More persistent weather causes a pronounced soil microbial legacy but does not impact subsequent plant communities

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Abstract

A soil history of exposure to extreme weather may impact future plant growth and microbial community assembly. Currently, little is known about whether and how previous precipitation regime (PR)-induced changes in soil microbial communities influence plant and soil microbial community responses to a subsequent PR. We exposed grassland mesocosms to either an ambient PR (1 day wet-dry alternation) or a persistent PR (30 days consecutive wet-dry alternation) for one year. This conditioned soil was then inoculated as a 10% fraction into 90% sterilized "native" soil, after which new plant communities were established and subjected to either the ambient or persistent PR for 60 days. We assessed whether past persistent weather-induced changes in soil microbial community composition affect soil microbial and plant community responses to subsequent weather persistence. The historical regimes caused enduring effects on fungal communities and only temporary effects on bacterial communities, but did not trigger soil microbial legacy effects on plant productivity when exposed to either current PR. This study provides experimental evidence for soil legacy of climate persistence on grassland ecosystems in response to subsequent climate persistence, helping to understand and predict the influences of future climate change on soil biota.

Keywords: Plant-soil interactions, Legacy effects, Climate change, Weather persistence, Soil inoculum, Microbial community

1 Introduction

Climate extremes have critical effects on ecosystem functioning and services (Anderegg *et al.*, 2015). The increasing frequency and intensity of extreme weather due to climate change can significantly affect both belowground and aboveground communities and processes (Maestre *et al.*, 2015, Na *et al.*, 2019). For instance, drought can reduce terrestrial carbon uptake (Gampe *et al.*, 2021), threaten plant performance and crop production (Tito *et al.*, 2018), and alter soil microbial community composition and diversity (Maestre *et al.*, 2015). Some effects of climate extremes on ecosystems can persist after the extreme events, known as "legacy effects" (Cuddington, 2012). These legacies can affect how microorganisms respond to current environmental factors by persisting abiotic changes or selecting a more tolerant microbial community (Evans & Wallenstein, 2011). Furthermore, soil legacies can have a major impact on ecosystem responses to subsequent climatic conditions (Bouskill *et al.*, 2013, Hannula *et al.*, 2021). For instance, a history of long-term increasing precipitation can have positive legacy effects on aboveground net primary productivity and soil respiration in tallgrass prairie, reducing their sensitivity to drought (Broderick *et al.*, 2022). In addition, drought has long-term impacts on soil microbial communities, reducing plant growth and changing competitive interactions when faced with subsequent drought (Kaisermann *et al.*, 2017). Because soil microbial communities with prior exposure to drought are more resistant to subsequent drought events (Bouskill *et al.*, 2013, Evans & Wallenstein, 2014), soil legacies can be a crucial determinant of ecosystem functioning and stability, especially under climate change. A relatively novel fingerprint of climate change is the increasing weather persistence in mid-latitude regions (Pfleiderer *et al.*, 2019), with prolonged periods of both dry and wet spells attributed to more stationary weather zones. This poses challenges for ecosystems but also for society as longer dry and wet periods can lead to widespread damages such as crop losses, flooding, and wildfires. The 'water crisis' is thus more complex than anticipating changes in one direction (e.g. generally drier conditions). Previous studies have suggested that more persistent weather, simultaneous lengthening in the duration of both dry and wet spells, reduces plant biodiversity (Reynaert *et al.*, 2021) and alters soil microbial community assembly (Li *et al.*, 2023).

However, although the soil legacy effects of drought on ecological communities have been studied extensively, it is not yet clear whether and how such persistent weather results in legacy effects especially on plant and microbial communities.

Legacy effects can be modified or overruled by current soil conditions, which is why the duration of these legacies is an important consideration when projecting future climate change. Previous studies have shown that the persistence of soil legacies can vary depending on the type of legacy and the microbial community involved (Grove *et al.*, 2015, Hannula *et al.*, 2021). For instance, soil legacies arising from differing plant species persistently affected fungal communities for at least five months but faded away rapidly in bacterial communities (Hannula *et al.*, 2021). In contrast, climate legacies induced by altered rainfall persisted for up to 4.5 years in grassland soils (Hawkes *et al.*, 2020). Given the variation in persistence across different types of soil legacies, it is important to consider both the current environment and the type of legacy when assessing the duration of soil legacy effects.

Soil legacy can be attributed to changes in soil physical and chemical properties (Zhou *et al.*, 2020), specific plant species (Hannula *et al.*, 2021), and soil microbial communities (Meisner *et al.*, 2013). Among these factors, soil microbes are critical drivers of soil nutrient cycling (Wagg *et al.*, 2019), which supports primary productivity and plant performance (van der Heijden *et al.*, 2008), making them crucial to soil legacy. Many studies have reported that soil microbial communities can acclimate and shift from sensitive bacterial-dominated communities to tolerant fungal-dominated communities after long-term exposure to drought (Evans & Wallenstein, 2011, Bouskill *et al.*, 2013). These changes in soil microbial composition can modulate plant performance and alter plant-soil feedback, thereby indeed giving rise to soil microbial legacy effects (Xi *et al.*, 2022). They may also affect plant responses to future extreme weather (Meisner *et al.*, 2013). However, experimental evidence for the soil legacy of climate extremes in response to subsequent extremes is still lacking. Given the potential importance of soil microorganisms in soil legacy, exploring their ecological role in plant growth and their response

to subsequent environments is crucial for understanding and predicting the effects of global climate change, yet remains challenging.

To address this challenge, we investigate whether and how changes in soil microbial communities induced by more persistent weather, impact plant growth and soil microbial community assembly under subsequent persistent weather events in an artificial grassland ecosystem. We conducted a two-phase mesocosm experiment. In the first phase, a mixture of twelve plant species was grown together under either an ambient (1 day wet-dry alternation) or a persistent PR (30 days consecutive wet–dry alternation) for one year to generate "conditioned" soil. In the second phase, new plant communities were established with soil inoculums from the first phase, and subjected to either the ambient or persistent PR (Figure 1). We hypothesize that (1) the soil histories cause legacy effects on the subsequent development of soil microbial communities, and the legacies are modified by current PRs; (2) the soil legacies fade away faster in bacterial communities than in fungal communities; (3) the historical PRs of the soil microbial communities affect plant community composition and productivity.

2 Materials and methods

2.1 Experiment design

A two-phase experiment with grassland soils was set up at Campus "Drie Eiken" of the University of Antwerp, Belgium (51°09′41″N, 04°24′09″E). The experimental site has a temperate oceanic climate, with an average air temperature of 10.6 °C and mean annual precipitation of 852.4 mm (KMI, 2019). Soil microbial communities were conditioned by PRs and plant communities in phase I, and the soil legacies were tested using a newly established plant community in phase II.

Phase I: Soil conditioning

The design in phase I is part of an experimentally manipulated precipitation gradient in grassland soil (Li *et al.*, 2023). Briefly, PVC containers (29.5 cm inner diameter and 50 cm depth) were filled with sandy soil (pH=7.0-7.2). The mesocosms were shielded from natural rain by movable rain-out covers

so that watering regimes were entirely controlled through the irrigation system with drippers. In this way, mesocosms were subjected to either an ambient PR (1 day wet-dry alternation, most commonly observed throughout the year and close to Belgian weather) or a persistent PR (30 days consecutive wet-dry alternation), while having received the same cumulative precipitation at the end of experiment. Each treatment included four replicates. The experiment started on 2 July 2019 with a wet period. After one year, by 25 June 2020, all mesocosms had received a total of 180 watering events, which only differed in temporal distribution according to the two PRs described above. In each event, the same water volume of 6.87 L m^{-2} was applied regardless of treatment. In each mesocosm, twelve common perennial temperate grassland species with three individuals each were transplanted. These plant species covered three functional groups: six grasses (*Agrostis capillaris L.*, *Anthoxanthum odoratum L.*, *Deschampsia cespitosa L.*, *Phleum pratense L.*, *Poa pratensis L.*, *Holcus lanatus L.*); three N -fixer forbs (hereinafter as N-fixers; *Lotus corniculatus L.*, *Trifolium pratense L.*, *Trifolium medium L.*); and three non N-fixer forbs (hereinafter as forbs; *Centaurea jacea L.*, *Lychnis flos-cuculi L.*, *Plantago lanceolata L.*).

At harvest of phase I, all plants including roots were removed from the mesocosms. The soil was destructively sampled (0-20 cm depth) from each mesocosm and mixed. Around 20 g of soil from each mesocosm was then freeze-dried and stored for microbial DNA analyses. The rest of the soil from each treatment (4 replicates) was pooled together and sieved with 2 mm meshes to remove fine roots, to serve as soil inoculums for phase II.

Phase II: Inoculum experiment

The same original soil used in phase I was steam-sterilized with an electric soil sterilizer (Pro-Grow supply corp.) at 80 °C for 24h, which was repeated three consecutive times separated by 24hrs of storage to effectively eliminate viable populations of most soil microorganisms. We recognize that some microorganisms like spore-forming bacteria may survive this treatment and microbial death could potentially lead to the release of nutrients. However, absolute sterility of the soil and the

potential nutrient addition are not required to meet or alter the objectives of this experiment given that our comparisons are between soil organisms that predominantly differ in the selection history exposed, not a comparison with sterilized soil. The experiment was conducted in PVC containers (diameter: 19.5 cm, height: 39 cm) that were filled with 90% (by volume) sterilized soil and 10% conditioned soil (inoculums) (Pineda *et al.*, 2020, Xi *et al.*, 2022). In accordance with these other studies, this dilution of "conditioned soil" at a ratio of 1:9 strikes a balance, minimizing abiotic confounders on one hand, while ensuring sufficient microbial propagules to evaluate their effects on the other. Mesocosms were created with four plant species including two grasses species (*H. lanatus* and *P. pratense*), one N-fixer (*L. corniculatus*), and one forb (*P. lanceolata*), which were a subset of species from phase I with a similar proportion of each functional group. Seeds from commercial suppliers were first surface sterilized by 3% NaClO for 2 min and washed 3 times with distilled water, then germinated in sterilized soil. Twelve two-week-old seedlings of these four species with three individuals each, were transplanted into the mesocosms at the same plant density as in phase I. These seedlings were regularly watered for one week to promote seedling establishment before the treatments began.

The water regimes started on 3 July 2020 and lasted for 60 days, starting with a wet period as in phase I. All the mesocosms were subjected either again to the same ambient or persistent PR as in Phase I, or switched to the contrasting PR condition, keeping the total precipitation constant across all treatments. This generated four different treatments over phase I and phase II: ambient PR in both phases (A_A), persistent PR in both phases (P_P), ambient PR in phase I then persistent PR in phase II (A_P), persistent PR in phase I then ambient PR in phase II (P_A) (Figure 1). Each treatment had six replicates. We measured plant physiological response by species with chlorophyll fluorescence (Roháček *et al.*, 2008) using a Plant Efficiency Analyser (Hansatech Ltd) at 30, 42, and 60 days regime exposure (RE). Maximum potential quantum efficiency of photosystem II is expressed as:

$F_v/F_m = \frac{Fm-F0}{Fm}$,

where F_m = maximum fluorescence, F_0 = initial fluorescence, and F_v = variable fluorescence (F_m-F_0). The leaves of *L. corniculatus* were too small to be measured. Five soil cores were taken in each mesocosm at 30 and 60 days regime exposure (RE) with an auger (1.5 cm diameter and 0-10 cm depth) and pooled together to represent one mesocosm for microbial analyses. Aboveground plant biomass was harvested separately for each species at day 60, then dried at 70 °C in the oven for three days and weighed. The total biomass and biomass of each functional group (grass, forb, and N-fixer; so the two grasses were combined) were calculated for each mesocosm in g per m^2.

2.2 Amplicon sequencing

Soil DNA was extracted from freeze-dried samples using the DNeasy PowerSoil Kit following the manufacturer's instructions (Qiagen, Venlo, the Netherlands). We used the universal primers 515F and 806R (Caporaso et al., 2011) targeting the prokaryotic V4 hypervariable region for 16S rRNA gene, and ITS1f (Gardes & Bruns, 1993) and ITS2 (White et al., 1990) targeting the fungal ITS1 region for internal transcribed spacer (ITS). All the PCR products were checked on a 1.5 % agarose gel. In total, 48 samples (two soil histories, two current PRs and two sampling times, with six replicates) were then pooled into a single library for 16S and ITS respectively, and subjected to a gel extraction using a QIAquick Gel Extraction Kit (Qiagen). The libraries were quantified with quantitative PCR (Kapa Library Quantification Kits; Kapa Biosystems, Wilmington, MA, USA) and sequenced on the Illumina MiSeq platform (Illumina Inc.) with 300 cycles for forward and reverse reads.

2.3 Bioinformatical and statistical analyses

Amplicon sequence analysis was conducted with the USEARCH pipeline (Edgar, 2010). The paired-end sequences were merged and primers were removed. Then the sequences were quality filtered with a minimal length of 150 bp and discarded if the read contained a Q score <15. After dereplication, operational taxonomic units (OTUs) with 97 % similarity were clustered using UPARSE (Edgar, 2013). Representative 16S and ITS sequences for each OTU were taxonomically assigned using the SILVA and UNITE databases, respectively. Singletons and sequences that were not assigned to bacteria or fungi

were removed. Finally, to minimize the bias of sampling depth on the further analyses as well as to make the reads of individual OTUs comparable across all treatments and sampling times, the clean sequences were normalized to 10474 reads for each bacterial sample and 11077 reads for each fungal sample, based on the minimum read count in bacterial and fungal samples, respectively. To perform functional annotation of bacterial taxa, bacterial OTUs were analysed using the FAPROTAX pipeline (Louca *et al.*, 2016). To annotate fungal ecological lifestyles, fungal OTUs were assigned using the FungalTraits pipeline (Põlme et al., 2021).

To reveal the distribution of microbial community composition and functional groups across treatments, non-metric multidimensional scaling (NMDS) ordination with the function *metaMDS* was conducted based on both weighted and unweighted Bray-Curtis dissimilarity of microbial communities at each sampling time. Significant differences in microbial communities between two soil histories, between two current PRs, and their interactions were evaluated by Permutational multivariate analysis of variance (PERMANOVA) test with the function *adonis* (Bray-Curtis distance, Permutation = 999). We used a Wilcoxon rank sum test to compare responses of individual OTUs between two soil histories in the same current PR at each sampling time. Of these responsive OTUs (P<0.05) we then calculated the fold change in relative abundance between two soil histories.

3 Results

3.1 Soil microbial community responses to soil histories and current PRs

At the end of phase I soil conditioning, both bacterial and fungal communities substantially differed between ambient and persistent PRs (Figure S1), indicating that soil microbial communities were different at the onset of phase II. In phase II, the bacterial communities (weighted Bray-Curtis distance) were significantly affected by both soil histories (PERMANOVA, R^2=0.09, P<0.001) and current PRs (R^2=0.08, P<0.001) at 30 days RE but not at 60 days after a full wet and dry cycle (P>0.05 for both soil histories and current PRs) (Table 1, Figure 2). Similar results were observed based on unweighted Bray-Curtis distances at 30 days RE, but not at 60 days when soil histories (R^2=0.08, P<0.001) and current

PRs slightly but significantly (R^2=0.06, *P*<0.049) affected bacterial communities (Figure S2). Similar to bacteria, fungal community composition was significantly affected by soil histories at 30 days RE (R^2=0.11, *P*=0.007, weighted Bray-Curtis distance), and this effect size was larger at 60 days RE (R^2=0.14, *P*=0.013), but unlike bacteria, they were not significantly affected by current PRs (*P*>0.05) at 30 or 60 days RE (Table 1). Similar results were observed by unweighted Bray-Curtis distance. At both 30 days and 60 days RE, the interaction between soil histories and current PRs did not significantly affect either bacterial or fungal communities. Collectively, bacterial communities were shaped more strongly by soil histories than current PRs, while fungal communities were affected exclusively by soil histories. Furthermore, soil histories had more pronounced impacts on fungal communities than on bacterial communities, especially at 60 days RE, and the size of effects increased over time (e.g. from 0.11 to 0.14 based on weighted Bray-Curtis distance) for fungal communities (Table 1).

Unlike microbial community composition, the profile of predicted bacterial community function was not significantly affected (*P*>0.05) by soil histories or current PRs either at 30 days or 60 days RE (Figure S3 and Table S1). Similar results were observed in the fungal lifestyles, except at 30 days RE when fungal lifestyles were significantly affected by soil histories (R^2=0.11, *P*=0.042) based on weighted Bray-Curtis distance (Table S1).

3.2 Soil history - responsive microbial taxa

Soil histories and current PRs did not affect bacterial and fungal communities at the phylum level (Figure S4). At the finer taxonomical level, *Rhodanobacter* and *Bryobacter*, two bacterial genera ranked within the top 10 abundant genera, were slightly but significantly affected by the treatments at 30 days RE (Figure S5). *Rhodanobacter* had the highest abundance (ANOVA, *F*=8.73, *P*=0.001) under current ambient PR with ambient PR history, while *Bryobacter* had the lowest abundance (*F*=4.14, *P*=0.02) under current persistent PR with persistent PR history. At 60 days RE, the abundance of the dominant bacterial genera (top 10) was not affected (*P*>0.5) by treatments. For fungal genera, *Penicillium* had a significantly higher abundance in persistent PR history than ambient PR history at 30

231 days (F=8.39, P=0.001) and 60 days RE (F=15.52, P<0.001) under either of the current PRs. Similarly,
232 we also observed a higher abundance of plant pathogens under persistent PR history than ambient PR
233 history (F=8.09, P=0.01) under either of the current PRs at 60 days RE (Figure S6).
234 To further explore the effect of soil microbial legacy on current microbial community composition, we
235 here define as "legacy OTUs" those that are differentially abundant (Wilcoxon rank test, P<0.05)
236 between ambient and persistent PR soil histories in either of the current PRs (Figure 3). In the current
237 ambient PR, there were 82 bacterial legacy OTUs at 30 days RE and the number decreased to 50 at 60
238 days RE. Surprisingly, only 9 OTUs were shared between 30 and 60 days RE, which were mainly
239 Proteobacteria. The current persistent PR had a similar trend between 30 days and 60 days RE, with a
240 reduction in the number of legacy OTUs from 47 to 32, and low overlap between time points. We
241 observed a contrasting shift in the fungal legacy OTUs primarily composed of Ascomycota between
242 time points compared to bacteria. In current ambient PR, there were 23 fungal legacy OTUs at 30 days
243 RE, which increased to 31 at 60 days RE. Eleven legacy OTUs mainly from *Penicillium* were shared
244 between 30 days and 60 days RE. In current persistent PR, there was only one fungal legacy OTU at 30
245 days RE but this increased to 29 legacy OTUs at 60 days RE (the single OTU at 30 days RE was also
246 presented at 60 days RE). The fungal legacy OTUs with higher abundance had larger variations
247 between the two soil histories (i.e. higher fold change). Together, the number of bacterial legacy OTUs
248 decreased while that of fungal legacy OTUs increased over time in both current PRs.

249 3.3 Plant community responses to soil histories and current PRs

250 In phase I, the plant aboveground biomass was higher under ambient than persistent PR (Figure S7).
251 In phase II, the total plant aboveground biomass of mesocosms which were harvested at 60 days RE,
252 did not significantly differ between soil histories (Figure 4). Total aboveground biomass only
253 significantly (Wilcoxon rank test; P<0.001) differed between the current persistent (180.4 g m^{-2}) and
254 ambient PR (381.4 g m^{-2}). Similar results were observed when the biomass was aggregated by the two
255 grasses, the forb or the N-fixer across soil histories and current PRs (Figure 4).

Similar to aboveground biomass, plant chlorophyll fluorescence evaluated by the F_v/F_m value of mesocosms, as a sensitive indicator of the plant physiological status under environmental stressors, was not significantly different (P>0.05) between two soil histories (ambient vs persistent PR) under current persistent (0.26 vs 0.37) or ambient PR (0.81 vs 0.80), but was significantly lower (P<0.001) under current persistent PR than current ambient PR at 60 days RE (Figure S8). Under current persistent PR, there was a slightly but none significantly higher F_v/F_m value in persistent PR history than in ambient PR history for the whole plant community. Similar results were observed when species/functional groups were analysed separately. Altogether, those results indicated that, unlike current PRs, soil microbial histories did not significantly affect plant productivity and might have small effects on performance.

4 Discussion

Based on our inoculation trial using soil that had been conditioned under two contrasting watering regimes, we elucidated that these regimes caused a more enduring soil microbial legacy effect on soil fungal communities than bacterial communities, having small impacts on plant performance under current persistent PR. These findings boost our understanding of soil microbial legacies from historical PRs.

4.1 Soil legacy effects on microbial communities

The bacterial and fungal communities differing in phase II due to the historical PRs conditioning in phase I, caused soil legacies, which is in line with previous studies where a history of drying-rewetting affected the soil microbiome (Meisner *et al.*, 2021). Such soil legacy effects may be explained by the priority effect where earlier established species influence the establishment of later arrived species, thus determining the development of community (Debray *et al.*, 2022). Our finding is not in line with a recent study (Goldford *et al.*, 2018) which found that 12 different microbial communities that were cultured under the same nutrient conditions converged to a similar community structure in well-controlled minimal synthetic media. The disagreement is probably because PR is a more complex

281 variable than a nutrient, as the former would influence water content, redox potential and other soil
282 factors besides nutrients. Moreover, soil is a complex system that likely contains many slow-growing
283 microbes that may not have been included in the cultures of Goldford *et al.* (2018).

284 Soil legacy had larger impacts on fungal than on bacterial communities regardless of the current PRs,
285 especially at 60 days RE. Fungal growth rates are slower than those of bacteria (Rousk & Bååth, 2007).
286 As a consequence, fungi are more stable and less affected by temporary variability in an environment
287 (Hannula *et al.*, 2019). This may explain why fungal communities were not affected by current PRs in
288 our study. Furthermore, the slow growth rates of fungi might result in a hysteresis of fungal
289 community response to environmental changes (Suz *et al.*, 2021), probably contributing to more
290 pronounced legacy effects in fungal communities than bacterial communities. Schimel *et al.* (2007)
291 suggest that historical environmental stress could select tolerant microbial taxa, which is in line with
292 our study. For instance, we observed that *Penicillium*, which is relatively tolerant to drought stress
293 (Srinivasan *et al.*, 2020), had a higher abundance in persistent than in ambient PR soil history. We also
294 observed more abundant plant pathogens when the community historically experienced persistent
295 PR. Those findings indicate that fungal communities were significantly affected by soil histories,
296 regardless of the current PRs, and current PRs did not impact the magnitude of soil legacy. In contrast
297 to fungi, bacterial communities were simultaneously affected by both soil histories and current PRs.
298 Bacteria are often considered to be more sensitive to environmental changes compared to fungi
299 (Jansson & Hofmockel, 2020) and to have high resilience after water stress (Li *et al.*, 2021). This can
300 explain why bacteria were relatively less affected by soil histories than fungi, especially at 60 days RE.

301 The legacy effects on fungi grew over time while those on bacteria faded away, which is partly
302 consistent with our hypothesis. This result is robust, as the same pattern was observed not only at the
303 community level but also at the individual species level. More fungal legacy OTUs were detected at 60
304 days than 30 days RE, but the opposite pattern was found for bacteria. This is in line with a previous
305 study where soil legacies from previous plants could be detected in the soil fungal communities at

least for 5 months but faded away rapidly in the bacterial communities (Hannula *et al.*, 2021). The different persistence of soil legacy effects may originate from the different traits between fungi and bacteria. As we mentioned above, fungal taxa need more time to develop due to the slow growth rate, cascading into new communities. Unexpectedly, there was only one fungal legacy OTU under current persistent PR at 30 days RE. This might be explained by historical PRs selecting certain fungal taxa, which need more time to adapt to the new environment (watering occurred daily during the first 30 days RE). Furthermore, only a few common legacy OTUs between 30 days and 60 days RE were observed in both bacterial and fungal communities at each current PR. This indicated that the effects of the previous precipitation persistence on microbes were not conserved over time. This evidence further proved that microbial communities either were still establishing (for fungi) or had a fast response to a new environment (for bacteria). Our results indicate that when soil microbes experienced persistent weather, the microbial legacy could last for months, especially for fungi. We can only speculate how long this legacy will last, as 60 days of current PRs following one year of previous PRs was not enough for the fungal legacy effects to fade away. This finding has implications for understanding the dynamics of soil microbial community in response to persistent weather, something that has been previously overlooked.

4.2 Soil microbial legacy effect on plant performance

For either of the current PRs, the plant performance in growth and photosynthesis did not significantly vary according to ambient or persistent soil histories. This finding indicates that plant productivity and performance were barely affected by soil legacy, unlike previous studies that found positive (de Vries et al., 2012) or negative (Kaisermann et al., 2017) impacts of drought legacy on plant growth. Changes in soil properties and microbial communities caused by historical exposure are the main determinants of soil legacy effects (De Long et al., 2019). For instance, drought can change soil properties such as increasing soil water retention and soil fertility (Bloor & Bardgett, 2012), and this legacy could have cascading effects on ecosystem functioning during subsequent development. However, in our study,

331 the soil consisted of 10% conditioned soil and 90% sterilized soil. The abiotic properties of soil were
332 thus majorly determined by the sterilized soil and consequently were similar across all treatments at
333 the starting point of the experiment. The only difference between the treatments was caused by
334 changes in the microbial communities from the conditioned soil, generating the soil legacy effects in
335 this study. In other words, our experimental design thus allowed us to specifically focus on the roles
336 of the microbial communities in soil legacy effects, by controlling for plant or abiotic-mediated effects.
337 Soil microbes play critical roles in plant performance and productivity as mutualists, pathogens and
338 decomposers of organic matter and nutrient cycling (van der Heijden et al., 2008, Yang et al., 2017).
339 For these reasons, we anticipated impacts of soil legacy on plant productivity through changes in
340 microbial communities historically exposed to persistent weather. Unexpectedly, in either of the
341 current PRs, plant biomass did not significantly differ between different soil histories, indicating that
342 soil microorganisms did not cause a significant soil legacy effect on plant productivity in the context
343 of subsequent climate change. It is worth noting that at the end of the treatment, plants with a history
344 of persistent PR showed a slightly but not significantly higher chlorophyll fluorescence (an
345 instantaneous parameter), compared to biomass (a cumulative parameter). This indicates that soil
346 microbial history may have the potential to affect plant performance, as the soil microbial legacy of
347 persistent weather could increase the tolerance of plants when exposed to subsequent persistent
348 weather. However, future studies are warranted to provide further evidence. Furthermore, bacterial
349 community potential functions or fungal community lifestyles barely or weakly differed between
350 different soil histories in either PR, which might also explain the above finding. These results are partly
351 in line with a previous study that suggests drought soil legacies do not affect aboveground biomass (Xi
352 et al., 2022). This confirms that soil legacy effects on different aspects of plant communities are
353 context dependent, such as duration and severity of the treatments. Another reason why our results
354 differ from previous studies (de Vries et al., 2012, Kaisermann et al., 2017) may be because the soil
355 legacy in our experiment is due to more persistent alternating dry and wet spells, rather than

exclusively drought. This weather persistence is relevant though, as it is thought to be on the rise in several temperate regions (Pfleiderer et al., 2019).

Our findings demonstrated that soil microorganisms that experienced previous climate extremes can significantly alter the subsequent development of soil microbial communities, regardless of the current environment. The increasing weather persistence can give rise to other legacy effects than drought or precipitation increase only, and that would affect soil function and may increase plant pathogens.

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Data availability statement

Data that support the findings of this study are available from the corresponding author upon request.

Reference

Anderegg WR, Schwalm C, Biondi F, Camarero JJ, Koch G, Litvak M, Ogle K, Shaw JD, Shevliakova E & Williams A (2015) Pervasive drought legacies in forest ecosystems and their implications for carbon cycle models. *Science* **349**: 528-532.

Bloor JMG & Bardgett RD (2012) Stability of above-ground and below-ground processes to extreme drought in model grassland ecosystems: Interactions with plant species diversity and soil nitrogen availability. *Perspectives in Plant Ecology, Evolution and Systematics* **14**: 193-204.

Bouskill NJ, Lim HC, Borglin S, Salve R, Wood TE, Silver WL & Brodie EL (2013) Pre-exposure to drought increases the resistance of tropical forest soil bacterial communities to extended drought. *The ISME Journal* **7**: 384-394.

Broderick CM, Wilkins K, Smith MD & Blair JM (2022) Climate legacies determine grassland responses to future rainfall regimes. *Global Change Biology* **28**: 2639-2656.

Cuddington K (2012) Legacy Effects: The Persistent Impact of Ecological Interactions. *Biological Theory* **6**: 203-210.

De Long JR, Semchenko M, Pritchard WJ*, et al.* (2019) Drought soil legacy overrides maternal effects on plant growth. *Functional Ecology* **33**: 1400-1410.

de Vries FT, Liiri ME, Bjornlund L, Setala HM, Christensen S & Bardgett RD (2012) Legacy effects of drought on plant growth and the soil food web. *Oecologia* **170**: 821-833.

Debray R, Herbert RA, Jaffe AL, Crits-Christoph A, Power ME & Koskella B (2022) Priority effects in microbiome assembly. *Nature Reviews Microbiology* **20**: 109-121.

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**: 2460-2461.

Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* **10**: 996.

Evans SE & Wallenstein MD (2011) Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter? *Biogeochemistry* **109**: 101-116.

Evans SE & Wallenstein MD (2014) Climate change alters ecological strategies of soil bacteria. *Ecology Letters* **17**: 155-164.

Gampe D, Zscheischler J, Reichstein M, O'Sullivan M, Smith WK, Sitch S & Buermann W (2021) Increasing impact of warm droughts on northern ecosystem productivity over recent decades. *Nature Climate Change* **11**: 772-779.

Goldford JE, Lu N, Bajić D, Estrela S, Tikhonov M, Sanchez-Gorostiaga A, Segrè D, Mehta P & Sanchez A (2018) Emergent simplicity in microbial community assembly. *Science* **361**: 469-474.

Grove S, Parker IM & Haubensak KA (2015) Persistence of a soil legacy following removal of a nitrogen-fixing invader. *Biological Invasions* **17**: 2621-2631.

Hannula SE, Heinen R, Huberty M, Steinauer K, De Long JR, Jongen R & Bezemer TM (2021) Persistence of plant-mediated microbial soil legacy effects in soil and inside roots. *Nature Climate Change* **12**: 5686.

Hannula SE, Kielak AM, Steinauer K, Huberty M, Jongen R, De Long JR, Heinen R & Bezemer TM (2019) Time after time: temporal variation in the effects of grass and forb species on soil bacterial and fungal communities. *MBio* **10**: e02635-02619.

Hawkes CV, Shinada M & Kivlin SN (2020) Historical climate legacies on soil respiration persist despite extreme changes in rainfall. *Soil Biology and Biochemistry* **143**.

Jansson JK & Hofmockel KS (2020) Soil microbiomes and climate change. *Nature Reviews Microbiology*.

Kaisermann A, de Vries FT, Griffiths RI & Bardgett RD (2017) Legacy effects of drought on plant-soil feedbacks and plant-plant interactions. *New Phytologist* **215**: 1413-1424.

KMI (2019) Klimatologisch jaaroverzicht, jaar 2019.

Li L, Preece C, Lin Q, Brechet LM, Stahl C, Courtois EA & Verbruggen E (2021) Resistance and resilience of soil prokaryotic communities in response to prolonged drought in a tropical forest. *FEMS Microbiol Ecol* **97**.

Li L, Nijs I, De Boeck H, Vinduskova O, Reynaert S, Donnelly C, Zi L & Verbruggen E (2023) Longer dry and wet spells alter the stochasticity of microbial community assembly in grassland soils. *Soil Biology and Biochemistry*.

428 Louca S, Parfrey LW & Doebeli M (2016) Decoupling function and taxonomy in the global ocean
429 microbiome. *Science* **353**: 1272-1277.
430 Maestre FT, Delgado-Baquerizo M, Jeffries TC*, et al.* (2015) Increasing aridity reduces soil microbial
431 diversity and abundance in global drylands. *Proceedings of the National Academy of Sciences of the*
432 *United States of America* **112**: 15684-15689.
433 Meisner A, De Deyn GB, de Boer W & van der Putten WH (2013) Soil biotic legacy effects of extreme
434 weather events influence plant invasiveness. *Proceedings of the National Academy of Sciences of the*
435 *United States of America* **110**: 9835-9838.
436 Meisner A, Snoek BL, Nesme J, Dent E, Jacquiod S, Classen AT & Prieme A (2021) Soil microbial
437 legacies differ following drying-rewetting and freezing-thawing cycles. *The ISME Journal*.
438 Na X, Yu H, Wang P, Zhu W, Niu Y & Huang J (2019) Vegetation biomass and soil moisture coregulate
439 bacterial community succession under altered precipitation regimes in a desert steppe in
440 northwestern China. *Soil Biology and Biochemistry* **136**.
441 Pfleiderer P, Schleussner C-F, Kornhuber K & Coumou D (2019) Summer weather becomes more
442 persistent in a 2° C world. *Nature Climate Change* **9**: 666-671.
443 Pineda A, Kaplan I, Hannula SE, Ghanem W & Bezemer TM (2020) Conditioning the soil microbiome
444 through plant-soil feedbacks suppresses an aboveground insect pest. *New Phytologist* **226**: 595-608.
445 Reynaert S, De Boeck HJ, Verbruggen E, Verlinden M, Flowers N & Nijs I (2021) Risk of short-term
446 biodiversity loss under more persistent precipitation regimes. *Global Change Biology* **27**.
447 Roháček K, Soukupová J & Barták M (2008) Chlorophyll fluorescence: a wonderful tool to study plant
448 physiology and plant stress. *Plant Cell Compartments-Selected Topics Research Signpost, Kerala,*
449 *India* 41-104.
450 Rousk J & Bååth E (2007) Fungal biomass production and turnover in soil estimated using the
451 acetate-in-ergosterol technique. *Soil Biology and Biochemistry* **39**: 2173-2177.
452 Schimel J, Balser TC & Wallenstein M (2007) Microbial stress‐response physiology and its
453 implications for ecosystem function. *Ecology* **88**: 1386-1394.

454 Srinivasan R, Prabhu G, Prasad M, Mishra M, Chaudhary M & Srivastava R (2020) Penicillium.
455 *Beneficial Microbes in Agro-Ecology,*p.^pp. 651-667.
456 Suz LM, Bidartondo MI, van der Linde S & Kuyper TW (2021) Ectomycorrhizas and tipping points in
457 forest ecosystems. *New Phytologist* **231**: 1700-1707.
458 Tito R, Vasconcelos HL & Feeley KJ (2018) Global climate change increases risk of crop yield losses
459 and food insecurity in the tropical Andes. *Global Change Biology* **24**: e592-e602.
460 van der Heijden MGA, Bardgett RD & van Straalen NM (2008) The unseen majority: soil microbes as
461 drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* **11**: 296-310.
462 Wagg C, Schlaeppi K, Banerjee S, Kuramae EE & van der Heijden MGA (2019) Fungal-bacterial
463 diversity and microbiome complexity predict ecosystem functioning. *Nature Climate Change* **10**:
464 4841.
465 Xi N, Chen D, Bahn M, Wu H, Chu C, Cadotte MW & Bloor JM (2022) Drought soil legacy alters drivers
466 of plant diversity-productivity relationships in oldfield systems. *Science Advances* **8**: eabn3368.
467 Yang T, Adams JM, Shi Y, He JS, Jing X, Chen L, Tedersoo L & Chu H (2017) Soil fungal diversity in
468 natural grasslands of the Tibetan Plateau: associations with plant diversity and productivity. *New*
469 *Phytologist* **215**: 756-765.
470 Zhou Y, Wang L, Xiao T, Chen Y, Beiyuan J, She J, Zhou Y, Yin M, Liu J & Liu Y (2020) Legacy of multiple
471 heavy metal (loid) s contamination and ecological risks in farmland soils from a historical artisanal
472 zinc smelting area. *Science of the Total Environment* **720**: 137541.

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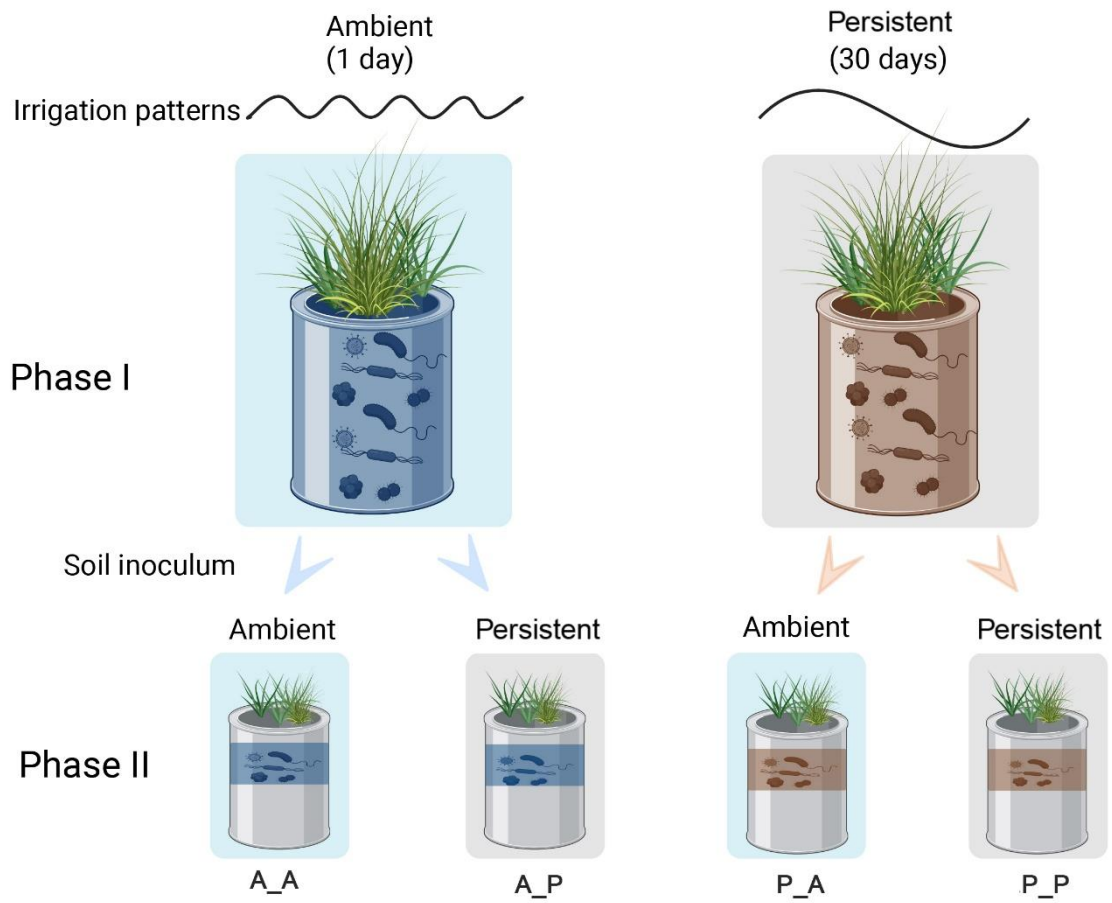
475 # Figures



476

477 **Figure 1.** Set up of the experiment. In phase I (conditioning experiment), plant communities (twelve
478 species) were grown in homogeneous soil under ambient PR (1 day wet-dry cycles) and persistent PR
479 (30 days consecutive wet-dry cycles) for one year to generate soil microbial legacies (PRs induced-
480 changes in microbial communities). In phase II (inoculum experiment), the effects of soil microbial
481 legacies were tested on new plant communities (four out of twelve species) grown under ambient
482 PR or persistent PR, using soil inoculums (1:9 proportion) conditioned in phase I.

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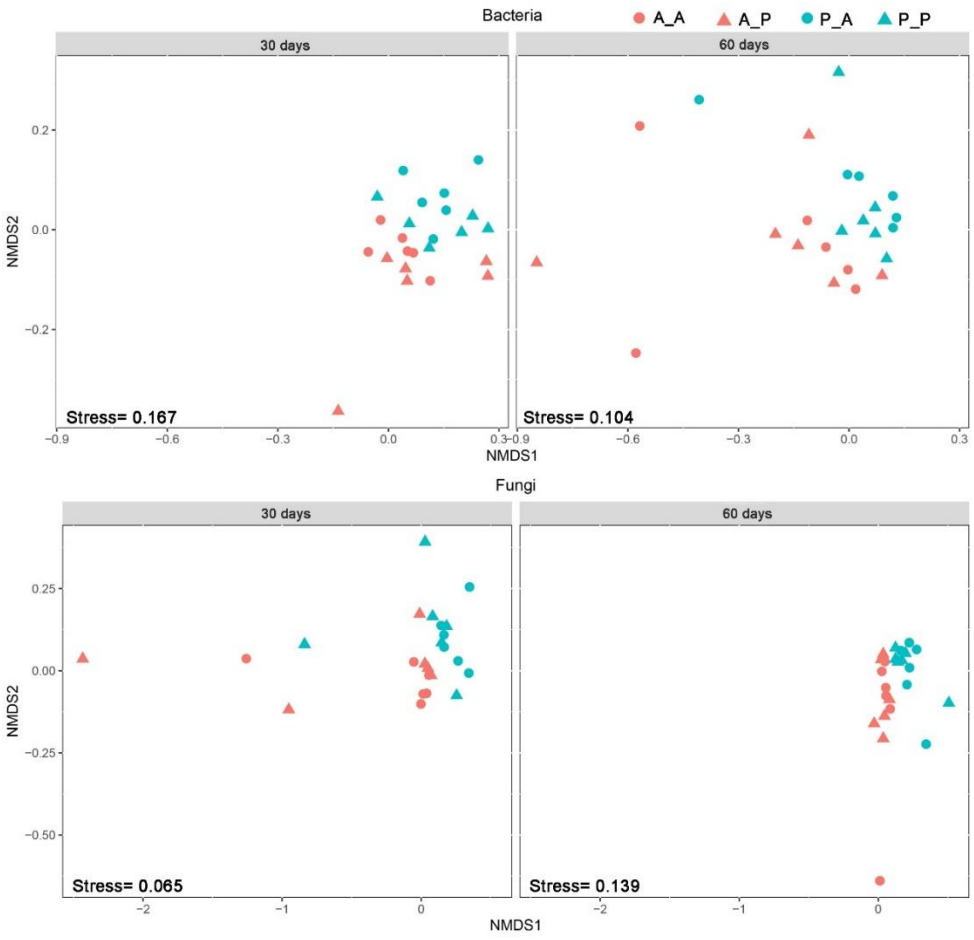

484

485 **Figure 2.** Bacterial and fungal non-metric multidimensional scaling (NMDS) ordination on weighted
486 Bray-Curtis dissimilarity in phase II after 30 and 60 days RE. The color indicates different soil histories
487 (PRs in phase I) and the shape indicates different current treatments (PRs in phase II). See Figure 1
488 for treatment codes.

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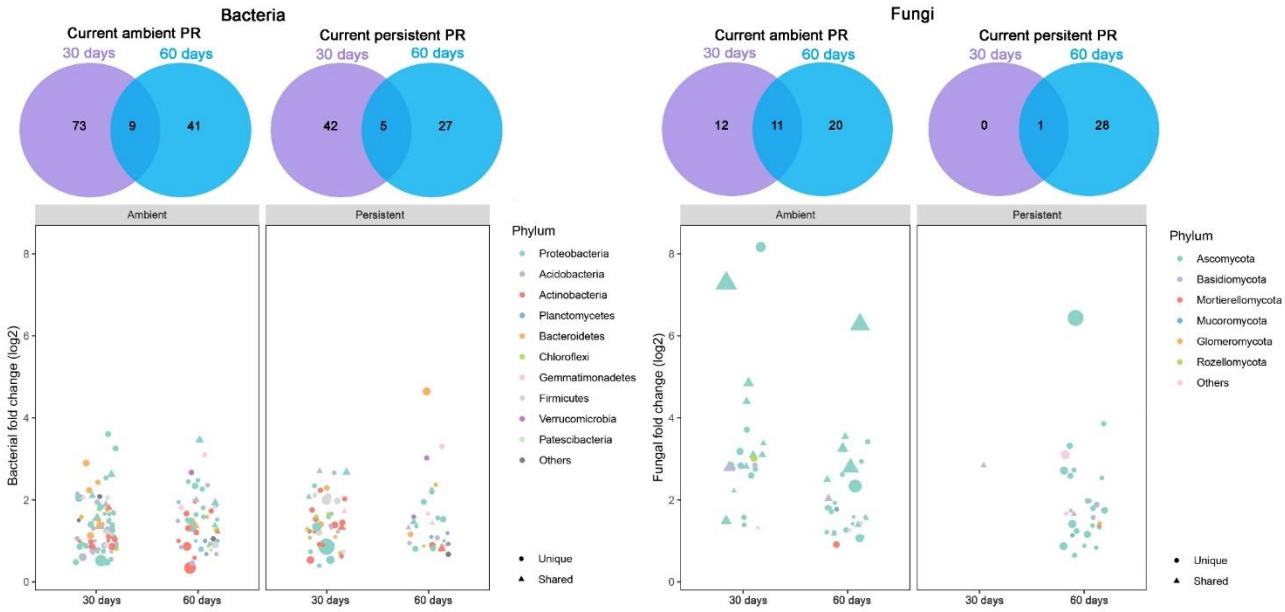
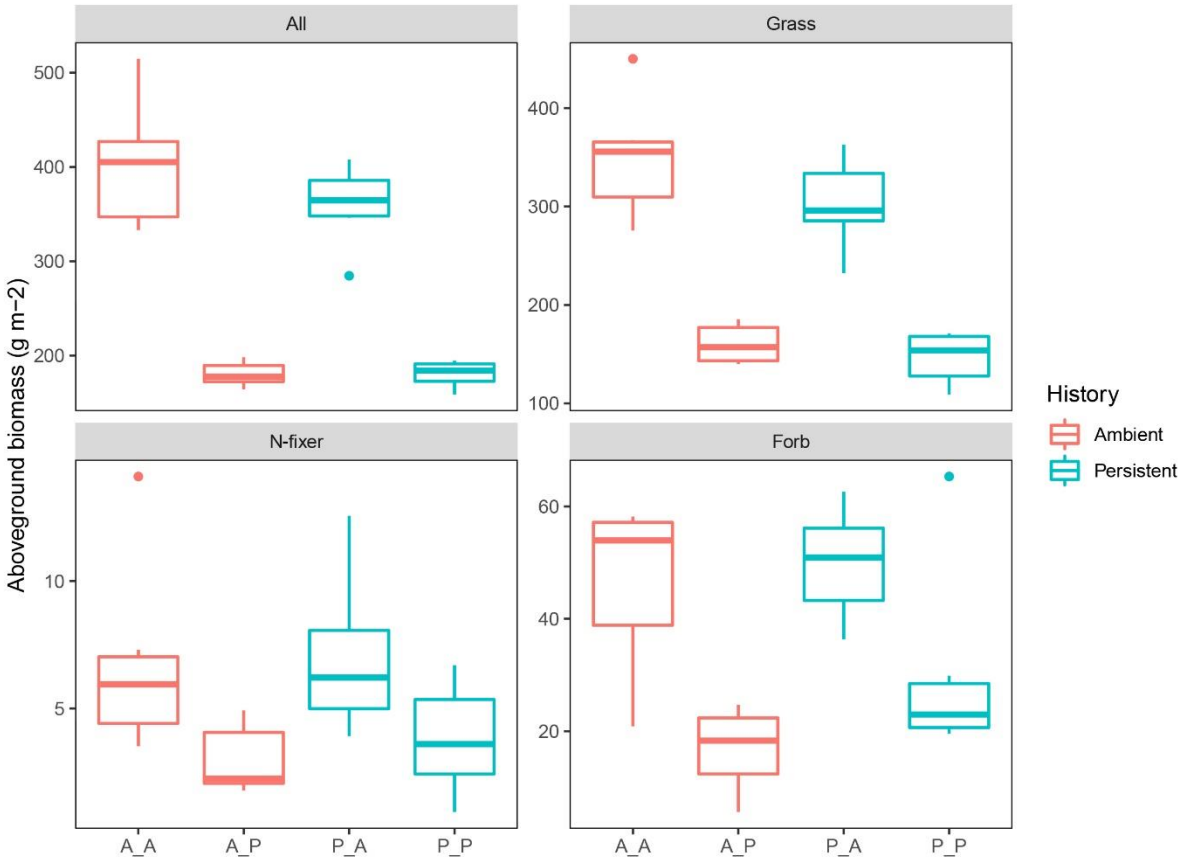


Figure 3. OTUs with significantly different relative abundance between ambient and persistent-PR soil history ("legacy OTUs"). The legacy OTUs are based on comparisons between two soil histories by Wilcoxon rank sum test (P<0.05) for each current PR (Ambient/Persistent) at 30 days and 60 days RE, respectively. Each point indicates an OTU which is sized by relative abundance and colored by phylum. The shape indicates whether they are unique or shared OTUs between 30 days and 60 days RE. Fold change = $|\log_2(\text{abundance(ambient)}/\text{abundance(persistent)})|$.



498

499 **Figure 4.** Plant aboveground biomass at the end of phase II for combinations of different historical
500 and current precipitation regimes (treatment codes: see Figure 1), for all species combined and
501 functional groups. The color indicates two soil histories. (n=6)

502

503

Table

Table 1. Results of PERMANOVA of bacterial and fungal communities at 30 days and 60 days RE of phase II based on weighted and unweighted Bray-Curtis distance. History: historical precipitation regimes from phase I; Current: present precipitation regimes from phase II.

			Weighted		Unweighted	
			Df	R^2	Df	R^2
Bacteria	30 days	History	1	0.09***	1	0.09***
		Current	1	0.08**	1	0.07***
		History*Current	1	0.04	1	0.04
	60 days	History	1	0.06	1	0.08***
		Current	1	0.05	1	0.06*
		History*Current	1	0.04	1	0.04
Fungi	30 days	History	1	0.11**	1	0.08*
		Current	1	0.06	1	0.05
		History*Current	1	0.03	1	0.04
	60 days	History	1	0.14*	1	0.10**
		Current	1	0.02	1	0.04
		History*Current	1	0.03	1	0.04