BACTERIAL COMMUNITIES IN SOIL BECOME SENSITIVE TO DROUGHT UNDER INTENSIVE GRAZING

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ABSTRACT

Increasing climatic and anthropogenic pressures on soil ecosystems are expected to create a global patchwork of disturbance scenarios. Some regions will be strongly impacted by climate change, others by agricultural intensification, and others by both. Soil microbial communities are integral components of terrestrial ecosystems, but their responses to multiple perturbations are poorly understood. Here, we exposed soils from sustainably- or intensively-managed grasslands in a cork oak agroecosystem to month-long extreme drought and flood simulations in a controlled mesocosm setting. We monitored the response of the bacterial communities at the end of one month as well as during the following month of recovery. The bacteriomes in sustainably-managed plots under all precipitation regimes were richer and more diverse than those in intensively-managed plots, and contained a lower proportion of rapidly-growing taxa. Soils from both land managements exhibited changes in bacterial community composition in response to flooding. In contrast, sustainably-managed soils were unaffected by drought. Intensively-managed soils exhibited faster post-disturbance dynamics than sustainably-managed ones. Finally, the rate of response of several taxa (i.e. Chloracidobacteria RB41, Janthinobacterium sp.) to precipitation depended on land management. Our findings show that strong climatic and anthropogenic pressures have synergistic effects on soil bacterial communities.

INTRODUCTION

The increased frequency and severity of soil flood and drought events predicted from climate change (Donat et al., 2016) and growing anthropogenic pressures (i.e., agricultural intensification and pollution) are expected to create a patchwork of distribution of environmental changes. The manner in which these stressors will affect local communities poses a further complication to our understanding of the effects of climate change. In soil, microbial communities are key regulators of nutrient cycles, with the potential to alter global carbon, nitrogen and greenhouse gas budgets (Jeffery et al., 2010). However, their response to this patchwork of stressors has not been explored.

In soil, floods and droughts strain the local microbial communities by creating physiological challenges, including maintaining osmotic balance and avoiding desiccation (Schimel et al., 2007). At a community level, the potential for altered precipitation patterns to alter soil microbial community structure and function has been explored in some previous studies (Singh et al., 2010; Wallenstein and Hall, 2012). For example, drying and rewetting may result in long-term changes to microbial community structure (Fierer et al., 2003; Pesaro et al., 2004). Furthermore, bacterial communities pre-exposed to climate-related perturbations (i.e. changes in precipitation and temperature) were found to be more resistant to similar stresses in the future than those that did not experience such perturbations (Bérard et al., 2012; Evans and Wallenstein, 2012). In contrast, fungal communities were less affected (Barnard et al., 2013).

The effect of land management has also been explored. Soil microbial communities under intensive agricultural management exhibit different community structures and higher biomass than those under sustainable management (Bissett, Richardson, Baker, & Thrall, 2011; Garbeva, Van Veen, & Van Elsas, 2003; Montecchia et al., 2011). In one case, the microbial communities underlying a harvested forest exhibited a significant, 16%, decrease in biomass decomposition genes even 12 years after harvesting as measured by metagenomics (Cardenas et al., 2015).

The combination of the aforementioned two types of stressors, which results in a form of compounded perturbation, may have multiplicative consequences for soil ecosystems (Paine et al., 1998). However, the response of
soil microbial communities to compounded perturbation is still a significant gap in our knowledge (Franciska T De Vries & Shade, 2014). In soil, changes in land management present long-term environmental factors that require the community to adapt to new conditions, while flood and drought are transient, allowing the system to return to its pre-disturbance state (Shade et al., 2012).

To our knowledge, the combined effect of changes in land management and precipitation has not been examined, however a multiplicative effect of compounded perturbation has been found when stressors present different physiological challenges (Kuan et al., 2006; Müller et al., 2002; Tobor-Kaplon et al., 2005). For example, studies examining the response of microbiomes in heavy metal-contaminated soils to transient heat stress found that the combination of both disturbances results in decreased recovery of substrate utilization capacity relative to controls as well as alterations in community structures, as evaluated by PCR-DGGE (Kuan et al., 2006; Müller et al., 2002; Tobor-Kaplon et al., 2005). In one case, the microbial community (as evaluated by PCR-DGGE) in an organo-mineral pasture soil exhibited less changes in response to benzene amendment than a mineral, arable soil (Girvan et al., 2005). This multiplicative effect may result from the reduction in diversity caused by the initial perturbation, which would result in a reduced number of ‘resistant’ or ‘tolerant’ bacterial populations capable of responding to the second disturbance.

We studied whether the management regime of an oak agroecosystem soil affects the soil microbial community’s response to extreme precipitation events similar to those expected from climate change. We define resilience as the process of reorganization following disturbance, and describe it in terms of resistance and recovery (Hodgson et al., 2015). Resistance is the (inverse of the) instantaneous effect of the disturbance, or the extent to which the community changes in immediate response to the disturbance. Recovery is the process by which the disturbed system returns to an equilibrium (Hodgson et al., 2015). Intensively-managed plots were subjected to regular grazing by sheep, as well as agrochemical inputs to improve yield. It is estimated that 60% of the world’s agricultural lands are used for grazing (Houghton, 1994).

Previous studies have shown that intensive grazing affects the soil microbial community structure and function (Ingram et al., 2008; Klumpp et al., 2009; Le Roux et al., 2007), that altered vegetation affects the underlying microbiota’s response to drought (Franciska Trijntje de Vries et al., 2012), and that soils without agrochemical inputs are better able to retain nutrients during drying-rewetting events than high-productivity soils supplemented with fertilizer (Gordon et al., 2008).

We hypothesized that sustainably-managed soils will contain more diverse microbial communities, and that such communities will be more resistant/tolerant to the precipitation treatments due to a greater level of functional redundancy. We also expect that the intensively-managed, grazed soils will recover from the treatments more rapidly than sustainably-managed counterparts as they are regularly exposed to more extreme environmental fluctuation due to the diminished vegetation cover and higher frequency of compaction.

We applied extreme precipitation regimes by selecting precipitation maxima and minima from the last two decades of local precipitation data, creating wetness conditions that were incrementally stronger than those in previous years. We thus avoided exposing the soil microbial communities to completely novel ecological challenges, which may elicit more drastic responses (Hawkes and Keitt, 2015). In fact, the gradual intensification of precipitation regimes across the globe is expected to promote gradual community adaptation in the long term (Wallenstein and Hall, 2012). We exposed intact field-collected soil cores from intensively-grazed versus sustainably-managed grasslands to month-long extreme drought and flood scenarios, and studied the bacterial communities for an additional month as they recovered. Our findings suggest that land management indeed is a crucial factor in determining soil responses to climate change.

**MATERIALS AND METHODS**

**FIELD SITE**

Samples were taken from an agro-silvo-pastoral oak woodland (Quercus suber and Quercus ilex) located in the Alentejo region of Portugal. The area is located ~290 m above sea level and exhibits a Mediterranean climate, with a mean annual precipitation of between 600 and 1000 mm. Our sampling site consisted of two contiguous plots: a sustainably-managed plot (Sustainable, 38°42’7”N, 8°18’29”W, pH ~5.9) and an adjacent, intensively-managed grazed
plot (Intensive, 38°42’14"N, 8°20’19"W, pH ~5.6). The sustainably-managed plot was characterized by the exclusion of grazing by sheep and natural vegetation for > 20 years, whereas there was regular grazing by sheep as well as active plowing and sowing of palatable herbs for grazing in the intensive-ly-managed plot. The soil from both plots was sandy loam. Further information about each plot’s nutrient contents and a list of vegetation is available in Supplementary Information, S1.

**SIMULATION OF EXTREME PRECIPITATION EVENTS**

In order to simulate extreme month-long rainfall and drought events, we selected the most extreme precipitation events between 1994 and 2013 from regional precipitation records from the Mitra station, in the Évora Geophysics Center database (http://www.cge.uevora.pt/pt/component/cge_bd/?cge_bd_e_first=mit). The drought regime was developed by calculating the mean daily precipitation during summer months (June, July, and August, 0.33 mm/day). The flood regime was developed by calculating the mean daily precipitation for winter months (November, December, and January, 4.7 mm/day), excluding months for which the mean daily precipitation was lower than the 20-year average for these months. The control rain regime was developed by averaging the yearly mean daily precipitation for the 20 years of data available (~1.76 mm/day). These were applied to cores from each management regime, in triplicate.

**MESOCOSM EXPERIMENT**

We employed Terrestrial Model Ecosystems, in which a large cylinder of soil is extracted from the land and moved to a laboratory setting, preserving over-laying vegetation and soil structure (after Rombke et al., 2004; J D Van Elsas, Dijkstra, Govaert, & Van Veen, 1986). Eighteen intact soil cores were extracted in March 2014 using a soil core extractor, which contained a high-density polyethylene tube that also served as an encasement for the cores. Cores measured 40 cm in length and 16.5 cm in diameter. Nine cores were extracted from each of the Sustainable and Intensive plots. Gauze separated the cores from the bottom of the cylinder, which was fitted with PVC pipe, allowing soil cores to drain. Cores were transported to temperature-controlled carts in a controlled chamber immediately after extraction and were partially isolated from each other by clear plastic enclosures at the soil surface. All cores were kept at control precipitation conditions (~1.76 mm/day) for 20 days, and were then subjected to treatment or control regimes for 28 days: 6 cores (3 Sustainable and 3 Intensive) were subjected to the drought regime (0.33 mm/day), 6 were subjected to the flood regime (4.7 mm/day), and 6 were maintained under control precipitation (~1.76 mm/day), resulting in triplicate cores for each source x treatment combination. Finally, all cores were returned to control precipitation regimes (~1.76 mm/day) for 26 days of recovery. Throughout the experiment, grasses in Intensive mesocosms were partially removed by plucking every ~10 days to simulate grazing. Watering took place three times per week, using artificial rain water prepared according to Velthorst (1993), and soil moisture content was monitored by inserting soil probes (Decagon devices Inc., Pullman, WA) into one core per source x treatment combination. Soil moisture data is available in Supplementary Information, S2.

Soil samples were obtained from all cores on the 30th and 32nd day of the extreme precipitation treatment to study resistance, as well as on the 5th, 12th, and 26th days of recovery. At each sampling time, 3 small cores (diameter 2 cm; length 15 cm) were collected from each core at a random location, avoiding the moisture probe and the edges of the core. The three soil cores from each mesocosm were pooled, homogenized and sieved through a ~2 mm sieve prior to analyses. Holes resulting from sampling were filled with PVC pipes to reduce disturbances in the structure of the soils.

For each time point and each core, DNA was extracted from 0.5 g of fresh soil using the MoBio PowerSoil DNA Extraction Kit (MoBio Laboratories, Carlsbad, CA, U.S.A.) following the manufacturer’s instructions, with three additional rounds of bead-beating for 30 sec using a Tissue Lyser LT equipment (Qiagen, Hilden, Germany). The concentration and band size of the resulting products were quantified by electrophoresis using a 0.8% agarose gel with a SmartLadder (Eurogentec, Liege, Belgium). One field sample from the Sustainable and one from the Intensive plots were also processed using the same procedure.
CHAPTER 7: BACTERIAL COMMUNITIES IN SOIL BECOME SENSITIVE TO DROUGHT UNDER INTENSIVE GRAZING

16S rRNA COPY NUMBER QUANTIFICATION

The number of 16S rRNA gene copies in the total community DNA were used to estimate the number of bacteria throughout the experiment. Copy number quantification was performed using an ABI PRISM 7300 Cycler (Applied Biosystems, Germany) as previously described (Pereira e Silva, Dias, van Elsas, & Salles, 2012) by targeting the 264-bp V5-V6 region of the 16S rRNA gene (primers 16SFP/16SRP (Bach, Tomanova, Schloter, & Munch, 2002)). A standard curve spanning six orders of magnitude \((10^{-2}-10^{8})\) was generated using linearized plasmids containing a 16S rRNA gene cloned from a \emph{Burkholderia} species. Amplification efficiency (E) was calculated according to the equation, \(E = (10^{-1/\text{slope}} - 1)\). For both runs, \(E = 100 \pm 10\%.\) All data are shown as log gene copy number g\(^{-1}\) soil.

16S rRNA GENE AMPLICON SEQUENCING AND ANALYSES

16S rRNA gene amplicon sequences were used to monitor bacterial community composition. Sequencing was performed using an Illumina MiSeq system at Argonne National Laboratory (Argonne, IL, U.S.A) as previously described (Caporaso et al., 2012). The raw sequences were analyzed using QIIME 1.9.0 (Caporaso et al., 2010c). Briefly, forward and reverse paired-end sequences were joined using the join_paired_ends.py command, and FastA headers were made QIIME-compatible with the split_libraries_fastq.py command. Operational taxonomic unit (OTU) picking was performed with the pick_open_reference_otus.py command, using the default uclust method and the Greengenes database, with the suppression of step 4 of the command.

Further analyses were performed in R 3.2.3 (R Core Team, 2014b) using the Phyloseq (McMurdie & Holmes, 2013) package. Singletons were removed and all samples were rarefied to 29300 reads per sample using rarefy_even_depth from the Phyloseq Package (seed.number = 266315), which led to the loss of 3 samples from the 12\(^{\text{th}}\) day of recovery. An outlier was also removed after examining its composition. The final dataset contained 2 549 100 reads distributed into 87 samples and 13 402 OTUs.

STATISTICAL ANALYSES

\(\alpha\)-diversity was calculated using the Shannon-Wiener diversity index and Pielou’s evenness, in the R environment, using the vegan package (Oksanen et al., 2007). Differences between treatments were evaluated with Kruskal-Wallis tests for \(\alpha\)-diversity as well as for the total 16S rRNA gene copy counts. Pairwise differences were evaluated using a post-hoc Tukey test after Nemeyi from package PMCMR (Pohlert, 2014).

To characterize the bacteriomes of both plots in the absence of precipitation extremes, we pooled all samples from control mesocosms for the sustainably-and intensively-managed plots. To evaluate changes in community composition in response to the extreme precipitation treatments we created ternary plots of taxa with average abundances greater than 0.1% in samples pooled from the 30\(^{\text{th}}\) and 32\(^{\text{nd}}\) day of treatment for each management × treatment combination, using package ggtern (Hamilton, 2015). A principal coordinates analysis (PCoA) of Bray-Curtis distances was used to visualize the relative differences between soil source, experimental treatments, sampling time (pooled into Treatment or Recovery phases), and between core and field samples. PERMANOVAs of Bray-Curtis distances between samples were performed using RVAideMemoire (Hervé, 2012). Principal response curves (PRCs) were used to follow microbial community recovery (Van den Brink and Ter Braak, 1999). We selected 79 taxa with species scores > 2 in a PRC including all managements, only sustainably-managed or only intensively-managed soils for further analyses. For the heat map, these taxa were centered and scaled, Euclidean distances were calculated and taxa were clustered using Ward’s method.

RESULTS

BACTERIAL ABUNDANCE AND \(\alpha\)-DIVERSITY

Total bacteria, quantified in terms of numbers of copies of bacterial 16S rRNA genes, exhibited no significant effect of either soil management, treatment or whether the samples were taken during treatment or recovery (Kruskal-Wallis
\( \chi^2 \) test, \( p > 0.4 \) for all comparisons, Supplementary Information, S3). Thus, using the total bacterial community DNA, similar sizes of bacterial biomass were estimated across all samples, regardless of treatment or core source.

In contrast, the \( \alpha \)-diversity values differed between the soils from the different treatments and sources (Figure 1). For all samples, sustainably-managed soils were richer (1-tailed \( t \)-test of Shannon’s \( H \), \( p = 0.006 \)) and more even (1-tailed \( t \)-test of Pielou’s \( J \), \( p = 0.022 \)) than the intensively-managed ones. Kruskal-Wallis \( \chi^2 \) tests also revealed significant effects of management \( \times \) treatment on richness (\( \chi^2 = 14.6871 \), \( p = 0.0403 \)) and evenness (\( \chi^2 = 16.582 \), \( p = 0.020 \)), as well as of source \( \times \) time \( \times \) treatment on richness (\( \chi^2 = 16.102 \), \( p = 0.003 \)) and evenness (\( \chi^2 = 18.183 \), \( p = 0.001 \)), suggesting that the communities changed in response to the treatments.

**RESISTANCE OF THE BACTERIAL COMMUNITY TO TREATMENT EFFECTS**

To characterize the (control) microbial communities in both management regimes, we analyzed the pooled soil samples collected in the control mesocosms from all time points in each source plot. The bacteriomes in the soils from both plots were similar at the higher taxonomic levels, and dominated by members of the Proteobacteria, Acidobacteria, Verrucomicrobia, Actinobacteria and Bacteroidetes. One-tailed \( t \)-tests between cores from sustainably- and intensively-managed soils revealed a significantly higher proportion of Actinobacteria, Verrucomicrobia, Firmicutes, Nitrospira and Planctomycetes in sustainably-managed cores, and a significantly higher proportion of Proteobacteria (mostly \( \alpha \)-Proteobacteria), Bacteroidetes, Gemmatimonadetes and Cyanobacteria in intensively-managed cores (Supplementary Information, S4, \( p < 0.01 \) for cases with significant differences).

In order to quantify the resistance of the bacteriomes to the impact of the treatment, we compared control samples with those exposed to extreme precipitation regimes from days 30 and 32 of treatment (Figure 2). The analysis showed that the effects of the drought and flood treatments on the communities were largely dependent on land management. Sustainably-managed soils exposed to flood exhibited increases in the relative abundances of Koribacter...
CHAPTER 7: BACTERIAL COMMUNITIES IN SOIL BECOME SENSITIVE TO DROUGHT UNDER INTENSIVE GRAZING

sp. (Acidobacteria), Pedobacter sp. and several unclassified members of the Chitinophagaceae (Bacteroidetes), as well as several proteobacterial OTUs including Acinetobacter sp., Janthinobacterium sp. and Luteibacter sp. No major changes were observed from exposure to the drought treatment.

On the other hand, the intensively-managed soils exposed to flood exhibited increases in the relative abundances of Sphingobacterium sp. (Bacteroidetes) as well as proteobacterial OTUs assigned to Comamonadaceae (Proteobacteria) and a single OTU classified as a Janthinobacterium sp. Intensively-managed soils exposed to drought exhibited increases in the relative abundances of Streptomyces sp. and Glycomyces sp. (Actinobacteria); Acinetobacter sp. and an unclassified Xanthomonadaceae OTU (Proteobacteria); Sphingobacterium sp. and Chitinophaga sp. (Bacteroidetes).

BACTERIAL COMMUNITY RECOVERY

No differences were observed in the structures of the dominant portion of the bacterial communities from specific time points (Figure 3), but clear and significant differences were observed between the communities sampled during treatment and recovery for each mesocosm, suggesting that the community underwent post-disturbance reorganization. A PERMANOVA of the Bray-Curtis distances of all bacteriomes revealed significant differences between the community structures from intensively- and sustainably-managed plots exposed to flooding during recovery. Moreover, the communities in intensively-managed soils subjected to drought were different from those in the controls ($p < 0.01$ for all pairwise comparisons, Figure 3), whereas no such differences were observed for the drought treatments in the sustainably-managed soils, further supporting the tenet that the microbial communities in intensively-managed soils are vulnerable to drought, but the ones from sustainably-managed soils are not.

We performed a principal response curve (PRC), which showed a clear separation between soil management types, but the effect of precipitation treatments over time was less apparent (Supplementary Information, S5). To further explore the community dynamics, we selected OTUs with species scores greater than 2 or smaller than $-2$ in the PRC for inspection, as these are expected to play a more substantial role in the temporal patterns observed in community composition (Figure 4). These 79 OTUs made up 34% of the communities on average, and could be grouped into two main clusters: (1) OTUs which were predominantly present in sustainably-managed soils and (2) those which were mostly present in intensively-managed soils. For example, 7 OTUs classified as different strains of Chthoniobacter DA101 were most abundant in sustainably-managed soils under control conditions, (together, 8% of the community on average). They decreased under flood and drought conditions (5% and 6% of the community on average, respectively), but were at lower relative abundances in intensively-managed soils under all conditions (2% of the community on average, Figure 4). In contrast, 5 OTUs classified as different strains of Kaistobacter sp. exhibited highest relative abundances in intensively-managed soils exposed to flooding and drought, particularly on the 5th day of recovery (together, 14% of the community on average for both treatments), slightly lower relative abundances in control soils (9.5% of the community on average), and much lower ones in sustainably-managed soils (2% of the community on average for all treatments, Figure 4). Notably, 13 OTUs assigned to different strains of Koribacter or Koribacteraceae exhibited different patterns. Those which were most abundant in intensively-managed soils were negatively affected by drought. For example, one OTU exhibited an average relative abundance of 0.5% in intensively-managed control soils, 0.7% in soils exposed to flood, and 0.2% in soils exposed to drought. Five OTUs assigned...
to Sphingobacteriaceae and Pedobacter sp. were more abundant in intensively-managed control soils than in sustainably-managed soils (1.5% and 0.5% of the community in total, respectively), but increased in sustainably-managed soils exposed to flooding (1.5% of the community in total), particularly during the flooding, and decreased during recovery back to control levels. In both soil management types, several of these OTUs exhibited highest relative abundances during treatment, and rapidly decreased during recovery. In contrast, three members of the Burkholderiales (Janthinobacterium sp., Ralstonia sp., and an unclassified OTU) had similar relative abundances in both soil management types (0.3% of the community in total), but increased to a greater degree in sustainably-managed soils when exposed to flooding (4% and 0.8% of the community, respectively). Finally, the response of some lowly-abundant taxa (< 0.5% of the community) to flood depended on the source soil. For example, an unclassified Acidobacteria OTU decreased from 0.6% to 0.3% in the sustainably-managed flood treatment but remained low (0.2%) in all intensively-managed mesocosms. In another example, an OTU matching Chloracidobacteria RB41 decreased relative to the controls from 0.3% to < 0.1% of the community in the flood treatment for sustainably-managed mesocosms, but significantly increased relative to the controls from 0.2% to 0.4% of the community in flood and drought treatments for the intensively-managed mesocosms (Figure 4).

**DISCUSSION**

Given the increase in anthropogenic pressures worldwide and the forecasted intensification of precipitation patterns, it is crucial to understand whether the resilience of soil ecosystems under stress is impaired. Our findings show that, in a cork oak ecosystem with differential grazing intensity, the level of response of soil bacterial communities to extreme precipitation events clearly depends on the land management. The overlying vegetation, which was maintained for the duration of the experiment, may explain the higher moisture content in the sustainably-managed soils under all treatments, as these had a thicker layer of overlying vegetation (Supplementary Information, S2). As has been documented in other studies (Fierer et al., 2003), this vegetation may have buffered the soils against changes in precipitation regimes.

The higher rate of fluctuation in moisture in the intensively-managed soils may partially explain the differences found in the original microbial community compositions of the two management types. The larger proportion of α-Proteobacteria and Bacteroidetes—which contain many copiotrophic phyla with potentially fast growth responses (Fierer et al., 2007)—in the intensively-managed cores points to an effect of the larger fluctuations of moisture conditions favoring rapid growth and adaptation in these soils. On the other hand, the higher proportion of Actinobacteria, Verrucomicrobia, Firmicutes, Nitrospira and Planctomycetes, which contain slow-growing or sensitive taxa (Attard et al., 2010; Buckley et al., 2006; Fierer, 2015), supports the notion that the sustainably-managed soils were exposed to less environmental change, favoring specialists.
It was intriguing that only the bacteriomes in the intensively-managed mesocosms exhibited changes in response to the drought treatment. These were characterized by increases in the relative abundances of Actinobacteria compared to the controls, specifically taxa belonging to Streptomyces (Figures 2 and 3). Actinobacteria have been reported to flourish upon changes of moisture in soil (Barnard et al., 2013), and drying of soil has also been shown to favor the growth of Streptomyces (Williams et al., 1972). Drought also resulted in an increase of rapid-growing, opportunistic taxa with the ability to metabolize complex hydrocarbons such as Acinetobacter sp. (Dougari et al., 2011), Sphingobacterium sp., and Chitinophaga sp. (Normand et al., 2014). This indicates that a lack of connectivity between soil pores may have induced parallel evolution of different responding soil bacteria. In sustainably-managed soil mesocosms, no significant changes were observed from the drought relative to the controls, which may be because these mesocosms already contained a high abundance of these taxa under control conditions. Thus, the increased vulnerability of intensively-managed soils to drought is consistent with the lower alpha diversity found in these soils as compared to the sustainably-managed soils. Once the environment changes, more resistant taxa are already found in the more diverse soil, so adapting to change requires less shifts in community composition. It is important to note, however, that cells inactivated by drought with intact DNA may have been detected as present by the molecular methods employed.

Conversely, communities from sustainably-managed soils became more similar to those of intensively-managed soils during and after flooding (Supplementary Information, S5). This corresponds with the increase observed for several members of Burkholderiales (i.e., Burkholderia sp., Bradyrhizobium sp., Ralilibacter sp.) in the sustainably-managed soils after the flood treatment. In intensively-managed soils, these taxa were already present in higher abundances prior to flooding. A strong effect of flooding was observed for soil bacteriomes from both management types, however. This may have resulted from the redistribution of resources within the soil matrix, favoring the growth of copiotrophs such as Proteobacteria and Bacteroidetes (Fierer et al., 2007; Schimel et al., 2007). Alternatively, the shifts may have resulted in the selection of flood-resistant taxa. For example, Janthinobacterium sp., which increased in relative abundance after flooding of the soils from both management types, belongs to a genus that contains efficient genes for osmotic stress tolerance (Shoemaker et al., 2015).

Our findings highlight the importance of the “pre-disturbance” community conformation for the modulation of the community responses to perturbation. The more diverse bacteriomes from the sustainably-managed soil plots contained a higher proportion of disturbance-tolerant taxa, and thus the system had less open niche space during and after the drought, and probably witnessed weaker successional dynamics for the colonization of these niches. In the less diverse, intensively-managed soils, adapting to drought required more drastic changes in the community. These results align with previous findings which revealed that community-wide tolerance (the added tolerance ranges of all community members) largely determines the outcome of perturbation (Awasthi et al., 2014). In this sense, our findings support the notion that biodiversity acts as an insurance against environmental change in the soil microbial system (Wittebolle et al., 2009; Yachi and Loreau, 1999). The pattern observed for flooding (i.e. that sustainably-managed soils become more similar to intensively-managed soils) aligns with the hypothesis that systems with a higher proportion of rapid-growing taxa are expected to exhibit an increased rate of recovery (Franciska T De Vries & Shade, 2014). Climate change is expected to increase the frequency and magnitude of both flood and drought events, and our results should be considered in conjunction. The bacteriomes of sustainably-managed soils are not affected by drought, but begin to resemble those of intensively-managed soils after flooding. Further research is necessary to determine whether multiple drying-rewetting cycles will make the bacterial communities of soils under different management regimes more similar, or whether there are thresholds to community tolerance after which community structure assembly becomes stochastic, as was found after diluting soil bacterial communities at increasing frequencies (Kim et al., 2013). It is likely that individual responses to perturbation are modulated by soil microbial community composition, as has been previously shown (van Elsas et al., 2006b). We found that the response of several lowly abundant taxa to flooding was dependent on land management type. Furthermore, recent findings have shown that individual bacteria alter their ecological strategies after multiple drying-rewetting cycles (Evans and Wallenstein, 2014). This adds a further complication to determining the outcome of soil disturbance on the
community, as the responses may be dependent on the microbial community structure at the time of perturbation.

It is still a matter of debate to what extent soil microbial community structure plays a role in the provisioning of ecosystem services (McGuire and Treseder, 2010; Nemergut et al., 2014). Recent experimental evidence suggests that altered soil microbial community composition can affect aggregate or emergent community traits, such as a community's propensity to invasion (van Elsas et al., 2012) and resilience, which depends on all community members and may be driven by direct or indirect interactions (Fiegna et al., 2015; Mallon et al., 2015). Our results show that the effect of precipitation extremes on soil microbial communities depends on land management, where intensively-managed grasslands containing less diverse communities were more vulnerable to drought. The ability of soils to continue to function under long-term fluctuations constitutes a fundamental component of soil quality (Seybold et al., 1999). From this perspective, the increased sensitivity, in terms of lower resistance, of intensively-managed soils signifies a loss of soil quality. Such a loss of quality may affect the ability of soils to cope with future stress. A recent theoretical model showed that the sensitivity of soil microbial communities to environmental change is dependent on whether the soils were subjected to disturbances of similar magnitudes in the past (Hawkes and Keitt, 2015). On the other hand, microcosm experiments showed that soils exposed to increasing frequencies of re-inoculation (every 7-56 days) into sterile soil collapsed at the highest frequency, becoming completely stochastic (Kim et al., 2013). The gradual intensification of disturbances expected from climate change may thus allow soil microbial communities to adapt. However the increasing frequency of extreme events eventually may lead to a catastrophic shift, in which the community drastically changes to a new conformation (Scheffer et al., 2001). The potential of microbial communities for exhibiting such shifts is poorly understood, and requires further study.

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