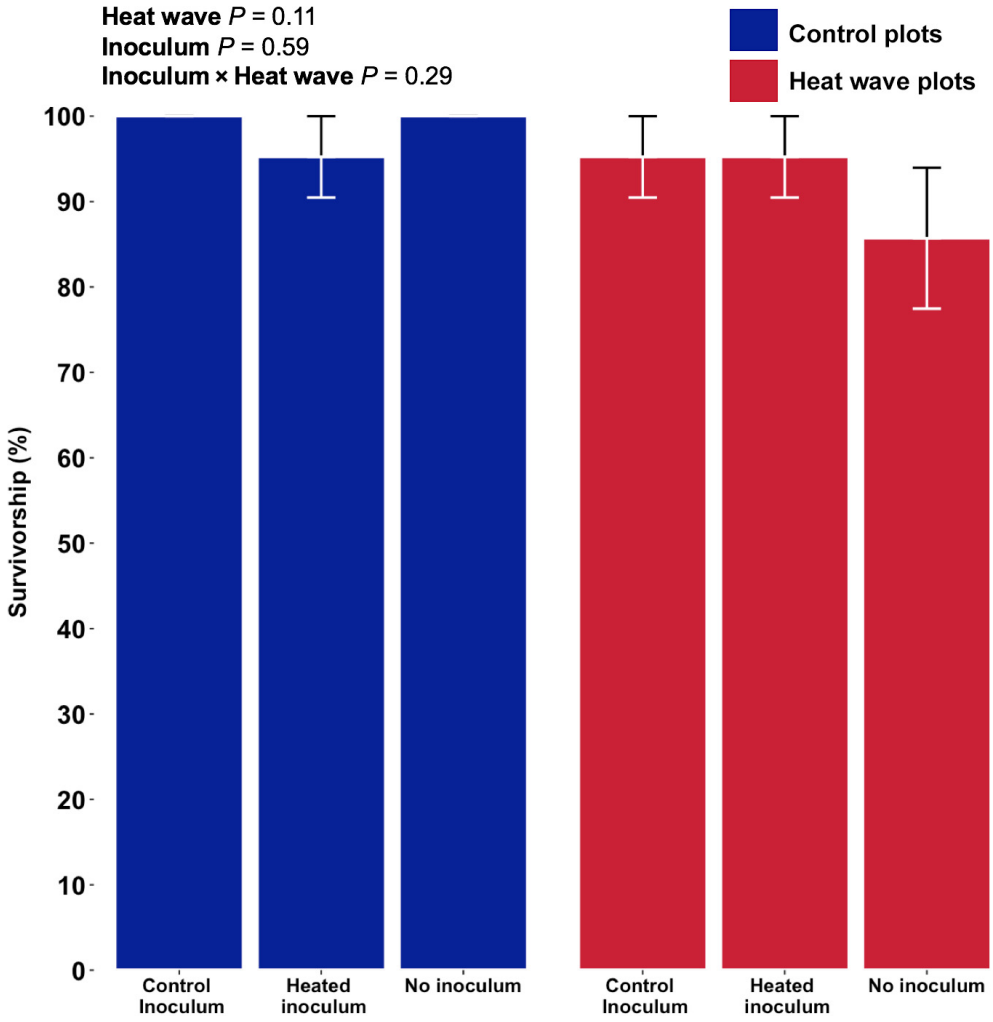

FIG. 4. Effect of field heat wave and inoculum treatments on survivorship for blue grama. Graph depicts percent survival per plot (mean ± SE). Neither the heat wave nor the inoculum treatments altered survivorship for blue grama.

suggesting a negative legacy effect of soil heating in which plant growth and performance were impaired under non-stressful conditions. One possibility is that the heat wave reduced the abundance or activity of certain guilds of phosphorus-solubilizing or nitrogen-fixing bacteria (Lau and Lennon 2012). Alternatively, it may have altered the ratio of mutualists to pathogens in the soil community. For example, short-term soil heating of disease-suppressive soils caused a significant increase in the alpha diversity of the rhizobacterial community, leading to partial or complete loss of disease protection in sugar beets (van der Voort et al. 2016). Future work should address additional mechanisms that may be responsible for a reduction in plant growth when plants are grown with a drought or heat-stricken microbial community.

In our study, AMF root colonization was unaffected by soil heating for both plant species in the glasshouse, indicating that the reduction in blue grama growth could have been driven by a shift in the bacterial community rather than changes in AMF colonization. One caveat is that AMF root colonization is only one index of AMF abundance; extraradical hyphal length may be more associated with mycorrhizal function than percent root colonization. In a *Plantago lanceolata*/*Holcus lanatus* mesocosm study, *Glomus mosseae* root colonization was unaffected by 8°C of soil warming, whereas the length of extraradical mycelium increased significantly (Heinemeyer et al. 2004). We were unable to conduct this additional assay due to the large particle size of the commercial potting mix, a background substrate we chose to mimic typical nursery practices in restoration.

Plants possess a variety of endogenous strategies to cope with heat and drought stress, which differs across plant species (Porras-Alfaro et al. 2008, Berg and Smalla 2009, Wang et al. 2012), life-history strategies, and functional groups. C_3 grasses can outgrow C_4 grasses in favorable growth conditions, but possess a lower water use efficiency compared with C_4 grasses (Pearcy and Ehleringer 1984). Our study indicates that the C_3 grass Arizona fescue and the C_4 grass blue grama also exhibit differences in their relationship with their soil microbiome: not only did each species engineer a distinct soil community, but Arizona fescue had lower AMF root colonization rates and higher non-AM fungi root colonization rates compared with blue grama. A difference in mycorrhizal dependency among C_3 and C_4 grasses has been previously described in prairie grasses (Hetrick et al. 1988,

1990, Wilson and Hartnett 1997, 1998). We propose that future research integrates soil microbiomes into our understanding of C_3-C_4 vegetation trajectories; for example, does reduced microbial dependency of fast-growing C_3 species provide an advantage under degraded soil microbial conditions and non-stressful climates?

Typically, heat waves are accompanied by drought, making it difficult to distinguish the effects of warming from the effects of soil drying (Mazdiyasni and AghaKouchak 2015). Furthermore, the individual effects of drought are usually stronger than the individual effects of heating: in a European grassland, plant mortality was correlated with background aridity rather than an experimental heat wave (Poirier et al. 2012). Similarly, in an alpine grassland, heat waves had no influence on fluorescence (a stress indicator), senescence, or aboveground productivity if irrigation was provided, but produced a significant effect when heat waves coincided with drought (De Boeck et al. 2015). In Experiment 1, soil heating was associated with soil drying, leading to marked changes in the soil microbial community that negatively affected blue grama growth in the glasshouse. In our field study, however, the heat wave did not impose a drought, which could explain why blue grama did not appear to be stressed, and even accumulated more leaves in the heat wave plots than in the control plots for the control inoculum treatment. Our study raises an important consideration for restoration: if available, supplemental watering may mitigate the effects of severe heat waves, both for plants and for their associated microbiomes.

We found no evidence that plant performance or survival could be influenced by microbiome transplants, which could be due to dilution by indigenous microbial populations. In our study, we left the topsoil in place, allowing donor inoculum to interact directly with the recipient site. Removing topsoil before planting has been shown to enhance the efficacy of soil microbiome transplants, particularly in sites with high densities of invasive species. For example, in an old field ecosystem, the impact of introduced inoculum on plant and soil community composition was most pronounced when the existing topsoil layer was removed (Wubs et al. 2016). Similarly, in an abandoned orchard, topsoil removal enhanced the effects of introduced inocula on the diversity and abundance of native target herbaceous species (Jaunatre et al. 2014). While topsoil removal has had promising results for inocula-assisted restoration, the decision to remove topsoil must be considered carefully (van Andel and Aronson 2006), because the cost of soil erosion may exceed the benefits of topsoil removal.

In contrast to blue grama, which had an average mortality of 8%, 78% of Arizona fescue plants died from the heat wave, suggesting a top-kill effect independent of soil moisture, possibly due to oxidative damage to aboveground membranes (Kotak et al. 2007). A difference in survival between C_3 and C_4 grasses under heat stress is to be expected, given that C_4 grasses possess a CO_2 concentrating mechanism that decreases photosynthetic dependence on intercellular CO_2 relative to C_3 grasses (Pearcy and Ehleringer 1984). In our study, Arizona fescue leaf temperature in heated plots was 49°C, similar to the 48°C temperature threshold of photosynthesis for the C_3 plant *Phaseolus vulgaris* (Hüve et al. 2010) and slightly lower than the 55°C temperature threshold for photosynthesis of C_3 shrub, *Larrea divaricata* (Pearcy and Ehleringer 1984). Blue grama exhibited a slightly lower leaf temperature (44°C), corresponding with the temperature optimum of photosynthesis for the C_4 plant *Tidestromia oblongifolia* (Pearcy and Ehleringer 1984). Due to stark differences in temperature optimums for each plant species, trade-offs between fast growth (C_3 species) and heat wave survival (C_4 species) should be prioritized when selecting seed mixes for ecological restoration.

While agriculture and agribusiness have promoted the use of commercial microbial inocula, the restoration field has been cautious to adopt this technique, due to concerns over weedy and/or invasive inocula (Schwartz et al. 2006). To minimize this risk, seeds and microbes should be sourced from the same location, due to local adaptation with the plant host and the environment (Smith et al. 2012, Emam 2015, Maltz and Treseder 2015, Rúa et al. 2016). Second, quantifying the relative abundance of these microbes within the natural communities they were sourced from, as in Hartman et al. (2017), could reveal microbial foundation species, species that possess the majority of the plant growth promotion potential.

Finally, under a scenario of increasing extreme events, plants will rely increasingly on their adaptations, including their ability to form effective mutualisms. We show that short-term soil heating can restructure the rhizosphere microbial community and negatively impact plant growth. However, inoculum had no effect under a field heat wave, either due to the sheer magnitude of the mortality event or because inoculum was diluted by indigenous mutualistic organisms at the recipient site. Additional research is needed to understand which plant species and soil conditions promote inoculum effectiveness in the field; armed with this, scientists and managers can begin to engineer rhizospheres to ameliorate the combined effects of land degradation and global change.

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Supporting Information

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/eap.1763/full