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Drought consistently alters the composition of soil fungal and bacterial communities in grasslands from two continents

Running head: Consistent drought effects on soil microbes

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41 **ABSTRACT**

42 The effects of short-term drought on soil microbial communities remain largely unexplored,
43 particularly at large scales and under field conditions. We used seven experimental sites from
44 two continents (North America and Australia) to evaluate the impacts of imposed extreme
45 drought on the abundance, community composition, richness and function of soil bacterial
46 and fungal communities. The sites encompassed different grassland ecosystems spanning a
47 wide range of climatic and soil properties. Drought significantly altered the community
48 composition of soil bacteria and, to a lesser extent, fungi in grasslands from two continents.
49 The magnitude of the fungal community change was directly proportional to the precipitation
50 gradient. This greater fungal sensitivity to drought at more mesic sites contrasts with the
51 generally observed pattern of greater drought sensitivity of plant communities in more arid
52 grasslands, suggesting that plant and microbial communities may respond differently along
53 precipitation gradients. Actinobateria, and Chloroflexi, bacterial phyla typically dominant in
54 dry environments, increased their relative abundance in response to drought, whereas
55 Glomeromycetes, a fungal class regarded as widely symbiotic, decreased in relative
56 abundance. The response of Chlamydiae and Tenericutes, two phyla of mostly pathogenic
57 species, decreased and increased along the precipitation gradient, respectively. Soil enzyme
58 activity consistently increased under drought, a response that was attributed to drought-
59 induced changes in microbial community structure rather than to changes in abundance and
60 diversity. Our results provide evidence that drought has a widespread effect on the assembly
61 of microbial communities, one of the major drivers of soil function in terrestrial ecosystems.
62 Such responses may have important implications for the provision of key ecosystem services,
63 including nutrient cycling, and may result in the weakening of plant-microbial interactions
64 and a greater incidence of certain soil-borne diseases.

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67 borne pathogens.

68

69 INTRODUCTION

70 Microorganisms play fundamental roles as primary producers and decomposers and provide
71 important ecosystem services such as organic matter decomposition and nutrient cycling and
72 storage (van der Heijden *et al.*, 2008; Bardgett & van der Putten, 2014). The structure and
73 activity of microbial communities in soils is influenced by substrate properties, particularly
74 soil pH and organic matter content, and vegetation type (Fierer & Jackson, 2006; Delgado-
75 Baquerizo *et al.*, 2016a); at larger spatial and temporal scales long-term climatic phenomena
76 such as increasing aridity have also been associated with lower bacterial and fungal
77 abundance and lower microbial diversity (Maestre *et al.*, 2015). However, much less is
78 known about how short-term climatic processes, including the more extreme drought events
79 forecast (Cook *et al.*, 2015), will affect microbial communities and the ecosystem services
80 they mediate.

81 Climate models forecast widespread changes in precipitation regimes, including
82 longer, more intense droughts (McLaughlin, 2014), causing desertification and promoting the
83 expansion of drylands globally (Huang *et al.*, 2016). Field-based climate change studies have
84 traditionally focused on aboveground responses (e.g., plant productivity, biomass and
85 community composition) of local studies, whereas belowground responses, particularly those
86 of microbial communities, have received much less attention (Wilcox *et al.*, 2015, 2017).
87 Experimental approaches across multiple sites and continents, where contrasting climatic and
88 edaphic conditions may mediate the impacts of changing precipitation regimes, are equally
89 needed to accurately predict the response of microbial communities to extreme drought
90 (Grime *et al.*, 2008; Fridley *et al.*, 2011). Drought experiments and meta-analyses
91 consistently predict negative impacts of drought on the diversity and abundance of soil
92 microbial communities (Wu *et al.*, 2011), with bacteria typically considered more sensitive
93 than fungi (Evans & Wallenstein, 2012; Fry *et al.*, 2016). Given the strong link between
94 microbial communities and soil functioning, any alteration in the composition of microbial
95 communities due to climate change might disrupt the functioning of soil, and thus the supply
96 of ecosystem services (Bellard *et al.*, 2012; McLaughlin, 2014). Because of this, improving
97 our understanding of the role of altered precipitation regimes in the regulation of soil
98 microbial communities is of paramount importance to accurately predict changes in terrestrial

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ecosystem processes linked to future climate change scenarios (Maestre *et al.*, 2015; Delgado-Baquerizo *et al.*, 2016b).

Grasslands are critically important components of terrestrial ecosystem feedbacks to climate change. They represent ca. 40% of the total land surface, store ca. 3.4 t C ha^{-1} yr^{-1} and provide multiple ecosystem services (McLaughlin, 2014). Grasslands also greatly contribute to regulate the inter-annual variability in the soil C sink at the global scale (Poulter *et al.*, 2014; Ahlström *et al.*, 2015) and, therefore, understanding how microbial community structure and functioning responds to drought is essential for predicting impacts of climate change on the global C cycle. Climate models for the Central and Southwest US, where grasslands dominate the landscape, predict an intensification of the hydrological cycle, with high inter-annual precipitation variability and fewer but larger rain events (Cook *et al.*, 2015). Similarly, for south-eastern Australia, where land conversion has transformed more than ninety percent of native woodlands into semi-natural grasslands, the most recent climate models predict an increase in the occurrence of extreme precipitation events interspersed with longer droughts and shifts in precipitation seasonality but little change in total precipitation (McLaughlin, 2014).

In this study, we evaluated the impacts of comparable extreme drought simulation experiments (50% precipitation reduction) conducted at seven grasslands located in two continents, North America and Australia (Table 1 and Figure 1). We sampled each site during the 2nd or 3rd year of imposed drought and measured (i) soil microbial community richness (number of phylotypes) and composition (relative abundance of phylotypes), (ii) microbial abundance, and (iii) the potential activities of enzymes associated with decomposition and nutrient cycling by soil microbial communities. The consistency of treatment types between sites in the USA (66% precipitation reduction during the growing season, equivalent to an annual reduction of 50%) and Australia (50% year-round reduction; Power *et al.*, 2016) allows for evaluation of responses to experimental drought across continents. We hypothesized that, at both inter-continental and local scales, experimental drought will significantly alter the assembly of microbial communities in grasslands, with bacterial communities being more sensitive than fungi to water limitation (Austin *et al.*, 2004; Clark *et al.*, 2009). Specifically, we predicted that the relative abundance of bacterial taxa such as Chloroflexi and Actinobacteria, known to be adapted to arid conditions (Acosta-Martínez *et al.*, 2014; Maestre *et al.*, 2015), would increase in response to drought events. In contrast, we predicted an increase in potential enzymatic activity in droughted plots, as a result of enzyme

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132 and substrate accumulation during periods of low soil moisture (Austin *et al.*, 2004). An
133 increase in potential enzymatic activity could also be linked to reduced competition with
134 plants for soil resources (Schwinning & Sala, 2004) and/or to greater inputs of organic matter
135 associated with the death of roots and shedding of foliage (Sinsabaugh *et al.*, 2008).
136
137 **MATERIALS & METHODS**

138 **Study sites.** The seven sites considered in this study encompass different types of grassland
139 ecosystems and span a wide range of climatic and soil conditions (Figure 1, Table 1). None
140 has been grazed for the last 15 years. Six experimental sites were selected across the Central
141 and Southwest US along a large precipitation gradient (242-860 mm). These sites are part of
142 the EDGE (Extreme Drought in Grasslands Experiment) experimental platform
143 (http://edge.biology.colostate.edu/). They included desert grasslands, shortgrass and tall grass
144 prairies, and mixed grasslands. Soil texture varied from sandy to clay loams (Knapp *et al.*,
145 2015), while soil pH ranged from slightly acidic (6.25) at the High Plains Grasslands
146 Research Center, Wyoming, to basic (8.82) at Sevilleta National Wildlife Refuge, New
147 Mexico. Konza Prairie, Kansas, is the only site that is burned annually, while the rest have
148 not burned in recent times.

149 In addition to the six sites in the US, another study site was selected in Eastern
150 Australia (DRI-Grass; Drought and Root Herbivore Interactions in a Grassland). The site is a
151 mesic grassland near Richmond, NSW, Australia, at an elevation of 25 m a.s.l. Mean annual
152 precipitation is 800 mm (Australian Government Bureau of Meteorology, Richmond – WSU
153 Hawkesbury Station1). The soil is a Blackendon Sand, with a sandy loam texture and pH of
154 6.38. The most abundant species include C4 grasses such as *Axonopus fissifolius*, *Cynodon*
155 *dactylon*, *Cymbopogon refractus*, *Eragrostis curvula*, and *Paspalum dilatatum*, C3 grasses
156 including *Microlaena stipoides* and *Lolium perenne*, and C3 forbs such as *Hypochaeris*
157 *radicata* and *Plantago lanceolata*. Although the sites encompass different types of grassland
158 ecosystems and span a wide range of edapho-climatic conditions, DRI-Grass is most
159 comparable to the mesic American grassland (Konza; Figure 1).

160 **Experimental treatments.** The EDGE platform was set up in spring 2013 at Sevilleta and
161 2014 at the other four sites and uses rainout shelters to impose a drought by reducing each
162 precipitation event by 66% for the entire growing season, the latter varying between sites.
163 This is roughly equivalent to a year-round reduction of 50% precipitation. Each experimental
164 treatment is replicated ten times at each site. Plots are 3 x 4 m and are hydrologically isolated

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165 from the surrounding soil matrix by aluminum flashing installed to varying depth depending
166 on site.

167 The DRI-Grass experiment started in June 2013 and consists of sixty plots (1.9 x 2.5
168 m) and five precipitation treatments. Plots are covered with fixed rainout shelters, which
169 exclude ambient precipitation inputs. In this study, we used a sub-set of two treatments
170 ("ambient" and "reduced amount" – a reduction of 50 % compared to ambient) replicated six
171 times, for a total of twelve plots. The shelters have open sides and are covered with UV-
172 transparent Perspex roofs sloped at an angle of 18°. Both treatments involve water re-
173 application through a programmable automated irrigation system. Soil moisture and
174 temperature were continuously recorded using sensors installed in almost all plots. More
175 detailed information on the field site and experimental design can be found elsewhere (Power
176 *et al.*, 2016).

177 **Soil sampling.** Soils at all sites were collected in 2015 during the main growing season
178 (March in Australia and July in the US sites). Eight to ten soil samples were collected from
179 each plot at a depth of 0-10 cm and bulked. Once in the laboratory, samples were kept at 4ºC
180 until further processing within a few days. A small subsample was also immediately frozen at
181 -20ºC for soil microbial analyses.

182 **Soil properties and microbial extracellular enzyme activity analyses.** Gravimetric soil
183 water content (%) was measured after drying a known amount of soil at 70ºC and then
184 weighing it. Soil pH was measured using a 1:2.5 ratio of fresh bulk soil to deionized water.
185 Soils were assayed for: β-1,4-glucosidase (BG), and β-D-cellobiohydrolase (CBH) enzymes,
186 involved in the degradation of cellulose and other beta-linked glucans (the major components
187 of plant cell walls), β-1,4-N-acetylglucosaminidase (NAG), associated with the degradation
188 of chitin and peptidoglycan (major microbial cell wall components), and phosphatase (PHOS;
189 phosphorus mineralization) for the P cycle. Briefly, assays were conducted by homogenizing
190 1 g of soil in 30 ml of pH-adjusted 50 mM sodium acetate buffer. The pH of the buffer was
191 adjusted to match the soil pH of each site (Figure 1). The homogenized solutions were then
192 added to a 96-deep-well (2 ml) microplate. Replicate soil slurry controls and 4-
193 methylumbellfferone (MUB) standard curves of 0-100 µm were included in each sample.
194 Fluorometric substrates (Sigma-Aldrich, reference numbers: M3633 for BG, M6018 for CBH,
195 M2133 for NAG, and M8883 for PHOS) were added to soil slurries and then incubated for
196 1.5 h at 35 °C. Following incubation, the supernatant solution was transferred into
197 corresponding wells in a black, flat-bottomed 96-well plate. The plates were then scanned on

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198 a microplate fluorometer using an excitation wavelength of 365 nm and an emission
199 wavelength of 450 nm. Enzyme assays for the Australian samples were carried out on the
200 same day at the Hawkesbury Institute for the Environment, Western Sydney University,
201 Australia using a 2300, EnSpire® Multilabel Reader (PerkinElmer, Boston, MA, USA),
202 while samples from all EDGE sites were analyzed on the same day at the University of New
203 Mexico.

204 **Quantitative PCR.** All molecular analyses were undertaken at the Hawkesbury Institute of
205 the Environment on a subset of three replicates per EDGE site and on all six DRI-Grass
206 replicates. First, we extracted the DNA from each soil sample using the Power Soil kit (Mo
207 Bio Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. Soils from
208 DRI-Grass were extracted and stored at -80ºC until further analyses, while soils from the
209 EDGE sites were extracted at the University of New Mexico, frozen, and then shipped to
210 Australia. Once there, all samples were defrosted and qPCR reactions carried out using 96-
211 well plates. Reactions consisted of 5 µl of polymerase mix, 1 µl of template DNA, 0.3 µl of
212 each primer, and 3.4 µl of H_2O, giving a final volume of 10 µl. Bacterial 16S rRNA gene and
213 fungal internal transcribed spacer (ITS) were amplified with the Eub 338-Eub 518 and ITS 1-
214 5.8S primer sets (Evans & Wallenstein, 2012). The abundance of fungi and bacteria were
215 then expressed as the number of ITS or 16S rRNA gene copies g^{-1} soil, respectively.

216 **Amplicon sequencing.** DNA samples were analyzed using Illumina MiSeq 2x 301 bp
217 (bacteria) or 2x 280 bp (fungi) paired end sequencing (Caporaso *et al.*, 2012) and the
218 341F/805R (bacteria) and FITS7/ITS4 (fungi) primer sets (Herlemann *et al.*, 2011; Ihrmark *et*
219 *al.*, 2012). The quality of all Illumina R1 and R2 reads was assessed using FastQC (Andrews,
220 2010), low quality regions (Q<20) were trimmed from the 5' end of the sequences (0 bp from
221 R1 and 22 bp from R2 for primer set 341F/805R; 5 bp from R1 and 50 bp from R2 for primer
222 set FITS7-ITS4R) using SEQTK (https://github.com/lh3/seqtk). The paired ends were
223 subsequently joined using FLASH (Magoč & Salzberg, 2011). Primers were removed from
224 the resulting sequences using SEQTK and a further round of quality control was conducted in
225 MOTHUR (Schloss *et al.*, 2009) to discard short sequences (<380 bp for primer set 341F-
226 805R; <150 bp for primer set FITS7-ITS4R), as well as sequences with ambiguous characters
227 or more than 8 homopolymers. Operational Taxonomic Units (OTUs) were built at 97%
228 sequence similarity using UPARSE (Edgar, 2013). Singletons were discarded, as well as
229 chimeric sequences identified by the UCHIME algorithm using the recommended SILVA
230 gold 16S rRNA gene or UNITE reference databases for bacteria and fungi, respectively

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231 (Edgar *et al.*, 2011). OTU abundance tables were constructed by running the usearch_global
232 command (http://www.drive5.com/). Taxonomy was assigned to OTUs in MOTHUR using
233 the naïve Bayesian classifier (Wang *et al.*, 2007) with a minimum bootstrap support of 60%
234 and the Greengenes database version 13_8 (DeSantis *et al.*, 2006; McDonald *et al.*, 2012) for
235 bacteria or the dynamic UNITE version 6 dataset (Kõljalg *et al.*, 2013) for fungi. The OTU
236 abundance tables were rarefied to an even number of sequences per sample to ensure equal
237 sampling depth (8115 sequences for bacteria and and 34403 sequences for fungi), prior to
238 calculating alpha diversity metrics using MOTHUR (Schloss *et al.*, 2009).

239 **Statistical analyses and numeric calculations.** All analyses reported were carried out in R
240 version 3.4.0 (R Core Team, 2017). Enzyme activity data, fungal and bacterial abundance
241 (log-transformed), richness, diversity, and the dominance of all taxa with a mean relative
242 abundance higher than 1% were analyzed using linear mixed-effects models, with drought
243 treatment as the fixed factor and location as a random effect. We also carried out linear
244 models at the site level with experimental treatment as a fixed factor. Analyses were
245 performed using the ´lme´ and ´lm´ functions from the *nmle* and *stats* packages, respectively.

246 We analyzed changes in the composition and structure of bacterial and fungal
247 communities by means of permutational analyses of the variance (9999 permutations) using
248 the ´adonis´ function in *vegan*. Samples were nested within sites using the ´strata´ argument.
249 Results from the permutational multivariate analyses were visualized by means of two non-
250 metric multidimensional scaling (NMDS) analyses using fungal and bacterial OTU data. For
251 this, we used the ´metaMDS´ function of the *vegan* package.

252 To investigate how microbial responses to drought may change along environmental
253 gradients, we calculated an Effect Size (ES) of each microbial variable considered, including
254 the two first components of the NMDS for bacterial and fungal communities. We defined the
255 ES as the absolute difference between the droughted and control plots for each site. We then
256 carried out non-parametric Spearman-rank correlation analyses (n = 7 sites) between the ESs
257 and climate (MAP and MAT) and soil pH. A significant correlation between a predictor
258 variable and the ESs indicates that the magnitude of the response of the dependent microbial
259 variable to drought is proportional to the environmental variable.

260 To build a more holistic understanding of the responses of bacterial and fungal
261 microbial communities to drought and environmental variation, we carried out structural
262 equation models (Grace, 2006) using the ´sem´ function from the *lavaan* package. All *a*

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263 *priori* models and citations for all hypothetical paths are depicted and referenced in
264 Supplementary Figure 9 and Appendix A, respectively. Microbial activity was the final
265 response variable and was computed as the average of the z-score of each individual soil
266 enzyme (i.e., equivalent to the simple multifunctionality index as described in Maestre *et al.*,
267 2012). In our model, climate (MAP) and drought affected all variables but, given that drought
268 is an experimental treatment, these were independent of each other (i.e., they are exogenous
269 variables). Mean annual precipitation was consistently used as our climatic variable over
270 MAT because we wanted to be able to better predict SWC, which we presumed was a key
271 variable in our model. Exploratory analyses also showed that models considering MAP had a
272 consistently better goodness of fit than models using MAT. Soil water content and pH were
273 hypothesized to have a direct effect on microbial community attributes (structure, abundance,
274 diversity and richness in separate models) and microbial activity. Finally, microbial
275 community attributes directly influenced microbial activity. Model fit was considered good
276 when the χ^2 test and its associated *P*-value were low (<2) and high (>0.05), respectively. The
277 root-mean-square error of approximation (RMSEA) was also used to evaluate the goodness
278 of fit. A model has a good fit when RMSEA is <0.05 and its associated *P*-value is >0.05.

279

280 **RESULTS**

281 Across all sites, bacterial communities were consistently dominated by globally distributed
282 bacterial phyla (Figure 2), including: Actinobacteria (26.1 %), Proteobacteria (23.4 %),
283 Acidobacteria (18.0 %), Planctomycetes (6.6 %), Verrocomicrobia (6.4 %), Chloroflexi
284 (6.1 %), Bacteroidetes (4.5 %), Gemmatimonadetes (2.9 %), and Firmicutes (1.8 %).
285 Dominant fungal taxa included: Ascomycota (55.0 %), Basidiomycota (24.5 %),
286 Chytridiomycota (1.8 %), Glomeromycota (2.8 %) and Zygomycota (5.3 %). This microbial
287 community composition is similar to that reported for global drylands (Maestre *et al.*, 2015),
288 but contrasts with previous studies in which Acidobacteria and Basidiomycota were found to
289 be the dominant bacterial and fungal phyla, respectively, at the global scale (Ramirez *et al.*,
290 2014; Tedersoo *et al.*, 2014). Despite common patterns in the relative abundance of the main
291 taxa at the highest taxonomic level (classes/phyla), microbial communities, particularly for
292 fungi, differed widely between sites at the OTU level (Figure 2).

293 Experimental drought significantly altered the assembly of soil bacterial (*P* < 0.001)
294 and, to a lesser extent, fungal communities (*P* = 0.090) across seven sites in two continents

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295 (Figures 2 and 3 and Supplementary Figures 1-5 and Supplementary Table 1). The magnitude
296 of the fungal community response to drought at the species (OTU) level was proportional to
297 the amount of ambient precipitation (Spearman's rho = 0.81, P = 0.027; Supplementary
298 Figure 6 and Supplementary Table 2); i.e., we found the largest absolute differences between
299 control and droughted plots at the most mesic end of the precipitation gradient, represented
300 by KNZ and HAYS, in the US, and the Australian site (Figure 2).

301 Of all taxa with a mean relative abundance higher than 1%, three bacterial phyla
302 (Actinobacteria, Chloroflexi and Gemmatimonadetes), seven classes (Rubrobacteria,
303 Acidobacteriia, Deltaproteobacteria, Thermoleophilia, Chloroflexi, Actinobacteria,
304 Pedospherae) and one genus (*Rubrobacter*), and one fungal class (Glomeromycetes) were
305 consistently affected by drought (P < 0.05; Figure 2 and Supplementary Table 1).
306 Glomeromycetes and Gemmatimonadetes decreased, whereas Actinobacteria (genus
307 *Rubrobacter*, in particular) and Chloroflexi, generally described as more dominant in
308 drylands, increased.

309 In parallel with consistent microbial responses in terms of community assembly and
310 relative abundance of some taxa, other microbial community attributes and taxa responded in
311 a site-dependent manner, thus highlighting the context dependency of some drought effects
312 (Supplementary Figures 1-5 and Supplementary Table 2). For example, fungal abundance
313 and richness increased in WYO, whereas fungal and bacterial richness decreased at the driest
314 location (SEV). Analyzed as effect sizes, the relative abundance of Chytridiomycota
315 decreased more at the warmest sites (Spearman's rho = -0.76, P = 0.049; Supplementary
316 Figure 6). In contrast, Chloroflexi (Spearman's rho = -0.85, P = 0.016) and Rubrobacteria
317 (Spearman's rho = -0.81, P = 0.027) increased in response to drought at the drier sites,
318 whereas Dothideomycetes (Spearman's rho = 0.82, P = 0.023), Rubrobacteria (Spearman's
319 rho = 0.86, P = 0.014) and Acidobacteria (Spearman's rho = 0.96, P < 0.001) were more
320 positively affected at the most acidic sites (Supplementary Figure 6). Tenericutes
321 (Spearman's rho = 0.87, P = 0.010) and Chlamydiae (Spearman's rho = -0.76, P = 0.049)
322 responded more positively and negatively to drought, respectively, toward the wettest end of
323 the precipitation gradient (Supplementary Figure 6).

324 Changes in soil microbial community composition in response to drought occurred in
325 parallel with changes in potential microbial enzyme activity, with responses of the latter
326 being strongly site-dependent (Figure 3, Supplementary Figure 7 and Supplementary Table 3).
327 For example, two C-degrading enzymes and one N-degrading enzyme increased in Australia

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328 in response to drought, whereas the C-degrading enzymes ß-glucosidase and
329 cellobiohydrolase increased in SGS and KNZ, respectively. Similar to fungal community
330 composition, drought-sensitivity of enzyme activity was only apparent at the more mesic sites.
331 Potential enzyme activity was also highly significantly related to soil pH at the inter-
332 continental scale (Figure 3 and Supplementary Figure 8), peaking at neutral pH, results that
333 are in line with previous global studies evaluating soil enzyme relationships with soil pH
334 (Sinsabaugh *et al.*, 2008).

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335 The SEM for the fungal community structure explained 97% of the variance in the first NMDS axis and 89% of potential microbial activity (Figure 3a). Similarly, the SEM for the bacterial community structure explained 94% of the variance contained in the first NMDS axis and 85% of potential microbial activity (Figure 3b). In contrast, models using bacterial and fungal richness, Shannon diversity, and abundance (qPCR) data explained a much lower proportion of the total variance in potential enzyme activity (Supplementary Figure 10). Indeed, models constructed using the relative abundance of major bacterial and fungal phyla also consistently explained a lower proportion of the variance in microbial activity than OTU-level analyses. In these models, the relationship between soil enzyme activity and microbial relative abundance was, however, significantly positive in the case of Acidobacteria, Verrucomicrobia, Glomeromycota and Basidiomycota, while it was negative in th e case o f α-Proteobacteria, ß-Proteobacteria, δ-Proteobacteria, Gemmatimonadetes, Zygomycota and Chytridiomycota (Supplementary Table 4). As expected, experimental drought reduced soil water content which, in turn, greatly influenced the structure of fungal and bacterial communities, as defined by the NMDS axes. The SEM analyses also indicate a significant increase in fungal richness in response to drought (Supplementary Figure 10e). In addition, SEMs indicate that the composition of both fungal and bacterial communities are also greatly driven by variations in MAP and soil pH. In contrast, the effect of drought was only statistically significant for bacterial, but not fungal, community composition, findings that support our previous analysis.

355

356 **DISCUSSION**

357 Our study provides novel experimental evidence that drought is a major climatic driver of the assembly of soil microbial communities. This is in agreement with a previous observational study that suggested that microbial communities are highly responsive to long-term climatic changes such as those from increases in aridity (Maestre *et al.*, 2015). However, our study provides, to the best of our knowledge, the first widespread evidence (i.e., from multiple sites spanning a precipitation gradient and two continents) that the assembly of microbial communities is also highly vulnerable to short-term climatic changes (i.e., 2-3 years of experimentally imposed drought), which may affect the provision of key microbially-mediated ecosystem services such as decomposition and nutrient cycling.

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366 The magnitude of the fungal community response to drought at the species (OTU)
367 level was proportional to the amount of ambient precipitation, whereby the largest absolute
368 differences between control and droughted plots were found at the most mesic end of the
369 precipitation gradient, represented by KNZ and HAYS, in the US, and the Australian site.
370 This suggests a common fungal community response pattern to drought in locations that are
371 thousands of kilometers apart and that show large differences in terms of microbial
372 community composition, as is particularly well-illustrated by the large site separation along
373 the second NMDS axis. This greater microbial sensitivity to drought at more mesic sites
374 contrasts with the generally observed pattern of greater drought sensitivity of aboveground
375 productivity in more arid grasslands (Knapp *et al.*, 2015), which suggests that plant and
376 microbial communities may respond differently along precipitation gradients.

377 At the higher taxonomic level (phylum and class), some consistent response patterns
378 also emerged. For example, Glomeromycetes and Gemmatimonadetes decreased, whereas
379 Actinobacteria (genus *Rubrobacter*, in particular) and Chloroflexi, generally described as
380 more dominant in drylands, increased. These bacterial taxa are highly resistant to desiccation
381 and low resource conditions, which may allow them to outcompete other microbial taxa
382 under extreme drought (Battistuzzi & Hedges, 2009). Particularly relevant was the response
383 of Tenericutes and Chlamydiae, two widely distributed bacterial phyla known for containing
384 species that can cause serious plant and animal diseases, and that responded more positively
385 and negatively to drought, respectively, toward the wettest end of the precipitation gradient.
386 Some studies have suggested an increase in soil pathogenicity under climate change scenarios
387 (van der Putten *et al.*, 2010) whereas our results suggest more complex, taxa-dependent
388 interactions between altered precipitation regimes and soil-borne pathogens.

389 We assessed microbial community functioning through using a high-throughput
390 analysis of soil extracellular enzyme profiles. Extracellular enzymes decompose soil organic
391 matter and reflect microbial nutrient demand (Sinsabaugh *et al.*, 2008). Changes in soil
392 microbial community composition in response to drought occurred in parallel with changes in
393 potential microbial enzyme activity, with responses of the latter being strongly site-dependent.
394 Similar to fungal community composition, drought-sensitivity of enzyme activity was only
395 apparent at the more mesic sites, suggesting that drought-driven alterations in soil microbial
396 communities may further impact the functioning of essential ecosystem services such as
397 nutrient cycling and decomposition, particularly at wetter locations.

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398 The use of SEMs allowed us to build a more holistic understanding of the responses
399 of bacterial and fungal microbial communities and soil functions to experimental drought. In
400 the case of both bacteria and fungi, our SEMs explained an enormous portion of the variation
401 in the distribution of microbial communities and enzyme activities (>85%). In contrast,
402 models using bacterial and fungal richness, Shannon diversity, and abundance (qPCR) data
403 explained a much lower proportion of the total variance in potential enzyme activity.
404 Acidobacteria, Verrucomicrobia, Glomeromycota and Basidiomycota showed a positive
405 effect on enzyme activities, while it was negative in the case of α-Proteobacteria, ß-
406 Proteobacteria, δ-Proteobacteria, Gemmatimonadetes, Zygomycota and Chytridiomycota
407 (Supplementary Table 4), highlighting a strong link between microbial community
408 composition and soil enzyme activities. This result further suggests that not all major taxa are
409 equally important for maintaining highly functional grassland soils.

410 Strikingly, both extreme drought and higher soil water content, the latter mainly
411 explained by MAP (positively) and drought treatment (negatively), enhanced potential
412 microbial activity, suggesting that the effects of long-term and short-term climatic
413 phenomena may operate through different mechanisms. For example, greater enzyme activity
414 may be associated with greater organic matter inputs and rhizosphere activity at the wetter
415 end of the precipitation gradient (Sinsabaugh *et al.*, 2008), but substrate accumulation may
416 drive enzyme response under more droughted conditions (Austin *et al.*, 2004). Greater
417 enzyme activity may also be due to reduced competition with plants, given that the levels at
418 which microbes become water-limited are typically much lower than those for plants
419 (Schwinning & Sala, 2004; Delgado-Baquerizo *et al.*, 2013), or to extra organic matter inputs
420 associated with the death of fine roots and shedding of foliage. Striking also was the direct
421 negative link between MAP and microbial activity, which may be due to the fact that most of
422 the positive effects of MAP on microbial activity are indirect (e.g., via increased SWC and
423 variations in soil pH and microbial community composition). In addition, SEM models
424 indicate that the composition of both fungal and bacterial communities are also greatly driven
425 by variations in MAP and soil pH. In contrast, the effect of drought was only statistically
426 significant for bacterial, but not fungal, community composition, findings that support our
427 previous analysis. Taken together, our results suggest that drought-induced changes in soil
428 microbial community composition and structure, rather than changes in abundance and
429 diversity, are likely to have the most important consequences in terms of ecosystem

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430 functioning and, therefore, affect the ability of these systems to provide key services on
431 which our societies and economies critically depend.

432 Our results provide unequivocal evidence that as little as 2-3 years of drought can
433 alter the assembly of microbial communities in grasslands from two continents, with clear
434 implications for ecosystem functioning. In particular, our study reinforces the role of
435 distributed networks of comparable experiments to study the impacts of drought (Fraser *et al.*,
436 2013; Tielbörger *et al.*, 2014) and unveiled consistent responses in contrasting grassland
437 ecosystems in Australia and North America that share similar climatic and edaphic conditions.
438 In response to drought, we found a greater abundance of drought-resistant bacterial taxa
439 (Actinobacteria and Chloroflexi) and lower abundance of a widely symbiotic, mycorrhizal-
440 forming fungal class (Glomeromycetes). Climate-change driven impacts on soil microbial
441 communities were modulated by the local environmental context, including an increase and
442 decrease in the relative abundance of two pathogenic taxa along a gradient of increasing
443 precipitation. However, unlike aboveground responses, many of the belowground variables
444 evaluated (for example, fungal community composition) exhibited a particularly high degree
445 of resistance to drought at the driest end of the gradient. This pattern suggests that plant and
446 microbial communities may respond differently to drought along precipitation gradients,
447 which opens new questions about the potential role of the disruption or weakening of plant-
448 microbial interactions under climate change scenarios due to a decoupling in the response of
449 both groups.

450

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460

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600 **Figure. 1.** (a) Location of study sites; (b) location of sites along the regional precipitation
601 gradient (color scale is in mm); (c) picture of study site at Sevilleta (EDGE); (d) picture of
602 DRI-Grass experimental facility. Site legend is as in Table 1.

603 **Figure 2.** Drought effects on microbial community composition and major bacterial and
604 fungal taxa. Blue bars = control; red bars = drought.

605 **Figure 3.** Structural equation model depicting the direct and indirect effects of drought and
606 environmental conditions on microbial community composition and activity. (a) Fungal
607 community, represented by the first two axes of the NMDS. (b) Bacterial community,
608 represented by the first two axes of the NMDS. SWC = soil water content. MAP = mean
609 annual precipitation. $*P < 0.05$; $**P < 0.01$, $***P < 0.001$.

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610 **Supplementary Figures**

611 **Supplementary Figure 1.** Site-level effects of drought on microbial community attributes.
612 H' = Shannon-Wiener diversity index

613 **Supplementary Figures 2-5.** Site-level effects of drought on major fungal and bacterial
614 phyla.

615 **Supplementary Figure 6.** Significant relationships between site-level treatment effect sizes
616 (Response Ratio, RR) and environmental drivers for microbial taxa and community attributes.

617 **Supplementary Figure 7.** Site-level effects of drought on: (a) soil water content (SWC), (b)
618 soil pH, (c) ß-glucosidase activity, (d) cellobiohydrolase activity, (e) N-acetyl-
619 glucosaminidase activity, and (f) phosphatase activity. The six american sites are ordered
620 based on the precipitation gradient, whereas the Australian site (DG) is most comparable to
621 KNZ.

622 **Supplementary Figure 8.** Relationship between potential soil microbial activity and pH.

623 **Supplementary Figure 9.** General structure of *a priori* structural equation models. For
624 references supporting predicted pathways see Appendix A.

625 **Supplementary Figure 10.** Structural equation model depicting the direct and indirect
626 effects of drought and environmental conditions on fungal and bacterial community attributes
627 (abundance, diversity and richness) and microbial activity.

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Table 1. Environmental characteristics of study sites. MAP = mean annual precipitation; MAT = mean annual temperature.

Site name	Code	Grassland type	MAP (mm)	MAT (ºC)	pH
Sevilleta National Wildlife Refuge	SEV Black	Desert	242	13.3	8.5
Sevilleta National Wildlife Refuge	SEV Blue	Shortgrass	242	13.3	8.8
Central Plains Experimental Range	SGS	Shortgrass	342	8.6	6.2
High Plains Grasslands Research Center	WYO	Mixed	384	7.6	7.1
Hays Agricultural Research Center	HAYS	Mixed	577	12.0	7.2
Konza Prairie Biological Station	KNZ	Tallgrass	860	12.9	6.4
DRI-Grass Experimental Site	DG	Australian grassland	800	17.0	6.6

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(a)

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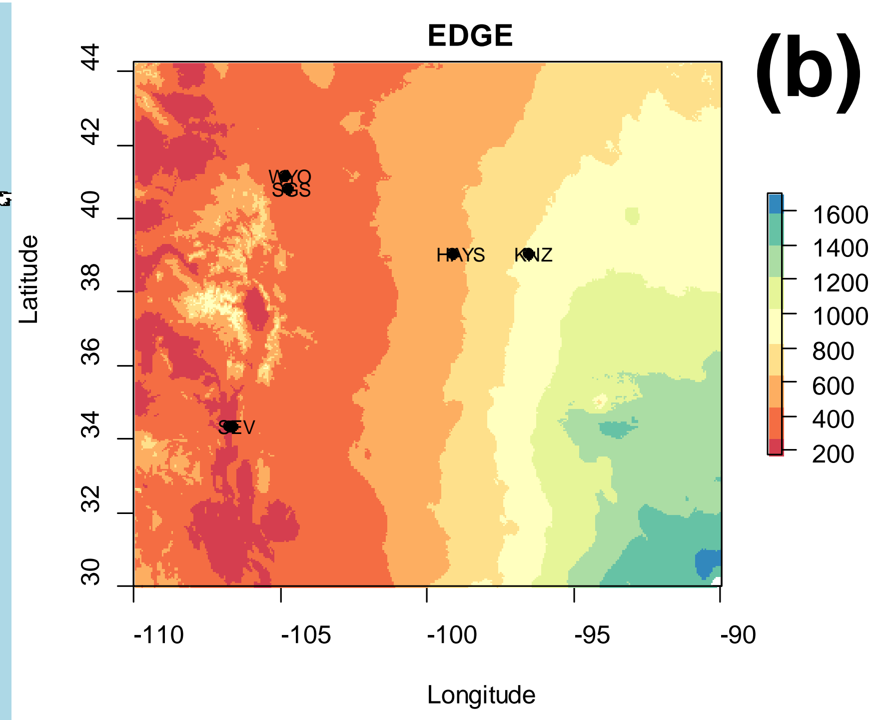
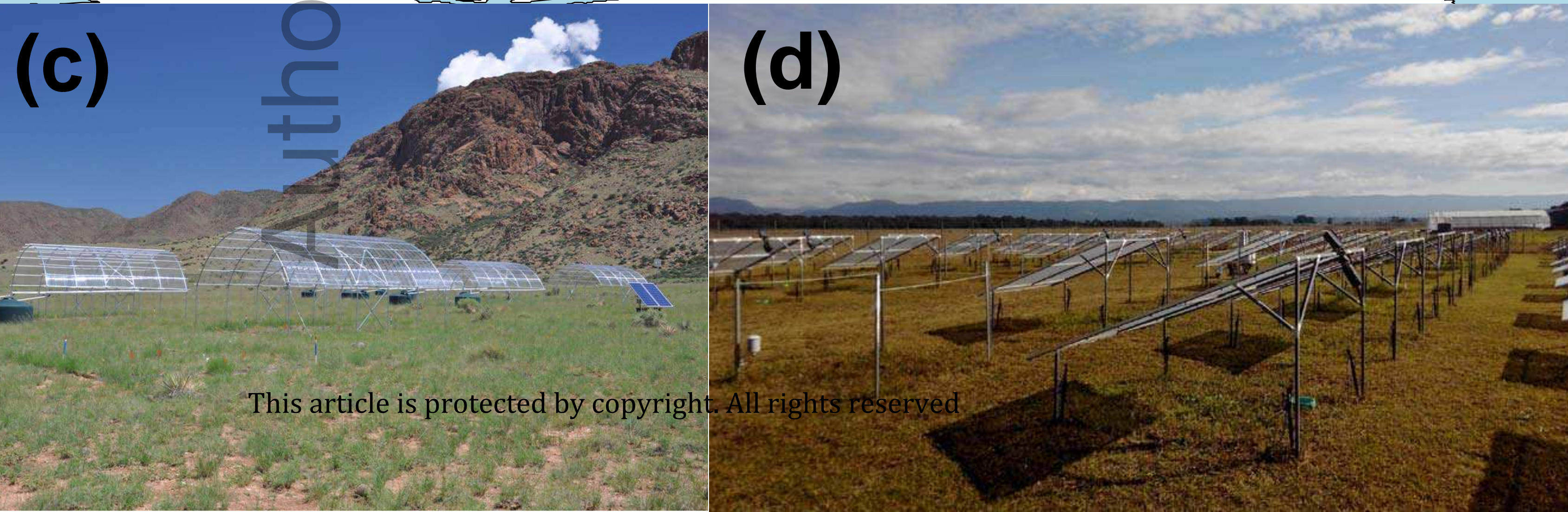
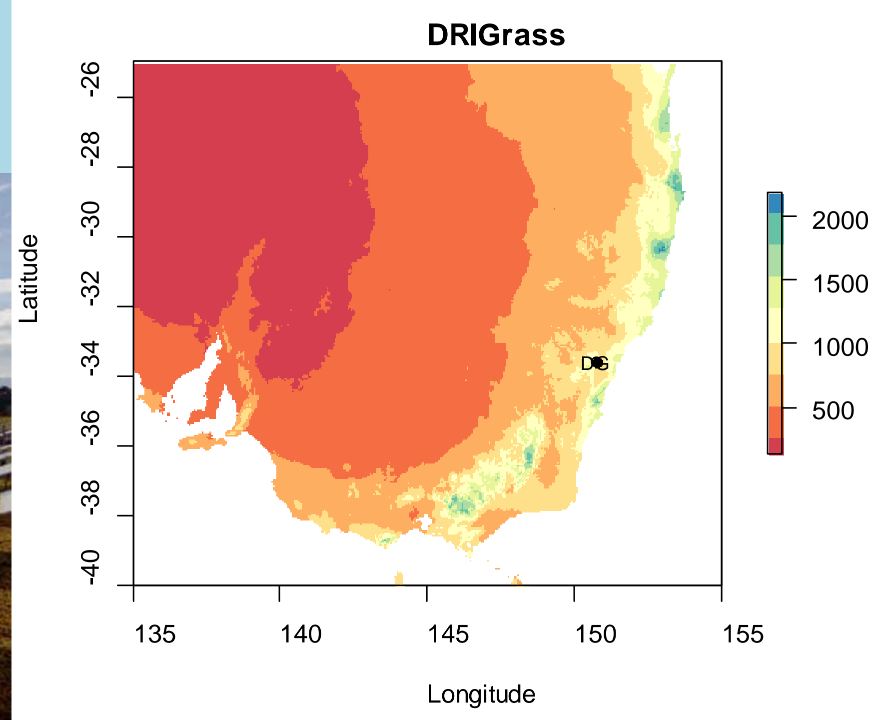
EDGE

DRIGrass







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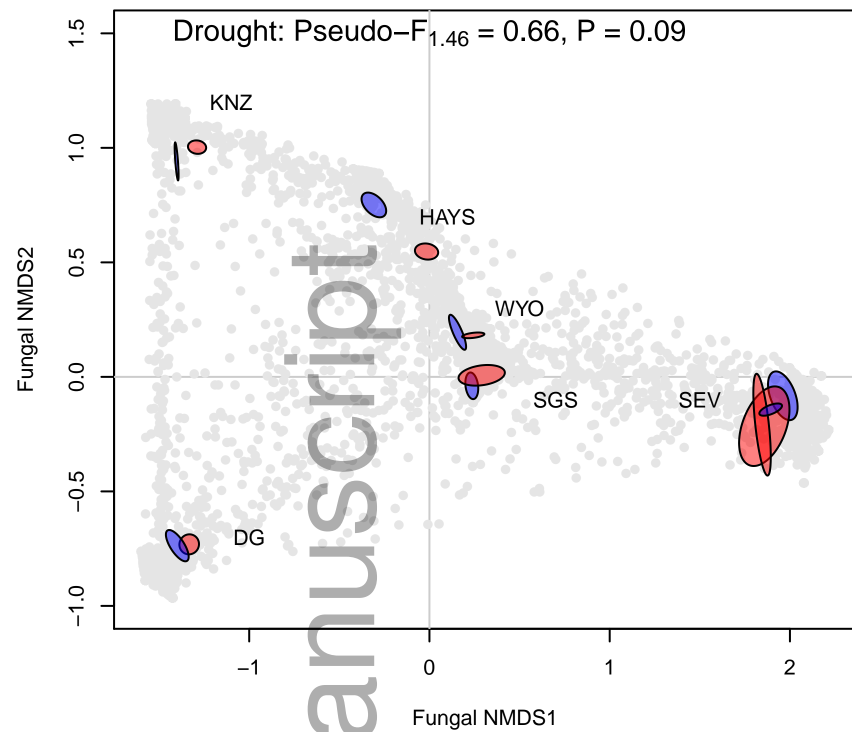

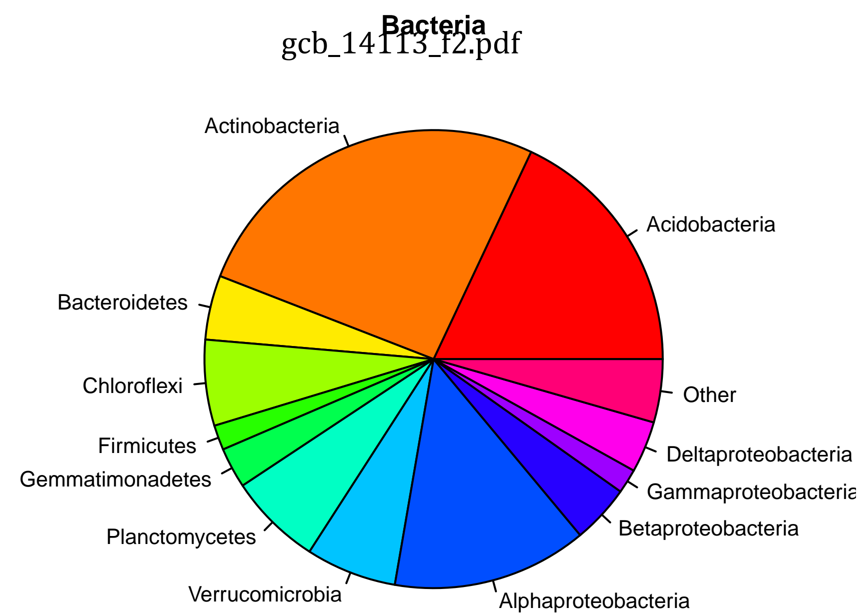

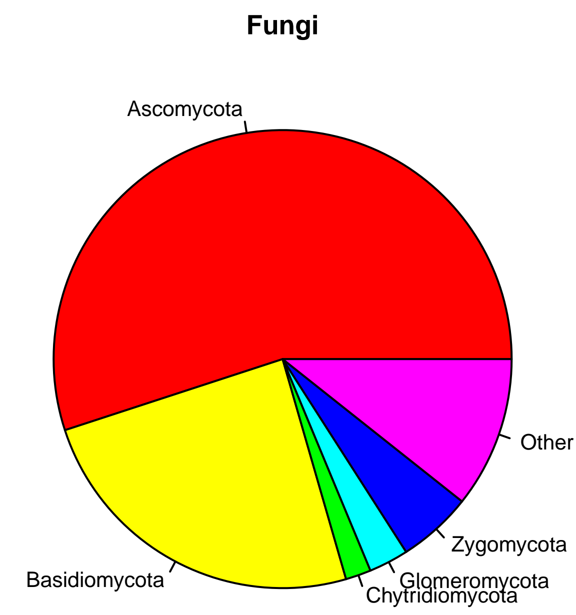

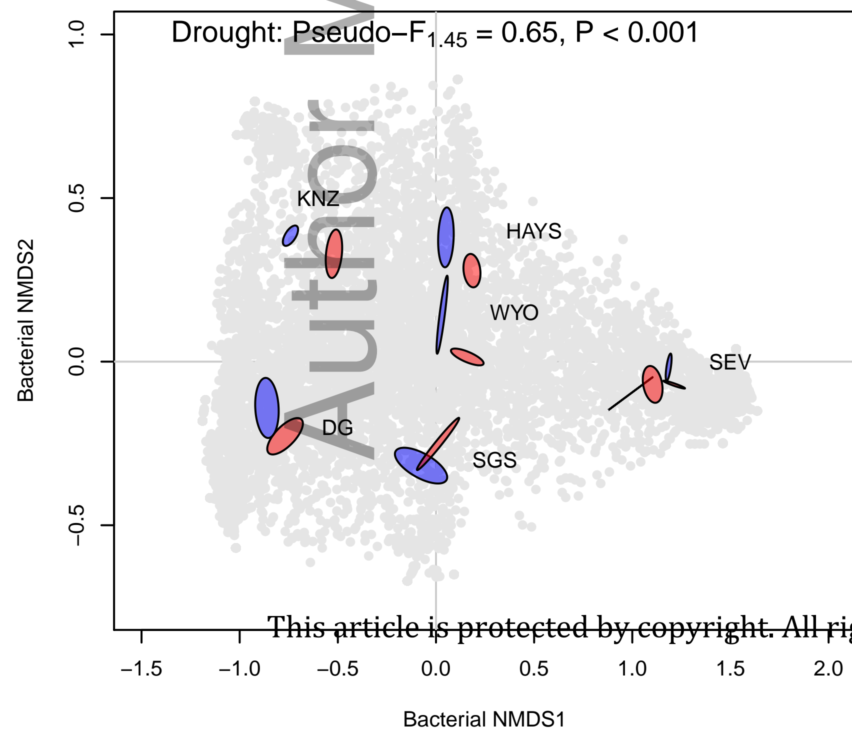

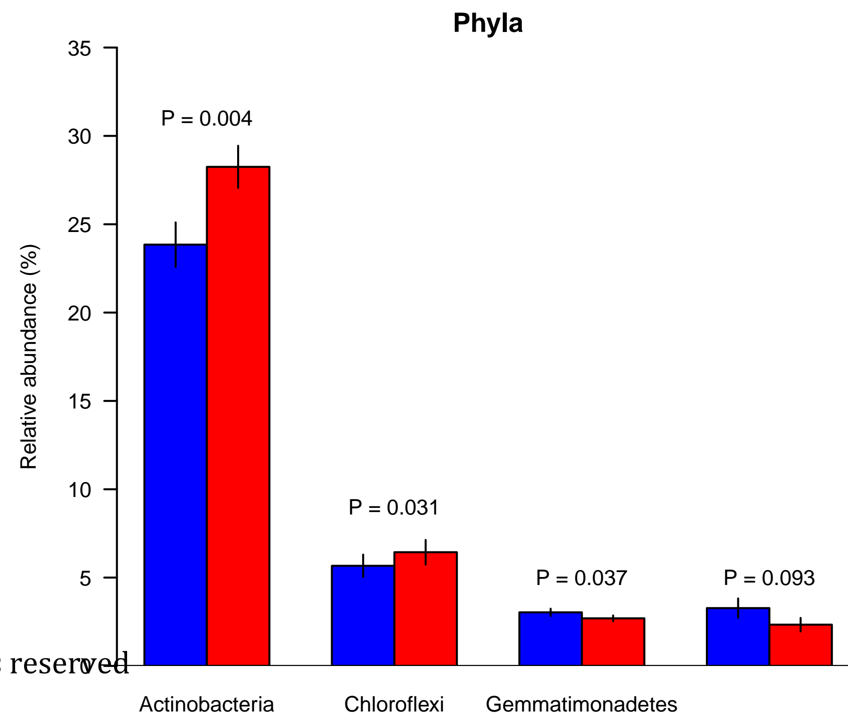

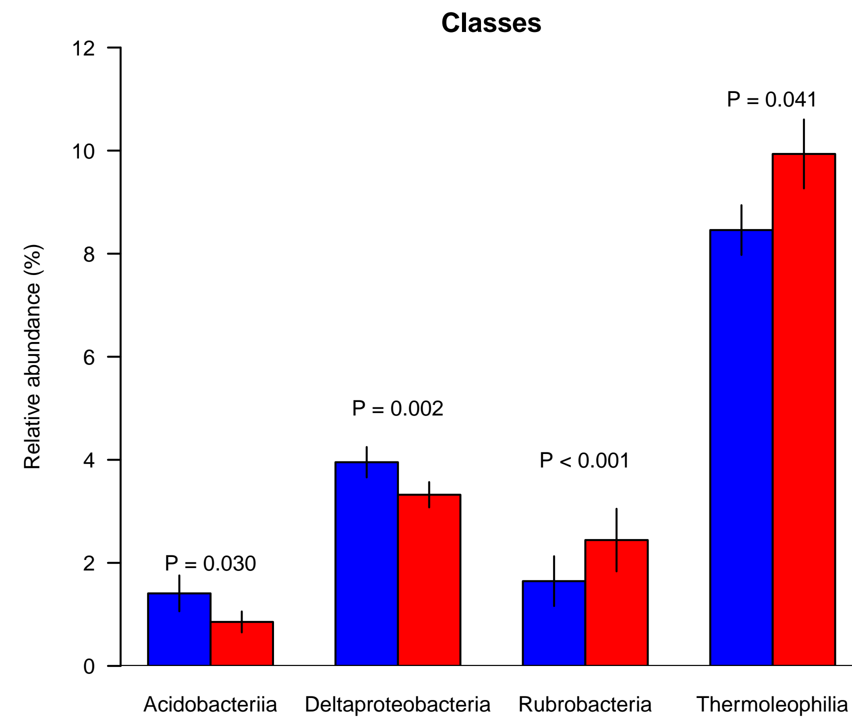

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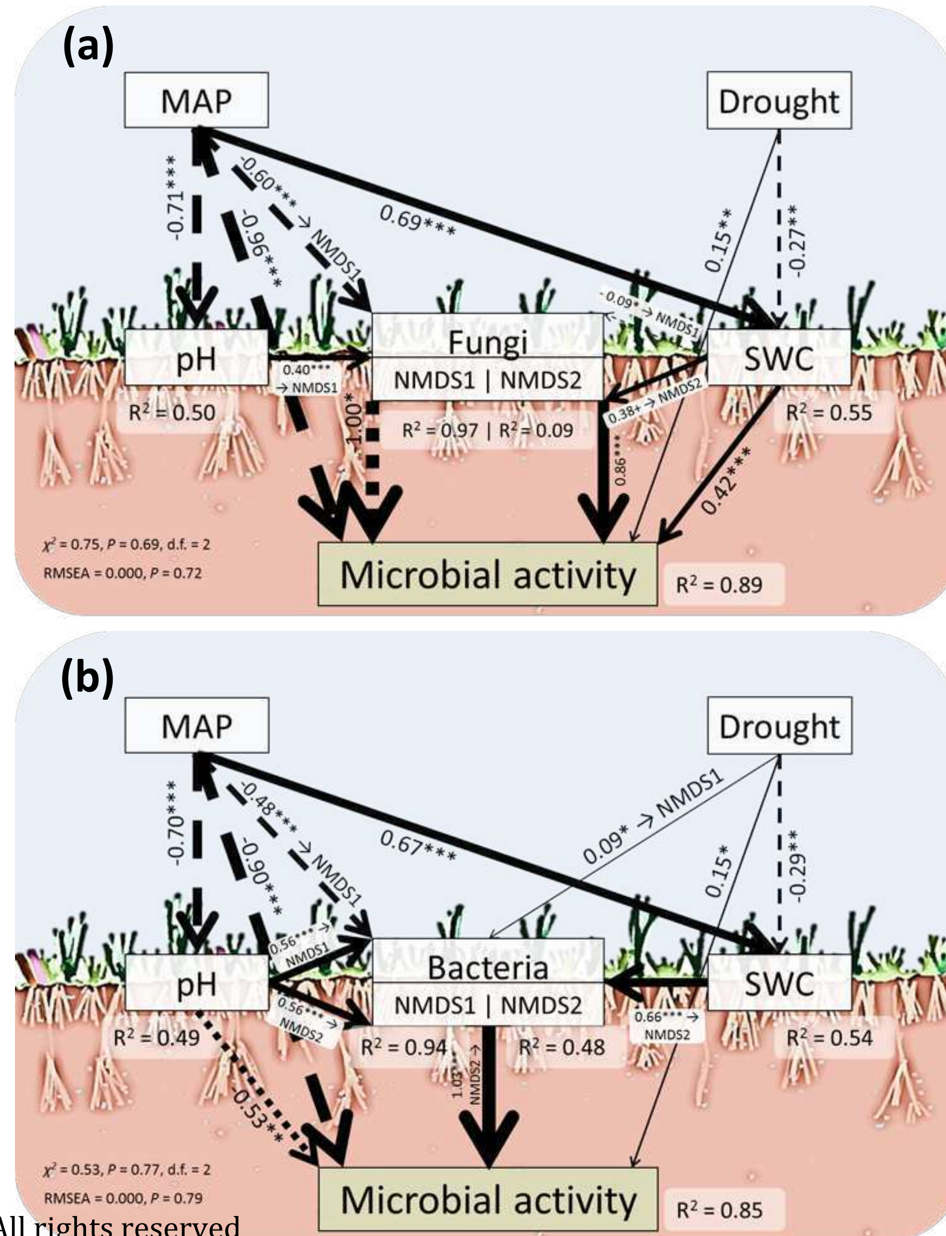

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