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Abstract

The timing and magnitude of rainfall events are expected to change in future decades, resulting in longer drought periods and larger rainfall events. Although microbial community composition and function are both sensitive to changes in rainfall, it is unclear whether this is because taxa adopt strategies that maximise fitness under new regimes. We assessed whether bacteria exhibited phylogenetically conserved ecological strategies in response to drying-rewetting, and whether these strategies were altered by historical exposure to experimentally intensified rainfall patterns. By clustering relative abundance patterns, we identified three discrete ecological strategies and found that tolerance to drying-rewetting increased with exposure to intensified rainfall patterns. Changes in strategy were primarily due to changes in community composition, but also to strategy shifts within taxa. These moisture regime-selected ecological strategies may be predictable from disturbance history, and are likely to be linked to traits that influence the functional potential of microbial communities.

Keywords

Birch effect, drying-rewetting, historical legacy, microbial life history, moisture niche, moisture stress, precipitation manipulation, rainfall pulses, rainfall timing, tallgrass prairie.

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INTRODUCTION

One of the fundamental pursuits in ecology is to understand how organisms respond to environmental change – a particularly urgent and important need given recent evidence of rapid climate change. Predicting how these organismal responses will influence the ecosystem process they mediate requires an approach that links changes in fitness of individuals to population dynamics, community composition and function. While responses of macroorganism communities to novel conditions are often reported, it has been more difficult to study these responses in microbial communities due to their extreme diversity, rapid turnover and the technical challenges that come with observing them. As a result, we do not know if microbes follow the same patterns of responses as other organisms. Because microbial communities are mediators of biogeochemical cycles, their responses to novel climates will be important for efforts to maintain ecosystem services and predict biogeochemical feedbacks to the climate system (Nie *et al.* 2013).

The availability of water is among the most important controls on rates of microbially mediated biogeochemical processes in soils (Parton *et al.* 1987; Paul & Clark 1996). Future climates will likely be characterised by more variable rainfall patterns; both drought duration and rainfall event size are expected to increase (Huntington 2006; IPCC 2007). Sudden changes in soil moisture, such as those resulting from drought and subsequent rain events, can induce a physiological stress on bacteria due to the rapid change in water potential (Harris 1981). Some bacteria may be able to tolerate these stresses

because they possess certain adaptive traits, like a thicker cell wall to withstand osmotic pressure (Kieft *et al.* 1987; Schimel *et al.* 2007). However, tolerating stress also requires an investment in resources (Schimel *et al.* 2007) such that taxa investing in this stress-tolerance strategy should have reduced relative fitness (including as a result of competitive interactions) when stress events are rare.

Microbial community composition and structure appear to be sensitive to experimental alterations of precipitation regimes (Allison & Martiny 2008; Clark *et al.* 2009; Evans & Wallenstein 2012; Evans *et al.* 2013) and also to change across precipitation gradients (Hawkes *et al.* 2011). These climate-driven shifts in community composition could be due to the favouring of certain taxa with ecological traits that increase their ability to thrive under a particular climate regime. There is indirect evidence that microbial communities do become more resistant and function more optimally under their historical rainfall regime. Microbial communities that have already experienced drying and rewetting can be more resistant to this type of stress than those that have not historically been subjected to it, in terms of both overall microbial biomass and community composition (Fierer *et al.* 2003; Evans & Wallenstein 2012; Bouskill *et al.* 2013). This suggests that certain moisture patterns select for species – or traits within species – that maximise the survival and growth of microorganisms under those conditions.

Organisms develop ecological strategies to maximise their chances of survival under a given set of biotic and abiotic conditions (MacArthur & Wilson 1967; Pianka 1970; Grime 1977). In addition to the widely-applied *r*- and *K*-selection

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delineation (MacArthur & Wilson 1967), Grime (1977) proposed that plants could be organised into strategies based on tolerance to stress and disturbance, and that the distribution of these strategies were determined by the frequency of stressors in the environment. Ecological strategies have long been identified in bacterial cultures (Winogradsky 1924; Golovlev 2001), but they have only recently been applied to diverse microbial communities to reveal how historical conditions of a site influence the ecological properties of many taxa in a field setting (Fierer *et al.* 2003; Placella *et al.* 2012; Barnard *et al.* 2013). For example, Fierer *et al.* (2012) report that taxa previously identified as r-strategists (Fierer *et al.* 2007), as well as traits associated with this strategy (identified by genomic markers), increased with greater soil nutrient concentration, consistent with *r-K* theory (MacArthur & Wilson 1967). Here, we include competitive interactions in our definition of individuals' fitness and therefore consider life strategy as a way to survive and grow under both climatic stress and competition (McGill *et al.* 2006).

Like nutrient availability, moisture stress has also been shown to drive tradeoffs among groups of traits in bacterial isolates (Lennon *et al.* 2012). This suggests that the assembly of diverse communities is likely to be influenced by more than just neutral drivers, and microbes may develop specific and conserved strategies for dealing with moisture stress. There is evidence that bacteria adopt highly conserved resuscitation strategies when responding to a single short-term moisture event (wet-up) (Placella *et al.* 2012), and in response to seasonal rainfall patterns (Barnard *et al.* 2013). However, whether altered rainfall patterns select strategies that are more optimal for that new rainfall regime has not been tested.

A change in the distribution of ecological strategies in a community could occur through a shift in community structure (i.e. new species colonise, increase in abundance or out-compete other species) or through a shift in the physiology of individual species. Ecological strategies and other microbial traits can be related to phylogeny at various, and sometimes broad taxonomic levels (Fierer *et al.* 2007; Philippot *et al.* 2010; Placella *et al.* 2012; Zimmerman *et al.* 2013). Highly conserved ecological strategies might suggest that the former mechanism (shift in community structure) is the primary cause of changes in ecological properties of microbial communities. However, ecological niche of a single microbial strain can also vary based on geographic origin or previous stress exposure (Kvitek *et al.* 2008; Wang *et al.* 2010). Microbes can also transfer genes horizontally and evolve quickly (Vasi *et al.* 1994). Thus, shifts in ecological strategies are also possible independent of shifts in community composition, and may occur at different rates or mean that shifts in ecological properties are controlled by different factors.

In this study, we used an approach to classify diverse bacterial communities into ecological strategies, analogous to those developed by Grime (1977) for plants, based on changes in the relative abundance of individual populations in response to drying and rewetting stress. Relative abundance integrates competitive ability and response to moisture to provide an indicator of relative fitness for each taxa. We then tested whether the distribution of ecological strategies is altered under a decade of more variable rainfall that increased the

frequency of drying-rewetting stress. Previously, we showed that changes in rainfall timing altered both the species composition and the functional potential of the microbial community (Evans & Wallenstein 2012), but it is not clear whether this was due to changes in the abundance of species employing certain moisture-response strategies. We addressed the following research questions:

- 1) Do microorganisms express phylogenetically conserved strategies when responding to drying and rewetting stress?
- 2) Does long-term exposure to a disturbance regime (more intense rainfall patterns) result in a shift towards strategies that are optimised to this moisture regime?
- 3) Is this shift in life strategies primarily explained by shifts in the species (and strategies) present in the community, or do species also change their strategy?

METHODS

Experimental design and pyrosequencing

We collected soils from the Rainfall Manipulation Plot Study (RaMPS) in the US tallgrass prairie, where the timing and quantity of precipitation events had been experimentally altered to simulate a more extreme rainfall regime (fewer, larger rainfall events separated by longer dry periods) for the previous 10 years (Fay *et al.* 2000; Harper *et al.* 2005). Soils were collected in late December 2007 from the manipulated-rainfall plots (hereafter 'Delayed'), and from control plots (hereafter 'Ambient') ($N = 6$) (Fig. 1a). Soils were sieved (<2 mm) and stored at -10°C until initiation of the laboratory experiment (Evans & Wallenstein 2012).

In 2009, the collected soils were pre-incubated for 5 days, then subject to four drying-rewetting cycles over 100 days in root-free laboratory incubations, mimicking soil moisture fluctuations measured in manipulated plots in the field (Fig. 1b) (Evans & Wallenstein 2012). Incubations were destructively harvested after the pre-incubation at the beginning of the laboratory experiment (Initial), and on the third day of the 1st (Pulse 1) and 4th (Pulse 4) rewetting pulse, resulting in a time series with three points (Initial, Pulse 1, Pulse 4). After DNA was extracted from soil at these time points, we used 454 pyrosequencing to sequence the 27–338 portion of the 16S rRNA gene, and used the QIIME pipeline to pre-process data (Fierer *et al.* 2008; Caporaso *et al.* 2010; Evans & Wallenstein 2012) (Fig. 1c, additional detail in Appendix S1). Operational taxonomic units (OTUs) were grouped at 97% similarity, which defines what we call 'species' in this study. We use these two terms (species and OTU) interchangeably hereafter.

Filtering

Before using cluster analysis to detect life strategies, we filtered OTUs to reduce noise and identify those species that responded to drying and rewetting pulses. We excluded rare (occurring in less than three samples total) and low-abundance (less than three sequences per OTU) OTUs, as well as those OTUs that displayed inconsistent responses among time point replicates. Specifically, we deemed the OTU 'present' at

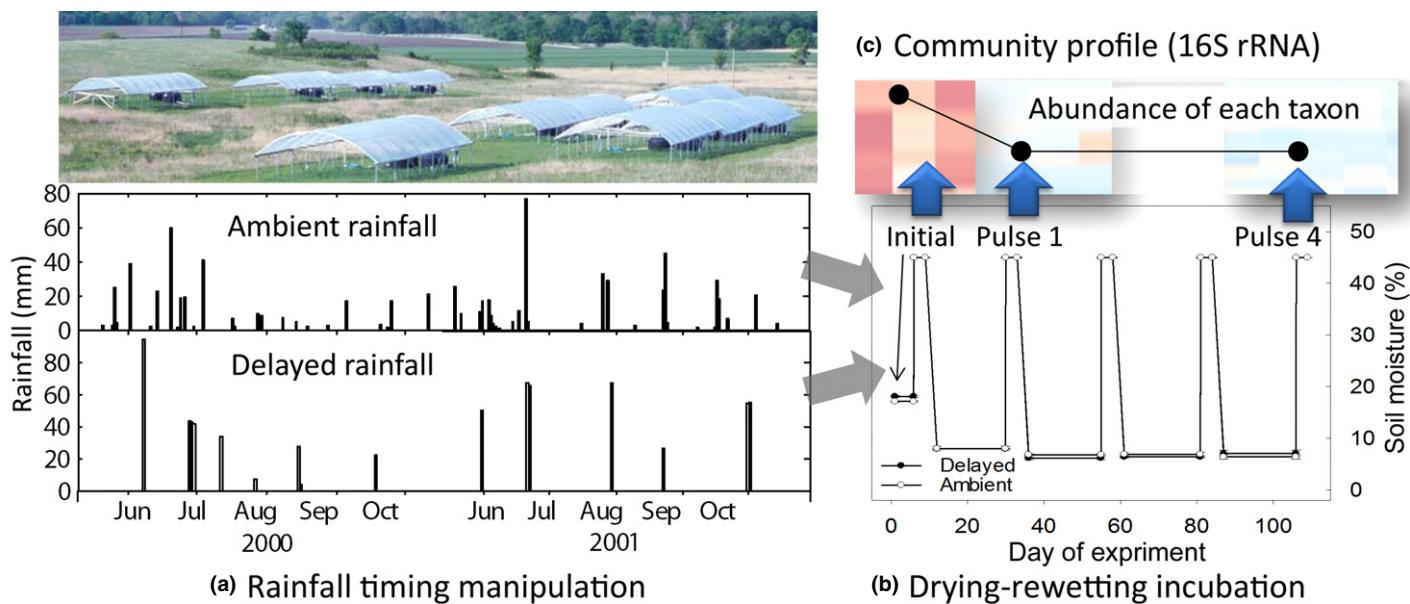


Figure 1 Experimental design. Soils were collected from plots receiving ambient rainfall and delayed-timing rainfall (a) (Fay et al. 2000), and each subject to a series of drying and rewetting events in laboratory incubations that mimicked higher-intensity rainfall regimes in the field (b) (Evans & Wallenstein 2012). Laboratory samples were assessed for the relative abundance of 16S rRNA genes at the beginning of the incubation, after the first moisture pulse, and after the last moisture pulse (c).

a particular time point if over half of the replicates (e.g. four of six) had a relative abundance greater than 0, and ‘absent’ if over half of the replicates’ relative abundance was equal to 0, as is used to filter gene expression microarray data before clustering analyses (McClintick & Edenberg 2006). We retained species that were either present or absent in all three time points, excluding any deemed absent in all three. This filter did not retain all types of bacterial ecological responses. For example, it would exclude a species that was highly abundant initially, but responded inconsistently among treatment replicates in other time points, perhaps because its abundance was driven by other factors. However, in order to determine whether strategies related to drying-rewetting were phylogenetically conserved and shifted with changes in drying-rewetting intensity, we first had to isolate only those species that responded to drying-rewetting pulses.

Clustering and phylogenetic analysis

We used a hierarchical clustering-based analysis to group species according to their response to moisture pulses over time (Eisen *et al.* 1998; Placella *et al.* 2012). All analyses were performed in R version 2.14.1 (R Development Core Team 2010). To each set of species filtered from Ambient and Delayed soils, we applied agglomerative hierarchical clustering using a 1 – Pearson correlation dissimilarity metric and an average clustering criteria. We cut dendograms where the variance explained began to diminish with increasing clusters (the ‘elbow’ of the curve) (Everitt *et al.* 2011) (see Fig. S1), and described the life strategies exhibited by these clusters. We verified differences among life strategies within each field treatment using multivariate analysis of similarity (ANOSIM) in Primer v6 (Clarke 1993) using a Pearson distance metric. We used global and pairwise R statistic and associated

P-values to determine whether mean similarity ranks were significantly smaller within than between life strategy groups.

We also verified these results by clustering OTUs on correlations that took into account the relative nature of our OTU abundance data. Compositional data describing relative abundance can be inherently correlated, and when correlations analyses (such as Pearson’s) are applied to relativised data, spurious patterns can emerge (Aitchison 2003; Friedman & Alm 2012). Because our data were relativised by total sequences, but then subset, it was less correlated than purely relativised data. Still, we tested the robustness of our life strategy groups by calculating correlations based on the ratio of log components of OTU abundances, using SparCC (Friedman & Alm 2012). We then based our clustering analysis and ANOSIM tests for significance on these correlations.

We used net relatedness index (NRI) and nearest taxon index (NTI) to determine whether the species in a particular life strategy are more phylogenetically related to one another than to other species found in that soil. The degree of phylogenetic relatedness in a community can indicate the processes that influenced its assembly, like the extent a community is shaped by environmental filtering (life strategy is clustered by phylogeny) or competitive interactions (life strategy is phylogenetically evenly distributed) (Webb *et al.* 2002). We indexed phylogenetic clustering by calculating the mean phylogenetic distance of all pairs of taxa (in NRI), and the minimal distance between taxa (in NTI), and standardising this number based on the number of taxa in each community. Due to their slightly different algorithms for determining phylogenetic distance, NTI is a better indicator of the extent of terminal clustering, or clustering near the tips of the tree, while NRI measures how life strategy is clustered overall (Webb 2000). We constructed a null model by shuffling tip labels (1000 random permutations) among all species we detected in that soil

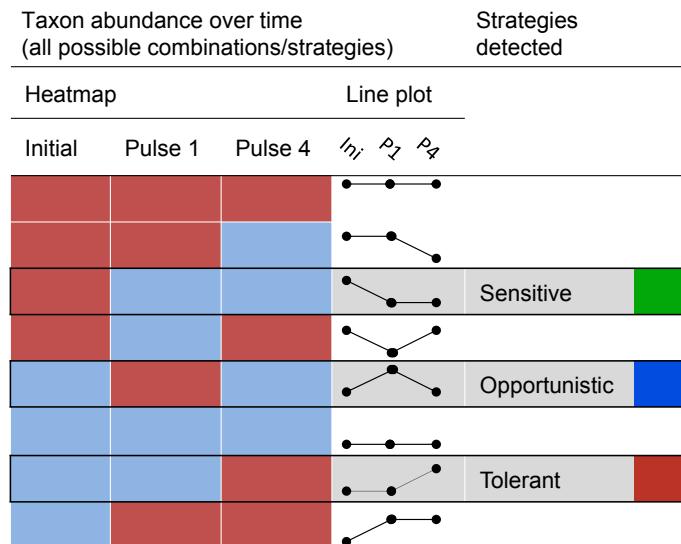


Figure 2 We determined life strategy based on the relative abundance of microbial taxa at three time points throughout a drying and rewetting incubation (Initial, Pulse 1, Pulse 4). Our cluster analyses revealed taxa grouped into three strategies (shaded in gray, outlined in box). Here, we show all possible combinations of high or low taxa relative abundance (colours) at each of three time points, represented by a theoretical heatmap (red = high abundance, blue = low), line plots of relative abundance over time. The last column shows those strategies we detected in communities under ambient and altered rainfall timing in the tall grass prairie.

after first filtering of low-abundance OTUs, then performed a two-tailed significance test ($\alpha = 0.05$) to determine whether the observed distances between taxa were greater or less than expected by the null model. Low P -values (and negative indices) indicate that species are more phylogenetically related than expected by chance (clustered), and high P -values (and positive indices) indicate that species with that life strategy are less closely related than expected by chance (overdispersed).

RESULTS

Grouping of taxa into life strategies

There was a sharp decrease in the variance explained by additional clusters after grouping species in Ambient and Delayed into three groups (forming a clear 'elbow' in Fig. S1). On the basis of the different response patterns of the three groups, we labelled the three life strategies opportunistic (which had low relative abundance at the start of the experiment, high after one pulse, and low after four pulses), tolerant (low, low, high) and sensitive (high, low, low) ecological strategies. Strategy groups were significantly different from one another in both Ambient (global R -statistic = 0.675, $P = 0.01$) and Delayed (0.775, $P = 0.01$) treatments. These three groups exhibited the same drying-rewetting responses in Ambient and Delayed (Figs 2 and 3), even though many combinations of abundances (and groups) were possible (see Fig. 2 for all possible response patterns). Clusters (and OTU cluster assignment) were robust

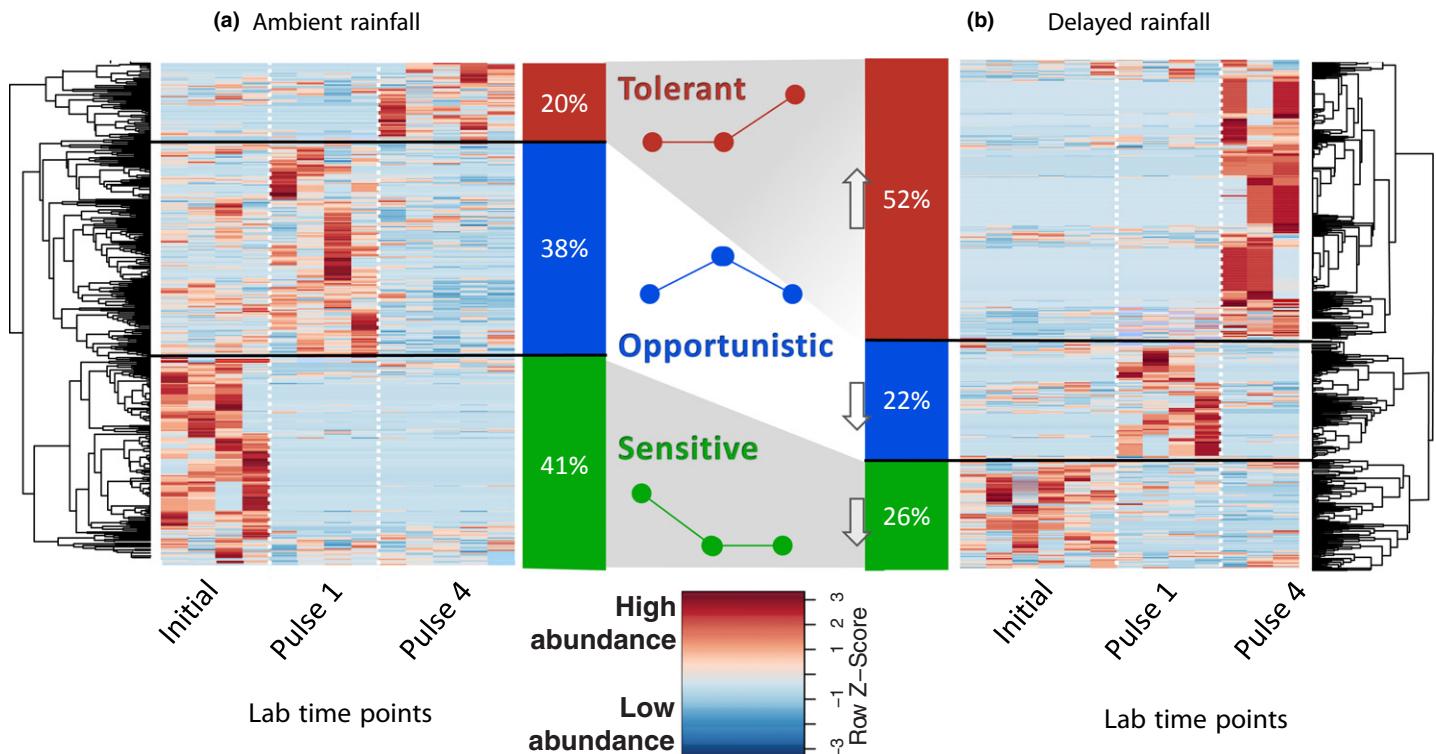


Figure 3 Heatmaps showing the relative abundance of each bacterial operational taxonomic unit (OTU) (rows) at three time points (Initial, Pulse 1, Pulse 4, x-axis, grouped in replicates) in a drying-rewetting incubation. Soils were collected from plots subject to Ambient rainfall (a) and Delayed rainfall timing (b). Responses over time were classified into ecological strategies (given labels in the centre) using cluster analysis dendograms, shown on the outside of each heatmap. The proportion of species that employed each ecological strategy (coloured bars, white percentage) differed in communities that had been exposed to Ambient rainfall (a) and those that had been exposed to more intense rainfall in the field (b).

Table 1 Phylogenetic structure indices of groups of life strategies

Index	Field treatment	Strategy	Num taxa	NRI or NTI value	P-value*
NRI					
Ambient	Sensitive	168	7.24	0.001	
	Opportunistic	156	2.23	0.012	
	Tolerant	85	-2.45	0.994	
Delayed	Sensitive	130	4.07	0.001	
	Opportunistic	107	-0.14	0.551	
	Tolerant	255	-0.97	0.834	
Changed strategy	-	104	1.71	0.058	
NTI					
Ambient	Sensitive	168	5.58	0.001	
	Opportunistic	156	1.71	0.042	
	Tolerant	85	2.84	0.002	
Delayed	Sensitive	130	2.82	0.001	
	Opportunistic	107	0.46	0.325	
	Tolerant	255	4.18	0.001	
Changed strategy	-	104	-0.02	0.499	

*P-value based on comparison of phylogenetic distance observed and that based on a 1000 permutations of a null model. Values in bold indicate $0.025 > P > 0.975$. Low P-values (and negative indices) indicate that species are more phylogenetically related than expected by chance (clustered), and high P-values (and positive indices) indicate that species with that life strategy are less closely related than expected by chance (over-dispersed). NRI, net relatedness index ; NTI, nearest taxon index.

($P < 0.01$ for the 3 clusters in Ambient and Delayed) when tested with alternative method developed by (Friedman & Alm 2012) that accounts for bias presented by composition data, although we did see slight changes in clustering at very fine scales ($P > 0.05$ for these groups, data not shown).

Filtering to exclude rare and low-abundance sequences resulted in a group of 3113 OTUs from the Ambient field treatment, and 2953 from the Delayed treatment. From this pool, we identified 404 species in Ambient and 492 species in Delayed that responded to drying-rewetting pulses in the laboratory, based on consistency in response among replicates. Some samples from the original six field replicates were excluded in downstream molecular analysis because they either failed to amplify, had less than 250 sequences or were outliers in our Non-metric Multidimensional Scaling analysis (resulting in, e.g. four replicates in Initial time point of Ambient field treatment, see Fig. 2). Additional details on pyrosequencing results are reported in Appendix S1 and Evans & Wallenstein (2012).

Phylogenetic relatedness and shifts in strategy

Species isolated from Delayed treatments clustered into the same three strategies, but the percentage of species employing sensitive strategies after exposure to drying-rewetting dropped to 26% of species in the Delayed species pool (from 41% in Ambient), while the percentage of tolerant species increased from 20% in Ambient to 50% in Delayed (Fig. 3). Groups of life strategies varied in the degree they were phylogenetically clustered (Table 1). Overall, taxa with sensitive and opportu-

nistic strategies clustered at deeper phylogenetic branches (NRI and NTI > 0 , $P < 0.05$), while tolerant taxa were over-dispersed across the entire tree (NRI < 0 , $P > 0.95$) but more clustered near the tips of the tree (NTI > 0 , $P < 0.05$) (Table 1; Fig. 4). These conclusions were robust when we narrowed the species pool to only those that responded to drying and rewetting (data not shown).

Life strategies varied among dominant phyla, and some phyla showed similar distribution under Ambient and Delayed treatments, while others did not (Fig. 4, pie charts). For instance, more Acidobacteria employed a tolerant strategy (42% in Ambient), especially with a history of drying-rewetting (64% in Delayed), while a higher percentage of Proteobacteria were opportunists under Ambient rainfall (42%) but tolerant (45%) after experiencing more intense rainfall patterns (Fig. 4, pie charts).

Of the 404 Ambient and 492 Delayed species that responded to drying-rewetting in the laboratory (i.e. were retained after filtering), 127 were present in both pools (Fig. 5). The remaining species in each treatment were either absent in the other treatment, too rare to be detected or non-responsive to drying rewetting according to our filtering criteria. Of these 127 species identified in both Ambient and Delayed species pools, 104 were assigned a different strategy if they originated from Ambient plots than if they originated from Delayed plots (Fig. 5b, plotted on phylogenetic tree). That is, part of the shift in strategy we observed was due to a within-species change in ecological strategy (Table S1 summarises within-species shifts). Neither presence in both treatments nor strategy plasticity (whether or not a species was present in both, or changed strategy) was related to phylogeny (as tested by NRI and NTI, data not shown, but see yellow- and black-labelled outer rings in Figs 4 and 5b).

DISCUSSION

Both bacterial community composition and function have been shown to change with experimental manipulation of precipitation timing (Fierer *et al.* 2003; Clark *et al.* 2009) including in the RaMPS experiment examined here (Evans & Wallenstein 2012), but most previous studies have not identified the ecological mechanisms driving these bacterial responses. In this study, we were able to attribute changes in species composition to shifts in the distribution of moisture-stress related ecological strategies within the community. We found that a decade of exposure to more frequent drying-rewetting stress resulted in a greater proportion of taxa exhibiting a stress tolerant strategy compared to communities exposed to the Ambient precipitation regime, which contained a greater abundance of pulse- or drought-sensitive organisms, consistent with our predictions. Characterising the ecological strategies of microbial taxa represents a potential way to predict responses to changes in rainfall regime or types of environmental change, which could be linked to specific effect traits that are likely to affect function (Barnard *et al.* 2013).

We identified ecological strategies based on changes in the relative abundance of taxa following moisture pulses in the lab, which integrates population growth, death, survival and

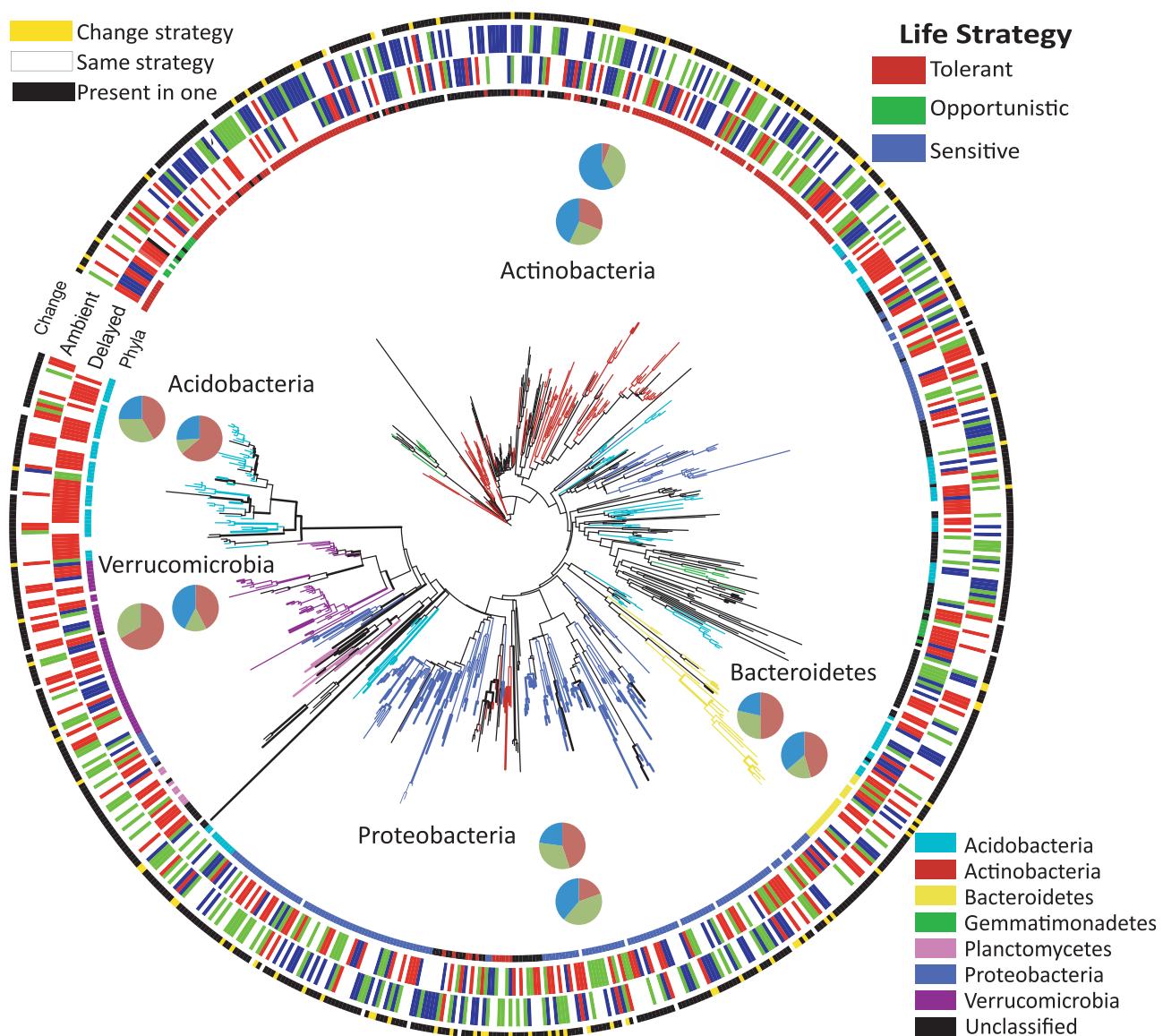


Figure 4 Phylogeny of bacterial taxa, and each taxon's assigned life strategy (colours in wider rings, corresponding to Fig. 2) in plots receiving ambient rainfall (outer wide ring) and delayed-timing rainfall (inner wide ring). Phyla are indicated by branch colours and the innermost ring, and the outer ring indicates whether taxa changed strategy with (Ambient to Delayed) rainfall history. Pie charts show the distribution of strategy within the most dominant phyla in Ambient (inner pie charts) and Delayed (outer pie charts). Taxa not responsive to drying and rewetting were included in phylogenetic statistical analyses (Table 1), but not shown for clarity.

reproduction over a period of time. These 'realised' strategies may differ from strategies exhibited by isolated bacteria in the absence of ecological interactions. Relative abundance is an indicator of fitness, but does not reveal the specific traits that influence microbial population dynamics under moisture stress. For example, we detected three strategies – microbes tolerant, opportunistic and sensitive in the face of moisture pulses. The lack of detection of other strategies (see Fig. 2 for all possible combinations) could suggest that these three strategies are fitness optima under drying and rewetting stress. However, we do not know the specific life history traits associated with the strategies, or whether they are the direct result of moisture response or competitive interactions. Still, we

have shown that when rainfall intensity alone is altered, strategies that are more optimal under these conditions increase.

Because life strategies incur intrinsic tradeoffs in resource allocation, a shift in the distribution of strategies is likely to affect the functional potential of communities (Litchman *et al.* 2007; Treseder *et al.* 2011). The changes in strategy distribution we observed may explain some functional measurements reported by Evans & Wallenstein (2012) using the same experiment, and provide insight into how the mechanisms driving large pulses of respiration following soil rewetting might vary with community composition. Evans & Wallenstein (2012) found that soils from Ambient plots had higher respiration rates than soils from Delayed plots after the first two labora-

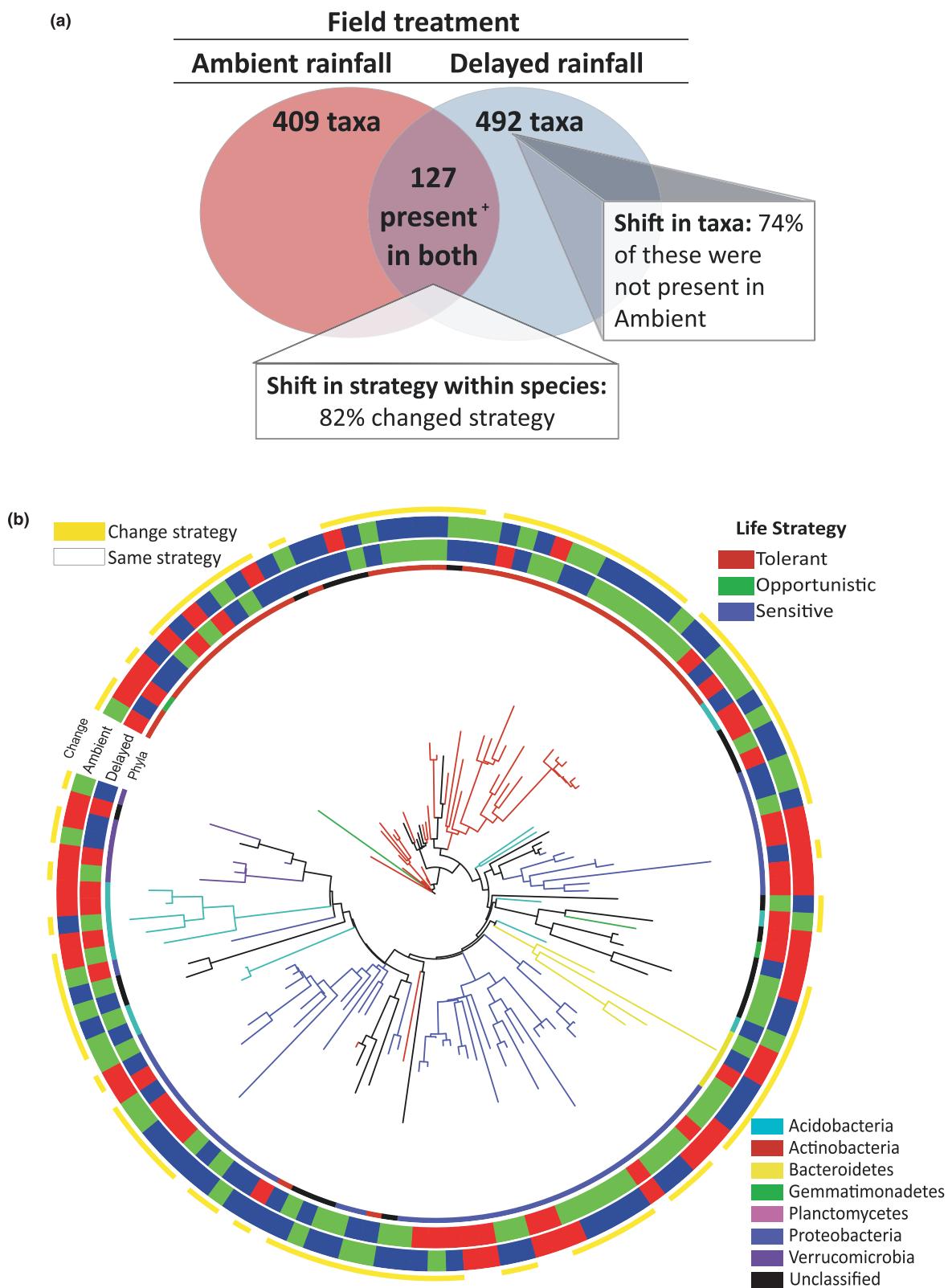


Figure 5 We found that changes in the abundance of certain life strategies, which occurred after communities were exposed to more intense rainfall (Delayed treatment), was primarily because species previously present were absent, or not abundant enough to be detected (a). However, of those 127 species that were present in both treatments [whose life strategies are plotted on the phylogenetic tree in (b)], most changed strategies (shown in yellow on outer ring), so within-species shift in strategy also contributed to the increased tolerance of the whole community to drying-rewetting.

tory drying-rewetting cycles. This functional response may be related to strategy distribution in two ways. First, taxa that are sensitive to moisture pulses may lyse upon rewetting, releasing labile carbon. The larger proportion of sensitive species in Ambient plots, and the higher dissolved organic carbon in these soils under drying-rewetting (Evans & Wallenstein 2012), suggest that shifts in life strategy could influence carbon flux through this mechanism. Second, species that are more tolerant of this moisture regime, which were more abundant in Delayed plots, could better maintain their allocation of carbon to growth pathways under stressful conditions (Schimel *et al.* 2007). This overall higher carbon use efficiency could contribute to the lower respiration pulse after rewetting observed initially in soils from Delayed plots. In our study, we did not measure 'effect traits' such as carbon use efficiency that might relate directly to function (Webb *et al.* 2010, Allison 2012). In addition, incubation design and conditions (e.g. soil storage, sieving) prevent us from fully extrapolating our results to field conditions [but see (Barnard *et al.* 2013)] or from accounting for legacies caused by physical factors (Manzoni *et al.* 2012; Navarro-Garcia *et al.* 2012)]. However, our results do suggest that the responses of taxa in a community (characterised by ecological strategy) are influenced by historical conditions, and that these responses are likely to overlap or link to traits that affect function.

The observed shift in life strategy between communities with a different precipitation history was accompanied by changes in the relative abundance of bacterial species which employed different strategies (Fig. 4). Other studies suggest that life history strategy, as well as other traits (Philippot *et al.* 2010), may be predicted by taxonomy at broad levels (Fierer *et al.* 2007; Placella *et al.* 2012), providing support for the hypothesis that shifts in species composition (e.g. as determined by 16S rRNA genes) are likely to alter the aggregate ecology, traits, or function, of whole communities (Wallenstein & Hall 2012). Our study provides overall support for these concepts. The changes in ecological strategy that we observed with moisture shifts were primarily explained by a change in species composition. Strategies were also related to taxonomy, though perhaps more weakly than in other studies (Placella *et al.* 2012), and we observed several Phyla-level responses to moisture that are consistent with previous reports (Fig. 4, pie charts) (Barnard *et al.* 2013). However, as only some strategies displayed phylogenetic coherence (Table 1), and some species switched strategy (discussed below), our findings do not suggest that extrapolation based on phylogeny is the best way to predict moisture response.

The phylogenetic relatedness of ecological properties can provide information on the ecological and evolutionary processes that influenced the emergence of these properties in taxonomic groups. We found that the extent to which ecological strategy was phylogenetically conserved varied by strategy, suggesting that different historical processes influenced the ways bacteria survive moisture stress. Species that employ traits for sensitive strategies, which were phylogenetically clustered (NRI and NTI high, $P < 0.05$, Table 1), may be more constrained by phylogeny. Therefore, the distribution of taxa we observed more likely emerged by selection through filtering (Webb *et al.* 2002), though this inference assumes that mois-

ture, the selection pressure we captured, is more important than other selection pressures like carbon limitation. In contrast, tolerant strategies were more evenly distributed throughout Phyla (NRI low, $P > 0.95$, Table 1), but clustered at finer taxonomic scales, near the tips of the phylogenetic tree (NTI high, $P < 0.05$, Table 1). The traits that give rise to tolerance to drying and rewetting may have emerged through competition between taxa with similar (conserved) traits or through more recent changes in genotypes (possibly through horizontal gene transfer). This pattern could also indicate that there are simply more ways (i.e. trait combinations) to be tolerant of drying and rewetting (Lennon *et al.* 2012; Placella *et al.* 2012), whereas the extent a species is sensitive to drying-rewetting may depend on a single trait which has evolved in only a few clades. Although phylogenetic metrics are sensitive to many factors (e.g. scale, regional pool, phylogenetic signal, see (Horner-Devine & Bohannan 2006) overall our results suggest that multiple mechanisms contribute to the way microbial moisture strategies assemble under a specific rainfall regime.

Although phylogenetic information suggests that some moisture life strategies are highly conserved, we also found evidence that the life strategy of a single bacterial species can vary depending on its climate history (and all indirect factors that accompany the shift in climate). When we examined the taxa that were present in both Ambient and Delayed treatments (Fig. 5a), we found that most of these taxa displayed different life strategies depending on the precipitation regime they had been exposed to during the previous 10 years (Fig. 5b). The observed change in strategy within a given species (with 'species' defined in this study by 97% similarity) could result from several processes. Change in strategy could be caused by phenotypic plasticity of organisms under new abiotic and biotic (competitive) conditions. However, as we did not observe any species plastic enough to remain abundant at all three time points, physiological plasticity may be unlikely to play a large role in strategy shifting, which may be influenced by many traits. It is also possible that variation in ecological strategy is expressed at a finer taxonomic level than 97% similarity, like other traits may be (Konstantinidis & Tiedje 2005; Zimmerman *et al.* 2013). In other words, patterns may have emerged from the ecological filtering of strains at a finer level of 'microdiversity' (Moore *et al.* 1998; Hunt *et al.* 2008) that we did not capture, but which future studies could quantify with new approaches (Martiny *et al.* 2013). Finally, these shifts in strategy within an organism may have emerged through evolution or horizontal transfer of genes related to moisture tolerance, without a change (or $< 3\%$ change) in the highly-conserved 16S rRNA gene that we targeted. This mechanism as an explanation for changes in ecological properties of communities has received less attention than shifts in community composition, but our results suggest this is a viable mechanism through which ecological change can occur.

Further studies are needed to evaluate the processes involved in the within-species changes in strategy we observed, but our results clearly show that shifts in ecological response to moisture stress can occur through mechanisms other than changes in the composition of taxa. Although recent studies have used metagenomics to correlatively link environmental variation (e.g. gradient of N-deposition) to both ecological properties of

microbes (as indicated by marker genes) and to microbial community composition (Fierer *et al.* 2012), whether the shift in ecological properties is caused by shifts in species composition or the strategy a species expresses remains unclear. Within-species and community shifts are controlled by different factors, and likely operate on different timescales, so their relative contribution to mediating changes in ecological properties could be important for predicting the responses of microbial communities to shifts in rainfall.

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AUTHORSHIP

SE developed the conceptual basis for this manuscript, performed the experiment and analysis, and wrote the manuscript. MW initiated soil sampling, helped conceive and set up the experiment, and edited the manuscript.

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