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ORIGINAL ARTICLE

Responses of soil bacterial and fungal communities to extreme desiccation and rewetting

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The microbial response to summer desiccation reflects adaptation strategies, setting the stage for a large rainfall-induced soil CO_2 pulse upon rewetting, an important component of the ecosystem carbon budget. In three California annual grasslands, the present (DNA-based) and potentially active (RNA-based) soil bacterial and fungal communities were tracked over a summer season and in response to controlled rewetting of intact soil cores. Phylogenetic marker genes for bacterial (16S) and fungal (28S) RNA and DNA were sequenced, and the abundances of these genes and transcripts were measured. Although bacterial community composition differed among sites, all sites shared a similar response pattern of the present and potentially active bacterial community to dry-down and wet-up. In contrast, the fungal community was not detectably different among sites, and was largely unaffected by dry-down, showing marked resistance to dessication. The potentially active bacterial community changed significantly as summer dry-down progressed, then returned to pre-dry-down composition within several hours of rewetting, displaying spectacular resilience. Upon rewetting, transcript copies of bacterial *rpoB* genes increased consistently, reflecting rapid activity resumption. *Acidobacteria* and *Actinobacteria* were the most abundant phyla present and potentially active, and showed the largest changes in relative abundance. The relative increase (*Actinobacteria*) and decrease (*Acidobacteria*) with dry-down, and the reverse responses to rewetting reflected a differential response, which was conserved at the phylum level and consistent across sites. These contrasting desiccation-related bacterial life-strategies suggest that predicted changes in precipitation patterns may affect soil nutrient and carbon cycling by differentially impacting activity patterns of microbial communities.
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Introduction

Global climate change is predicted to alter precipitation and drought patterns, resulting in more extreme conditions, especially for Mediterranean ecosystems (IPCC, 2007), which are characterised by hot dry summers and cool wet winters. Microbial mineralization of carbon substrates that are accumulated during the summer period fuels large mineralization pulses upon soil rewetting (Birch, 1958; Borken and Matzner, 2009; Inglima *et al.*, 2009). CO_2 pulses resulting from the rewetting of Mediterranean annual grasslands after the summer dry period account for a large part of the annual carbon they lose to the atmosphere (Xu *et al.*, 2004; Jarvis *et al.*, 2007). Thus, changes in dry-down patterns have potentially large consequences for these ecosystems' nutrient and carbon budgets (Waldrop and Firestone, 2006a; Sheik *et al.*, 2011; Vargas *et al.*, 2012).

The temporal distribution of precipitation in Mediterranean ecosystems, in which rainfall is almost entirely absent during the summer, probably selects for life-strategies to deal with the direct physiological effects of summer dry-down and sudden rewetting in the autumn. The characteristic patterns of soil water availability may select for indigenous soil microbes with physiological strategies that render them tolerant of a dynamic water potential environment, as well as of the indirect effects on water availability. Indeed, access to nutrients becomes more limited as the water film thickness is reduced by drought (Stark and Firestone, 1995); the rewetting of soils with autumn rains causes an abrupt flush of nutrients associated with the mineralization burst upon rewetting dry

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soils (Borken and Matzner, 2009; Inglima *et al.*, 2009). Both bacterial and fungal isolates have known strategies to survive desiccation and rewetting (Potts, 1994; Griffin, 1977), including: (i) the accumulation of compatible solutes (see review by Schimel *et al.*, 2007); (ii) exopolysaccharide production, extensively studied in *Pseudomonas sp.* (Roberson and Firestone, 1992; Chang *et al.*, 2007) and recently in *Acidobacteria* (Ward *et al.*, 2009); and (iii) the production of dormant life forms such as spores. Field-based evidence of different bacterial groups displaying contrasting desiccation-related life-strategies nevertheless remain scarce. Tolerance to desiccation may also result from morphological life form: fungi are generally considered more resistant to desiccation than bacteria (Gordon *et al.*, 2008; de Vries *et al.*, 2012), with hyphae that may cross air-filled soil pores to access nutrients and water.

Both soil bacteria and fungi include heterotrophic microorganisms that are capable of rapid activation upon wet-up, that is, of playing a role in the mineralization burst that is responsible for the soil CO_2 efflux pulse following a rewetting event (Fierer and Schimel, 2003; Placella *et al.*, 2012). Although microbial communities have sometimes been shown to shift seasonally in water-limited systems (Waldrop and Firestone, 2006b; Clark *et al.*, 2009; Cruz-Martinez *et al.*, 2009), the functional or taxonomic groups that drive the microbial response to dry-down and rewetting, their associated strategies and to what extent these responses may be generalised still remain largely unclear (Placella *et al.*, 2012).

Molecular techniques based on ribosomal RNA (rRNA) allow the phylogenetic characterisation of bacterial and fungal groups that are present (as rRNA genes) and that have the capacity to actively synthesise proteins (rRNA). The abundance of rRNA has commonly been used as an indicator of activity (for example, Schippers *et al.*, 2005; Jones and Lennon, 2010; see review by Blazewicz *et al.*, 2013). The present study investigated changes in the present and potentially active soil bacterial and fungal communities, in three Mediterranean annual grasslands in California, over a 5-month summer dry-down period and subsequent rewetting. The primary objective was to assess the temporal responses of the diverse components of the soil microbial community to extreme desiccation and subsequent rewetting across the three sites, in order to better understand the life history bases of below-ground response to dry-down and wet-up. The three sites are similar ecosystems, albeit with different soil properties and climatic conditions, thus allowing us to test for site-specific microbial community dynamics in addition to more general summer desiccation and rewetting responses. For this purpose, ribosomal RNA transcripts and genes from bacteria and fungi were analysed by 454 Titanium pyrosequencing, and the abundance of selected phylogenetic marker genes and transcripts was measured using quantitative PCR. We expected to learn how highly adapted indigenous bacterial and fungal communities were to extreme desiccation and wet-up, and whether the present and potentially active communities would differ among sites (due to edaphic and climatic differences) and thereby respond differently to dry-down and rewetting.

Materials and methods

Field sites

Three California grasslands sites with contrasting rainfall patterns and soil types (Supplementary Table S1) were chosen: (i) Hopland field station (University of California Hopland Research and Extension Center, 39°0'N, 123°3'W), (ii) Sierra field station (University of California Sierra Foothill Research and Extension Center 39°14'N, 121°17'W) and (iii) Sedgwick field station (University of California Sedgwick Reserve 34°42'N, 120°2'W). Soil from the Sedgwick site has lower organic matter and organic carbon content but higher clay content than the other two soils. The Hopland soil has a lower saturation percentage and cation-exchange capacity than the other sites.

An experimental plot ($5 \times 5\,m^2$) was established in each grassland, within a homogeneous fenced area. In each plot, 20 PVC cores (7.5 cm internal diameter, 15 cm long) were inserted in the soil, 15 cm deep, ~1 m apart, between the 5th and the 12th of April 2010, depending on the site. Core installation may have severed some plant roots and potentially altered rhizodeposition; however, plant photosynthetic activity does not exceed a few weeks after April at these sites, and most plants had set seed and were senescing by this time. Five cores were randomly chosen and removed from each site, three times over the spring-summer dry-down period: 5–12 April, 15–18 June and 2–8 September 2010. At each sampling date, each site was sampled within a day, and three sites were sampled within a week. To avoid edge effects, the PVC cores were internally cored (5 cm diameter, 10 cm depth). The internal core was sieved and subsampled to determine gravimetric water content (by comparing fresh with 105 °C dried soil weights), whereas the remaining sieved soil was stored at −80 °C, before nucleic acid extraction.

A composite of soil collected within each plot at the initial sampling date was analysed for soil chemical and physical properties (University of California, Davis, Analytical Laboratory). A soil–water retention curve was established on the basis of the soil–water potential measured using a constant preset atmospheric pressure potential (four points between −0.03 and −1 MPa) and an isopiestic method (four points between −4.6 and −88 MPa, using saturated solutions of copper sulphate, sucrose, sodium chloride and magnesium nitrate, Tokunaga *et al.*, 2003).

Wet-up
At the last sampling date (2–8 September 2010), five additional cores were taken and subjected, under controlled conditions, to a simulated first rainfall event after summer desiccation. The bottom of the cores was sealed, and a 1-L PVC chamber was fitted airtight on top of the core. Immediately after installing the chamber, distilled water was dispensed through a septum onto the soil surface over a 2-min period, while a needle through another septum ensured no pressure build-up. The amount of water delivered to each core approximated the moisture lost during dry-down in the soils of each site, based on the average weight loss of cores during dry-down and amounted to a ca. 25 mm rain event. The water added was left to penetrate the soils for 2 hours, thus amounting to 12.5 mm h^{-1}, which is near the upper limit of precipitation amounts at the sites. Two hours after rewetting, the cores were internally cored, and soil samples processed as above.

Soil nucleic acid extraction and purification
Both DNA and RNA were extracted from the soil samples, using the protocol adapted from Griffiths *et al.*, (2000) and Brodie *et al.*, (2002). All solutions and glassware were rendered RNase-free by diethyl pyrocarbonate (DEPC) treatment. In brief, for each soil sample, three 0.4 g dry-weight subsamples were extracted separately. Each aliquot was transferred to a 2-ml Lysing Matrix E tube (MP Biomedicals, Solon, OH, USA) and extracted twice as follows. Five hundred microlitre extraction buffer (5% CTAB, 0.5 M NaCl, 240 mM K_2HPO_4, pH 8.0) and 500 μl 25:24:1 phenol:chloroform:isoamyl alcohol were added before shaking (FastPrep24, MP Biomedicals; 30 s, 5.5 m s^{-1}). After spinning down the debris (16 100 *g*, 5 min, 4 °C), the nucleic acids were purified using pre-spun 2 ml Phase Lock Gel tubes (5 Prime, Gaithersburg, MD) with an equal volume of 24:1 chloroform:isoamyl alcohol, mixed and centrifuged (16 100 *g*, 2 min, 4 °C). The aqueous phases from both extractions were pooled, mixed with 1 ml 40% polyethylene glycol 6000 dissolved in 1.6 M NaCl and 3 μl linear acrylamide (5 mg ml^{-1}; Ambion, Grand Island, NY, USA), and incubated for 1 h at the room temperature. After centrifugation (16 100 *g*, 20 min, room temperature), the pellet was rinsed with 1 ml ice-cold 70% ethanol, air-dried, resuspended in 20 μl RNase-free water and stored at −80 °C. For each sample, the three subsample extracts were pooled during purification (AllPrep DNA/RNA Mini Kit, Qiagen, Valencia, CA, USA) according to the manufacturer's instructions.

Abundance of genes and transcripts
The extracted DNA and RNA were quantified using Quant-iT PicoGreen dsDNA and RiboGreen RNA reagents, respectively (Invitrogen, Grand Island, NY, USA) with a CFX96 thermocycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). cDNA was generated from 50 ng of the extracted RNA from each subsample using the QuantiTect Reverse Transcription Kit (Qiagen) and then pooled.

The abundance of genes and transcripts was assessed by quantitative polymerase chain reaction (qPCR). The genes encoding bacterial 16S, archaeal 16S and fungal 28S ribosomal components were selected as phylogenetic markers. The bacterial *rpoB* gene was also selected, as this single-copy gene encodes a sub-unit of the bacterial RNA polymerase and thus provides an estimation of transcriptional activity. The primers used were: EUB338 and EUB518 for bacterial 16S (Fierer *et al.*, 2005); NL1f and LS2r for fungal 28S (Bates and Garcia-Pichel, 2009); A364aF and A934b for archaeal 16S (Kemnitz *et al.*, 2005); and rpoB-f-4 and rpoB-r-2 for bacterial *rpoB* gene (Silkie and Nelson, 2009).

DNA and cDNA quantification was performed using a CFX96 thermocycler (Bio-Rad) in a total volume of 12 μl including 2 to 4 ng DNA or 2.5 ng cDNA, 10 μl SsoFast EvaGreen Supermix (Bio-Rad) and 300 μM of each primer. All qPCR assays were run for 30 s at 95 °C, and then 40 cycles, with plate-reading, of 95 °C for 5 s and 60 °C for 15 s, with a final melt-curve step from 75 °C to 95 °C. Standard curves were obtained using serial dilutions of plasmids containing the cloned genes and the efficiencies ranged between 91.9–108.7% (R^2 minimum 0.985).

Pyrosequencing of bacterial and fungal communities
DNA and cDNA samples from three randomly chosen replicate cores from each site and sampling date were sequenced by 8 bp tag-encoded FLX amplicon pyrosequencing, except for the DNA extracts from the second dry-down sampling date (due to lack of funds). In total, 36 cDNA samples and 27 DNA samples were sequenced (Research and Testing Laboratory, Lubbock, TX, USA; Roche 454 FLX Titanium). Initial generation of the sequencing libraries utilised a one-step PCR with a total of 30 cycles, a mixture of Hot Start and HotStar High-Fidelity Taq polymerases. The primers used were 939F (5′-TTGACGGGGGCCCGCAC-3′) and 1492R (5′-TACCTTGTTACGACTT-3′) to sequence the V6 region of the bacterial 16S, and the LR0R (5′-ACCCGCTGAACTTAAGC-3′) and LR3 (5′-CCGT GTTTCAAGACGGG-3′) to sequence the D1 domain of the fungal 28S. The sequence data were submitted to the NCBI Sequence Read Archive database (accession No. SRP016037). The pyrotag analysis was run using PyroTagger (Kunin and Hugenholtz, 2010) as follows. Sequences were denoised, quality filtered by removing reads with ⩾3% low-quality bases over a given length, length filtered at 200 nucleotides, clustered (uclust algorithm, Edgar, 2010) using the most abundant unique sequence as cluster representative. Taxonomy was assigned

using the greengenes and silva databases for bacteria and fungi, respectively.

Sequencing depth for rRNA and rRNA genes was analysed by analysis of variance and was not significantly affected by time, site or their interaction.

Phylogenetic community structure

Phylogenetic pairwise dissimilarity matrices were calculated using FastUniFrac (Hamady *et al.*, 2010), based on sequence abundance, without normalizing for differing mutation rates. Phylogenetic trees for bacterial 16S rRNA and fungal 28S rRNA gene sequences (4559 and 804 clusters, respectively) were constructed using a generalised time-reversible model of the nucleotide sequences in FastTree 2.1.1 (Price *et al.*, 2009), after aligning the representative sequences against the greengenes core set using the NAST algorithm (75% minimum sequence similarity, 200 nt minimum length, no gap character removal) on the greengenes website (DeSantis *et al.*, 2006).

The phylogenetic structure of the DNA- and RNA-based communities was evaluated using net relatedness index (NRI) on the basis of the mean phylogenetic distance (Webb, 2000; Webb *et al.*, 2002), well suited for terminally unresolved phylogenies (Swenson, 2009). A null model of random community phylogenetic relationships (picante package, 999 runs, not abundance weighted) among the sequences recovered from all sites and sampling times was used.

Statistical analyses

Statistical analyses were performed in R 2.15.0 (R Development Core Team, 2012). DNA and RNA data were analysed separately, and, within each of these data sets, the dry-down and wet-up data sets were analysed separately. The NRI data and log-transformed abundance of RNA genes and transcripts were analysed by analysis of variance, using as explanatory variables site, either dry-down or wet-up, and the interaction between site and either dry-down or wet-up. The effect of dry-down was determined over three time points (5–12 April, 15–18 June and 2–8 September 2010). The effect of wet-up was determined over two time points (pre-wet-up on 2–8 September 2010, then 2 h after wet-up).

UniFrac distances were compared by non-parametric permutational multivariate analysis of variance (adonis function of vegan package, Anderson, 2001), nested by site. The explanatory variables were site and dry-down or wet-up, and their interaction.

Relative abundances of bacterial and fungal groups were analysed by aggregating all taxa at the phylum and class levels. The analysis targeted the most abundant phyla and classes, by using the lowest relative abundance threshold that ensured that all taxa in the analysis were present in all the samples. For bacteria, this threshold corresponded to the phyla and classes accounting for more than 1% of the total number of OTUs sequenced in at least one sample at one sampling time and site. For fungal phyla and classes, the threshold was 0.5% and 1%, respectively. The relative abundance of the most abundant bacterial and fungal taxa was analysed by analysis of variance, after log-transformation of the data. The explanatory variables were site, taxonomic identity (phylum or class), either dry-down or wet-up, and their interactions. The statistical model respected the hierarchical structure of the experiment: the taxa identity level was nested within the soil core level.

Results

Soil water status

The three sites share a general Mediterranean climate precipitation pattern, with most rain during the winter months and a 3- to 5-month dry-summer period. However, they generally experience different yearly precipitation amounts, which during the 2009-2010 growth season ranged from 346 mm in Sedgwick to 932 mm in Hopland (Figure 1a). Despite several rain events between our first two sampling dates, soil water content showed a pronounced dry-down pattern over the sampling period, from above 20% in April to an average 4.2% in September 2010 (Figure 1b). By September, soil water potential had dropped to extremely low-negative values (average -22 MPa, Figure 1c). This dry-down pattern is normal for these sites.

A 5 mm rewetting event brought soils back to an average 13.6% water content (average water potential -1.4 MPa). Rewetting was slightly less in the Sedgwick soils, which appeared to have a lower wettability (Supplementary Table S1).

Bacterial and fungal phylogenetic community structure

The bacterial DNA and cDNA data sets had 62 565 and 86 674 pyrotag sequences remaining after curation, respectively, representing 4646 clusters (or OTUs) for each. The fungal DNA and cDNA data sets had 25 115 and 15 315 pyrotag sequences remaining after curation, respectively, representing 1347 clusters for each. An average $\pm$ s.e. of 2317 ± 139, 2408 ± 132, 930 ± 58 and 425 ± 27 pyrosequences were found per sample of bacterial DNA and cDNA and fungal DNA and cDNA, respectively.

As microbial DNA and RNA reflect different snapshots of the soil microbial community, here we distinguish between the bacterial and fungal communities on the basis of DNA (the present communities, assessed using the 16S rRNA gene) and the communities on the basis of RNA (the potentially active communities, assessed using 16S rRNA).

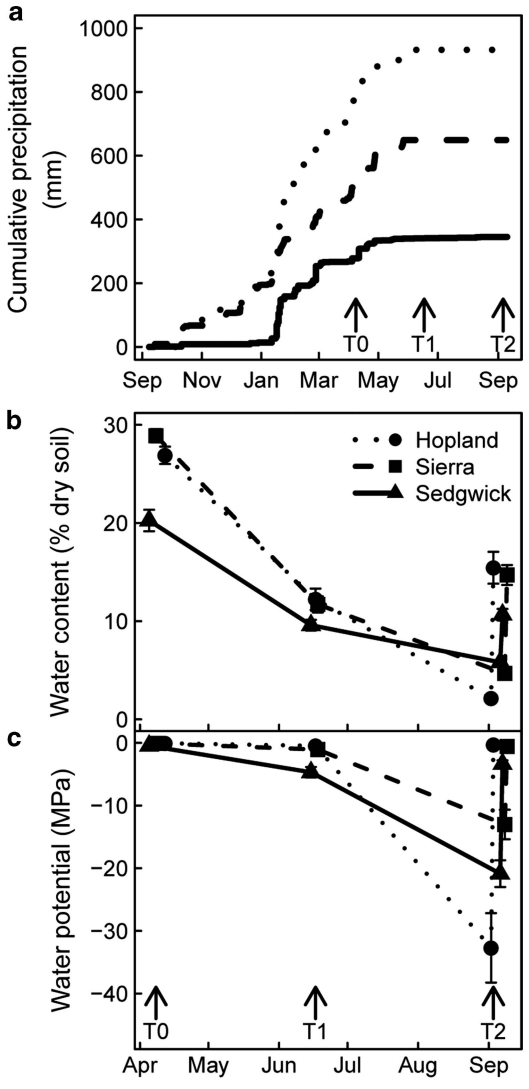

Figure 1 Dynamics of water conditions at the Hopland (dots), Sierra (dashes) and Sedgwick (full) experimental sites: cumulative precipitation from September 2009 to September 2010 (**a**), dynamics of soil water content (**b**) and potential (**c**) throughout summer dry-down and controlled wet-up. T0, T1 and T2 indicate field sampling dates, bars indicate ± 1s.e. ($n = 5$). Precipitation data source: California Irrigation Management Information System and University of California Santa Barbara Geography Department.

Soil bacterial communities present at the three sites in April 2010 were dominated in relative abundance by the *Acidobacteria* phylum (mostly *Acidobacteria* iii1–15 class), followed by the *Actinobacteria* (mostly *Rubrobacteridae* class) and *Proteobacteria* (primarily alpha, delta and gamma) phyla (Supplementary Figure S1). Although the present bacterial communities were significantly affected by both dry-down and site, the communities at the different sites did not differ significantly in their dry-down response (Figures 2a and b, Supplementary Table S2). The present bacterial community structure was unaffected by wet-up (Figure 2a). The potentially active bacterial communities were significantly affected by site, dry-down and wet-up, but the sites did not differ significantly in their dry-down or wet-up responses (Figure 2b, Supplementary Table S2).

The soil fungal communities present at the three sites in April 2010 were dominated in relative abundance by the *Ascomycota* and *Basidiomycota* phyla, whereas the distribution of fungal classes, dominated by *Agaricomycetes*, *Sordariomycetes* and *Eurotiomycetes* was more even within sites and variable among sites (Supplementary Figure S2). Over all sites, neither dry-down nor wet-up had a significant effect on the present (DNA-based) or potentially active (RNA-based) fungal communities (Figures 2c and d, Supplementary Table S3). In contrast, wet-up affected the rRNA-based fungal community differently at the different sites.

The RNA-based bacterial communities showed consistently significant phylogenetic clustering (overall NRI 8.22 ± 0.39 mean and standard error), which generally increased with dessication and decreased with rewetting (Supplementary Figure S3, Supplementary Table S4). The DNA-based bacterial communities were generally less clustered (overall NRI 3.88 ± 0.19) and not affected by changes in water availability. The phylogenetic structure of fungal communities was overall slightly overdispersed for the RNA-based and the DNA-based communities (-0.25 ± 0.17 and -0.96 ± 0.17, respectively). NRI responses to dry-down and wet-up were not significantly different among sites (Supplementary Table S4).

Response of bacterial groups to dry-down and wet-up
Bacterial 16S rRNA gene. Relative abundances of the bacteria present were analysed by aggregating all taxa at the phylum and class levels. Over all sites, dry-down affected the relative abundance of bacteria present, at the class level only (Figure 3, Supplementary Tables S5 and S6). Sites differed in the relative abundance of the bacteria present, at both the phylum and class levels, but the response to dry-down and wet-up did not differ among sites. The relative abundance of the different bacterial phyla and classes responded differently to dry-down over the three sites. The three-way interaction dry-down (or wet-up) × site × phylum (or class) evaluates differences in the dry-down (or wet-up) response of the different phyla (or classes) among sites. It was only found at the class level, and explained only 1.1% and 1.0%, of the variance of the dry-down and wet-up data, respectively. Adding a contrast term on the basis of taxa or groups of taxa to the ANOVA models did not enable us to relate changes associated with the response of individual taxa or groups of taxa.

Bacterial 16S rRNA. As the abundance of ribosomal 16S rRNA gene was much less responsive than that of rRNA, the rRNA transcript: ribosomal rRNA gene abundance ratio of the most abundant phyla

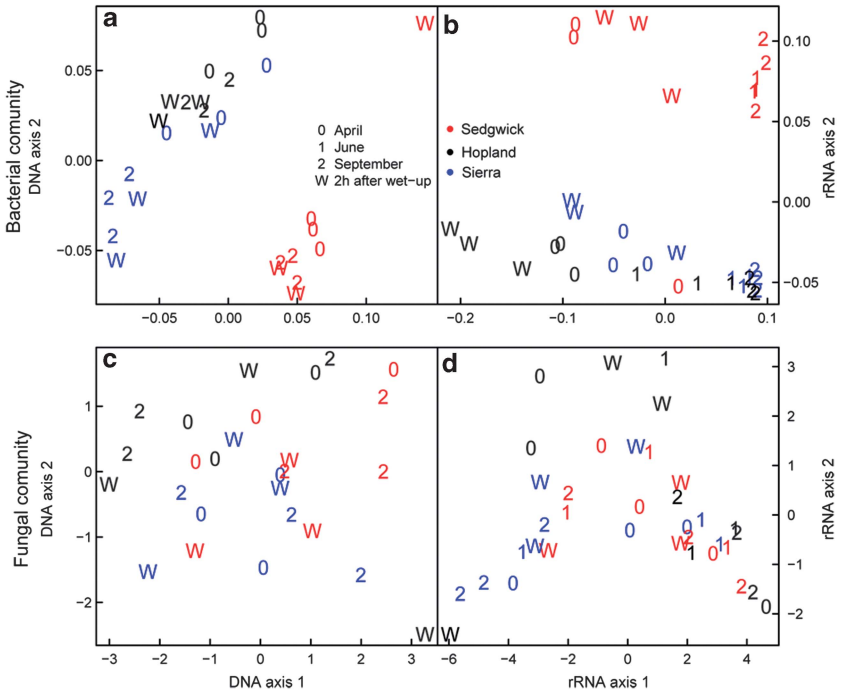

Figure 2 Principal coordinates analysis (PCoA) of the UniFrac pairwise dissimilarity of the relative abundance of bacterial and fungal pyrotag sequences based on 16S rRNA gene (**a**) and 16S rRNA (**b**) for bacteria and on 28S rRNA gene (**c**) and 28S rRNA (**d**) for fungi, over dry-down and wet-up in the experimental sites (Hopland: black, Sierra: blue, Sedgwick: red). The numbers indicate sampling dates over dry-down, W indicates data acquired 2 h after the end of wet-up.

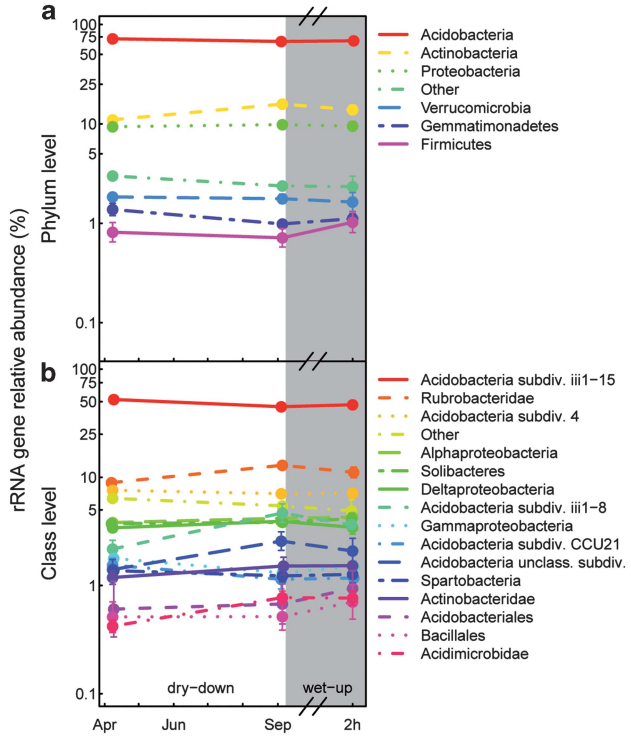

Figure 3 Dynamics of the 16S rRNA gene-based relative abundance of the main present bacterial groups at the phylum (**a**) and class (**b**) levels, sequenced during dry-down and wet-up (grey area, note the difference in x-axis scale) over the three experimental sites. Bars indicate ± 1s.e. ($n = 9$).

and classes followed the general pattern of rRNA (Supplementary Figure S4). Thus, changes in the relative abundance of rRNA appears to reflect changes in abundance of (potentially active) cellular transcriptional machinery. Both dry-down and wet-up affected the relative abundance of potentially active bacteria, at both phylum and class levels (Figure 4, Supplementary Tables S7 and S8). Differences among sites were only significant for the dry-down period at the phylum level (Supplementary Table S7). The different phyla and classes responded differently in relative abundance to dry-down and wet-up over the three sites. The three-way interaction was significant at the class level for dry-down, but explained only 1.1% of the variance of the data. Adding a contrast term on the basis of taxa or groups of taxa to the ANOVA models did not enable us to relate changes associated with the response of individual taxa or groups of taxa. Nevertheless, the community-dominating *Actinobacteria* (as rRNA) were the most responsive, increasing strongly with dry-down (from 62.5 to 82.9% relative abundance) and decreasing (to 61.7%) with wet-up (Figure 4a). In contrast, *Acidobacteria* and *Verrucomicrobia* followed an opposite pattern. At the class level, *Rubrobacteridae* (*Actinobacteria* phylum) were the most abundant and very responsive to changes in water availability, following the *Actinobacteria* pattern (Figure 4b). The main other responsive classes were the *iii1-15*, *4*, *Solibacteres* and 'other' classes of *Acidobacteria*, which followed the *Acidobacteria* pattern.

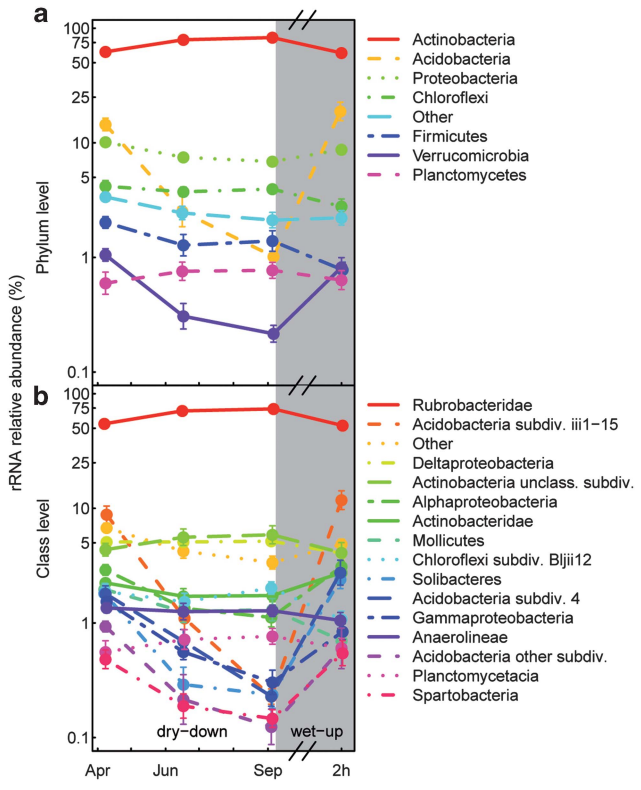

Figure 4 Dynamics of the 16S rRNA-based relative abundance of the main potentially active bacterial groups at the phylum (**a**) and class (**b**) levels, sequenced during dry-down and wet-up (grey area, note the difference in x-axis scale) over the three experimental sites. Bars indicate ± 1s.e. ($n = 9$). In the wet-up analysis, *Acidobacteriales*, *Acidobacteria* subdiv. 4 and *Acidobacteria* other subdiv. were grouped into one class due to missing values. The dry-down analysis did not include *Acidobacteriales*, *Acidobacteria* other subdiv., *Planctomycetacia* or *Spartobacteria*, which were below the abundance threshold.

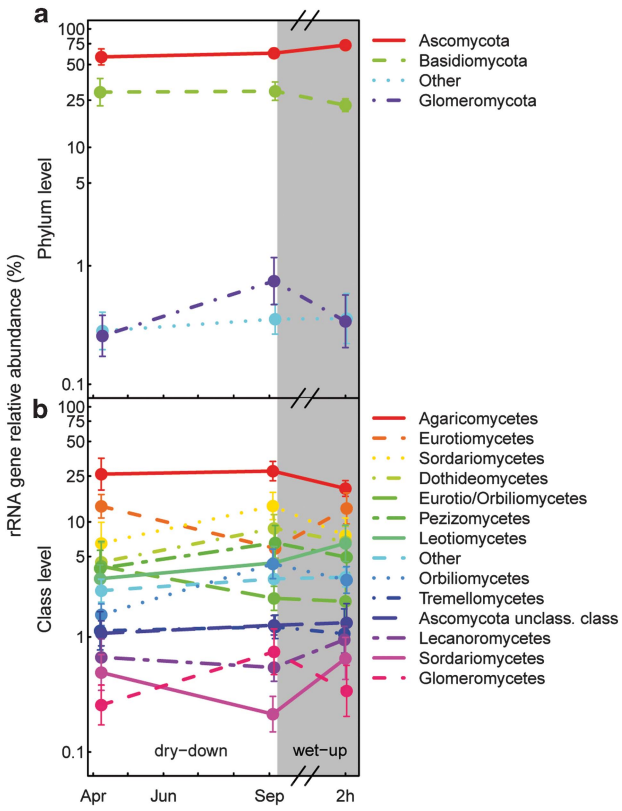

Figure 5 Dynamics of the 28S rRNA gene-based relative abundance of the main present fungal groups at the phylum (**a**) and class (**b**) levels, sequenced during dry-down and wet-up (grey area, note the difference in x-axis scale) over the three experimental sites. Bars indicate ± 1s.e. ($n = 9$).

The *Gammaproteobacteria* (*Proteobacteria* phylum) and *Spartobacteria* (*Verrucomicrobia* phylum) classes followed patterns similar to that of *Acidobacteria*.

Response of fungal groups to dry-down and wet-up

Fungal 28S rRNA gene. Dry-down had a significant effect only on the relative abundance of present fungi at the phylum level over all sites (Figure 5a, Supplementary Table S9). The relative abundance of the different fungal classes responded differently to dry-down over the three sites (Figure 5b, Supplementary Table S10). Wet-up had no significant effect on the relative abundance of the different fungal taxa, at either phylum or class level. However, wet-up affected differently the relative abundance of the different phyla and classes present at the different sites. The three-way interaction indicated a significantly different response of the different phyla to dry-down and wet-up among sites, but explained only 2.8% and 2.5% of the variance of the data at the phylum and class levels, respectively. No phylum or class appeared to statistically stand out in its response to dry-down or wet-up.

Fungal 28S rRNA. Over the taxa considered, neither dry-down nor wet-up affected the potentially active fungi over all three sites, at either phylum or class level (Figure 6, Supplementary Tables S11 and S12). We found differences among sites over the dry-down period at the phylum level for the most abundant taxa, with sites significantly differing in their response to dry-down. Note that this site × dry-down interaction was not significant when considering the UniFrac index of all taxa. Among taxa, both the different potentially active fungal phyla and classes differed in their relative abundance response to dry-down over the three sites but not to wet-up. The different phyla and classes did not change differently at the different sites over dry-down or wet-up. The three-way interaction was significant for dry-down at the class level, explaining 6.3% of the variance of the data. No phylum or class appeared to statistically stand out in its response to dry-down or wet-up.

Quantification of bacterial and fungal genes and transcripts

On the basis of qPCR data, the abundance of bacterial 16S, fungal 28S, archaeal 16S and bacterial *rpoB* genes (DNA) and transcripts (rRNA) differed among sites, both over dry-down and wet-up

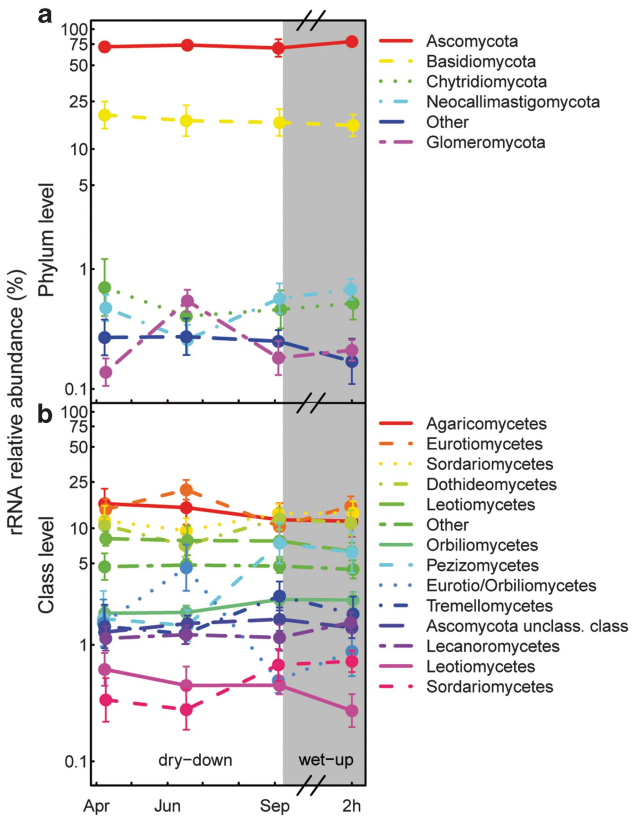

Figure 6 Dynamics of the 28S rRNA-based relative abundance of main potentially active fungal groups at the phylum (**a**) and class (**b**) levels, sequenced during dry-down and wet-up (grey area, note the difference in x-axis scale) over the three experimental sites. Bars indicate ±1s.e. ($n=9$). The dry-down analysis did not include *Leotiomycetes* or *Sordariomycetes*, which were below the abundance threshold.

(Figure 7, Supplementary Table S13). Over all sites, dry-down significantly increased the abundance of bacterial 16S, fungal 28S and bacterial *rpoB* genes by 93%, 298%, 88% and 98%, respectively (Figures 7a–d), with no significant difference in the response among sites. Dry-down had no significant effect on the abundance of bacterial 16S, fungal 28S or archaeal 16S transcripts, but affected the abundance of the bacterial *rpoB* transcripts, with a significant site × dry-down interaction driven by a strong decrease in the Hopland and Sierra sites, whereas the Sedgwick site was quite unresponsive (data not shown).

Over all sites, wet-up had no significant effect on the abundance of the genes measured using qPCR (Supplementary Table S13). The wet-up response of bacterial 16S and fungal 28S gene abundance was significantly different among sites (little response at Hopland, decrease at Sierra and increase at Sedgwick, data not shown). Wet-up significantly increased the abundance of fungal 28S and bacterial *rpoB* transcripts (average +34% and +3399%, respectively, Figures 7e–h) over all sites, with no significant difference in the response among sites.

Discussion

Bacterial and fungal community-level response

The composition of both the DNA-based (present) and rRNA-based (potentially active) bacterial communities at the three Californian grassland sites were site-specific. However, their compositional response to dry-down and wet-up did not differ among sites. The most prominent features of these responses to changes in water availability were (i) the relatively small magnitude of change of most present bacterial groups and (ii) the large relative response of several groups, in particular *Actinobacteria* (increased in relative abundance with desiccation, decreased with rewetting) and *Acidobacteria* (decreased in relative abundance with desiccation, increased with rewetting), the two most abundant phyla of both the present and potentially active communities. Thus, in these grassland systems, soil bacterial communities appear to follow parallel responses to changes in water availability, despite differences in community composition, soil type and local climate. In contrast to the bacterial community, neither the present nor the potentially active fungal communities in the field were detectably different among sites. In addition, these fungal communities remained unaffected by dry-down or wet-up, displaying a marked resistance to changes in water availability. The contrasted bacterial and fungal responses support the ecological distinction of fungi and bacteria (de Boer *et al.*, 2005), which here appear to occupy different water-related niches. The dry-down response of both the present and the potentially active fungal communities did not differ among sites, which is similar to our findings for bacteria. Our study is consistent with seasonal changes in the microbial community in arid or semi-arid systems, shown using ^{13}C-PLFA targeting the active microbial community (Waldrop and Firestone, 2006b), or a DNA-based approach targeting the present community (Clark *et al.*, 2009; Cruz-Martinez *et al.*, 2009).

The rRNA-based soil bacterial community structure in all three sites returned to pre-dry-down structure within 2 h of rewetting, highlighting the resilience of the potentially active soil bacterial communities in California semiarid grasslands. The phylogenetic structure of the RNA-based community reflected this resilience, as the desiccation-related increase in clustering was released upon wet-up, returning to pre-desiccation levels. In contrast, both the DNA-based bacterial community structure and NRI remained unaffected by changes in water availability. The dry-downs in our study constituted normal summer dry-downs for these sites, and thus we did not expect large changes in the taxonomic structure of the present communities over summer. On the other hand, the RNA-based community would be expected to be more variable, as RNA is known to be much more responsive to changing environmental conditions. Our results

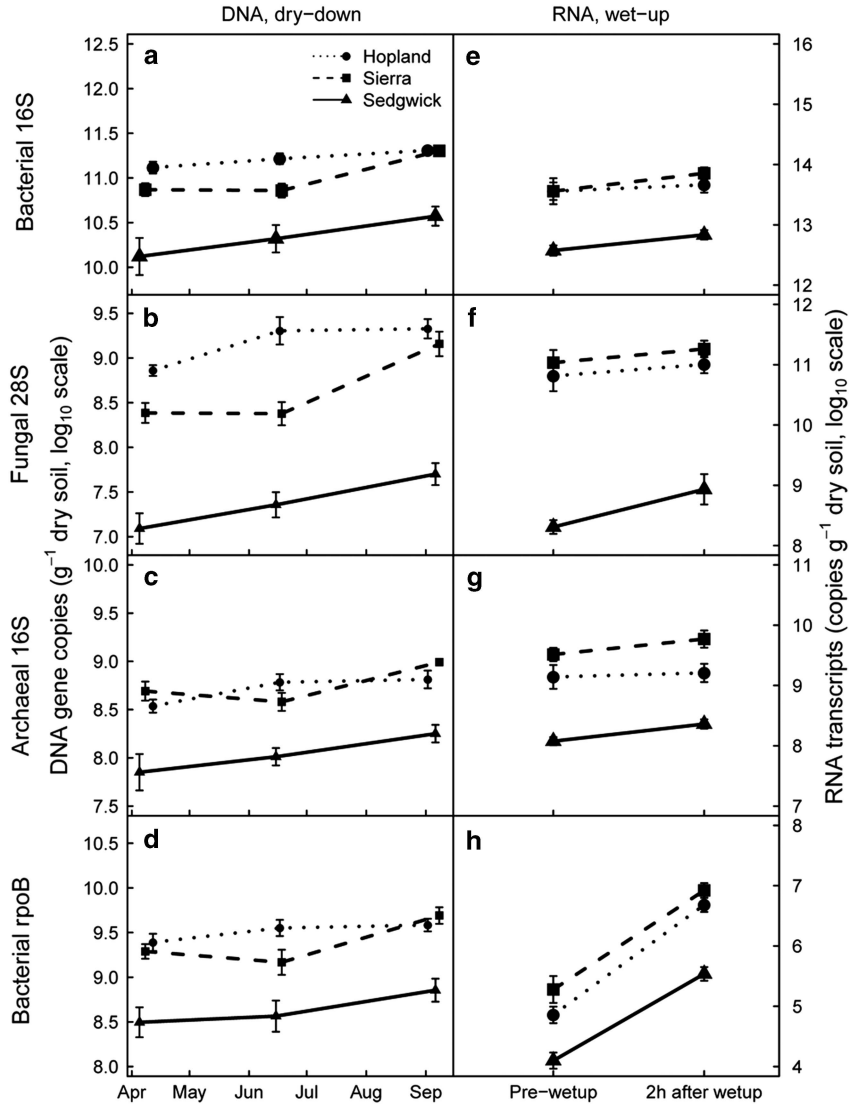

Figure 7 Abundance of selected genes over dry-down (left panels) and RNA transcripts over wet-up (right panels) for the experimental sites (Hopland: dots, Sierra: dashes, Sedgwick: full), obtained by qPCR. Bars indicate ± 1s.e. ($n = 3$).

point towards dynamic changes in the potential activity of several major bacterial taxa (in particular belonging to the *Actinobacteria* and *Acidobacteria* phyla), depending on soil moisture status. Further, the spectacular increase in *rpoB* transcript abundance, which was consistent across sites, reflects the rapid resumption of transcriptional activity in soil bacteria upon rewetting.

The gene abundances of bacterial 16S, fungal 28S and archaeal 16S increased during dry-down; however, their respective transcript abundances did not change. This suggests an increased number of microbial cells with less potential for activity per cell. The increase in number of microbial cells is consistent with the reported increase in microbial biomass in a California grassland over the dry summer (Parker and Schimel, 2011). This is also consistent with previous work showing bacterial cell division and down-sizing in response to desiccation as well as starvation (Potts, 1994; Alvarez *et al.*, 2004).

As a whole, the bacterial community appears to tolerate the rapid upshock in water potential (influx of water), which is known to cause lysis (plasmoptysis) in many bacterial cells that have adjusted to low water potential conditions (Kieft *et al.*, 1987; Halverson *et al.*, 2000). On the basis of the rapid (within 2 h) increase in rRNA in bacteria, archaea and fungi, the members of these soil communities appear to be poised for immediate response to rewetting (Placella *et al.*, 2012); this is consistent with the large pulses of CO_2 that result from wetting of dry soil (Borken and Matzner, 2009; Inglima *et al.*, 2009). The extremely dynamic response of the relative potential activity of the different bacterial phyla and classes to rapid changes in water availability that we found would not have been detectable using DNA-based methods, but the

combination of rRNA-based with DNA-based molecular analyses has the potential to further unveil the microbial mechanisms that drive the CO_2 pulse upon rewetting dry soils.

Water-related bacterial and fungal life-strategies

Changes in relative abundance of the most numerous bacterial taxa (by pyrosequencing) were mainly due to differing responses of different taxa rather than to site-driven responses, whether considering the present or the potentially active community. Here, we define a life-strategy as a set of traits driving the response to environmental and physiological conditions, and geared towards survival, growth and reproduction. The analysis at the phylum and class levels point towards different life-strategies within the potentially active bacterial community. In contrast, the fungal community generally remained unaffected by desiccation. Our direct characterisation of the stronger desiccation resistance of the potential activity of fungi compared with bacteria is consistent with the results from recent studies that used a PFLA approach to document the stability of fungal-based compared with bacterial-based food webs in grasslands (Gordon *et al.*, 2008; de Vries *et al.*, *et al.*, 2012).

In the bacterial community, changes in the ratio of rRNA transcript to ribosomal rRNA gene abundance of the most abundant phyla were driven by changes in the former in our experiment, indicating that changes in rRNA transcript abundance mainly reflected changes in the level of cellular transcriptional machinery, that is, ribosome synthesis in the cell. The response of the relative rRNA abundance of the main bacterial phyla to changes in water availability followed three distinct patterns in our experiment. Two response patterns were characterised by high responsiveness to changes in soil moisture but with contrasted strategies. First, the *Acidobacteria* and *Verrucomicrobia* phyla displayed a water-related opportunistic behaviour: these phyla showed a rapid decline in ribosomal synthesis as dry-down progressed, mirrored by a fast response of ribosome synthesis to rewetting. Second, the *Actinobacteria* phylum showed the opposite response pattern, their synthesis of ribosomes being stimulated by desiccation and reduced after rewetting. By accumulating ribosomes throughout the dry-down period, *Actinobacteria* may be able to gain a head start as soon as conditions become more favourable for nutrient acquisition. The observed decline in relative ribosome levels upon rewetting may possibly be explained by a competitive disadvantage during the first hours of rewetting. Numerous members of the Gram-positive, high-G+C content *Actinobacteria* phylum are drought resistant and have been shown to be able to grow under challenging dry conditions (Goodfellow and Williams, 1983; Zvyagintsev *et al.*, 2007). Nevertheless, the *Rubrobacteridae* class, which is abundant at our sites as well as in other water-limited soils, remains poorly characterised (Holmes *et al.*, 2000; Placella *et al.*, 2012). In contrast with the *Actinobacteria* phylum, the *Acidobacteria* and *Verrucomicrobia* phyla do not appear to invest in ribosome accumulation during desiccation, but to rely on extremely rapid ribosomal synthesis upon rewetting. The third response pattern was characterised by a resistant response pattern, with generally stable ribosome content throughout both dry-down and wet-up, in the phyla *Proteobacteria*, *Chloroflexi*, *Firmicutes*, *Gemmatimonadetes* and *Planctomycetes*.

The classification of bacterial groups with respect to nutrition and growth is a century-long effort (Winogradsky, 1924, see terminology overview by Langer *et al.*, 2004). Fierer *et al.*, (2007) have proposed an ecological classification on the basis of a few deep-rooted bacterial phyla along a copiotrophic (analogue to r-strategist: fast growing under high nutrient availability, highly variable population size) to oligotrophic range (analogue to K-strategist: slow-growing, more stable population size). Several studies have documented the oligotrophic life-strategy of *Acidobacteria* and *Verrucomicrobia*, contrasting with the copiotrophic *Actinobacteria*, along soil carbon and nitrogen availability gradients (Fierer *et al.*, 2007; Nemergut *et al.*, 2010; Fierer *et al.*, 2012; Ramirez *et al.*, 2012). In contrast, we find that when considering the water availability and time dimensions, a different ecological classification emerges, where (i) *Acidobacteria* and *Verrucomicrobia* follow a fast-response life-strategy, (ii) *Actinobacteria* displays a preparedness strategy and iii) *Proteobacteria*, *Chloroflexi*, *Firmicutes*, *Gemmatimonadetes* and *Planctomycetes* follow a more stable and resistant life-strategy. Our results show that niche dimension matters for bacteria: considering the soil water availability and time dimensions rather than the nutrient availability dimension involves a different set of traits and results in a different ecological life-strategy classification (for example, Lennon *et al.*, 2012). Our results support the ecological grouping of bacterial phyla along the lines of the life-history concepts initially developed for plants and animals (MacArthur and Wilson, 1967), but stress the need to include the life-strategies of bacterial phyla within a framework of multi-dimensional niche.

The clear responses that we determined were apparent at phylum and class levels, showing that patterns of bacterial response to changes in water availability were conserved at high phylogenetic groupings, supporting the recent hypothesis of ecological coherence among deeply rooted bacterial groups (Philippot *et al.*, 2010; Lennon *et al.*, 2012; Placella *et al.*, 2012). Such a coherence appears all the more surprising in that the most responsive bacterial phyla, *Actinobacteria* and *Acidobacteria*, form broad, diverse groups that likely cover a wide metabolic range (Quaiser *et al.*, 2003; Gao

and Gupta, 2012). Our findings suggest that deep taxonomic nodes may constitute meaningful ecological units for the response to drying and rewetting. Better understanding the evolutionary implications of the traits that underlie this coherence would be a valuable next step.

Conclusion

Our study shows that adaptation of soil bacteria to extreme soil desiccation and rapid rewetting in Mediterranean grasslands is expressed through different bacterial life-strategies, conferring a spectacular resilience to the potentially active bacterial communities. The similar response trajectories of these communities across sites, despite site-specific community composition and structure, suggest that these life-strategies may be indicative of general patterns. The desiccation response of fungi seemed to reflect a high degree of resistance to changes in water availability. Future trait-based approaches should be useful to provide insights into the response of ecosystem functions in relation to shifts in microbial groups. Changes in precipitation regimes that are predicted to have an impact on Mediterranean systems may affect the life-history envelope of soil microorganisms, which could in turn have an impact on the soil microbial community and its functions, with potential effects on carbon budgets and nutrient cycling on a large scale.

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