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Soil compartment is a major determinant of the impact of simulated rainfall on desert microbiota

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Summary

Although desert soils support functionally important microbial communities that affect plant growth and influence many biogeochemical processes, the impact of future changes in precipitation patterns on the microbiota and their activities is largely unknown. We performed *in-situ* experiments to investigate the effect of simulated rainfall on bacterial communities associated with the widespread perennial shrub, *Rhazya stricta* in Arabian desert soils. The bacterial community composition was distinct between three different soil compartments: surface biological crust, root-attached, and the broader rhizosphere. Simulated rainfall had no significant effect on the overall bacterial community composition, but some population-level responses were observed, especially in soil crusts where Betaproteobacteria, Sphingobacteria, and Bacilli became more abundant. Bacterial biomass in the nutrient-rich crust increased three-fold one week after watering, whereas it did not change in the rhizosphere, despite its much higher water retention. These findings indicate that between rainfall events, desert-soil microbial communities enter into stasis, with limited species turnover, and reactivate rapidly and relatively uniformly when water becomes available. However, microbiota in the crust, which was relatively enriched in nutrients and organic matter, were primarily water-limited, compared with the rhizosphere microbiota that were co-limited by nutrients and water.

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Introduction

Deserts are one of the most extensive terrestrial biomes, and desertification is an accelerating global problem (Makhalanyane *et al.*, 2015; Pointing and Belnap, 2012), especially in developing countries (Maestre *et al.*, 2012). However, one effect of climate change is that some deserts may have more rain in the future (Kharin *et al.*, 2007; Maestre *et al.*, 2012). There is also widespread interest in managed "greening of the desert" as a potential means to feed the growing human population, but intensive arable agriculture has a high demand for water that would generally be unsustainable in arid regions (Köberl *et al.*, 2011). However, lower intensities of watering can encourage growth of native xerotolerant plants, providing valuable food, livestock feed, biofuels, and pharmaceuticals (Köberl *et al.*, 2011). Microbes play significant roles in regulating productivity and ecological success of plants (Dimkpa *et al.*, 2009; Segura *et al.*, 2009), and water is the primary factor limiting microbial activity. Consequently, in deserts, biogeochemical processes together with soil stabilization and fertilization are predominantly regulated by the size, frequency and timing of precipitation pulses (Austin *et al.*, 2004; Büdel *et al.*, 2009; Noy-Meir, 1973; Steven *et al.*, 2015). However, the biogeochemical and bacterial responses to precipitation events *in situ* are largely unknown, and must be better understood in order to better manage future changes in water supply to arid land, whether due to altered rainfall patterns or deliberate applications to encourage the growth of plants.

One such xerotolerant plant species of medicinal and ecological importance is *Rhazya stricta* Decne (family Apocynaceae), a widely-distributed, perennial, evergreen shrub that frequently dominates arid soils in South Asia and the Middle East (Emad El-Deen, 2005; Gilani *et al.*, 2007; Marwat *et al.*, 2012). Many differentially expressed genes are regulated by the circadian clock of this C_3 plant, allowing it to adapt to an extreme but predictable environment (Yates *et al.*, 2014), where it maintains high photosynthetic capacity even when leaf temperatures

reach 48°C (Lawson *et al.*, 2014), consistent with its capacity to tap water via a deep root system. Over a hundred alkaloids have been identified as contributing to this species' medicinal properties (Marwat *et al.*, 2012). Despite the importance of *Rhazya stricta*, nothing is known about its associated soil microbiota.

In addition to their important interactions with plants, microbes play a vital role in the formation of desert soil crusts (Belnap and Lange, 2003). Carbon sequestration by desert soil crusts, together with cryptogamic cover in other biomes, is 7% of that by terrestrial plants; while biological crusts are responsible for half of the nitrogen fixation on land (Elbert *et al.*, 2012). Desert crusts have been described as "mantles of fertility" (Garcia-Pichel *et al.*, 2003), providing many ecosystem functions, including production of extracellular polymers that enhance soil water retention between and beneath plants. Soil crusts are typically dominated by photoautotrophic Cyanobacteria, but also contain a wide range of heterotrophic Bacteria, Archaea and Fungi (Abed *et al.*, 2010; Angel and Conrad, 2013; Steven *et al.*, 2013; 2014; 2015). Numerous factors have been shown to affect the distribution of microbes in deserts, including: proximity to plants (Ben-David *et al.*, 2011; Kuske *et al.*, 2002; Saul-Tcherkas *et al.*, 2013), seasonality (Saul-Tcherkas *et al.*, 2013; Yeager *et al.*, 2012), depth within and beneath soil crusts (Garcia-Pichel *et al.*, 2003; Kuske *et al.*, 2002; Steven *et al.*, 2013; 2014), and precipitation frequency (Belnap *et al.*, 2004; Steven *et al.*, 2015; Yeager *et al.*, 2012; Zelikova *et al.*, 2012). However, there are few controlled *in-situ* watering studies, with most having focussed on long-term seasonal responses (e.g. Yeager *et al.*, 2012) or specific processes, such as CO_2 flux (Sponseller, 2007).

The primary goal of this study was to understand how soil bacterial communities within the different soil compartments associated with *Rhazya stricta* respond to simulated rainfall events. We investigated a location (Baharah) in the Arabian Desert, one of the world's driest (60–250 mm annual precipitation; Noy-Meir, 1973) and most extensive (2,330,000 km^2) deserts. We focussed on Bacteria, because they dominate desert soils (Steven *et al.*, 2014; and analysis shown here). Respiration and photosynthesis commence in desert soils within an hour of wetting (Austin *et al.*, 2004), and so we hypothesise that bacterial communities after both single and repeated watering events would be compositionally similar to those in dry desert soils; i.e. because rainfall events are rare and episodic, and water is lost rapidly from the soil by evaporation and percolation, the capacity to withstand desiccation and grow immediately after hydration will be an innate and essential adaptation mechanism for desert microbes.

We tested this hypothesis in the context of other factors that have been shown to affect the distribution of microbes in deserts, comparing bacterial communities in three soil compartments: surface biological crust, within the root zone but not attached to roots (rhizosphere), and from root shavings of *Rhazya stricta*. Importantly, these experiments were performed *in situ* in order to: (1) allow natural evaporation and percolation processes, (2) maintain the soil's vertical structure, redox states and nutrient fluxes, and (3) to retain any connectivity with *Rhazya stricta*. We performed a suite of physico-chemical analyses in order to determine the potential drivers for bacterial community composition and diversity. Our specific hypothesis was that the bacterial community composition and richness would be more similar between watered and dry samples within soil compartments than across the different soil compartments (i.e. crust, rhizosphere and root shavings).

This hypothesis was tested using two watering regimes at the same location in different years (see Experimental Procedures and Fig. 1 for full details). In Experiment 1, soil was watered, and samples of crust, rhizosphere and root shavings were taken two days afterwards to investigate relatively short-term responses. In Experiment 2, soil was watered one, two or three times over a 3-week period prior to sampling crust and rhizosphere in order to investigate longer-term responses to repeated watering. In all cases, simultaneous dry control samples were taken, and each single watering event corresponded to 50 mm of rainfall, representing a typical, heavy downpour in late autumn or winter in this location.

Results

Comparison of dry crust, rhizosphere, and root - attached bacterial communities

The soils were made up primarily of sand and silt (60–70%) with lesser proportions of gravel and clay (Supporting Information Fig. S1a). There was significantly more clay (*t* test; $P < 0.001$) and less gravel (*t* test; $P < 0.05$) in the crust than in the rhizosphere (Supporting Information Fig. S1a). The rhizosphere and crust were (bio)chemically distinct soil compartments (Supporting Information Figs. S1b,c). Crusts had much higher concentrations of organic matter (dissolved organic carbon (DOC), 4-fold), and nitrogen (dissolved organic nitrogen, 14-fold; ammonia, 25-fold; nitrate, 26-fold; Supporting Information Figs. S1b,c). The pH of the crust (7.41 ± 0.06) was lower than that of the rhizosphere (8.42 ± 0.04), and the concentration of water-soluble major ions was higher in the crust (Supporting Information Figs. S1b,c). When acidified for DOC analysis, all crust samples effervesced, suggesting the presence of calcium carbonate.

Rarefied bacterial OTU richness was significantly lower in the crust than the rhizosphere (ANOVA, $F_{3,34} = 15.94$, $P < 0.001$; Tukey HSD, $P = 0.001$) and root-shaving samples ($P = 0.037$). The root-shaving OTU richness was not

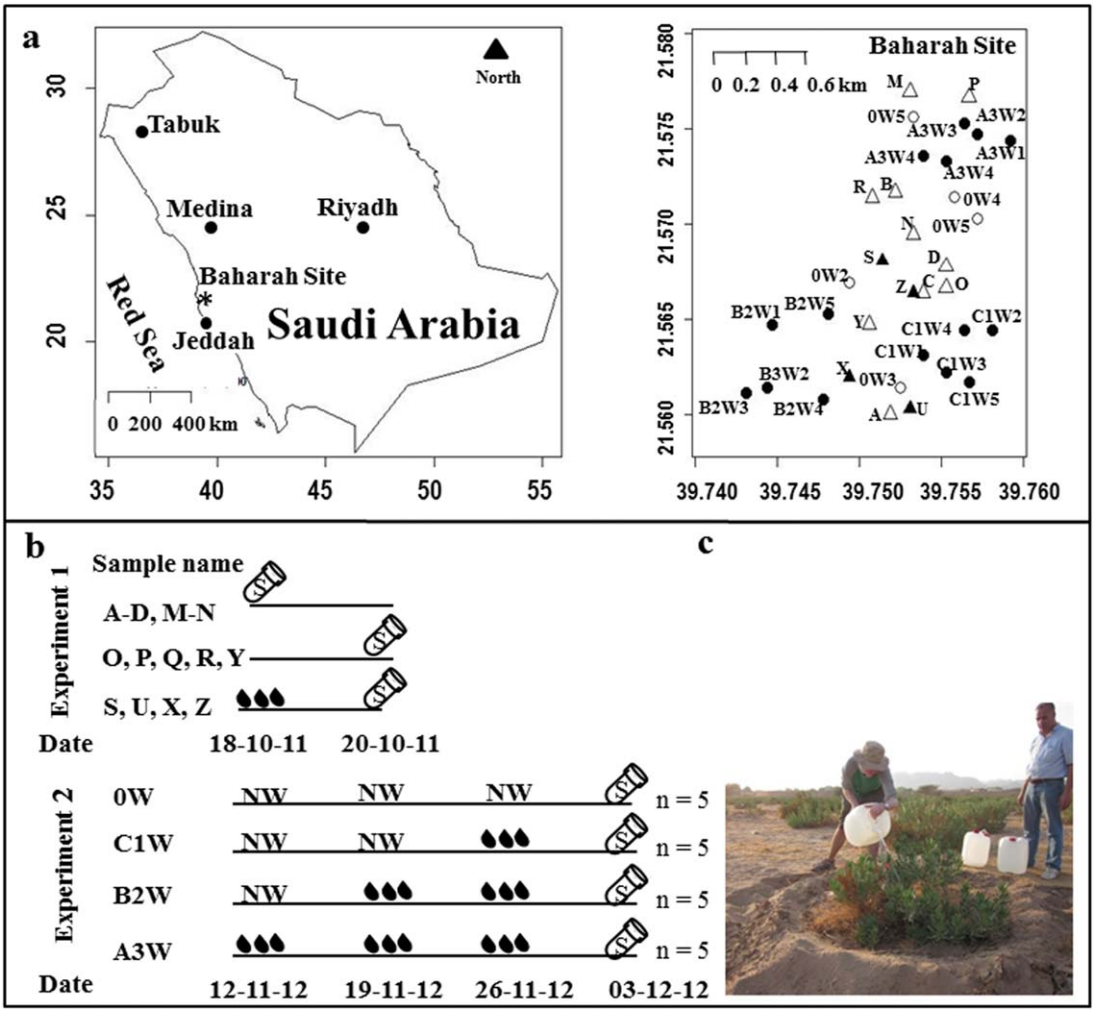

Fig. 1. Summary of the experimental design and location of sampling site: (**a**) Maps showing: the location of desert field site, Baharah ~55 km from Jeddah in Saudi Arabia; and the relative positions of the plants, beneath which soil samples were taken (triangles and circles represent soil sampled for Experiment 1 in 2011 and for Experiment 2 in 2012, respectively; filled symbols represent samples that were watered to simulate rainfall, while unfilled symbols represent samples that were not watered). (**b**) Schematic representation of the design of the watering experiments, and indication of the symbols used as prefixes to refer to samples (corresponding with plants) and their treatments. Raindrop symbols indicate a watering event (50 L per plant), NW indicates no watering, and the tube symbols show when the soil sample was taken. In Experiment 1 samples were taken from the surface biological crust (referred to as *Crust*), soil at ~6–20 cm below the surface within the broad root zone of the plant (referred to as rhizosphere soil, *Rhizo*), and from roots plus attached soil that were scraped with a scalpel (referred to as *Root*). In Experiment 2, only Rhizo and Crust samples were taken. Slight deviation in some reported numbers is due to sample loss in transit. (**c**) Illustration of a *Rhazya stricta* plant being watered; note that the soil around the plant was raised to ensure that the water was retained beneath the plant.

significantly different from that of the rhizosphere (Fig. 2a). Actinobacteria and Alphaproteobacteria were the dominant classes in the three soil compartments (Supporting Information Table S1). However, multidimensional scaling (MDS) analysis shows distinct bacterial communities in the crust, rhizosphere, and root shavings (Fig. 2b); and PERMANOVA analysis confirms that soil compartment is a significant factor in structuring the bacterial communities ($F_{3,34} = 7.648$, $P < 0.001$). The major phylum contributing to this difference (albeit with great variability) was the Cyanobacteria, almost exclusively from the order Oscillatoriales, with high abundance (12.1%) in the crust compared with other soil compartments (< 0.1%) (Supporting Information Fig. S2a; Table S1). Overall, there was no significant difference in the relative abundance of Actinobacteria between crust and rhizosphere (data not shown), but this masked the fact that Actinomycetales were much more abundant in the crust, while other actinobacterial orders were more abundant in the rhizosphere (Supporting Information Fig. S2a). In particular, Actinomycetales-related OTUs belonging to *Arthrobacter* and Microbacteriaceae were highly enriched in the crust

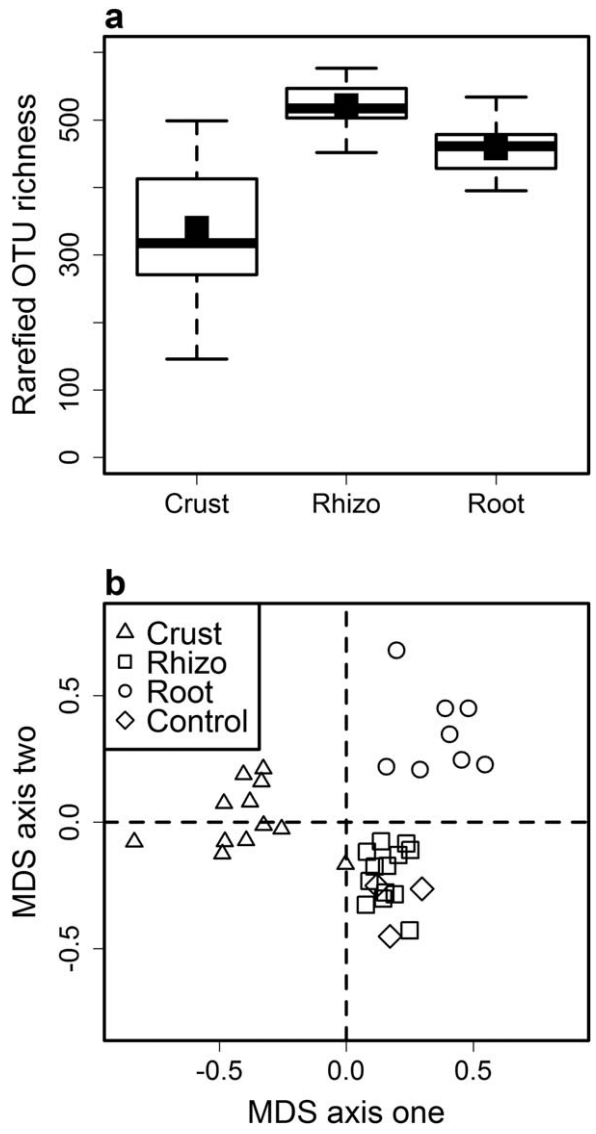

Fig. 2. Differences between bacterial communities from dry samples associated with *Rhazya stricta* from Experiments 1 and 2 combined, comparing sample compartments: surface soil (Crust), soil within the root zone (Rhizo), and root shavings (Root). **(a)** Bacterial rarefied OTU richness in which boxes represent the median and 25-75 percentiles, bars represent minima and maxima and filled squares represent the mean. **(b)** Multidimensional scaling (MDS) plot, based on Bray–Curtis distance of the OTU-abundance matrix, showing differences in bacterial community composition. The samples marked as "control" were taken at the same depth as the Rhizo samples, but were at least 2 m from any plant. Here, and throughout the paper, OTUs are defined as having $>95\%$ identity in 16S rRNA gene sequence.

(Supporting Information Fig. S3a). Sphingobacteria were also relatively more abundant in the crust (4.0%) compared to the rhizosphere (0.7%) (Supporting Information Fig. S2a; Table S1). In contrast, Acidobacteria were much more abundant in the rhizosphere (12.0%) than the crust (2.2%), despite the rhizosphere being more alkaline, as were the Planctomycetes (3.4% in rhizosphere, 0.6% in crust) (Supporting Information Fig. S2a; Table S1).

There was clear evidence that close association with, or attachment to, the roots of *Rhazya stricta* selected for specific bacterial groups, when compared with the general vicinity of the roots. Most notably, roots were dominated by Actinomycetales (37.6% in roots and 16.6% in rhizosphere), and were relatively diminished in Acidobacteria (2.7% in roots and 12.0% in rhizosphere) (Supporting Information Fig. S2b; Table S1). A very abundant Actinomycetales-related OTU belonging to the genus *Kibdelosporangium* was 8-fold more abundant on/in the roots (Supporting Information Fig. S3b). The Alphaproteobacteria as a whole were not significantly differentially abundant (Supporting Information Fig. S2b), but two OTUs belonging to the Rhizobiales were more abundant on/in the root than in the rhizosphere (Supporting Information Fig. S3b).

MDS analysis showed that bacterial communities from soils at the same depth as rhizosphere samples, but not associated with any plant, clustered with bacterial communities from the *Rhazya stricta* rhizosphere (Fig. 2b); and there was also no significant difference in physico-chemical properties (Supporting Information Table S2). However, microbial biomass was about 3.5 times higher in the rhizosphere (Supporting Information Table S2).

Factors influencing the bacterial community composition

A range of physico-chemical parameters was measured in soils before and after watering (see Fig. 3 for a selection). Mantel tests showed that soil pH was the parameter that correlated most significantly with bacterial community composition ($P < 0.001$; Fig. 4a), but see the Discussion for an appraisal of the relevance of this correlation. Interestingly, the instantaneous measurement of water content did not correlate with bacterial community composition (Fig. 4a). Redundancy analysis, which allows both the inclusion of watering as a presence–absence variable and factors that incorporate multiple variables, showed that the best predictor of bacterial community composition was soil compartment (Fig. 4b).

Impact of simulated rainfall on soil chemistry and bacterial communities

Watering of the soils (Fig. 1) affected the crust and rhizosphere in different ways. In the crusts there was a significant increase in water content ($\%H_2O$), from 1.6 to 6.5%, 2 days after watering (Fig. 3, Experiment 1). There was no observable change in crust water content in Experiment 2 when the interval between watering and sampling was 1 week, but the crusts had retained some moisture as

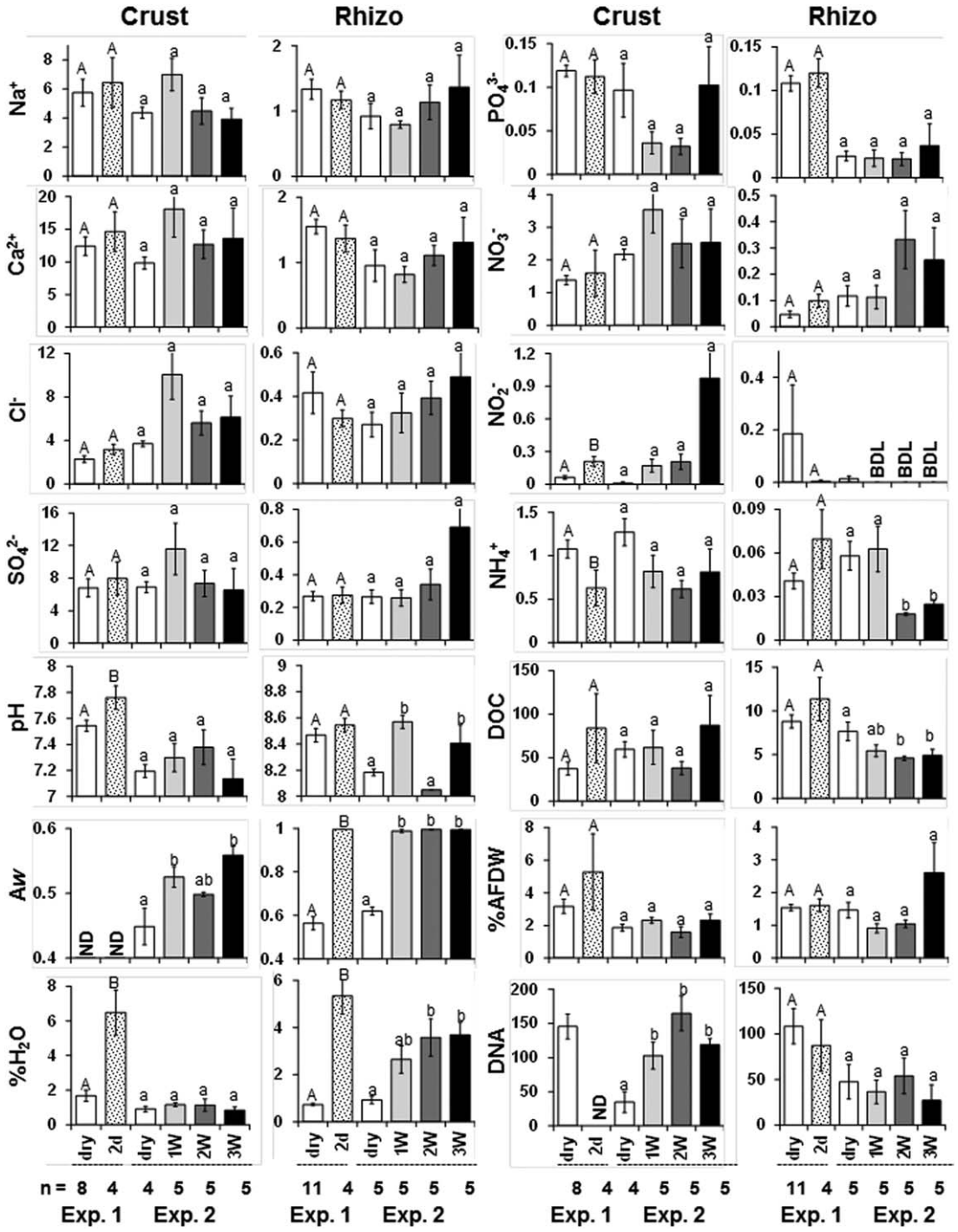

Fig. 3. Impact of watering in Experiment 1 and Experiment 2 on the chemistry of crust and rhizosphere (Rhizo) soils beneath *Rhazya stricta* (see Fig. 1 for details of the experimental design. Codes: 2d = sample taken 2 days after watering; 1W = sample taken 1 week after watering, 2W = sample taken 1 week after 2 weekly waterings, 3W = sample taken 1 week after 3 weekly waterings). Units are μmol g^{-1} dry soil for all ions and DOC (dissolved organic carbon). DNA concentration is given as ng g^{-1} dry soil. Significant differences ($P < 0.05$) among experimental conditions are shown by different letter codes (there is no significant difference between data with same letter code). Capital letters are used for Experiment 1, and small letters are used for Experiment 2. The number of samples for each treatment (*n*) is shown at the bottom of each bar and the error bars indicate the standard error; however, the value of n differed from the indicated values for the DNA concentrations: 6 for crust Exp-1 dry, 5 for crust Exp-2 dry, and 10 for rhizo Exp-1 dry. ND and BDL indicate that the measurements were not determined or below detection limit, respectively. Data for Mg^{2+} is not shown, but the pattern of its change in concentration almost exactly matches that for Ca^{2+}, and its concentration compared with Ca^{2+} is ~5-fold lower in the crust and ~10-fold less in the rhizosphere.

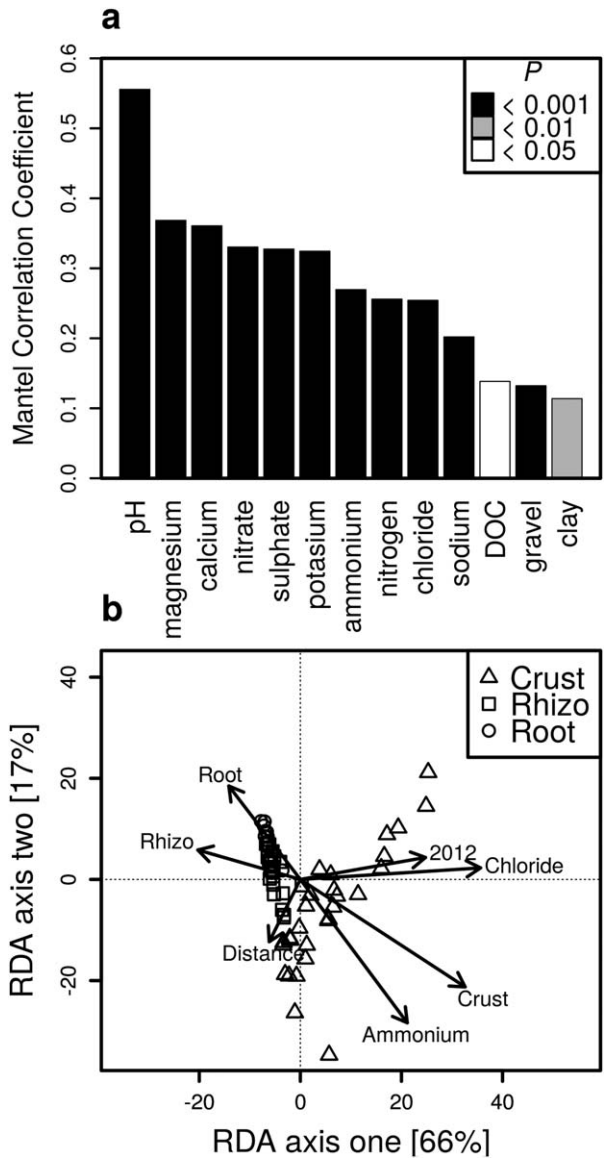

Fig. 4. Environmental variables significantly influencing bacterial community composition. **(a)** Mantel tests revealed significant correlations between changes in 13 separate environmental variables and turnover in bacterial communities between samples. Mantel correlation coefficients and associated significance values were calculated across factors (i.e. analyses were performed on all samples regardless of soil compartment (crust, rhizo, or root), watering treatment, or sampling year). Nitrogen = dissolved organic nitrogen. **(b)** Redundancy analysis (RDA) significantly explained 83% of the variation within the bacterial species-environment relationship across the first two ordination axes. Fixed factors (soil compartment, watering treatment and sampling year) and all other continuous environmental variables (distance, gravel, sand, silt, clay, pH, moisture, AFDW, sodium, potassium, magnesium, calcium, ammonium, nitrite, nitrate, sulfate, chloride) were included in the initial RDA. Examination of variance inflation factors and stepwise forward selection of explanatory variables revealed a parsimonious RDA model that included the following significant predictors of bacterial community composition: soil compartment ($F_{3,59} = 4.08$, $P = 0.01$), chloride ($F_{1,59} = 26.24$, $P = 0.01$), NH_4^+ ($F_{1,59} = 5.83$, $P = 0.02$), sampling year ($F_{1,59} = 2.52$, $P = 0.05$), distance from road ($F_{1,59} = 2.59$, $P = 0.08$).

evidenced by the small but significant increase in A_w (Fig. 3). In contrast, water content and A_w were significantly higher in the watered, compared with the dry, rhizosphere soils, with an A_w of 0.994 (Fig. 3) in the watered treatment in both Experiments 1 and 2. The only significant watering-induced changes in the soil crust chemistry were an increase in pH, and a decrease in ammonia and increase in nitrite concentrations [significant ($P < 0.01$) in Experiment 1 only; Fig. 3]. In the rhizosphere, significant changes in chemistry were seen only in Experiment 2 when samples had been watered twice or three times, in which there were fluctuations in pH, a decrease in concentrations of DOC and ammonia and an increase in nitrate (Fig. 3). In Experiment 2, DNA concentration (a proxy for microbial biomass) increased approximately threefold in the watered crusts, but did not change significantly in the watered rhizospheres (Fig. 3).

Watering elicited a small but significant change in rarefied bacterial OTU richness in Experiment 1 (ANOVA; $F_{1,37} = 5.98$, $P = 0.02$), which was due to the decrease in richness in the water-treated rhizosphere samples (Fig. 5a); and a borderline significant change due to watering was seen in Experiment 2 (ANOVA; $F_{1,26} = 4.39$, $P = 0.05$; Fig. 5b). Importantly, MDS analysis confirmed that the bacterial community composition was driven primarily by soil compartment (PERMANOVA; $F_{3,37} = 7.73$, $P < 0.001$ in Experiment 1 and $F_{1,26} = 19.04$, $P < 0.001$ in Experiment 2), compared with watering ($F_{1,37} = 1.82$, $P = 0.05$ in Experiment 1 and $F_{1,26} = 2.31$, $P = 0.05$ in Experiment 2; Figs. 5c,d).

In agreement with the overall minor effect of watering on bacterial community composition (Figs. 5c,d), far fewer taxa had significantly different abundances between wet and dry treatments (Fig. 6) compared with differences due to soil compartment (Supporting Information Fig. S2). More crust taxa (Figs. 6c and d) were affected by watering than rhizosphere taxa (Figs. 6a and b), and there was no significant water-induced change in root-attached taxa (hence there is no "root" panel in Fig. 6). The relatively modest effect of watering on bacterial community composition, especially on the root and rhizosphere samples, was even more apparent when examining differences in the relative abundance of OTUs (95% similarity level). For example, when comparing wet *versus* dry samples (combined years) the number of OTUs that were significantly differentially abundant ($P < 0.01$) were: 16 for crust, three for rhizosphere, and zero for root samples. This is in contrast with 49 differentially abundant OTUs between dry crust and dry rhizosphere samples.

After watering, the Betaproteobacteria (primarily Burkholderiales (Supporting Information Table S3) and especially the genus *Massilia*; Supporting Information Figs. S4c and d) increased in relative abundance,

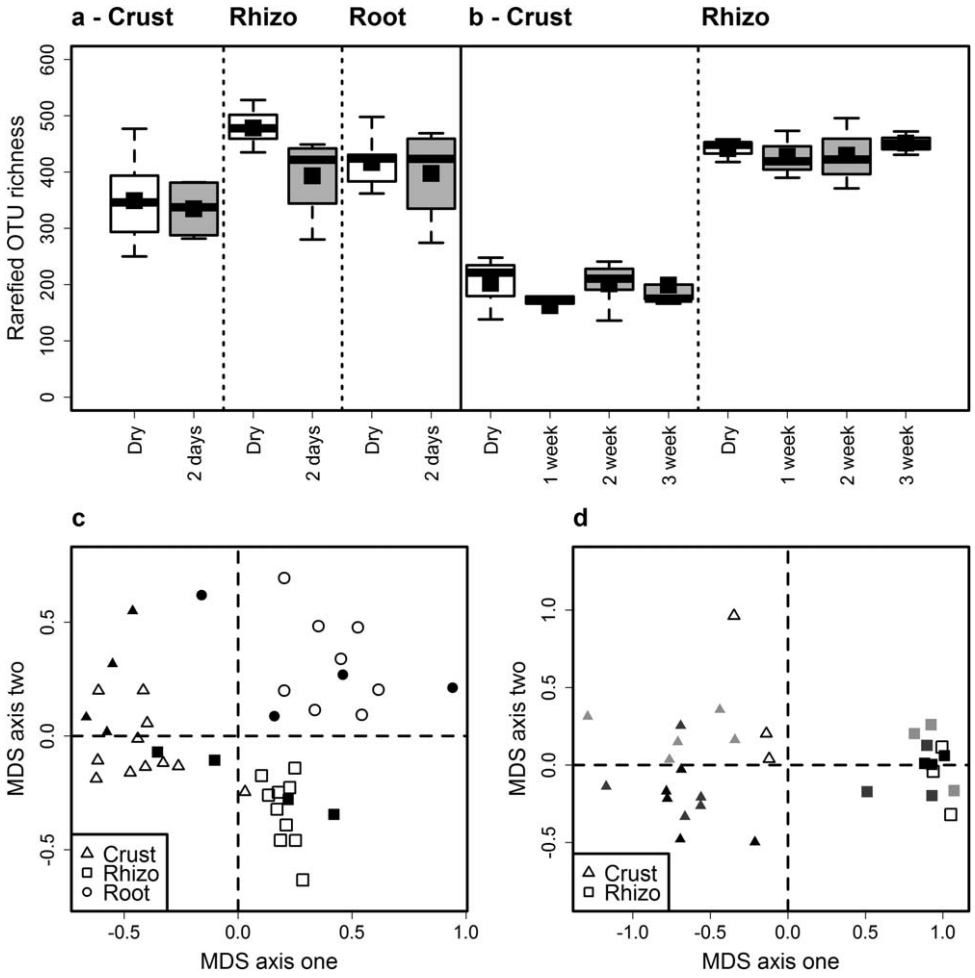

Fig. 5. Impact of watering on bacterial communities in soil associated with *Rhazya stricta* (see Fig. 1 for details of the Experimental Design). Impact of different watering regimes on rarified OTU richness in **(a)** Experiment 1: Crust, rhizosphere (Rhizo) and root shavings (Root), and **(b)** Experiment 2: Crust and Rhizo samples. Boxes represent the median and 25–75 percentiles, bars represent minima and maxima and filled squares represent the mean. Multidimensional scaling (MDS) plot illustrating the impact of watering on bacterial community composition in: **(c)** Experiment 1 (crust, rhizosphere and root shavings), and **(d)** Experiment 2 (crust and rhizosphere). Unfilled symbols represent dry samples. Filled symbols represent watered samples; shading (light gray, gray, black) in (d) indicates different degrees of watering (1, 2, and 3 weeks, respectively). Crust samples are shown as triangles, rhizosphere samples as squares and root shaving samples as circles.

especially in the crusts (Figs. 6c and d). The Sphingobacteria (primarily *Adhaerobacter* spp.; Supporting Information Fig. S4d) also increased significantly in relative abundance after simulated rainfall in the crust (both experiments) and rhizosphere (Experiment 2 only) (Fig. 6). In the crust, there was a large and significant increase in relative abundance of Bacilli in Experiment 2 (Fig. 6), from 11.0% in dry samples to 46.1% 1 week after a single watering event, followed by a decrease to 19.9% after further watering (Supporting Information Table S3). In Experiment 1, the approximate doubling of Bacilli in wet crusts (Supporting Information Table S3) was not statistically significant ($P = 0.175$). The Bacilli OTUs that increased most with watering were from the genera *Bacillus* and *Planomicrobium* (Supporting Information Fig. S4d). Also in the crust, in Experiment 1 there was a decrease in cyanobacterial relative abundance after watering, and in Experiment 2 cyanobacterial relative abundance decreased from 20.5% in dry samples to 1.0% 1 week after a single watering event (Supporting Information Table S3), but neither of these changes was statistically significant. However, one cyanobacterial OTU was significantly more abundant in the dry crust compared with the wet crust in Experiment 1 (Supporting Information Fig. S4c).

Metagenome analysis

In order to assess how the microbes inhabiting the crust from the Saudi Arabian desert compared with those from other locations, a sample was subjected to metagenomic sequencing and analysis of small-subunit rRNA gene fragments. The Baharah desert crust had a bacterial community composition that clustered within those from other deserts (Supporting Information Figs. S5 and S6), and that was most similar to North American desert bacterial communities (Nevada, Mojave, Chihuahua in Supporting Information Fig. S6). The majority of the metagenomic marker gene fragments were derived from Bacteria (92.5% of 9,989 small subunit ribosomal RNA gene fragments), with 0.06% from Archaea and 7.4% from Eukarya (mainly Fungi) (Supporting Information Fig. S7). This analysis allows us to be confident that the desert soil microbiome studied here is not atypical, and that we were justified in focussing our analysis on the bacterial community.

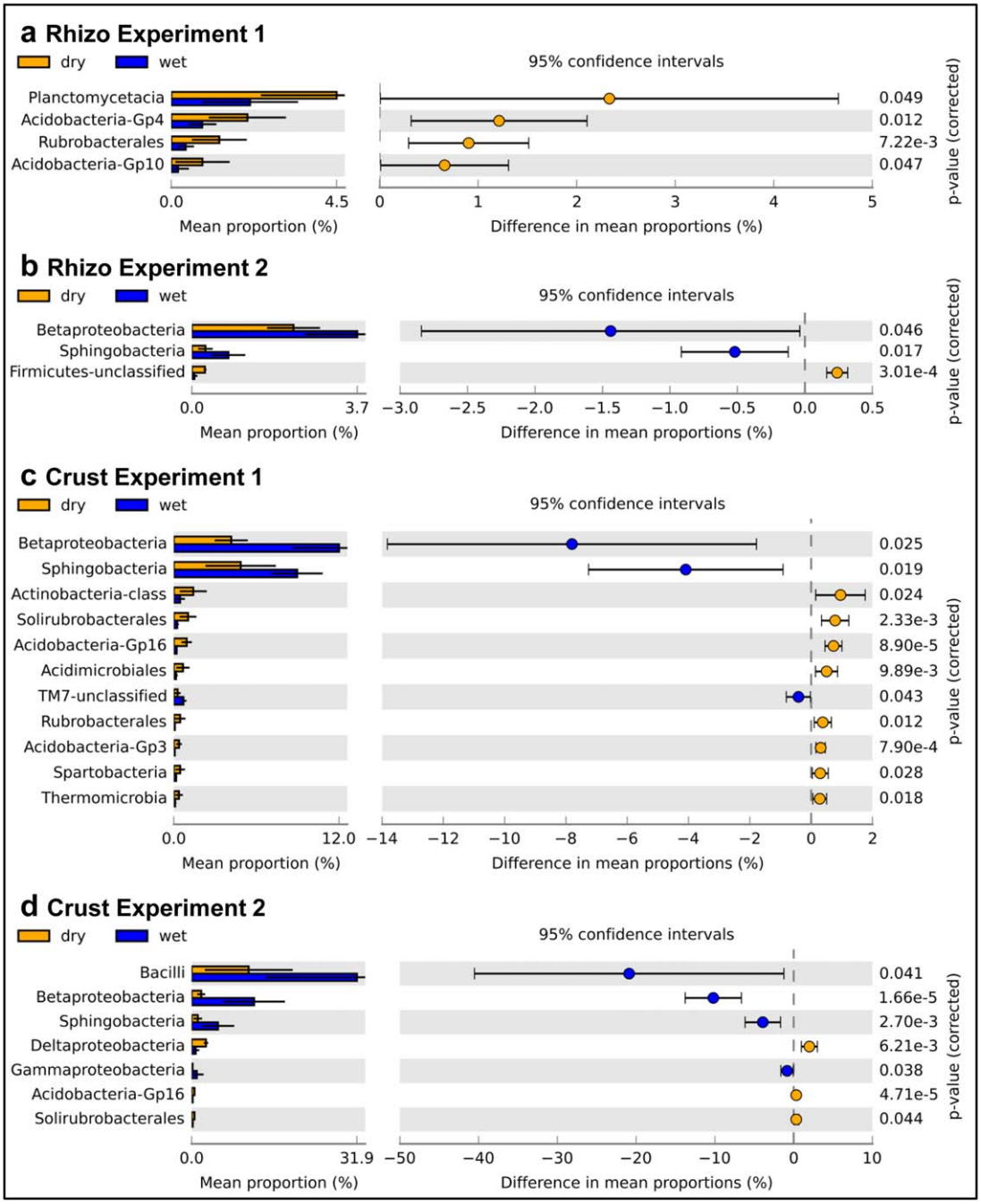

Fig. 6. Comparison of the taxa (hierarchy level 3; see Supporting Information Table S6) that are differentially abundant in wet versus dry soil, specifically: **(a)** Rhizosphere Experiment 1, **(b)** Rhizosphere Experiment 2, **(c)** Crust Experiment 1, **(d)** Crust Experiment 2. No taxa had significantly different abundance when wet and dry root shavings were compared. Analysis was performed using STAMP (Parks *et al.*, 2014) with default parameters, except that parameters for filtering out were: P value > 0.05; difference between proportions < 0.2 or difference between ratios < 1.5. Data were sorted according to effect size. Note the differences in the scale of the x axes.

Discussion

Factors driving niche partitioning in desert microbiota

Considering the dry soil data only, the pattern of high-level-taxon community composition observed is typical of most hot deserts across the globe, namely the dominance of Actinobacteria and Alphaproteobacteria in all sample types (collectively 48–64% of the Bacteria), and the more location- and time-specific abundance of Bacilli, Acidobacteria, Sphingobacteria, and Cyanobacteria (Abed *et al.*, 2012;

Andrew *et al.*, 2012; Angel and Conrad, 2013; Favet *et al.*, 2013; Fierer *et al.*, 2012; Köberl *et al.*, 2011; Makhalanyane *et al.*, 2015; Saul-Tcherkas and Steinberger, 2011; Saul-Tcherkas *et al.*, 2013; Steven *et al.*, 2013; 2014). Hot desert soils have a microbiota that is distinct from other soil types (Fierer *et al.*, 2012), and can be transported globally on wind-blown sediment particles (Favet *et al.*, 2013). Despite the capacity for global transport, metagenomic analysis revealed that the microbial community in the Baharah crust was distinct and equally different from communities in several north American deserts, and more different from the microbiota in extremely arid and saline deserts, such as the Atacama and Kutch.

We observed niche partitioning spatially, with the three soil compartments investigated—, crust, broad rhizosphere and root-attached—, housing distinct bacterial communities. Differences in community composition in these soil compartments were driven mainly by changes in the relative abundance of shared OTUs. Such differences have been reported between crust and deeper desert-soil communities (Kuske *et al.*, 2002; Steven *et al.*, 2013). Apart from the higher abundance of Cyanobacteria in the crust, the most notable difference in bacterial community composition was a six-fold lower abundance of Acidobacteria in the crust compared with the rhizosphere, most pronounced for the more alkalitolerant group 6 (Rousk *et al.*, 2010). Similarly, Steven *et al.* (2013) found that Acidobacteria (primarily groups 4 and 6) was the most depleted phylum in the crust compared with deeper desert soils. Superficially, this might suggest a direct impact of pH, which is approximately 1 unit greater in the deeper soil. However, it is more likely to be because members of this widespread, versatile and heterotrophic phylum are more competitive in low-nutrient soils (Fierer *et al.*, 2007), which is consistent with the presence of high-affinity ABC transporters for sugars in genome-sequenced isolates (Ward *et al.*, 2009). This conclusion is further supported by the observed ~4.5-fold decrease in Acidobacteria relative abundance attached to roots compared with the broader rhizosphere of *Rhazya stricta*.

The selection for specifically adapted endophytic and epiphytic bacteria in the root-attached samples compared with the broader rhizosphere is likely to have contributed to the difference in community composition, as found with *Agave* species (Coleman-Derr *et al.*, 2016; where they refer to the broad rhizosphere as "root-zone soil"). The main OTUs that were preferentially attached to or within the root compared with the broader rhizosphere belong to the orders Actinomycetales, Rhizobiales, and Sphingobacteriales, all of which were also more abundant in the crust than the rhizosphere, suggesting a preference for nutrient-rich environments, irrespective of whether the nutrients are supplied by Cyanobacteria in the crust or the roots of *Rhazya stricta*. The Rhizobiales and Sphingobacteriales were also the most enriched bacterial taxa on roots compared with the surrounding soil from a high-altitude cold desert (Angel *et al.*, 2016). The dominant root-associated OTU belongs to the genus *Kibdelosporangium*, which is found in diverse soils, specifically in association with plant roots, including in arid regions (Chowdhury *et al.*, 2009; Qin *et al.*, 2015). The nature of any beneficial (or even antagonistic) interaction between *Kibdelosporangium* species and *Rhazya stricta* merits further investigation. It may be that the variety and abundance of alkaloids and other metabolites (Marwat *et al.*, 2012) found within, or excreted from, *Rhazya stricta* roots select for particular microbes able to degrade or modify them, further encouraged by a local supply of moisture.

Based on the Mantel test, pH was a major correlating factor for the observed differences in bacterial community composition, as found in many studies (Andrew *et al.*, 2012; Dumbrell *et al.*, 2010; Lauber *et al.*, 2009). It is difficult to ascertain the extent to which pH is the cause or effect of differences in bacterial community composition, and the degree to which it correlates with other factors must be considered. When a stepwise model reduction approach was used (i.e. RDA), removing highly covarying factors, the significance of pH was diminished. Indeed, a multitude of inter-related factors will be responsible for shaping these microbial communities, including light, which drives photosynthesis that in turn produces organic carbon, enhances cyanobacterial nitrogen fixation, and leads to sediment stabilisation by EPS (Mazor *et al.*, 1996). All these factors create a distinct and more nutrient-rich environment in the crust compared with the soil below (Garcia-Pichel *et al.*, 2003; Yu *et al.*, 2014). Both fine-grained sediments and EPS inhibit water movement through the soil (Mazor *et al.*, 1996), reducing nutrient loss and compartmentalising microbes, as seen in our study. Thus, topographical features of exposed and flat desert soils, as studied here, allow build-up of fine-grained sediments, and promote the creation of distinct niches and microbial communities.

The presence of plants may influence the microbiota below by providing organic matter from root exudates and leaf falls (Saul-Tcherkas *et al.*, 2013). Although we detected a 3.5-fold higher microbial biomass in the rhizosphere of *Rhazya stricta* compared with similarly deep soil 2 m away from a plant, there was no significant difference in bacterial community composition. Contrasting results have been found in studies from other deserts. For example, soil bacterial communities beneath shrubs (Ben-David *et al.*, 2011) and grasses (Kuske *et al.*, 2002) differed from those in the inter-shrub soils. However, geographic location (Andrew *et al.*, 2012) and season (Saul-Tcherkas *et al.*, 2013) had a much greater influence on bacterial community composition than whether the sample was from the rhizosphere. Also, Nagy *et al.* (2005) found that desert

crust communities were the same beneath and between shrubs. Thus, *Rhazya stricta* affects bacterial community composition, but its zone of influence is restricted to the immediate vicinity of its roots, as found with *Agave* species (Coleman-Derr *et al.*, 2016).

Impact of simulated rainfall on soil bacterial communities

We proposed that watering would not greatly alter the desert soil bacterial community composition, based on the notion that successful desert Bacteria (the dominant biota) will be adapted to pulses of rain and respond rapidly and equally to its arrival, and survive its absence. Our data support the hypothesis that desert soil compartment (i.e. crust, broad rhizosphere and root shavings) is a bigger driver of bacterial community composition and richness than whether soil has received large pulses of water. However, simulated rainfall resulted in chemical changes and population-level responses that differed between the soil compartments under investigation, as discussed below.

Are heterotrophic bacteria water-limited in the crust, and (after water addition) primarily limited by organic carbon and nutrients in the rhizosphere? Microbial biomass was approximately threefold higher in the watered, compared with the dry, crust (data available only for Experiment 2). The concentration of extractable DNA, a proxy for microbial biomass, has been shown to correspond well with direct cell counts in desert soils (Garcia-Pichel *et al.*, 2003). This increase in biomass is likely to be caused primarily by an initial burst of cyanobacterial activity upon watering, leading to release of extracellular polymeric substances (EPS), compatible solutes and other organic molecules from viable and lysed cells (Borken and Matzner, 2009; Yeager *et al.*, 2012), which would fuel the growth of heterotrophic Bacteria. OTUs that became more abundant after hydration were from chemoorganoheterotrophic taxa, most notably Bacilli, Burkholderiales, and Sphingobacteria, each of which more than doubled its relative number of sequences after watering (in Experiment 2), corresponding with a greater than six-fold increase in absolute sequence abundance. The main taxa that increased with hydration were *Massilia* (Burkholderiales), *Adhaeribacter* (Sphingobacteria), *Planomicrobium*, and *Bacillus* (Bacilli). Representatives of these four genera have been found in diverse environments, including deserts, and they can use a wide variety of organic compounds as sources of carbon and energy. The biggest water-induced increase was found for an OTU belonging to *Massilia*. Our study provides further evidence for resilience of *Massilia* species, which do not form spores, yet can withstand extremely arid conditions (see Ofek *et al.*, 2012) and were common in desert hypoliths (Pointing *et al.*, 2007). *Massilia* are also commonly associated with (and presumably feed on the products from) the rhizosphere (Ofek *et al.*, 2012) and cyanobacteria (Salomon *et al.*, 2003), and they are generally considered to be fast-growing microbes, capable of moving rapidly through sandy soil microcosms (Wolf *et al.*, 2015). Combinations of these features are likely to contribute to the rapid water-induced reactivation of *Massilia* in the crust.

Focussing primarily on the three-week watering experiment (Experiment 2); in contrast to the crust, there was no significant increase in microbial biomass in the rhizosphere and far less change in the relative abundance of particular bacterial taxa upon hydration (although small, significant increases in Betaproteobacteria and Sphingobacteria were seen). This is despite the fact that water was not a limiting factor, with a high soil A_w of 0.994 even 7 days after watering; having increased from a value of 0.62, which is close to the threshold of 0.61, below which microbial growth has not been detected (Stevenson *et al.*, 2015). The OTUs that increased in abundance in the watered crust (described above), were also present in the rhizosphere (data not shown), but did not become more abundant after watering in this deeper region of the soil. Taken together, the evidence supports the idea that increased availability of phototroph-derived organic matter fuelled the growth of the aforementioned taxa in the watered crust, but was insufficient to allow growth to anything like the same extent in the rhizosphere.

Using samples of arid desert crust, Angel and Conrad (2013) observed no change in microbial biomass after laboratory watering, but did see a marked change in active microbial community composition. Thus, the lack of increase in microbial biomass in the rhizosphere after watering does not necessarily imply microbial inactivity. However, the absence of net microbial growth in the rhizosphere corresponds with no significant change in community composition and few significant population changes, despite being fully hydrated. We are confident that the negligible change observed is not a consequence of using DNA, rather than ribosomal RNA, as the basis of our community analysis, because: (1) our method was sensitive enough to detect more significant population changes in the crust after watering; (2) sufficient time had elapsed in the water-saturated rhizosphere soils to allow microbial growth and so multiplication of DNA; (3) community changes similar to those seen in our study (e.g. hydration-induced increase in Burkholderiales) were observed in another desert crust using ^{18}O-labelled-water RNA-stable-isotope-probing (Angel and Conrad, 2013); and (4) in arid environments DNA-based community analysis has proven to be as robust as that derived from ribosomal RNA (Angel *et al.*, 2013). There are thus two explanations, which are not mutually exclusive, as to why the bacterial community did not change significantly in the watered rhizosphere: either there was a limiting factor

other than water, or cells were active but not multiplying. The soluble inorganic nitrogen concentrations in the rhizosphere were 20 times lower than in the crust, and the inorganic N:P ratio was 8 in the rhizosphere and 32 in the crust, indicating that N is more likely than P to be a limiting nutrient in the rhizosphere. This is consistent with the near absence in the rhizosphere of cyanobacteria, a major contributor to N_2 fixation in desert soils (Abed *et al.*, 2010; Johnson *et al.*, 2005).

Evidence for bacterial activity in the rhizosphere comes from the water-induced fluctuation in pH of 0.5 units and the significant decrease in DOC concentration. After watering, concentrations of ammonia in the rhizosphere also decreased significantly, nitrite decreased to below the limit of detection, and there was a corresponding non-significant increase in nitrate, which are collectively indicative of nitrification. In the rhizosphere, *Nitrospira*, which can oxidise nitrite and potentially carry out complete nitrification from ammonia (Daims *et al.*, 2015), was present (0.12–0.34%), as was the ammonia-oxidising genus, *Nitrosospira* (but in low abundance); Archaea were not targeted and so the presence of ammonia-oxidizing Archaea cannot be ruled out, especially as they are as abundant as bacterial ammonia oxidizers in warmer arid soils (Marusenko *et al.*, 2013). The water-induced stimulation of nitrification in the rhizosphere indicates that this process is not restricted to the crust, in contrast with the findings of Johnson *et al.* (2005). The non-significant decrease in the concentration of biologically inactive ions such as chloride in the watered crust, and their tendency to increase in the rhizosphere, provide tentative evidence that organic matter and nutrients leach from the crust and provide a means to sustain microbial activity in the rhizosphere, as proposed by Yu *et al.* (2014). It can be surmised that desert rhizosphere Bacteria are collectively adapted and poised to respond to rain, resulting in no major shift in community composition, and that low nutrient levels prevent rapid growth when water is available.

Why didn't cyanobacteria become dominant in watered desert crusts? In laboratory experiments, Cyanobacteria, the main primary producers in most desert crusts, become more abundant when intact soil crusts are placed in permanently water-saturated conditions (Abed *et al.*, 2014; Angel and Conrad, 2013). This was not observed in our field study, despite three, weekly watering events. Cyanobacterial populations may not have flourished *in situ* due to a water-induced burst of activity resulting in a net carbon (C) imbalance caused by respiratory C loss exceeding photosynthetic C fixation (cells would not have been able to photosynthesise until about 12 h after watering, as this was carried out at dusk), a mechanism previously proposed to explain cyanobacterial biomass reduction in hydrated desert soils (Yeager *et al.*, 2012), and observed in laboratory studies (Belnap *et al.*, 2004; Lange *et al.*, 1998). Hydration results in a favourable environment for respiration, as well as osmotic shock, both of which lead to carbon losses (Belnap *et al.*, 2004), e.g. by mineralisation to CO_2 in the case of the former and leaching (e.g. of osmolytes that have leaked from burst cells) in the latter. Moreover, in *Microcoleus vaginatus*, frequently the dominant cyanobacterial species in desert crusts (Garcia-Pichel and Belnap, 1996), hydration resulted in the expression of genes involved in the production of extracellular polymeric substances (EPS), a potential anticipatory response to ensuing desiccation (Rajeev *et al.* 2013). Increased water availability also allows photosynthesis and thus C fixation to resume (Belnap *et al.*, 2004; Rajeev *et al.*, 2013), but there is a delay before C fixation compensates for C loss (Belnap *et al.*, 2004). Water added to a sandy soil is rapidly lost from the surface by percolation into deeper soil and by evaporation. If this water loss is too rapid then the compensation point will not be reached, resulting in the demise of the Cyanobacteria. One week after the final water addition in Experiment 2, the A_w of the crusts had decreased to 0.498–0.559 (Fig. 3); such loss of surface water would be exacerbated by the temperatures that exceeded 40°C. Consistent with this idea, Steven *et al.* (2015) found an increase in cyanobacterial abundance in desert crusts at the end of multiple watering over 6 years compared with dry controls. However, with a simultaneous increase in watering and warming there was a massive decline in cyanobacterial abundance (Steven *et al.*, 2015).

Hot-desert bacterial communities, including the Arabian desert microbiota investigated here (Supporting Information Fig. S6), are distinct from other soil communities, and are shaped more by adaptation to abiotic stresses than competitive interactions (Fierer *et al.*, 2012). The minimal effect that watering of desert soils had on the bacterial community composition, especially in the broader rhizosphere, demonstrates that resistance and resilience to desiccation and wetting are fundamental features of the desert microbiota. On top of this are adaptations to specific soil niches, as well as differential influences of water addition on microbial populations, biomass and activity in the rhizosphere and crust. These insights, together with results from experiments building on these findings, should contribute to improved predictions of the effects of changing precipitation patterns on desert microbial communities and provide more rational strategies to green the desert. For example, microbial activities and thus nutrient supply to plants will be influenced by the diel and seasonal timing as well as the depth of the added water.

Experimental procedures

Field site

In October 2011 and December 2012, experiments were performed and samples were collected from a desert site close to

Baharah (N21°26.4' E39°31.8'), near Jeddah, Saudi Arabia (Fig. 1), where *Rhazya stricta* was the dominant plant. From January 2011 to December 2012, the Baharah site received an average of 50 mm rainfall per month. In 2011, a couple of days of torrential rain in January contributed about 70% of the annual precipitation, with little further rainfall before fieldwork in the following October. In contrast, rainfall throughout 2012 was more evenly distributed (Supporting Information Table S4).

Experimental design and sampling

In order to investigate the diversity of plant- and soil-associated Bacteria, and their temporal responses to watering (Fig. 1), soil samples were taken under plant canopies. In Experiment 1 (2011), samples of surface biological crust, rhizosphere soil and root shavings were taken from six randomly selected *Rhazya stricta* bushes of a similar size (at least 0.5 × 0.5 m ground cover). Under a further nine bushes, soil surrounding an area of $\sim$1 m^2 was raised to prevent water run-off (Fig. 1b). Four of these plants were watered with 50 l of distilled water, while five plants were not watered. Two days later soil samples were collected from beneath these nine plants (Fig. 1c).

Experiment 2 was performed in 2012 at the same location but with different plants. Twenty *Rhazya stricta* bushes were divided randomly into four groups. The soil beneath five plants (A3W) received 50 l of distilled water each on 12th November. A week later, the first set of plants (A3W) along with a second set of five plants (B2W) received 50 l of water each. On 26th November, a third set of five plants (C1W) was similarly watered along with A3W and B2W, while five control plants (0W) were not watered (Fig. 1c). One week later, samples of crust and rhizosphere were collected from beneath these plants.

Soil crusts were collected under plant canopies by first removing fallen plant material from the ground, and lifting surface crusts (3–5 mm thick) with a sterile scalpel and placing them into sterile containers. The rhizosphere (defined as soils within the root mass of *Rhazya stricta*), was sampled at a depth of 6–20 cm; while nonplant associated soils were collected at the same depth, but at least 2 m away from any plant. For root shavings, plants were dug out, and soil attached to the roots along with some root material, including fine root hairs, were scraped from the roots using a sterile scalpel. Samples were either frozen in the field on dry ice for genomic analysis or maintained at 4°C on ice for other analyses, and maintained at those temperatures during storage and transit. However, a small number of frozen samples were lost in transit, which is why the actual numbers analysed are lower than the number sampled in a few cases.

DNA extraction, PCR, pyrosequencing, and data analysis

DNA was extracted using the MoBio PowerSoil DNA isolation kit using the manufacturer's protocol, and PCR was performed using the bacterial 16S rRNA gene primers: Bakt_341F (5′-CCTACGGGNGGCWGCAG-3′ and Bakt_805R (5′-ACH VGGGTATCTAATCC-3′) (Herlemann *et al*., 2011). GS FLX Titanium adaptors were at the 5′-end of the Bakt primers: adaptor A for the forward primer (5′-CGTATCGCCTCCCTC GCGCCATCAG-3′) and B for the reverse primer (5′-CTATGCGCCTTGCCAGCCCGCTCAG-3′). Sample-specific 10 bp barcodes were located between the B adaptor and Bakt_805R (Supporting Information Table S5). PCR mixtures (50 μl) contained 1–2 μl of extracted DNA; 1× reaction buffer (Thermo Scientific, Epsom, UK); 100 μM dNTPs and 2.5 U DreamTaq DNA polymerase (Thermo Scientific, Epsom, UK) and 0.4 μM primers. In order to reduce the inhibitory effect of humic acids, 1 μg of T4 gene 32 protein (Roche Molecular Biochemicals, Mannheim, Germany) was added to each PCR mix. Amplifications were performed in Applied Biosystems 2720 Thermal Cyclers as follows: 95°C for 5 min, 30–35 cycles of 95°C for 30 s, 62°C for 30 s and 72°C for 45 s, then 72°C for 10 min (cycles were adjusted to obtain a similar quantity of PCR product). Duplicate PCR amplicons were pooled and then cleaned using GenElute PCR clean-up kit (Sigma, Gillingham, UK), followed by gel extraction using QIAquick Gel extraction kit (Qiagen, Crawley, UK). Amplicons were quantified using the Quant-iT dsDNA HS assay with a Qubit fluorometer (Invitrogen, Carlsbad, USA), mixed in equimolar amounts and sequenced from the reverse primer direction using the GS FLX 454 Titanium sequencer (Roche Applied Biosystems, Nutley, USA) at Plant Research International, Wageningen, The Netherlands.

Sequences were analysed using the QIIME pipeline and associated modules (Caporaso *et al*., 2010). Pyrosequencing data were fully denoised using AmpliconNoise (Quince *et al*., 2011). Sequences were removed if they: had errors in the 10-bp barcodes and taxon-specific primers, were $<$ 450 bp, had low quality scores ($<$ 25) and $\geq$7 bp homopolymer inserts. Pyrosequences were clustered into operational taxonomic units (OTUs) at the 95% similarity level (approximately corresponding with genus-level discrimination) using USearch (Edgar, 2010); and the associated *de novo* chimera checker (Edgar *et al*., 2011) was used to detect and remove remaining chimeras and OTUs represented by fewer than four sequences across all samples. Representative sequences from each OTU were assigned to a taxonomic group using the RDP classifier algorithm (Wang *et al*., 2007). Rarefaction analysis revealed the total microbial community had been asymptotically sampled, but sequence coverage was unequal across samples. Thus, we removed samples with fewer than 1000 reads and conducted further analyses (except STAMP analysis) on rarefied data that were normalised to the sample with the fewest reads. Overall, after denoising, 218,019 sequences were obtained from 74 samples. The mean number of OTUs per sample was 537.08 (SD 190.83), and the mean number of reads per sample was 2946.2 (SD 748.66).

Metagenomics

DNA was extracted from 1.5 g of a crust soil sample collected 2 days after watering (sample Z11) as described above, and was sequenced using Illumina MiSeq platform (San Diego, USA) with 150 bp paired-end sequencing, using Nextera library preparation at the Institute of Biological, Environmental and Rural Sciences (IBERS, Aberystwyth, Wales, UK). Analytical approaches can be found in Supporting Information.

Physico-chemical analysis of soil

Soil samples were collected to measure soil texture (by sieving) and a range of other parameters. Soil pH was measured using a Jenway 3150 pH meter on soil extracts (two volumes of water were added to soil samples, incubated at 30°C with shaking at 120 rpm for 3 h). Ammonia concentrations were determined spectrophotometrically (Solórzano, 1969). Other ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-}, PO_4^{3-}, NO_3^-, and NO_2^-) were analysed by ion exchange chromatography (Dionex ICS-3000). Samples were filtered through 0.2 μm filters before injection. For cations, the column, Ionpac AS 18 Analytical (2 mm × 50 mm) with an Ionpac AS 18 guard column, was used. For anions, the column, Ionpac CS12 A (4 mm × 250 mm) with an Ionpac CG12A guard column, was used. A gradient of 0–30 mM KOH with a flow rate of 0.25 ml min^{-1} was used over a period of 95 min to separate cations, and 20 mM methylsulfonic acid with a flow rate of 1 ml min^{-1} over a period of 60 min was used to separate anions. Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were measured on a Shimadzu TOC-VCSH Total Organic Carbon autoanalyzer (Shimadzu TOC-VCSH; Japan) with an attached Total Dissolved Nitrogen measuring unit (Shimadzu TNM-1; Japan) as described by McKew *et al.* (2011). Water activity (A_w), moisture content (%H_2O) and Ash free dry weight (%AFDW) were determined as described by McKew *et al.* (2011).

Data analysis

For the comparison of the physico-chemical data, significant differences between sample types were determined using *t* test and analysis of variance (ANOVA, with Tukey *post hoc* tests). Where data showed significant deviation from normality, log10 ($n + 1$) (for concentration data) or arcsin (for proportion data) transformations were used. ANOVA and *t* tests were performed in SPSS 19.0.

Changes in bacterial species richness (number of OTUs) were evaluated using ANOVA, followed by Tukey's HSD *post hoc* tests, and based on rarefied species–abundance data. Compositional changes in bacterial communities were examined using PERMANOVA and visualised using nonmetric multidimensional scaling (NMDS), both based on Bray–Curtis distances of the rarefied species–abundance matrix. Correlations between turnover in bacterial communities (evaluated by Bray–Curtis index) and changes in measured environmental parameters (evaluated based on Euclidean distance) were examined using Mantel tests and ranked according to Mantel correlation coefficients, significance testing was based on 10,000 randomisations of the data. Redundancy analysis (RDA) was used to examine significant drivers of bacterial community composition. A full RDA model based on Hellinger transformed rarefied species–abundance data, and which incorporated all the measured environmental variables and fixed factors, was evaluated using variance inflation factors and subjected to stepwise forward selection of explanatory variables. Significance of model fit and relationship between environmental variables and bacterial composition data were examined using Monte-Carlo permutation tests, based on 10,000 randomizations. All of the above community analyses were performed using the R statistical language (R Development Core Team, 2007) with the 'vegan' library. STAMP (Parks *et al.*, 2014) was used to identify OTUs (95% level) or specified phyla that changed significantly in relative abundance with the watering treatment or across soil location. The parameters used are indicated in the relevant figure legends.

Accession numbers

Metagenomic sequence data and associated metadata for sample Z11 can be found at the MG-RAST site with the ID: 4539732.3. Raw pyrosequences of amplified bacterial 16S rRNA genes from all of the samples investigated can be extracted from the European Nucleotide Archive (ENA) under accession number PRJEB14112. Supporting Information Table S5 provides the information required to identify the relationship between sample and sequences.

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Conflict of interest

Authors certify that there is no conflict of interest with any financial or nonfinancial organisation regarding the subject matter or materials discussed in this manuscript.

References

Abed, R.M.M., Polerecky, L., Al-Habsi, A., Oetjen, J., Strous, M., and de Beer, D. (2014) Rapid recovery of cyanobacterial pigments in desiccated biological soil crusts following addition of water. *PLoS ONE* **9:** e112372.

Abed, R.M.M., Ramette, A., Hübner, V., De Deckker, P., and de Beer, D. (2012) Microbial diversity of eolian dust sources from saline lake sediments and biological soil crusts in arid Southern Australia. *FEMS Microbiol Ecol* **80:** 294–304.

Abed, R.M.M., Al Kharusi, S., Schramm, A., and Robinson, M.D. (2010) Bacterial diversity, pigments and nitrogen fixation of biological desert crusts from the Sultanate of Oman. *FEMS Microbiol Ecol* **72:** 418–428.

Andrew, D.R., Fitak, R.R., Munguia-Vega, A., Racolta, A., Martinson, V.G., and Dontsova, K. (2012) Abiotic factors shape microbial diversity in Sonoran Desert soils. *Appl Environ Microbiol* **78:** 7527–7537.

Angel, R., and Conrad, R. (2013) Elucidating the microbial resuscitation cascade in biological soil crusts following a simulated rain event. *Environ Microbiol* **15:** 2799–2815.

Angel, R., Pasternak, Z., Soares, M.M., Conrad, R., and Gillor, O. (2013) Active and total prokaryotic communities in dryland soils. *FEMS Microbiol Ecol* **86:** 130–138.

Angel, R., Conrad, R., Dvorsky, M., Kopecky, M., Kotilínek, M., Hiiesalu, I., Schweingruber, F., and Doležal, J. (2016)

The root-associated microbial community of the world's highest growing vascular plants. *Microb Ecol.* DOI 10.1007/s00248-016-0779-8

Austin, A.T., Yahdjian, L., Stark, J.M., Belnap, J., Porporato, A., Norton, U., et al. (2004) Water pulses and biogeochemical cycles in arid and semiarid ecosystems. *Oecologia* **141:** 221–235.

Belnap, J., and Lange, O.L. (eds) (2003) *Biological Soil Crusts: Structure, Function, and Management*. Berlin: Springer.

Belnap, J., Phillips, S.L., and Miller, M.E. (2004) Response of desert biological soil crusts to alterations in precipitation frequency. *Oecologia* **141:** 306–316.

Ben-David, E.A., Zaady, E., Sher, Y., and Nejidat, A. (2011) Assessment of the spatial distribution of soil microbial communities in patchy arid and semi-arid landscapes of the Negev Desert using combined PLFA and DGGE analyses. *FEMS Microbiol Ecol* **76:** 492–503.

Borken, W., and Matzner, E. (2009) Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. *Global Change Biol* **15:** 808–824.

Büdel, B., Darienko, T., Deutschewitz, K., Dojani, S., Friedl, T., Mohr, K.I., *et al*. (2009) Southern African biological soil crusts are ubiquitous and highly diverse in drylands, being restricted by rainfall frequency. *Microb Ecol* **57:** 229–247.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., *et al*. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7:** 335–336.

Chowdhury, S.P., Schmid, M., Hartmann, A., and Tripathi, A.K. (2009) Diversity of 16S-rRNA and *nifH* genes derived from rhizosphere soil and roots of an endemic drought tolerant grass, *Lasiurus sindicus*. *Eur J Soil Biol* **45:** 114–122.

Coleman-Derr, D., Desgarennes, D., Fonseca-Garcia, C., Gross, S., Clingenpeel, S., Woyke, T., *et al*. (2016) Plant compartment and biogeography affect microbiome composition in cultivated and native Agave species. *New Phytol* **209:** 798–811.

Daims, H., Lebedeva, E.V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., *et al*. (2015) Complete nitrification by *Nitrospira* bacteria. *Nature* **528:** 504–509.

Dimkpa, C., Weinand, T., and Asch, F. (2009) Plant–rhizobacteria interactions alleviate abiotic stress conditions. *Plant Cell Environ* **32:** 1682–1694.

Dumbrell, A.J., Nelson, M., Helgason, T., Dytham, C., and Fitter, A.H. (2010) Relative roles of niche and neutral processes in structuring a soil microbial Community. *ISME J* **4:** 337–345.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., and Knight, R. (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27:** 2194–2200.

Edgar, R.C. (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26:** 2460–2461.

Elbert, W., Weber, B., Burrows, S., Steinkamp, J., Büdel, B., Andreae, M.O., and Pöschl, U. (2012) Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. *Nat Geosci* **5:** 459–462.

Emad El-Deen, H.M. (2005) Population ecology of *Rhazya stricta* Decne in western Saudi Arabia. *Int J Agric Biol* **7:** 932–938.

Favet, J., Lapanje, A., Giongo, A., Kennedy, S., Aung, Y.Y., Cattaneo, A., *et al*. (2013) Microbial hitchhikers on intercontinental dust: catching a lift in Chad. *ISME J* **7:** 850–867.

Fierer, N., Bradford, M.A., and Jackson, R.B. (2007) Toward an ecological classification of soil bacteria. *Ecology* **88:** 1354–1364.

Fierer, N., Leff, J.W., Adams, B.J., Nielsen, U.N., Bates, S.T., *et al*. (2012) Cross-biome metagenomics analyses of soil microbial communities and their functional attributes. *Proc Natl Acad Sci USA* **109:** 21390–21395.

Garcia-Pichel, F., and Belnap, J. (1996) Microenvironments and microscale productivity of cyanobacterial desert crusts. *J Phycol* **32:** 774–782.

Garcia-Pichel, F., Johnson, S.L., Youngkin, D., and Belnap, J. (2003) Small-scale vertical distribution of bacterial biomass and diversity in biological soil crusts from arid lands in the Colorado Plateau. *Microb Ecol* **46:** 312–321.

Gilani, S.A., Kikuchi, A., Shinwari, Z.K., Khattak, Z.I., and Watanabe, K.N. (2007) Phytochemical, pharmacological and ethnobotanical studies of *Rhazya stricta* Decne. *Phytother Res* **21:** 301–307.

Herlemann, D.P.R., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J.J., and Andersson, A.F. (2011) Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME J* **5:** 1571–1579.

Johnson, S.L., Budinoff, C.R., Belnap, J., and Garcia-Pichel, F. (2005) Relevance of ammonium oxidation within biological soil crust communities. *Environ Microbiol* **7:** 1–12.

Kharin, V.V., Zwiers, F.W., Zhang, X., and Hegerl, G.C. (2007) Changes in temperature and precipitation extremes in the IPCC ensemble of global coupled model simulations. *J Climate* **20:** 1419–1443.

Köberl, M., Müller, H., Ramadan, E.M., and Berg, G. (2011) Desert farming benefits from microbial potential in arid soils and promotes diversity and plant health. *PLoS ONE* **6:** e24452–e24452.

Kuske, C.R., Ticknor, L.O., Miller, M.E., Dunbar, J.M., Davis, J.A., Barns, S.M., and Belnap, J. (2002) Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in arid grassland. *Appl Environ Microbiol* **68:** 1854–1863.

Lange, O.L., Belnap, J., and Reichenberger, H. (1998) Photosynthesis of the cyanobacterial soil–crust lichen *Collema tenax* from arid lands in southern Utah, USA: Role of water content on light and temperature responses of CO_2 exchange. *Funct Ecol* **12:** 195–202.

Lauber, C., Hamady, M., Knight, R., and Fierer, N. (2009) Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl Environ Microbiol* **75:** 5111–5120.

Lawson, T., Davey, P.A., Yates, S.A., Bechtold, U., Baeshen, M., Baeshen, N., Sabir, J., and Mullineaux, P.M. (2014) C3 photosynthesis in the desert plant *Rhazya stricta* is fully functional at high temperatures and light intensities. *New Phytol* **201:** 862–873.

Maestre, F.T., Salguero-Gómez, R., and Quero, J.L. (2012) It is getting hotter in here: Determining and projecting the impacts of global environmental change on drylands. *Philos Trans Roy Soc B* **367:** 3062–3075.

Makhalanyane, T.P., Valverde, A., Gunnigle, E., Frossard, A., Ramond, J.-B., and Cowan, D.A. (2015) Microbial ecology of hot desert edaphic systems. *FEMS Microbiol Rev* **39:** 203–221.

Marusenko, Y., Bates, S.T., Anderson, I., Johnson, S.L., Soule, T., and Garcia-Pichel, F. (2013) Ammonia oxidizing archaea and bacteria are structured by geography in biological soil crusts across North American arid lands. *Ecol Proc* **2:** 9.

Marwat, S.K., Fazal, U.R., Usman, K., Shah, S.S., and Anwar, N Ullah, I. (2012) A review of phytochemistry, bioactivities and ethno medicinal uses of *Rhazya stricta* Decsne (Apocynaceae). *Afr J Microbiol Res* **6:** 1629–1641.

Mazor, G., Kidron, G.J., Vanshak, A., and Abeliovich, A. (1996) The role of cyanobacterial exopolysaccharides in structuring desert microbial crusts. *FEMS Microbiol Ecol* **21:** 121–130.

McKew, B.A., Taylor, J.D., McGenity, T.J., and Underwood, G.J.C. (2011) Resistance and resilience of benthic biofilm communities from a temperate saltmarsh to desiccation and rewetting. *ISME J* **5:** 30–41.

Nagy, M., Perez, A., and Garcia-Pichel, F. (2005) The prokaryotic diversity of biological soil crusts in the Sonoran Desert of Arizona. *FEMS Microbiol Ecol* **54:** 233–245.

Noy-Meir, I. (1973) Desert ecosystems: Environment and producers. *Ann Rev Ecol Syst* **4:** 25–51.

Ofek, M., Hadar, Y., and Minz, D. (2012) Ecology of root colonizing *Massilia* (Oxalobacteraceae). *PLoS ONE* **7:** e4011.

Parks, D.H., Tyson, G.W., Hugenholtz, P., and Beiko, R.G. (2014) STAMP: Statistical analysis of taxonomic and functional profiles. *Bioinformatics*. doi: 10.1093/bioinformatics/btu494.

Pointing, S.B., and Belnap, J. (2012) Microbial colonization and controls in dryland systems. *Nat Rev Microbiol* **10:** 551–562.

Pointing, S.B., Warren-Rhodes, K., Lacap, D.C., Rhodes, K.L., and McKay, C.P. (2007) Hypolithic community shifts occur as a result of liquid water availability along environmental gradients in China's hot and cold hyperarid deserts. *Environ Microbiol* **9:** 414–424.

Qin, S., Miao, Q., Feng, W.W., Wang, Y., Zhu, X., Xing, K., and Jiang, J.H. (2015) Biodiversity and plant growth promoting traits of culturable endophytic actinobacteria associated with *Jatropha curcas* L. growing in Panxi dry-hot valley soil. *Appl Soil Ecol* **93:** 47–55.

Quince, C., Lanzen, A., Davenport, R.J., and Turnbaugh, P.J. (2011) Removing noise from pyrosequenced amplicons. *BMC Bioinform* **12:** 38.

R Development Core Team (2005) R: a language and environment for statistical computing. *R Foundation for Statistical Computing*. Vienna, Austria. URL http://www.R-project.org.

Rajeev, L., Da Rocha, U.N., Klitgord, N., Luning, E.G., Fortney, J., Axen, S.D., *et al.* (2013) Dynamic cyanobacterial response to hydration and dehydration in a desert biological soil crust. *ISME J* **7:** 2178–2191.

Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., and Fierer, N. (2010) Soil bacterial and fungal communities across a pH gradient in an arable Soil. *ISME J* **4:** 1340–1351.

Salomon, P.S., Janson, S., and Graneli, E. (2003) Molecular identification of bacteria associated with filaments of *Nodularia spumigena* and their effect on the cyanobacterial growth. *Harmful Algae* **2:** 261–272.

Saul-Tcherkas, V., and Steinberger, Y. (2011) Soil microbial diversity in the vicinity of Negev desert shrub—*Reaumuria negevensis*. *Microb Ecol* **61:** 64–81.

Saul-Tcherkas, V., Unc, A., and Steinberger, Y. (2013) Soil microbial diversity in the vicinity of desert shrubs. *Microb Ecol* **65:** 689–699.

Segura, A., de Wit, P., and Preston, G.M. (2009) Life of microbes that interact with plants. *Microb Biotechnol* **2:** 412–415.

Solórzano, L. (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnol Oceanogr* **14:** 799–801.

Sponseller, R.A. (2007) Precipitation pulses and soil CO_2 flux in a Sonoran desert ecosystem. *Global Change Biol* **13:** 426–436.

Steven, B., Kuske, C.R., Gallegos-Graves, L.V., Reed, S.C., and Belnap, J. (2015) Climate change and physical disturbance manipulations result in distinct biological soil crust communities. *Appl Environ Microbiol* **81:** 7448–7459.

Steven, B., Gallegos-Graves, L.V., Yeager, C., Belnap, J., and Kuske, C.R. (2014) Common and distinguishing features of the bacterial and fungal communities in biological soil crusts and shrub root zone soils. *Soil Biol Biochem* **69:** 302–312.

Steven, B., Gallegos-Graves, L.V., Belnap, J., and Kuske, C.R. (2013) Dryland soil microbial communities display spatial biogeographic patterns associated with soil depth and soil parent material. *FEMS Microbiol Ecol* **86:** 101–113.

Stevenson, A., Cray, J.A., Williams, J.P., Santos, R., Sahay, R., Neuenkirchen, N., *et al.* (2015) Is there a common water-activity limit for the three domains of life? *ISME J* **9:** 1333–1351.

Wang, Q., Garrity, G.M., Tiedje, J.M., and Cole, J.R. (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* **73:** 5261–5267.

Ward, N.L., Challacombe, J.F., Janssen, P.H., Henrissat, B., Coutinho, P.M., Wu, M., *et al.* (2009) Three genomes from the phylum *Acidobacteria* provide insight into the lifestyles of these microorganisms in soils. *Appl Environ Microbiol* **75:** 2046–2056.

Wolf, A.B., Rudnick, M.B., de Boer, W., and Kowalchuk, G.A. (2015) Early colonizers of unoccupied habitats represent a minority of the soil bacterial community. *FEMS Microbiol Ecol* **91:** fiv024.

Yates, S.A., Chernukhin, I., Alvarez-Fernandez, R., Bechtold, U., Baeshen, M., Baeshen, N., *et al.* (2014) The temporal foliar transcriptome of the perennial C3 desert plant *Rhazya stricta* in its natural environment. *BMC Plant Biol* **14:** 2.

Yeager, C.M., Kuske, C.R., Carney, T.D., Johnson, S.L., Ticknor, L.O., and Belnap, J. (2012) Response of biological soil crust diazotrophs to season, altered summer precipitation, and year-round increased temperature in an arid grassland of the Colorado Plateau, USA. *Front Microbiol* **3:** 358.

Yu, J., Glazer, N., and Steinberger, Y. (2014) Carbon utilization, microbial biomass, and respiration in biological soil crusts in the Negev Desert. *Biol Fertil Soils* **50:** 285–293.

Zelikova, T.J., Housman, D.C., Grote, E.E., Neher, D.A., and Belnap, J. (2012) Warming and increased precipitation frequency on the Colorado Plateau: Implications for biological soil crusts and soil processes. *Plant Soil* **355:** 265–282.

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