Soil microbial communities drive the resistance of ecosystem multifunctionality to global change in drylands across the globe

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Data accessibility

The primary data have been deposited in figshare: https://figshare.com/s/8892a0ab3cfff186458e (DOI: 10.6084/m9.figshare.5089942). The raw sequence data have been deposited in the GenBank SRA database (BioProject accession no. PRJNA301533).

Abstract

The relationship between soil microbial communities and the resistance of multiple ecosystem functions (multifunctionality resistance) to global change has never been assessed globally in natural ecosystems. We collected soils from 59 dryland ecosystems worldwide to investigate the importance of microbial communities as predictor of multifunctionality resistance (C, N and P cycling) to climate change and nitrogen fertilization. Multifunctionality had a lower resistance to wetting-drying cycles than to warming or N deposition. Multifunctionality resistance was regulated by changes in microbial composition (relative abundance of phylotypes) but not by richness, total abundance of fungi and bacteria or the fungal: bacterial ratio. Our results suggest that positive effects of particular microbial taxa on multifunctionality resistance could potentially be controlled by altering soil pH. Together, our work demonstrates strong links between microbial community composition and multifunctionality resistance in dryland soils from six continents, and provide insights into the importance of microbial community composition for buffering effects of global change in drylands worldwide.

Keywords: Multifunctionality; Resistance; Carbon; Nitrogen; Phosphorus; Bacteria; Fungi

Introduction

Soil microbes are the most abundant and diverse organisms on Earth (Fierer & Jackson 2006; Locey & Lennon 2016). Recent experiments and observational studies have showed that, consistent with reported observations for plant communities (Cardinale et al. 2011; Maestre et al. 2012; Soliveres et al. 2016), soil microbial diversity plays an important role in maintaining multiple ecosystem functions simultaneously (i.e. multifunctionality) in terrestrial ecosystems (Philippot et al. 2013; Wagg et al. 2014; Delgado-Baquerizo et al. 2016). These functions include, but are not limited to, litter decomposition, nutrient cycling, primary production and the regulation of greenhouse emissions (Wagg et al. 2014; Philippot et al. 2013; Delgado-Baquerizo et al. 2016; Liu et al. 2017). Conversely, the role of microbial communities in regulating the resistance of multifunctionality (multifunctionality resistance hereafter) to global environmental change drivers remains largely unexplored and poorly understood (Orwin et al. 2006; de Vries et al. 2012; de Vries & Shade 2013). Identifying the major microbial drivers (composition, diversity, or abundance) of multifunctionality resistance is crucial for developing sustainable ecosystem management and conservation policies. Such knowledge will help in prioritizing future protection of microbial attributes involved in multifunctionality resistance, with implications to reduce impacts from climate change and land use intensification on terrestrial ecosystems.

Existing knowledge, based mostly on the results of small-scale controlled experiments, suggests that particular soil microbial attributes (e.g. fungal: bacterial ratio) might regulate the resistance of particular ecosystem functions (e.g. soil respiration or N mineralization) to global change drivers such as land use intensification and drought (Orwin et al. 2006; Downing & Leibold 2010; de Vries et al. 2012; de Vries & Shade 2013). However, we lack direct empirical evidence to identify how multiple microbial attributes, including the abundance, richness and composition of soil bacteria and fungi, regulate the response of multifunctionality to global change drivers, particularly at the global scale. Microbial attributes such as abundance, richness and community composition could play important roles in driving multifunctionality resistance to global change (MRGC hereafter), as they constitute important regulators of microbial growth, microbial interactions and key functional attributes belonging to particular taxa (e.g. nitrification). Further, little is known about how changes in the composition of microbial communities across such scales (e.g. dissimilarity across sites; β-diversity) affect MRGC,

125 particularly in drylands. These ecosystems already cover ~45% of Earth's land mass (Prăvălie
126 2016), and are expected to increase by up to 23% by the end of the 21st century due to forecasted
127 increases in aridity under climate change (Huang et al. 2016). Achieving a better understanding
128 of how dryland soil microbes drive MRGC is particularly important because: 1) microbial
129 communities are highly affected by changes in aridity (Maestre et al. 2015), 2) drylands are
130 overrepresented in developing countries (Huang et al. 2016), and 3) 38% of the global population
131 is highly reliant on the primary production of drylands (Powell & Agnew 2011).
132 Herein we assess the importance of soil microbial community composition and
133 abundance for MRGC, including warming, wetting-drying cycles and N fertilization. This has
134 never been assessed at the global scale. We aimed to do so using soils from 59 dryland
135 ecosystems from all continents except Antarctica (Fig. 1). Soils were incubated for 21 days under
136 different conditions to simulate expected impacts from temperature (control & 4.5ºC warming),
137 changes in water availability (control & wetting-drying cycles) and N fertilization (control & 20
138 kg N ha^{-1} $year^{-1}$), which were used as proxies of two major global change drivers (climate change
139 and N deposition; Fig. 2a). Following incubation, we measured eight soil variables (hereafter
140 "functions") related to carbon (starch and cellulose degradation and carbohydrate availability),
141 nitrogen (chitin degradation and availability of nitrate and ammonium) and phosphorus (P
142 mineralization and availability) cycling.
143
144 **Methods**
145 *Study area and soil sampling*
146 Field data were collected between 2006 and 2014 from 59 dryland sites located in 12 countries
147 from all continents except Antarctica (Fig. 1). All the surveyed sites had an aridity index (AI =
148 precipitation/potential evapotranspiration) between 0.05 and 0.65 (UNEP 1992). Locations for
149 this study were selected to cover a wide variety of natural and semi-natural ecosystem types
150 (including grasslands, shrublands and open woodlands) representative of dryland ecosystems
151 worldwide. Field surveys were conducted according to a standardized sampling protocol
152 (Maestre et al. 2012). In brief, a composite topsoil (0-7.5 cm) sample (collected from five
153 randomly selected plant interspaces) was obtained from each site and separated into two
154 portions. One portion was air-dried and used for soil biochemical and functional analyses. The
155 other portion of soil was immediately frozen at -20 ºC for molecular analyses. Note that previous

156 studies have found that air drying and further storage of dryland soils from do not alter the
157 biogeochemistry of these soils (i.e., enzyme activities and nutrient contents; Zornoza et al. 2009).
158 Similarly, previous studies have found a small effect, or no effect from air drying and further
159 storage of soils on the community composition of bacteria and fungi (Macdonald et al. 2008;
160 Lauber et al. 2010). For this reason, this storage approach is generally used in large-scale surveys
161 (e.g., Maestre et al. 2012; 2015).
162 *Environmental and physicochemical analyses.*
163 Air-dried soils were extracted in de-ionized water for 1h to achieve a 1:5 soil: water solution.
164 Soil pH was then determined using a combination pH electrode. Total soil organic carbon (TOC)
165 was determined using the Walkley-Black method as explained in Maestre et al. (2012). The
166 Aridity Index (AI; mean annual precipitation/potential evapotranspiration) was determined from
167 Zomer et al. (2008), and uses interpolations from the Worldclim database
168 (http://www.worldclim.org). For clarity, we used aridity [1–AI] instead of AI (Delgado-
169 Baquerizo et al. 2013a). We used aridity instead of mean annual precipitation in our study
170 because aridity includes both mean annual precipitation and potential evapotranspiration, and is
171 therefore a more accurate metric of the long-term water availability at each site.
172 *Characterizing soil microbial communities.*
173 DNA was extracted using the Powersoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad,
174 CA, USA) according to the instructions provided by the manufacturer. qPCR reactions were
175 performed in triplicate by using 96-well plates on an ABI 7300 Real-Time PCR (Applied
176 Biosystems). The bacterial 16S-rRNA and fungal ITS genes were amplified with the Eub 338-
177 Eub 518 and ITS 1-5.8S primer sets (Evans & Wallenstein 2011). The fungal: bacterial ratio was
178 calculated using qPCR data. Note that calculating this ratio using qPCR may be inaccurate in
179 terms of absolute values; however, it can still be useful for assessing its relationship with MRGC.
180 In addition, we obtained information on the richness and composition of soil bacteria and fungi
181 by performing 16S rRNA and ITS genes amplicon sequencing (Illumina MiSeq platform) and the
182 341F/805R and and FITS7/ITS4 primer sets, respectively (Herlemann et al. 2011; Ihrmark et al.
183 2012). Bioinformatic analyses were conducted using the QIIME package (See Maestre et al.
184 2015 for analytical details). Operational Taxonomic Units (OTUs) were picked at 97% sequence
185 similarity. The resultant OTU abundance tables from these analyses were rarefied to an even
186 number of sequences per samples to ensure equal sampling depth (11789 and 16222 for 16S

187 rDNA and ITS, respectively). Bacterial and fungal alpha diversity (i.e. number of phenotypes)
188 was calculated from these OTUs tables. We also obtained the diversity (i.e. number of
189 phenotypes) of common (the top 10% in terms of number of reads) and rare (the bottom 90%)
190 species as described in Soliveres et al. (2016). Rare species, which are highly vulnerable to
191 global change drivers, are being increasingly recognized as important drivers of ecosystem
192 functioning (Jousset et al. 2017).
193 *Experimental design: soil incubations*
194 Soils were incubated to evaluate the effects of warming, changes in water availability, i.e.
195 wetting-drying cycles, and N fertilization. In parallel, 5 g of soil from each site were placed in
196 four plastic containers, one for each global driver plus an environmental control. The levels of
197 the different treatments were selected to provide a realistic estimation of the response of
198 ecosystem functioning to climate change, and land use intensification such as N fertilization
199 from atmospheric N deposition and livestock dung in global drylands. Thus, the environmental
200 control was incubated at 25ºC, the average land surface temperature for all sites (see
201 https://neo.sci.gsfc.nasa.gov/), and 35% of water holding capacity (WHC). The amount of water
202 in the control was chosen to ensure a minimum of microbial activity during the incubation period
203 (Fig. 1 in Schwinning & Sala 2004; Delgado-Baquerizo et al. 2013b,c). The warming treatment
204 had similar water conditions as the environmental control but with increased temperature
205 (+4.5ºC; Fig. 2a). This temperature increase mimic global warming forecasts by the end of this
206 century (A2 scenario from IPPC 2013). The wetting-drying treatment was incubated at the same
207 temperature than the environmental control, but included four wetting-drying cycles. Each
208 wetting-drying cycle involved wetting until a 35% WHC was achieved and a subsequent natural
209 drying for five days. Soil samples were watered the first day of incubation (Fig. 2a). Rapid
210 changes in water availability, such as those from wetting-drying cycles, are expected to increase
211 with climate change in global drylands (IPPC 2013). Finally, the N fertilization treatment
212 includes the same temperature and water conditions as the environmental control plus the
213 equivalent to 20 kg N ha^{-1} $year^{-1}$ (Fig. 2a), which were added in the form of NH_4NO_3 during the
214 first watering. This amount was selected to simulate artificial N loads from N deposition and N
215 in manure from grazing, a major driver of land degradation in drylands worldwide (Eldridge &
216 Delgado-Baquerizo 2017). The levels applied at our study sites (Fig. 1) were predicted using
217 published mapping information (Dentener et al. 2006; Potter et al. 2008). Moisture content was

adjusted and maintained at 35% WHC during the duration of the experiment for all treatments other than the wetting-drying treatment. A total of 236 samples (59 sites x 4 treatments) were incubated under the different treatments for 21 days.

Assessing multiple ecosystem functions

After incubation, we measured in all soil samples eight functions related to C, N and P cycling: activity of β-glucosidase (starch degradation), β-D-celluliosidase (cellulose degradation), N-acetyl-β-glucosaminidase (chitin degradation) and phosphatase (organic phosphorus mineralization) and four measurements of C (dissolved carbohydrates), N (ammonium and nitrate) and P (inorganic P) availability. Extractable carbohydrates, ammonium and nitrate were obtained from K_2SO_4 extracts as explained in Delgado-Baquerizo et al. (2013a). Soil P availability was estimated from sodium bicarbonate extracts as described in Maestre et al. (2012). Extracellular soil enzyme activities were measured from 1g of soil by fluorometry as described in Bell et al. (2013). Overall, these variables constitute good proxies of processes driving nutrient cycling, biological productivity, and the buildup of nutrient pools (Maestre et al. 2012). In brief, carbohydrates are an essential source of energy for soil microbes and are used as an indicator of organic matter biodegradability (De Luca 1993). Extracellular enzymes such as those we measured are produced by soil microorganisms and are involved in the processing, stabilization, and destabilization of soil organic matter and nutrient cycling in terrestrial ecosystems (Bell et al. 2013). They are also considered a good indicator of nutrient demand by plants and soil microorganisms (Bell et al. 2013). Ammonium and nitrate are important N sources for both microorganisms and plants, and are produced by important ecosystem processes such as N mineralization and nitrification (Schimel & Bennett 2004). Inorganic P is the main P source for plants and microorganisms, and its availability is linked to the desorption and dissolution of P from soil minerals (Vitousek et al. 2004). We explicitly focused on the bioavailable pools of C, N and P (usually <1% of the total of their respective forms) because the total pools of these elements may not be relevant for the MRGC within our short-term incubation experiment.

Assessing the resistance of multiple ecosystem functions to global change drivers

We used the Orwin & Wardle (2004) index (RS) to evaluate the resistance of multiple functions as:

$$RS = 1 - \frac{(2 \cdot (D_0))}{((C_0) + (D_0))}$$

In this equation, D_0 is the difference between the environmental control (C_0; value of each functional variable in the absence of global change treatments) and the disturbed (P_0, warming, wetting-drying cycles and N fertilization treatments) soils after the incubation period. This index has the advantage of being: i) standardized by the control, and ii) bounded between -1 (lowest resistance) and +1 (maximal resistance) even when extreme values are encountered (Orwin & Wardle 2004). We calculated the resistance of each function independently for each global change driver. After this, and to evaluate MRGC, we averaged the resistance of the eight functions measured to obtain a standardized index of multifunctionality resistance. Similar approaches have been used to obtain multi-stability (Durán et al. 2017) and multifunctionality (Maestre et al. 2012; Wagg et al. 2014; Delgado-Baquerizo et al. 2016) indexes, as well as response ratios in meta-analysis (Eldridge & Delgado-Baquerizo 2016). Note that our study focuses on the simultaneous responses of multiple functions to global change rather than on the response of single functions that might not be representative of the overall functioning of a particular ecosystem.

Statistical analyses

Relationship between microbial community composition and multifunctionality resistance

We first explored the overall relationship between the β diversity of microbial communities and MRGC. To do this, we calculated microbial β-diversity using Bray–Curtis dissimilarity matrices at the OTU level independently for bacterial and fungal communities. Similarly, the Euclidean distance was used to create three independent distance matrices from the resistance of eight single functions. A matrix was constructed for each of the three global environmental drivers: warming, wetting-drying cycles and N fertilization. We then independently correlated the β-diversity of bacteria and fungi to the dissimilarity matrices from resistance measurements using Mantel correlations (Pearson). We also assessed all possible Mantel correlations (Pearson) among resistance multifunctionality to warming, drying-wetting cycles and N fertilization.

Random Forest modeling

To gain a mechanistic understanding of the drivers of MRGC, we conducted a classification Random Forest analysis (Breiman 2001) as described in Delgado-Baquerizo et al. (2016), which allowed us to identify common microbial predictors across sites. We used class-level information

in these analyses for two main reasons (1) information on microbial functional traits has become increasingly available at this taxonomic level (Fierer et al. 2007; Trivedi et al. 2013); and (2) unlike high taxonomic rank information (OTU/genus), class-level taxa are shared across all soil samples at the global scale, allowing us to infer general patterns in the role of microbial composition in predicting MRGC at this spatial scale. In addition to class-level predictors, we included in our models other microbial attributes such as abundance (qPCR), fungal: bacterial ratio and alpha diversity (richness of all, common and rare fungi and bacteria). The importance and statistical significance of each predictor were computed using the rfPermute package (Archer 2016) of the R statistical software, version 3.0.2 (http://cran.r-project.org/). We also used Spearman correlations between selected major microbial attributes from Random Forest analyses and the resistance of single functions to global change. The aim of this approach was to obtain insights into the relationships between the relative abundance of particular microbial taxa and the resistance of specific functions, complementing results from MRGC analyses.

Structural equation modeling

We used structural equation modeling (SEM; Grace 2006) to evaluate the direct and indirect relationships between geographical location (latitude and longitude), aridity, soil properties (pH and soil total organic carbon) and microbial attributes on MRGC based on expectations under an *a priori* model (Fig. S1). Microbial drivers included pre-selected major significant MRGC predictors from Random Forest analyses described above. Aridity and soil properties such as total organic carbon and pH are major drivers of microbial community composition in drylands (Fierer & Jackson 2006; Fierer et al. 2012; Maestre et al. 2015). These same drivers have been reported to strongly influence multifunctionality in global drylands (Delgado-Baquerizo et al. 2016). Geographical location was included in our models to control for spatial autocorrelation (Delgado-Baquerizo et al. 2013a). In our study, aridity does not represent a lack of available water because soils were watered during incubation. Rather, we included it to illustrate the legacy effects of aridity on soil properties and microbial communities. Microbial drivers and geographical location were included as composite variables in the SEM. The use of composite variables does not alter the underlying SEM model, but collapses the effects of multiple conceptually-related variables into a single composite effect, aiding to interpret model results (Grace 2006).

As some of the variables introduced were not normally distributed, the probability that a path coefficient differs from zero was tested using bootstraping. Bootstrapping is preferred to the classical maximum-likelihood estimation in these cases because probability assessments are not based on the assumption that the data conform to a specific theoretical distribution. Bootstrapped data were randomly sampled, with replacement, to derive estimates of standard errors associated with the distribution of the sample data. Following these data manipulations, we parameterized our model and tested its overall goodness-of-fit. There is no single universally accepted test of overall goodness-of-fit for SEM (Schermelleh-Engel et al. 2003). We used three metrics to quantify the goodness of fit of our model: (1) Chi-square test (χ^2; the model has a good fit when $0 \leq \chi^2/df \leq 2$ and $0.05 < P \leq 1.00$) (Schermelleh-Engel et al. 2003), (2) The root mean square error of approximation (RMSEA; the model has a good fit when $0 \leq RMSEA \leq 0.05$ and $0.10 < P \leq 1.00$) (Schermelleh-Engel et al. 2003) and (3) Bollen-Stine bootstrap test (the model has a good fit when 0.10 < Bollen-Stine bootstrap *P-value* ≤ 1.00). The different goodness-of-fit metrics used indicate that our *a priori* model was satisfactorily fitted to our data, and thus no post hoc alterations were made.

Finally, to aid interpretation of the SEM, we calculated the standardized total effects (STEs) of geographical location (latitude and longitude), aridity, soil properties (pH and soil total organic carbon) and microbial attributes on MRGC. The STEs, the net influence that one variable has upon another is calculated by summing all direct and indirect pathways between the two variables. If the model fits the data well, the total effect should approximate the bivariate correlation coefficient for that pair of variables.

Results

On average, multifunctionality showed the lowest and highest resistance values to wetting-drying cycles and N fertilization, respectively (Fig. 2b; $P < 0.001$). The resistance of single functions to global change drivers followed similar patterns to those observed for MRGC (Table S1; Fig. S2). Mantel tests revealed that the more similar the microbial communities between two sites, i.e. the more similar their β-diversity, the more similar their functional resistance to warming, wetting-drying cycles and N fertilization is (Fig. 3; $P < 0.05$). Interestingly, we also found significant positive relationships among multifunctionality resistance to warming and to wetting-drying cycles and N fertilization (Fig. S2; $P < 0.05$). Conversely, we failed to find any significant

relationship between the richness of fungi and bacteria and MRGC (Table S2). The abundance of bacteria was positively related (Spearman $\rho = 0.26$; $P = 0.05$) to multifunctionality resistance to warming (Table S2).

In general, the composition of fungi and bacteria were selected over other microbial drivers as the main predictors of MRGC (Fig. S3). We found that a relatively small proportion of bacterial and fungal taxa (2-10%) were major drivers of MRGC in our studied drylands (Fig. S3). Microbial attributes selected by Random Forest analyses as major predictors of MRGC were also significantly correlated with the resistance of single functions to the global change drivers evaluated (Table S3). The fungal: bacterial ratio was never selected as a major predictor of MRGC by our Random Forest models. Even so, we still found a positive correlation between this ratio and the resistance of particular functions such as nitrate (Spearman $\rho = 0.27$; $P = 0.04$) and carbohydrate availability (Spearman $\rho = 0.23$; $P = 0.08$).

Our SEM analyses provided further evidence that microbial taxa can have both positive and negative effects on MRGC via direct effects and that these effects are maintained after accounting for important drivers of soil microbial communities and ecosystem multifunctionality (Fig. 4; Appendix S1; Table 1). For example, the relative abundance of class Saprospirae (Bacteroidetes) was negatively related to the resistance of multifunctionality and labile C availability to warming (Fig. 4 and Tables 1 and S3). Conversely, the relative abundance of the classes Solibacteres and Spartobacteria (phyla Acidobacteria and Verrucomicrobia) were both positively related to the resistance of multifunctionality and starch degradation to drying-wetting cycles and warming, respectively (Fig. 4, Table 1; Appendix S1). Selected examples of specific effects from microbial taxa on MRGC are given in Table 1 and explained in detail in Appendix S1.

We also found that, compared with geographical location, soil carbon and aridity, only pH had a consistently net positive effect on MRGC (Fig. 4). This was an indirect effect driven via changes in the soil microbial composition induced by this variable (Fig. 4). For example, pH had a negative direct effect on the relative abundance of Spartobacteria and Saprospira, which were both negatively related to multifunctionality resistance to warming (Fig. 4; Table 1). Moreover, soil pH had a positive effect on the class Gitt-GS-136, which promotes multifunctionality resistance to drying-wetting cycles, and negatively related to the class Solibacteres, which reduced multifunctionality resistance to wetting-drying cycles (Fig. 4; Table

1). Finally, pH had a positive effect on the relative abundance of class Fibrobacteria, which increased the resistance of multifunctionality to N fertilization (Fig. 4; Table 1).

Discussion

Our study provides strong evidence for a link between the composition of bacterial- and fungal-communities and multifunctionality resistance to warming and fertilization in dryland soils from across the globe. Most importantly, we identified particular microbial taxa that are likely to be major drivers of the resistance of multifunctionality to these major global change drivers. In the short-term –while improvements in microbial isolation and culturing techniques take place–, our results suggest that MRCG could be promoted by altering soil properties such as pH, a major driver of microbial community composition (Fierer & Jackson 2006; Lauber et al. 2009). Notably, multifunctionality had a lower resistance to wetting-drying cycles than to warming or N deposition. This is an interesting point, as we should expect that wetting-drying cycles are the disturbances that these dryland soils are more likely to be adapted to. However, our results accord with the largely accepted notion that water availability is the principal driver of ecosystem functioning in drylands (Maestre et al. 2012). It further indicates that more intense wetting-drying cycles will reduce MRGC in drylands worldwide (Evans and Wallenstein 2014). Overall, our work provides new insights into the importance of microbial composition for buffering the negative effects of global change drivers.

Interestingly, we also detected significant positive relationships between multifunctionality resistance to warming and to wetting-drying cycles and N fertilization, suggesting some commonalities in the processes driving MRGC across the globe (Fig. S3). The importance of soil microbial communities as drivers of multifunctionality is supported by a number of small-scale experiments showing that total abundance of microbes controls the resistance of particular functions such as soil respiration or N mineralization to drought (de Vries et al. 2012; de Vries & Shade 2013; Downing & Leibold 2010). However, to the best of our knowledge, our results provide the first empirical evidence, based on experimental manipulation, that microbial community composition and multifunctionality resistance are linked at the global scale. Our findings indicate, therefore, that microbial community composition can be critical for maintaining MRGC, and that changes in this composition resulting from land use intensification

(Gossner et al. 2016) or climate change (Maestre et al. 2015) will likely alter the resistance of critical ecosystem functions to global change drivers in drylands across the globe.

Our Random Forest analysis allowed us to identify particular microbial taxa (class level) as major predictors of MRGC over other microbial attributes such as abundance, diversity and fungal: bacterial ratio. In particular, we found that a relatively small proportion of bacterial and fungal taxa (2-10%) were major drivers of MRGC. These included specific classes within phyla *Verrucomicrobia*, *Bacteroidetes*, *Chloroflexi*, *Acidobacteria*, *Firmicutes* and *Ascomycota*, which are globally distributed (Ramirez et al. 2014; Maestre et al. 2015). The same microbial taxa were also correlated with the resistance of single functions to global change (Table S2). These results imply that different microbial drivers govern multifunctionality and MRGC in dryland soils worldwide. Thus, while multifunctionality *per se* is likely to be driven by multiple microbial attributes (Appendix S2; Figs. S4 and S5), the effects of microbial attributes on MRGC are mostly limited to those from microbial composition via key microbial taxa. These results are consistent with novel soil ecological theories suggesting that key microbial taxa may control the resistance of soil functioning to global change (de Vries & Shade 2013). Conversely, we failed to find any significant relationship between abundance and richness (rare and common species) of fungi and bacteria and MRGC. Similarly, our results further suggest that the fungal:bacterial ratio, previously suggested to be a major predictor of ecosystem functions (de Vries et al. 2012), may be a poor predictor of MRGC. Note that, unlike de Vries et al. (2012), we used a qPCR approach to calculate the fungal: bacterial ratio. Thus, we would like to acknowledge that the use of different methods might also partially explain differences between de Vries et al. (2012) and our results. Nevertheless, we still found a positive correlation between this ratio and the resistance of particular functions such as nitrate, a proxy for nitrification rates, and carbohydrate availability. This finding supports results of a previous study demonstrating strong relationships between the fungal:bacterial ratio, and both N mineralization and soil respiration (de Vries et al. 2012).

Our SEM revealed a direct and significant relationship between the composition of microbial communities and MRGC after accounting for multiple drivers of this resistance. These results further support the notion that key microbial taxa play critical roles in supporting MRGC in dryland soils worldwide. We found that different microbial taxa were involved in the multifunctionality resistance of each global change factor. Given that multiple global change

drivers will occur simultaneously, our results suggest that preserving the diversity of soil microbial communities may be crucial to sustain the provision of ecosystem services in the future. Furthermore, we found both direct positive and negative effects from particular taxa on MRGC. We argue that many of the effects can be understood by drawing on our current knowledge of soil microbial communities. Of special interest is the role that microbial life-strategy (i.e., r- vs. k- strategists) might play in driving MRGC, with special references to C cycling (de Vries & Shade 2013). For example, the relative abundance of class Saprospirae (Bacteroidetes), classified as r-strategist or copiotrophs (Fierer et al. 2007) directly and negatively affected multifunctionality resistance and labile C availability resistance to warming, presumably due to their rapid growth. Conversely, the greatest net negative effect of a microbial taxon on the resistance of multifunctionality (i.e., to wetting-drying cycles) came from Solibacteres (Fig. 4; Table 1), which was positively related to functions associated with the C cycle (e.g. starch degradation) but negatively related to functions from N cycle (e.g. chitin degradation and N availability; Table S3). The positive effect of Solibacteres on the resistance of labile C mineralization is consistent with results from previous studies suggesting that oligotrophic communities (*sensu* Fierer et al. 2007; Trivedi et al. 2013) promote the resistance of functions related to C cycle (de Vries & Shade 2013). The negative effect of class Solibacteres may be related to the necessity of certain bacteria to immobilize/release large amounts of N in osmolytic forms to survive desiccation in response to wetting-drying cycles (Schimel & Balser 2007; Tables 1 and S3; de Vries & Shade 2013). The resistance of starch degradation appears to behave differently to the other functions. Thus, microbial taxa that are positively correlated with the resistance of starch degradation seem to be negatively correlated with the resistance of other functions. This intriguing result suggests that C preferences from microbial communities (labile vs. more recalcitrant) might influence the resistance of particular ecosystem functions to global change drivers.

Our SEM analyses further suggested that by adjusting soil pH we could potentially unleash the positive effects of microbial community composition on MRGC. Thus, pH was the only environmental predictor having a consistent net positive effect on MRGC either by suppressing or promoting taxa that were negatively (Spartobacteria, Saprospira and Solibacteres) and positively (Gitt-GS-136 and Fibrobacteria) related to MRGC, respectively. The importance of soil pH as a major driver of the composition of bacterial and fungal communities in terrestrial

ecosystems is well known (Fierer & Jackson 2006; Lauber et al. 2009). However, our study provides evidence, for the first time, that soil pH also indirectly regulates the effects of microbial community composition on MRGC. These results have implications for the understanding and management of MRGC in the field, as they suggest that we could still potentially increase MRGC by changing soil pH, thereby driving the composition of soil microbial communities in a specific direction. Future endeavors exploring the role of microbial composition in driving multifunctionality resistance may further test this hypothesis using experimental approaches including soil pH manipulations.

Altogether, we found a strong link between soil bacterial and fungal communities and MRGC in soils from global drylands. Our results suggest that key microbial taxa, rather than the richness, abundance and the ratio of bacteria and fungi, control MRGC. They also point to the potential role that manipulations in soil pH could have to buffer negative effects of global change drivers on multifunctionality resistance. Our findings imply that climate- and/or management-induced changes in the composition of soil bacterial and fungal communities may alter multifunctionality resistance, with concomitant effects on the provision of key ecosystem services than rely on them.

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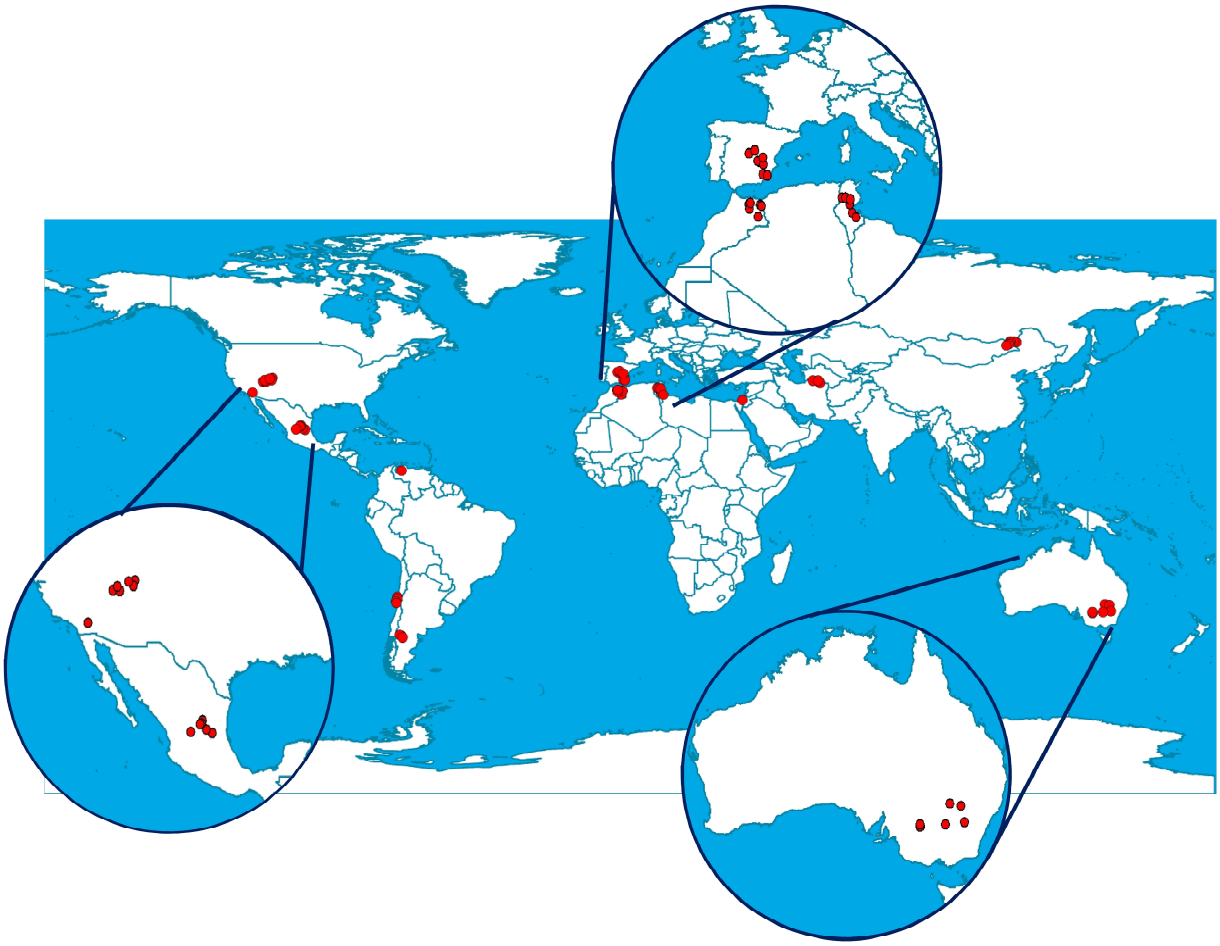


Figure 1. Locations of the 59 sites included in this study.

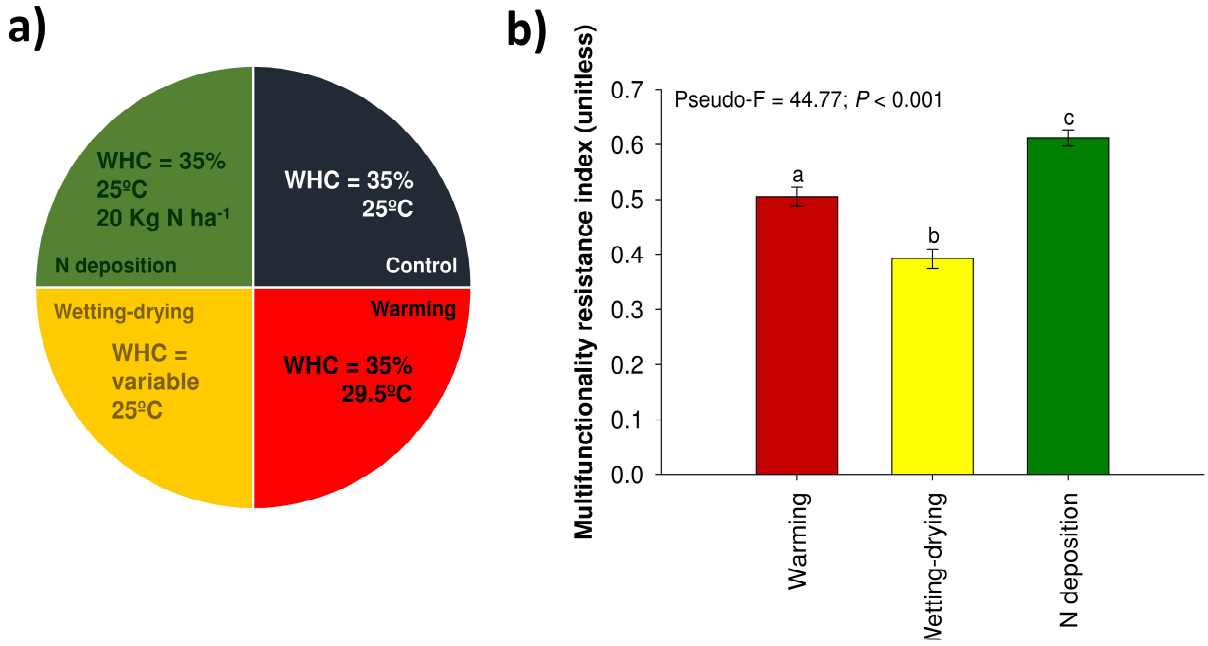


Figure 2. (a) Methodological framework explaining the conditions in all experimental treatments used. (b) Effects of warming, wetting-drying cycles and N fertilization on the multifunctionality resistance of dryland soils from across the globe. Data are means ± SE (n = 59).

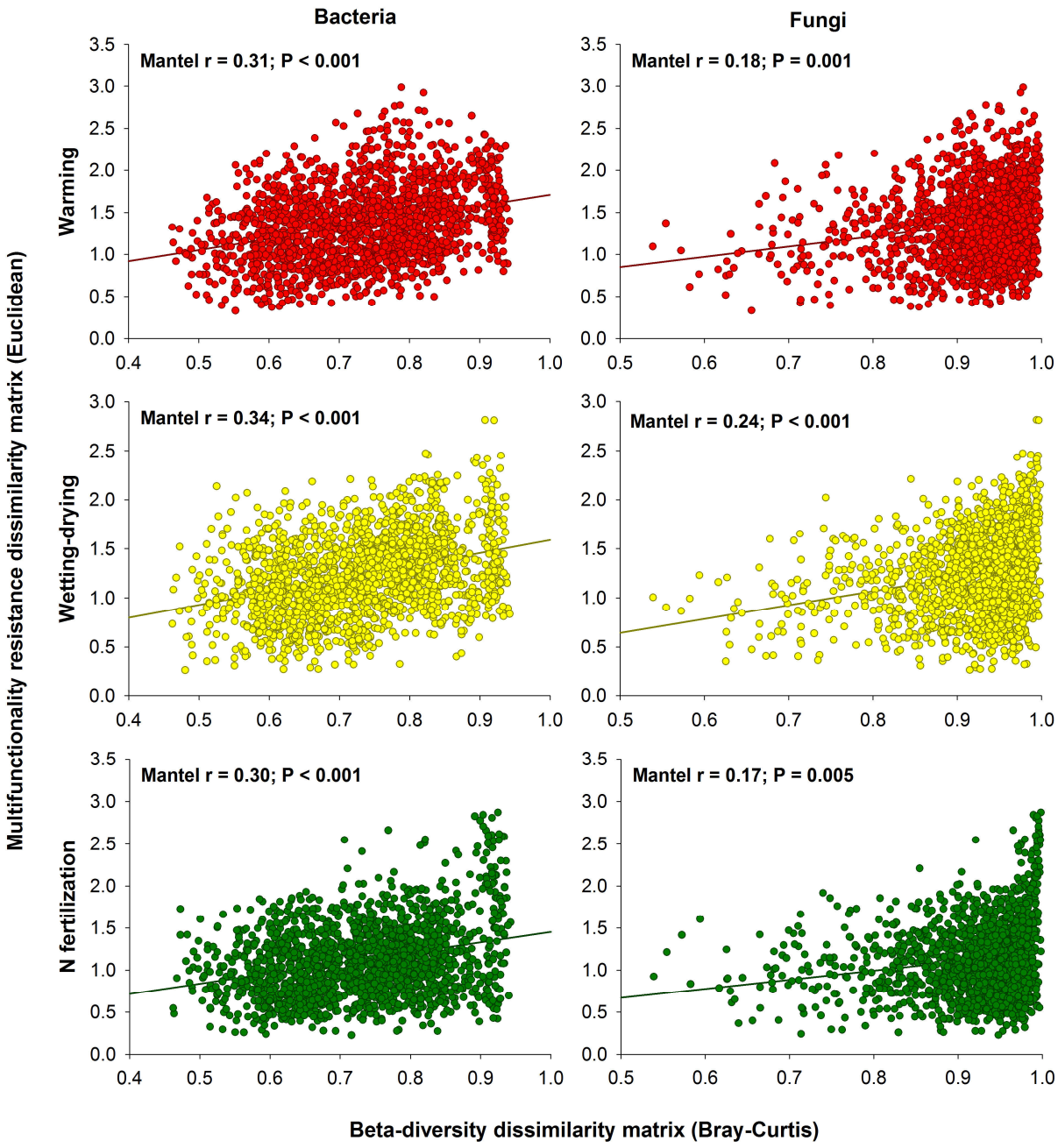
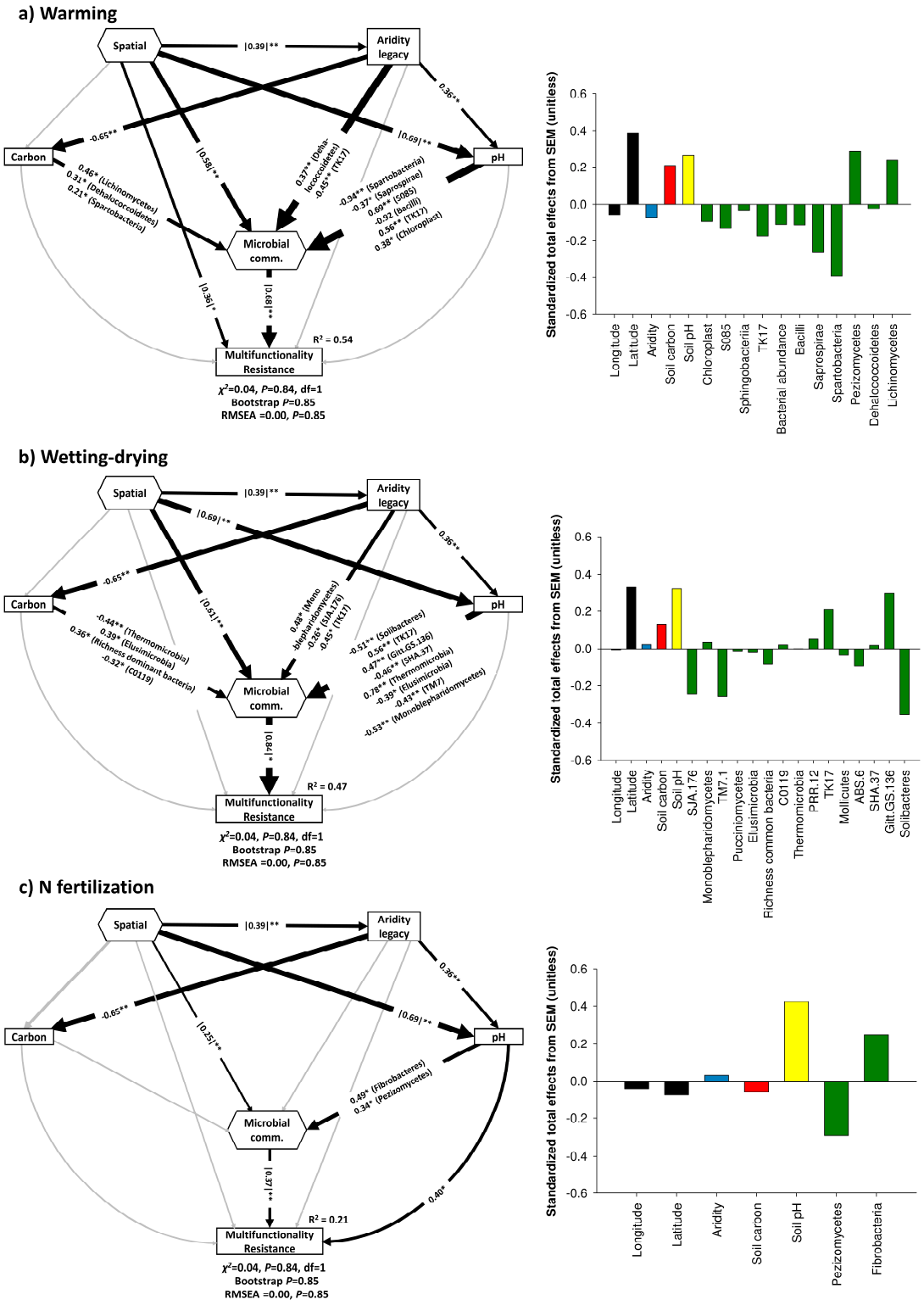


Figure 3. Relationship between community dissimilarity for community composition of bacteria and fungi and multifunctionality resistance to warming, wetting-drying cycles and N fertilization in soils from global drylands. The solid lines represent the fitted linear regressions.



687

688 **Figure 4.** Structural equation model describing the effects of multiple drivers on
689 multifunctionality resistance to warming, wetting-drying cycles and N fertilization. Numbers

adjacent to arrows are indicative of the effect size of the relationship. For simplicity, only significant direct effects are plotted ($P < 0.05$; see *a priori* model in Fig. S1). ($P < 0.05$; see *a priori* model in Fig. S1). Brackets includes information of the particular taxa related to MRGC. R^2 denotes the proportion of variance explained. Significance levels of each predictor are *$P < 0.05$, **$P < 0.01$. Bar graphs include total standardized effects (sum of direct and indirect effects) from SEM on multifunctionality resistance to warming, wetting-drying cycles and N fertilization. Grey lines represent tested, but not significant, paths.

Table 1. Selected examples of the positive and negative effects of differential microbial drivers on the resistance of multiple and single ecosystem functions. This table is derived from results in Fig. 4 and Table S2. An extended version of these examples with further explanations is available in Appendix S1.

Global change driver	Microbial driver	Effect	Function	Microbial trait
Temperature	Lychinomycetes		Multifunctionality, NH_4^+ availability, P mineralization	Ascomycota. Dominant phylum in dry environments. Highly adapted to extreme temperatures conditions (physical protection)
	Pezizomycetes		P mineralization	
	Spartobacteria		Starch degradation (+) Multifunctionality, Chitin degradation & P mineralization (-)	Verrucomicrobia – Saccharolytic. Oligotroph: slow C cycling.
	Saprospirae		Multifunctionality, Labile C availability, Chitin degradation & P mineralization	Bacteroidetes – copiotroph: fast C cycling.
Wetting-drying cycles	Gitt-GS-136		Multifunctionality, Labile C availability, cellulose and chitin degradation & NH4+ and NO3- availability (+) Starch degradation (-)	Chloroflexi – Prefer dry to humid ecosystems. Structural adaptations to desiccation. Resistant– life strategy vs. wetting-drying cycles. Slow-growing bacteria.
	TK17		Multifunctionality, NH4+ availability (+) & Starch degradation (-)	
	Solibacteres		Starch degradation (+), Multifunctionality, chitin degradation & NH4+ and NO3- availability (-)	Acidobacteria – Prefer humid to dry ecosystems. Oligotroph: slow C cycling. May need to immobilize/release large amounts of N (in osmolytes) to survive desiccation
N fertilization	Fibrobacteria		Multifunctionality, NH4+ and P availability	Obligatory anaerobic. Slow-growing bacteria in dry conditions.
	Pezizomycetes		Multifunctionality, Starch degradation	Dryland fungi – N use efficiency. Use N to produce C degradation enzymes.

Supplementary figure captions

Figure S1. *A priori* structural equation model including direct and indirect effects of geographical location, aridity, soil properties and microbial communities on the resistance of multifunctionality to global change.

Figure S2. Effects of warming, wetting-drying cycles and N fertilization on the resistance of eight single functions to global change. Data are means ± SE (n = 59).

Figure S3. Relationship between the matrix of dissimilarity (Euclidean) from multifunctionality resistance to warming, wetting-drying cycles and N fertilization in global drylands. The solid lines represent the fitted linear regressions.

Figure S4. Results from a Random Forest aiming to identify the main significant ($P < 0.05$) microbial predictors of multifunctionality resistance to warming, wetting-drying cycles and N fertilization in global drylands. Pie chart includes the relative abundance of selected taxa driving multifunctionality resistance to global change drivers in dryland soils from across the globe.

Figure S5. (a) Results from a Random Forest aiming to identify the main significant ($P < 0.05$) microbial predictors of multifunctionality in global drylands. **(b)** Pie chart includes the relative abundance of selected taxa driving multifunctionality in global drylands. **(c)** Structural equation model describing the effects of multiple drivers on multifunctionality. Numbers adjacent to arrows are indicative of the effect size of the relationship. Continuous and dashed arrows indicate positive and negative relationships, respectively. This model only includes the direct effects that were statistically significant ($P < 0.05$; see a priori model in Fig. S1). Brackets includes information of the particular taxa related to multifunctionality resistance to global change. R^2 denotes the proportion of variance explained. Significance levels of each predictor are *$P < 0.05$, **$P < 0.01$.

Supplementary Materials

Soil microbial communities drive the resistance of ecosystem multifunctionality to global change in drylands across the globe

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Appendices S1-S2

Tables S1-S3

Figures S1-S5

Appendix S1. Selected examples on specific effects from microbial taxa on MRGC.

Fungal classes *Lychinomycetes* and *Pezizomycetes*, highly adapted to extreme temperatures via physical protection (Paul 2015), had the highest net positive effect on multifunctionality resistance, and that of activity of phosphatase and/or ammonium availability to warming (Table 1; Table S2). *Saprospirae*, however, always had a significant negative effect on the resistance of multiple and single functions to changes in temperature (Fig. 4; Table 1). These results are in agreement with the notion that the resistance of soil functioning may decrease with increasing relative abundance of r-strategists (i.e., copiotrophs), such as those from phylum *Bacteroidetes*, vs. k-strategist (oligotrophs, Table 1, Fierer et al. 2007; Trivedi et al. 2013; de Vries & Shade 2013).

Chloroflexi classes Gitt-GS-136 and TK17, highly resistant to desiccation and wetting-drying cycles (Battistuzzi & Hedges 2009; Barnard et al. 2013), had the highest net positive effect on the resistance of multiple and single ecosystem functions to wetting-drying cycles (Fig. 4; Tables 1 and S2).

Finally, the bacteria class *Fibrobacteria* had the highest positive effect on multifunctionality resistance to N fertilization (Fig. 3; Table 1), as well as positive effects on the resistance of N and P availability (Table S1). These organisms are obligate anaerobes (Rahman et al. 2015), and presumably have a slow growth dynamics in drylands. Because of this, this class might immobilize both N and P during prolonged dry periods (Schimel & Balser 2007) promoting the stability of N and P cycles. On the contrary, the fungi class *Pezizomycetes* had the highest negative microbial effect on multifunctionality resistance and that of starch and cellulose degradation to N fertilization (Fig. 4c; Tables 1 and S1). This class might use N from fertilization to produce enzymes (N-rich molecules) aiming to decompose soil organic matter, reducing the stability of soil C cycle in response to N additions (Austin et al. 2004).

Appendix S2. Identifying major microbial drivers of multifunctionality.

Using the control treatment and information on eight soil functions (activity of β-glucosidase, cellobiosidase, N-Acetylglucosamine and phosphatase and carbohydrates, ammonium, nitrate and inorganic P), we calculated a multifunctionality index (Maestre et al. 2012). To obtain an averaging multifunctionality index for each sample, we first normalized (log-transformed when needed) and standardized each of our eight ecosystem functions using the Z-score transformation

as described in Maestre *et al.* (2012). Following this, the standardized ecosystem functions were averaged to obtain a multifunctionality index (Maestre *et al.* 2012). We then repeated analyses explained in the Methods section including Random Forest and Structural equation modeling to identify the major microbial drivers of multifunctionality. These analyses are independent to those from Delgado-Baquerizo et al. (2016), as here, we used functions measured in the treatment controls for this incubation experiment. Our Random Forest model (Fig. S4) supported the results from Delgado-Baquerizo et al. (2016), further suggesting that richness of bacteria and fungi are major drivers of multifunctionality in global drylands. In particular, our SEM results (Fig. S4) indicate that richness from common bacteria (calculated following Soliveres et al. 2016) and richness from all fungi positively relate to multifunctionality. In addition, our Random Forests selected other microbial attributes –not included in Delgado-Baquerizo et al. (2016)– as major drivers of multifunctionality including bacterial and fungal total abundance and the fungal: bacterial ratio –all of them positively related to multifunctionality– and bacterial and fungal composition –with both positive (e.g. *Ktedonobacteria*) and negative (e.g. *Solibacteres*) effects on multifunctionality–. Our SEM results further suggested that all effects from soil carbon, pH, aridity, latitude and longitude on multifunctionality are indirectly driven via changes in microbial community attributes; being soil organic C the environmental driver with the highest total positive effect (sum of directs and indirect effects from SEM) (Fig. S4).

Table S1. Spearman correlations between the resistance of multifunctionality and that from eight single functions to warming, wetting-drying cycles and N fertilization. P values below 0.05 are in bold. BG = β-glucosidase activity; CB = β-D-celluliosidase activity; PHOS = Phosphatase activity; NAG = N-acetyl-β-Glucosaminidase activity.

Resistance of	Parameters	Resistance of multifunctionality		
		Warming	Wetting-Drying	N fertilization
Carbohydrates	ρ	0.510	0.685	0.572
	P value	<0.001	<0.001	<0.001
Ammonium	ρ	0.533	0.473	0.490
	P value	<0.001	<0.001	<0.001
Nitrate	ρ	0.565	0.473	0.160
	P value	<0.001	<0.001	0.226
Available P	ρ	0.097	0.361	0.421
	P value	0.465	0.005	0.001
CB	ρ	0.276	0.416	0.099
	P value	0.034	0.001	0.457
BG	ρ	-0.090	-0.079	0.191
	P value	0.498	0.554	0.148
NAG	ρ	0.298	0.502	0.330
	P value	0.022	<0.001	0.011
PHOS	ρ	0.548	0.261	0.256
	P value	<0.001	0.046	0.051

Table S2. Spearman correlations between microbial abundance, diversity and the fungal: bacterial ratio and multifunctionality resistance to warming, wetting-drying cycles and N fertilization.

Microbial drivers	Parameter	Warming	Wetting-drying cycles	N fertilization
Fungal abundance	**ρ**	0.204	0.128	-0.048
	P-value	0.122	0.334	0.719
Bacterial abundance	**ρ**	0.256	0.095	-0.177
	P-value	0.051	0.473	0.181
Fungal: bacterial ratio	**ρ**	-0.103	0.059	0.079
	P-value	0.436	0.658	0.553
Richness all bacteria	**ρ**	0.184	0.008	-0.076
	P-value	0.163	0.951	0.565
Richness rare bacteria	**ρ**	0.017	-0.129	-0.12
	P-value	0.896	0.328	0.367
Richness common bacteria	**ρ**	0.215	0.051	-0.048
	P-value	0.103	0.7	0.72
Richness all fungi	**ρ**	0.118	0.03	-0.132
	P-value	0.371	0.823	0.317
Richness rare fungi	**ρ**	0.063	-0.023	-0.118
	P-value	0.637	0.86	0.375
Richness common fungi	**ρ**	0.144	0.098	-0.095
	P-value	0.276	0.462	0.474

883

884

885 **Table S3**. Spearman correlations between selected microbial variables from Random Forest
886 analyses and the resistance of eight single functions to warming, wetting-drying cycles and N
887 fertilization. P values below 0.05 are in bold. BG = β-glucosidase activity; CB = β-D-
888 celluliosidase activity; PHOS = Phosphatase activity; NAG = N-acetyl-β-Glucosaminidase
889 activity.

890

Global change drivers	Microbial variable	Parameter	BG	Carbohydrates	CB	NAG	Ammonium	Nitrate	PHOS	Available P
Warming	**Pezizomycetes**	**ρ**	-0.099	0.107	-0.068	0.201	0.433	0.249	0.34	-0.031
		P-value	0.456	0.42	0.611	0.127	**0.001**	0.057	**0.008**	0.816
	Spartobacteria	**ρ**	0.397	-0.205	0.002	-0.57	-0.148	-0.141	-0.268	-0.021
		P-value	**0.002**	0.119	0.990	**<0.001**	0.263	0.285	**0.040**	0.877
	Saprospirae	**ρ**	0.239	-0.263	0.094	-0.301	-0.219	-0.049	-0.407	-0.037
		P-value	0.069	**0.044**	0.478	**0.021**	0.096	0.711	**0.001**	0.778
	Bacilli	**ρ**	0.15	-0.184	-0.042	-0.327	-0.267	-0.146	-0.154	0.069
		P-value	0.258	0.164	0.75	**0.012**	**0.041**	0.271	0.243	0.606
	Bacterial abundance	**ρ**	-0.198	0.142	-0.09	0.162	0.211	0.318	-0.016	-0.064
		P-value	0.133	0.282	0.498	0.22	0.108	**0.014**	0.904	0.632
	TK17	**ρ**	-0.192	0.079	-0.187	0.41	-0.042	-0.106	0.414	0.105
		P-value	0.145	0.552	0.156	**0.001**	0.754	0.424	**0.001**	0.429
	Sphingobacteriia	**ρ**	0.15	-0.296	-0.021	-0.102	-0.24	-0.051	-0.241	-0.119
		P-value	0.258	**0.023**	0.872	0.442	0.067	0.701	0.066	0.37
	S085	**ρ**	-0.505	0.265	-0.191	0.42	0.176	0.206	0.104	0.097
		P-value	**<0.001**	**0.043**	0.148	**0.001**	0.183	0.117	0.435	0.465
	Chloroplast	**ρ**	-0.087	0.037	-0.329	0.079	-0.105	-0.097	0.045	-0.041
		P-value	0.512	0.778	**0.011**	0.551	0.427	0.464	0.736	0.761
	Lichinomycetes	**ρ**	0.141	0.024	0.06	-0.044	0.09	-0.043	0.31	-0.075
		P-value	0.286	0.857	0.652	0.742	0.496	0.746	**0.017**	0.574
	Dehalococcoidetes	**ρ**	-0.174	-0.028	-0.135	0.099	-0.055	-0.004	-0.091	0.059
		P-value	0.187	0.835	0.309	0.456	0.677	0.976	0.493	0.655
Wetting-Drying	**Solibacteres**	**ρ**	0.542	-0.178	-0.078	-0.337	-0.575	-0.561	0.177	-0.109
		P-value	<0.001	0.176	0.557	**0.009**	**<0.001**	**<0.001**	0.181	0.413
	Gitt.GS.136	**ρ**	-0.425	0.361	0.258	0.428	0.356	0.363	0.012	0.166
		P-value	**0.001**	**0.005**	**0.048**	**0.001**	**0.006**	**0.005**	0.926	0.209
	SHA.37	**ρ**	0.474	-0.258	-0.28	-0.456	-0.608	-0.358	0.034	0.014
		P-value	**<0.001**	**0.049**	**0.032**	**<0.001**	**<0.001**	**0.005**	0.796	0.918

	Thermomicrobia	**ρ**	-0.476	0.097	0.236	0.454	0.328	0.413	-0.134	0.295
		P-value	**<0.001**	0.467	0.072	**<0.001**	**0.011**	**0.001**	0.311	0.023
	C0119	**ρ**	0.371	-0.3	-0.1	-0.272	-0.252	-0.295	0.032	-0.198
		P-value	**0.004**	**0.021**	0.453	**0.037**	0.054	**0.023**	0.810	0.133
	Richness common bacteria	**ρ**	-0.367	-0.04	0.039	0.116	0.369	0.345	0.009	-0.197
		P-value	**0.004**	0.764	0.769	0.381	**0.004**	**0.007**	0.948	0.134
	Elusimicrobia	**ρ**	0.22	-0.096	-0.061	-0.169	-0.135	-0.281	-0.032	-0.306
		P-value	0.094	0.469	0.646	0.202	0.309	**0.031**	0.809	**0.019**
	PRR.12	**ρ**	0.113	0.119	0.031	0.019	0.187	0.094	0.119	-0.174
		P-value	0.395	0.369	0.817	0.886	0.155	0.480	0.371	0.187
	TM7.1	**ρ**	0.299	-0.132	-0.1	-0.128	-0.394	-0.333	0.028	-0.199
		P-value	**0.022**	0.320	0.453	0.333	**0.002**	**0.010**	0.830	0.130
	Mollicutes	**ρ**	-0.246	-0.222	-0.167	0.093	0.060	0.047	0.039	-0.164
		P-value	0.060	0.091	0.207	0.484	0.650	0.726	0.77	0.216
	TK17	**ρ**	-0.281	0.245	0.096	0.212	0.335	0.233	-0.102	0.207
		P-value	0.031	0.062	0.471	0.108	**0.010**	0.076	0.441	0.115
	Monoblepharidomycetes	**ρ**	0.287	-0.084	0.015	-0.148	-0.381	-0.481	-0.197	-0.051
		P-value	**0.028**	0.526	0.909	0.262	**0.003**	**<0.001**	0.134	0.703
	Pucciniomycetes	**ρ**	0.135	-0.142	-0.091	-0.135	0.086	-0.086	0.058	0.056
		P-value	0.307	0.283	0.495	0.309	0.518	0.515	0.66	0.674
	ABS.6	**ρ**	0.537	-0.095	-0.516	-0.24	-0.537	-0.3	0.27	-0.091
		P-value	**<0.001**	0.473	**<0.001**	0.067	**<0.001**	**0.021**	**0.039**	0.494
	SJA.176	**ρ**	0.352	-0.067	-0.212	-0.207	-0.368	-0.16	0.077	-0.33
		P-value	**0.006**	0.614	0.107	0.115	**0.004**	0.226	0.56	**0.011**
N fertilization	**Fibrobacteria**	**ρ**	-0.119	0.162	-0.186	-0.066	0.264	0.096	-0.159	0.252
		P-value	0.371	0.222	0.159	0.619	**0.043**	0.468	0.23	0.055
	Pezizomycetes	**ρ**	-0.263	0.082	-0.233	-0.192	-0.019	-0.096	-0.215	-0.112
		P-value	**0.044**	0.537	0.076	0.145	0.886	0.471	0.103	0.398

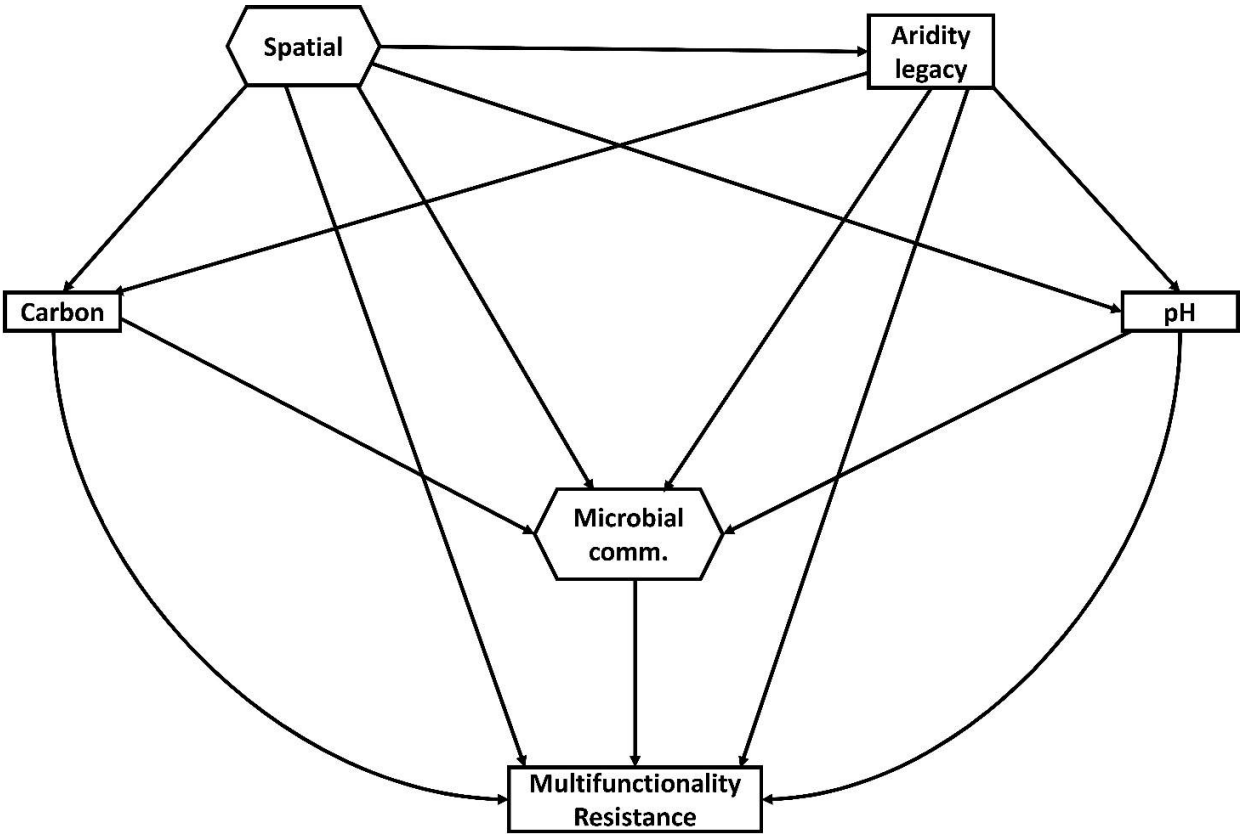
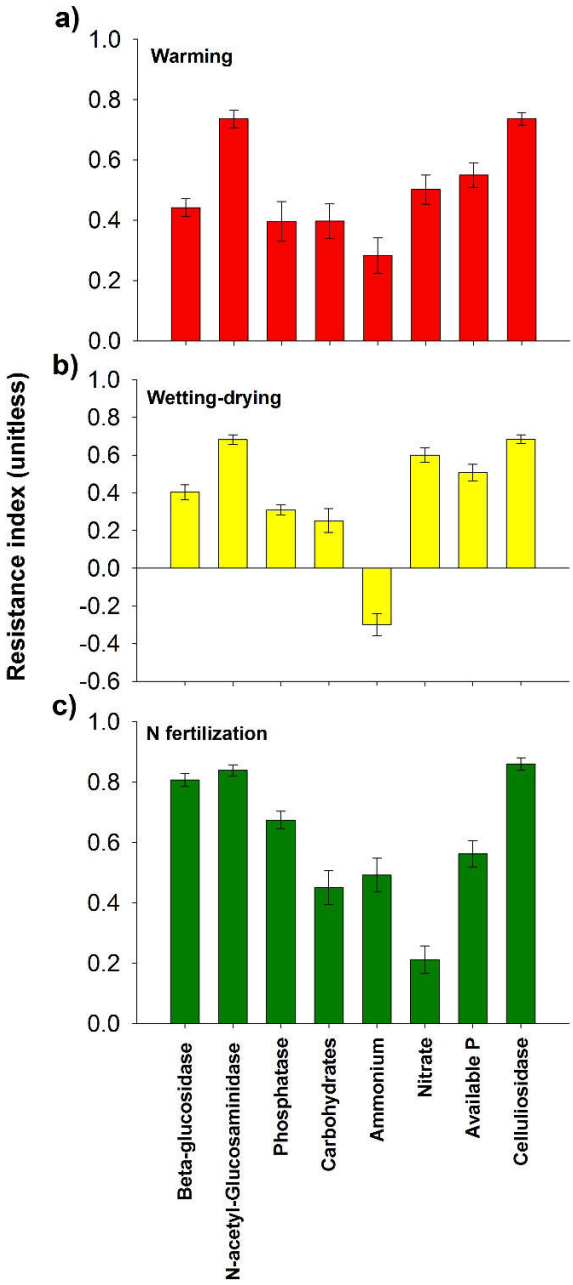


Figure S1. A priori structural equation model including direct and indirect effects of geographical location, aridity, soil properties and microbial communities on the resistance of multifunctionality to global change.



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909 **Figure S2**. Effects of warming, wetting-drying cycles and N fertilization on the resistance of

910 eight single functions to global change. Data are means ± SE (n = 59).

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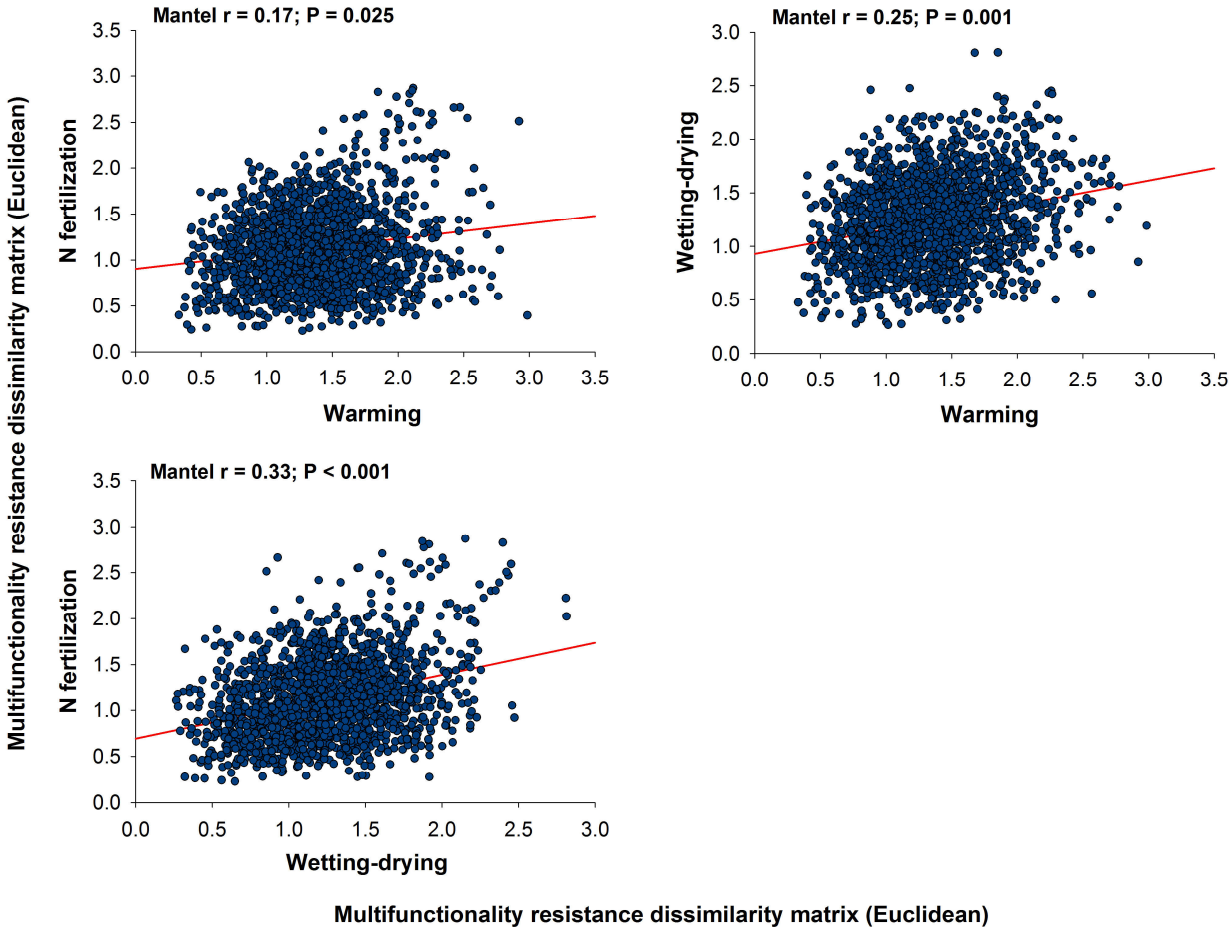


Figure S3. Relationship between the matrix of dissimilarity (Euclidean) from multifunctionality resistance to warming, wetting-drying cycles and N fertilization in global drylands. The solid lines represent the fitted linear regressions.

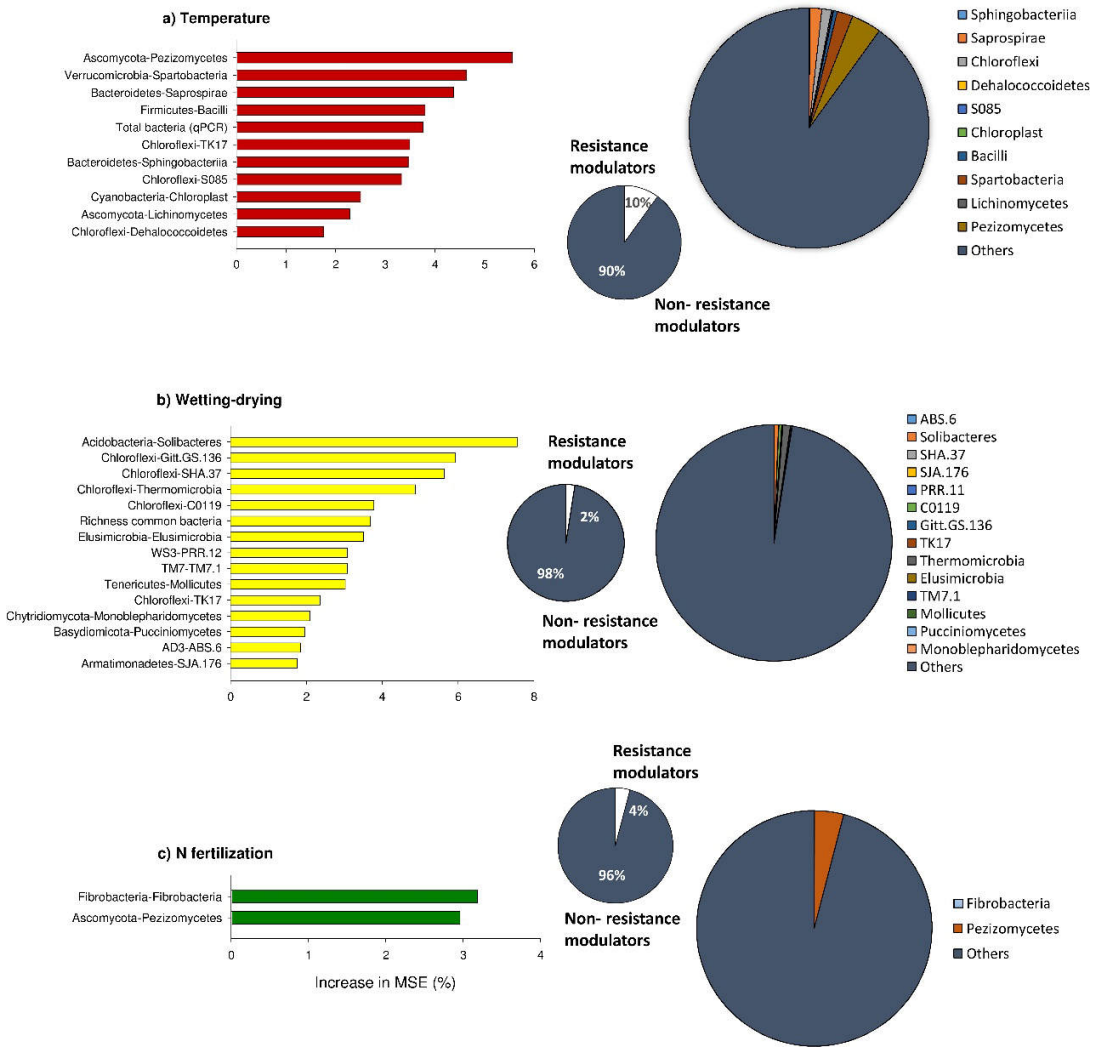
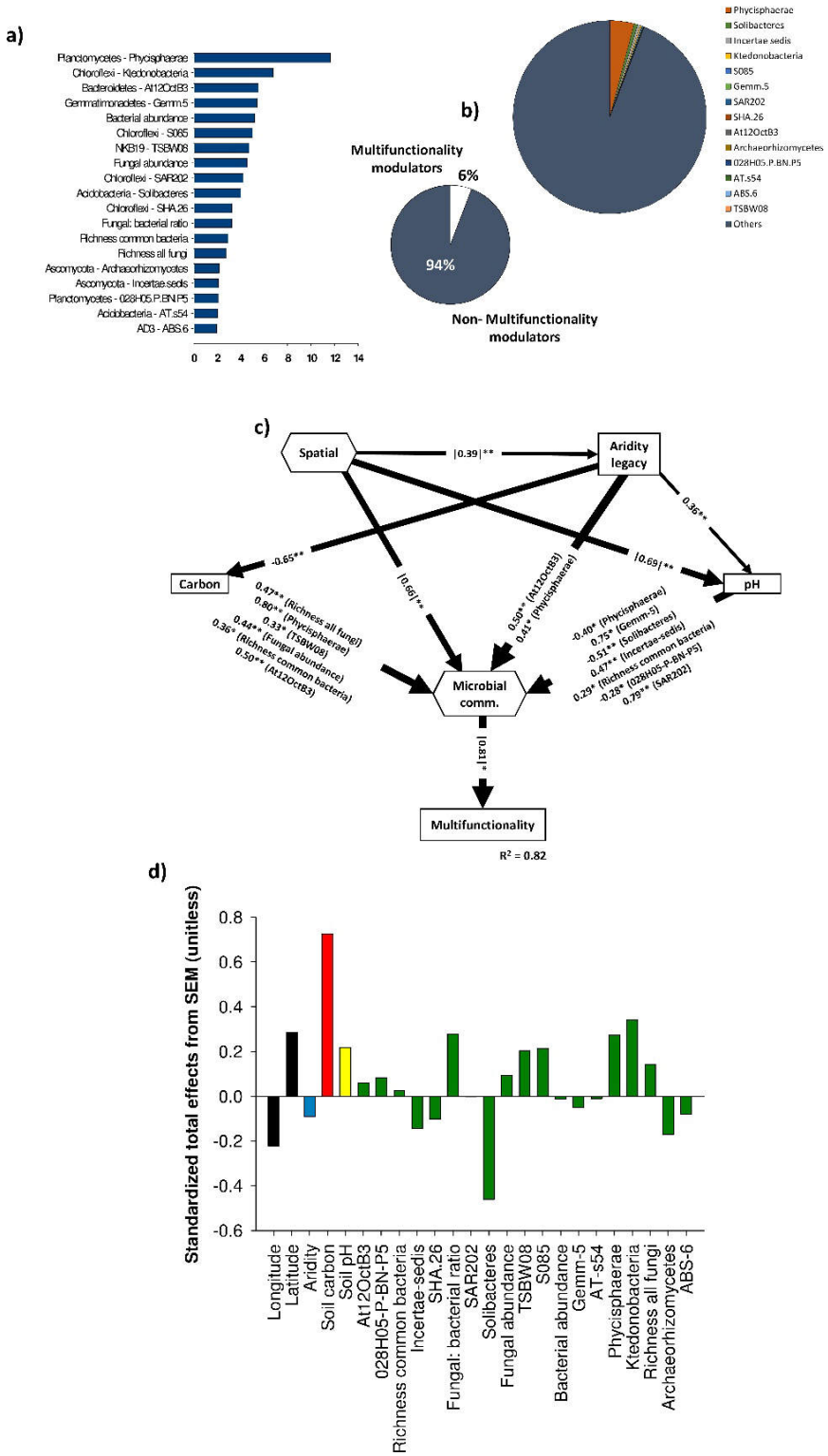


Figure S4. Results from a Random Forest aiming to identify the main significant ($P < 0.05$) microbial predictors of multifunctionality resistance to warming, wetting-drying cycles and N fertilization in global drylands. Pie chart includes the relative abundance of selected taxa driving multifunctionality resistance to global change drivers in dryland soils from across the globe.



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935 **Figure S5. (a)** Results from a Random Forest aiming to identify the main significant ($P < 0.05$)
936 microbial predictors of multifunctionality in global drylands. **(b)** Pie chart includes the relative

abundance of selected taxa driving multifunctionality in global drylands. **(c)** Structural equation model describing the effects of multiple drivers on multifunctionality. Numbers adjacent to arrows are indicative of the effect size of the relationship. Continuous and dashed arrows indicate positive and negative relationships, respectively. This model only includes the direct effects that were statistically significant ($P < 0.05$; see a priori model in Fig. S1). Brackets includes information of the particular taxa related to multifunctionality resistance to global change. R^2 denotes the proportion of variance explained. Significance levels of each predictor are *$P < 0.05$, **$P < 0.01$.

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