Identity of biocrust species and microbial communities drive the response of soil multifunctionality to simulated global change

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ARTICLE INFO

Article history:

Received 24 June 2016

Received in revised form

2 December 2016

Accepted 10 December 2016

Available online 23 January 2017

Keywords:

Microbial diversity

Bacterial community

Global change

Lichens

Ecosystem functioning

Drylands

ABSTRACT

Increasing N inputs and changing rainfall regimes will lead to drastic changes in multiple ecosystem functions such as nutrient cycling, organic matter decomposition and gas exchange in dryland ecosystems. As fundamental components of drylands, biological soil crusts (biocrusts) play important roles in the regulation of responses of multiple ecosystem functions to global environmental changes. Biocrusts are home to highly functional microbial communities; however little is known on the role of microbial communities associated with different biocrust species in regulating the response of multiple ecosystem functions to global change. Here, we conducted a microcosm experiment to evaluate the roles of biocrust-forming lichens (*Diploschistes thunbergianus*, *Psora crystallifera* and *Xanthoparmelia reptans*) in mediating the effects of simulated changes in rainfall frequency and nitrogen (N) addition on soil multifunctionality involving nutrient availability, greenhouse gas flux and enzyme activities. The three biocrust species supported different levels of soil bacterial diversity, and specific community composition as revealed by MiSeq sequencing. Biocrust species always promoted multiple functions related to carbon, nitrogen and phosphorus cycling compared to bare ground, with *X. reptans* having the highest effect on multifunctionality. Most importantly, the relative abundance of specific microbial communities associated with different lichen species modulates the response of multifunctionality to impacts of water frequency (negative) and N addition (positive). Our results suggest that biocrust species could regulate global change impacts on soil multifunctionality in drylands, although the strength and direction vary among the biocrust species. These findings highlight the importance of preserving biocrusts as hotspots of microbial genetic resources and ecosystem functioning in drylands.

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1. Introduction

Drylands ecosystems are one of the most important biomes for global sustainability (Reynolds et al., 2007; Canfora et al., 2014,

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2016). Predicted changes in rainfall regime could negatively influence soil biodiversity, essential ecosystem functions and services such as nutrient cycling and plant production in drylands (Wardle et al., 2004; Solomon, 2007; Maestre et al., 2015a; Delgado-Baquerizo et al., 2016a). In addition to climate change, elevated nitrogen (N) inputs from anthropogenic sources into drylands are simultaneously influencing ecosystems (Delgado-Baquerizo et al., 2016a), and reducing soil microbial diversity or altering community composition (Allison et al., 2008; Ramirez et al., 2010, 2012). Understanding the interactive effects of the global change drivers (e.g. altered rainfall patterns or N inputs) on soil microbial communities and ecosystem processes is essential to develop predictive

frameworks for protecting ecosystem functioning under changing environments in drylands (Roose et al., 2016).

Biological soil crusts (biocrusts hereafter) are communities of mosses, lichens, cyanobacteria, and heterotrophs that colonize the surfaces of dryland soils (Belnap, 2003; Canfora et al., 2016), that can constitute up to 70% of the biotic cover of drylands (Belnap, 2003; Maestre et al., 2013; Ferrenberg et al., 2015). A growing body of literature had demonstrated that biocrusts (i.e. species and cover) are essential for the stability and productivity of dryland ecosystems, as they drive multiple ecosystem functions such as nutrient cycling and organic matter decomposition (Eldridge and Greene, 1994; Belnap, 2003; Yoshitake et al., 2010; Maestre et al., 2012a). In addition, previous studies had indicated the effect of biocrust species in controlling the total abundance and composition of soil microbial communities (Bates et al., 2012; Maier et al., 2014; Maestre et al., 2015b). Most important to our topic, biocrusts have been shown to play an essential role in controlling the response of ecosystem functioning to climate change (Reed et al., 2012; Maestre et al., 2013). For instance, a recent study highlighted the important role of biocrust-forming mosses in mitigating the negative impacts of global change (increasing aridity) on ecosystem multifunctionality (Delgado-Baquerizo et al., 2016c). In addition, species identity of biocrust-forming lichens modulated the response of soil processes (i.e. N cycling) to global change drivers (Liu et al., 2016). However, much less is known on the role of identity of biocrust species in regulating the response of ecosystem multifunctionality to global change. The response of soil microbial diversity and ecosystem multifunctionality associated with different biocrust species to global change (e. g. N inputs and changing rainfall frequency) remains largely unknown.

Biodiversity is a key factor regulating ecosystem functioning (Tilman et al., 1996, 2001; Jing et al., 2015), and loss of biodiversity is considered a major threat to ecosystem services (Hector et al., 1999; Wagg et al., 2014). However, our understanding of the relationship between microbial diversity and ecosystem functioning is much more limited. An increasing number of studies have provided evidence that microbial communities directly influence ecosystem functions and services in terrestrial ecosystems (Berg and Smalla, 2009; Singh et al., 2010; Bodelier, 2011; Trivedi et al., 2013; Bardgett and van der Putten, 2014; Wagg et al., 2014; Delgado-Baquerizo et al., 2016b). These functions include, but are limited to, decomposition of organic matter, nutrient acquisition and the regulation of greenhouse gas emissions. The different microbial communities living under different species of biocrusts may then greatly influence the response of soil functioning to global environmental change. Thus, given the importance of biocrusts for the sustainability of global drylands (Elbert et al., 2012), understanding the role of the microbial communities inhabiting the biocrusts is of paramount importance to predict the response of multiple ecosystem functions to on-going global changes.

Herein, we hypothesized that: i) identity of biocrust species will regulate the response of soil bacterial community to changes in N addition and rainfall frequency; and ii) microbial diversity and composition associated with different biocrust species will differentially modulate the response of multiple ecosystems functions to global change impacts. To test these hypotheses, we conducted a microcosm experiment to examine the potential direct and indirect (via microbial communities) role of biocrusts species, including *Diploschistes thunbergianus*, *Psora crystallifera* and *Xanthoparmelia reptans*, in mediating responses of soil multifunctionality (i.e. 15 functions involved in nutrient cycling and the regulation of greenhouse gas emission) to simultaneous changes in N amendment and precipitation frequency.

2. Materials and methods

2.1. Experimental design

For the microcosm experiment, we collected 120 soil cores (0–5 cm depth) from a semiarid area of Nyngan (31°34', 147°12'E), New South Wales, Australia, where open areas between plant patches contained well-developed biocrust communities. The sampled soil cores covered with three lichens studied: *D. thunbergianus*, *P. crystallifera* and *X. reptans*. Each pot exclusively contains either bare ground, *D. thunbergianus*, *P. crystallifera* or *X. reptans*. Basic chemical characteristics of the soils before our incubation experiment are listed in Table S1. The experimental design had been described in detail previously in Liu et al. (2016). In brief, we established a microcosm study with a full factorial experimental design including three factors: identity of biocrusts species (*D. thunbergianus*, *P. crystallifera* and *X. reptans*), N amendment aligned with reported rates for N deposition (0 and 20 kg N ha⁻¹ year⁻¹) and altered water frequency (i.e. high frequency, 3.61 mm each 3 days; moderate frequency, 7.22 mm each 6 days; and low frequency, 14.44 mm each 12 days). Bare soil was also treated as a procedure control vs. lichen species under the simulated global change conditions. The total amount of watering to these soil cores by the end of the experiment is exactly the same regardless of the altered frequency, which was adjusted to mimic the rainfall level during the spring season of previous year under field conditions. Moreover, N was added at the beginning of the experiment by dissolving NH₄NO₃ in water. The N addition and watering treatments were established to test whether biocrust-forming lichens can potentially drive the response of below-ground bacterial community and ecosystem multifunctionality to the global change drivers. Five replicated cores per combination of treatments were incubated for 72 days in a climate-controlled glass-house. Full details of incubation conditions are provided in the methods description within the Supplementary information. After the incubation, soil samples (0–4 cm depth) from different treatments were collected, carefully removing the above biocrusts. All the soil samples were mixed homogeneously and passed through a 2.0 mm sieve, which were subsequently divided into two sub-samples. One sub-sample was stored at –20 °C for microbial DNA extraction, and another sub-sample was stored at 4 °C for the assessment of soil functions.

2.2. Single soil function analysis

In total, we tested for 15 soil variables as key ecosystem functions including (1) available nitrogen (AN), (2) potential nitrification rate (PNR), (3) microbial biomass N (MBN), (4) dissolved organic carbon (DOC), (5) microbial biomass carbon (MBC), (6) CO₂ flux, (7) CH₄ flux, (8) N₂O flux, (9) β-D-glucopyranoside (BG), (10) β-D-cellulosidase (CB), (11) β-xylosidase (XYL), (12) α-glucosidase (AG), (13) L-Leucine-7-amido-4-methylcoumarin hydrochloride (LAP), (14) phosphate (PHOS), and (15) N-acetyl-β-glucosaminidase (NAG). These selected variables are related to cycling of nitrogen (N), carbon (C) and phosphate (P), and the regulation of greenhouse gas emissions. Dissolved organic carbon and AN in the soils were extracted with 0.5 M K₂SO₄ in a ratio 1:5 by shaking at 200 rpm for 1 h and filtered using 0.45-μm Millipore filter paper (Liu et al., 2016). In parallel, the carbon in microbial biomass (MBC) and the N in microbial biomass (MBN) were evaluated using the fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). The selected enzyme activities including BG, CB, XYL, AG, LAP, PHOS and NAG were measured using 4-methyl umbelliferyl (MUB) substrate yielding the highly fluorescent cleavage products MUB upon hydrolysis (Wallenstein and Weintraub, 2008).

Greenhouse gas (CH₄, N₂O and CO₂) flux was monitored with a whole soil column including soil and biocrusts. Full details of these soil function measurements are provided in the methods description within the [Supplementary information](#).

2.3. Soil DNA extraction and quantification of 16S rRNA gene abundance

The total genomic DNA was isolated from 0.25 g of soil using the MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) following to the manufacturer's instructions. The concentration and quality of extracted DNA was checked photometrically using a NanoDrop® ND-2000c UV–Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Bacterial 16S rRNA gene abundance was quantified by qPCR on a CFX-96 thermocycler (Biorad, USA) using primer pairs of Eub338F/Eub518R ([Cregger et al., 2012](#)). The 10 µl reaction mixture contained 5 µl SensiMix SYBR No-ROX reagent (Bioline, Sydney, Australia), 0.3 µl of each primer (20 mM), 0.4 µl BSA (20 mM), and 1 µl of diluted template DNA (1–10 ng). Thermal-cycling conditions were as follows: 5 min initial denaturation at 95 °C, 40 cycles of 30 s at 95 °C, 30 s at 55 °C, 1 min at 72 °C, followed by a plate read at 83 °C. The PCR amplicons were ligated into a pGEM-T Easy vector (Promega, USA) and transformed into *Escherichia coli* JM109 cells. Plasmid containing 16S rRNA gene was used as standard template, and standard curve were generated with 10-fold serial dilutions of the plasmid. Melt curve analyses were conducted following each assay to verify the specificity of the amplification products, and the PCR efficiency of standard curve for different assays ranged between 89% and 99%.

2.4. Bacterial community composition analysis

To evaluate the bacterial community composition, we amplified the V4 region of the bacterial 16S rRNA gene with the primer pairs of 341F/805R on Illumina MiSeq platform of the Western Sydney University ([Herlemann et al., 2011](#); [Delgado-Baquerizo et al., 2016b](#)). Primer sequences were modified by adding Illumina adaptor A to the 5'-ends of the forward primers, and adaptor B followed by 12 nucleotide barcode sequences to the 3'-ends of the reverse primers. Raw sequences generated through MiSeq paired-end sequencing were merged using fast length adjustment of short reads (FLASH) ([Magoč and Salzberg, 2011](#)) with Q30 of clean full-length reads ranging from 95.0 to 95.8%. A chimera filtering approach (UPARSE), was used to pick operational taxonomic units (OTUs) at 97% similarity on the Bio-Linux platform. Representative sequences were processed using the QIIME pipeline ([Edgar, 2013](#)). PyNAST alignment, and ribosomal database project (RDP) assignment were carried out based on the latest Green genes database ([McDonald et al., 2012](#)). Resampling according to the minimum sequence numbers across all samples was performed before downstream analyses. Bacterial species richness and Shannon diversity index were calculated based on 97% OTU similarity of obtained bacterial sequences. The community composition provides the classification information at phylum level across the soils from different treatments.

2.5. Assessing multifunctionality

To obtain a quantitative multifunctionality index for each treatment, we first normalized (log-transformed when needed) and standardized each of the 15 functions measured using the Z-score transformation. These standardized ecosystem functions were then averaged to obtain a multifunctionality index as described in ([Maestre et al., 2012b](#)). This index is now largely used in the current literature and allows us to explore the role of biocrust

species, microbial communities and global change on multiple ecosystem functions simultaneously, instead of focusing on one or several functions that would not be representative of a natural ecosystem. A greater value of ecosystem functions reflects a more desirable aspect of the ecosystem. CH₄ and N₂O production were multiplied by –1 (inverted around the 0 mean) to maintain directional change with other ecosystem functions, such that a decline from their desirable state corresponds to increasingly negative values. By doing this the general difference among biocrusts and global change treatments in overall ecosystem functioning could be more easily assessed.

2.6. Statistical analysis

We used ANOVA to test for differences of the effect in identity of biocrust species, water frequency and N addition treatment on the bacterial community (total abundance, diversity and relative abundance of main phyla/classes) and soil multifunctionality. The 16S rRNA gene copy numbers were log-transformed prior to statistical analysis to meet normality assumptions. Due to the high amount of data derived from MiSeq sequence analyses, we first pre-selected those groups highly related to soil functionality, hence are able to explain an indirect impact of identity of biocrust species on multifunctionality via relative abundance of microbes. To do this, we conducted a Random Forest analysis to identify the important bacterial predictors (at a phylum/class level) of multifunctionality as explained previously ([Delgado-Baquerizo et al., 2016c](#); [Trivedi et al., 2016](#)). After this, Spearman's correlation analyses were performed to assess the relationships between relative abundance of major bacterial predictors of multifunctionality and single functions studied. All statistical analyses were performed using R.3.2 software (<http://www.r-project.org>). Note that we conducted our analysis at phyla/class level for two main reason: 1) the use of high bacterial taxonomic ranks (phylum and class) have been highly recommended to predict patterns in ecosystem functioning ([Philippot et al., 2010](#); [Trivedi et al., 2013](#)); 2) functional information has become increasingly available at these taxonomic levels ([Fierer et al., 2007](#); [Trivedi et al., 2013](#)).

We then used structural equation modelling (SEM) to evaluate the direct and indirect effect of the identity of biocrust species, global change (altered rainfall frequency and N amendment), and bacterial communities (i.e. composition, richness and abundance) on soil multifunctionality. The N treatments were set as categorical exogenous variables with two levels: 0 and 1 ([Grace, 2006](#)). In the model, the different biocrust-forming lichens species (*D. thunbergianus*, *P. crystallifera* and *X. reptans*) were categorical variables with two levels: 0 (specific species) and 1 (remaining species + bare soil). Bare soil was included as our procedural control in these models. This approach allowed us to compare the effect of a particular biocrust species (e. g. *D. thunbergianus*) on the bacterial community and multifunctionality compared with the average of the remaining microsites. Note that bare soil was selected as our baseline condition, and was not included explicitly in our model ([Grace, 2006](#)). We included bacterial abundance, richness and composition (i.e. relative abundance of bacterial communities pre-selected from Random Forest analyses) in the model. Bacterial composition was included as a composite variable in our model ([Grace, 2006](#)). The data matrix was fitted to the model using the maximum-likelihood estimation method. There is no single universally accepted test of overall goodness of fit for SEM. Thus, we used the Chi-square test (χ^2 ; the model has a good fit when $0 \leq \chi^2 \leq 2$ and $0.05 < P \leq 1.00$) and the root mean square error of approximation (RMSEA; the model has a good fit when $RMSEA \leq 0.05$ and $0.10 < P \leq 1.00$) ([Schermelleh-Engel et al., 2003](#)). The different goodness-of-fit metrics indicated that

our *a priori* model satisfactorily fit to our data, and thus no post hoc alterations were made. To aid with the final interpretation of the SEM, we also calculated the standardized total effects of the biocrust species, global change drivers and microbial community features on ecosystem multifunctionality. The net influence that a given variable has upon another was calculated by summing all direct and indirect pathways between these two variables. SEM analyses were done using AMOS 21.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Soil multifunctionality under the three biocrust-forming lichens

The results indicate that soils beneath different biocrust-forming lichens showed differential soil multifunctionality responses to simulated global change (Fig. 1), which correlated to most of the examined single functions associated with cycling of C, N and P (Table S2). Soils under biocrusts had a higher multifunctionality index than bare soils, with the highest multifunctionality index being under *X. reptans*. However, multifunctionality was also affected by added N and its interactions with altered rainfall frequency ($P < 0.001$, Fig. 1; Table S3). In particular, N addition increased soil multifunctionality under *D. thunbergianus* and *P. crystallifera*, while negative effects were found for *X. reptans* under low water frequency ($P < 0.05$, Fig. 1; Table S4).

3.2. Soil bacterial diversity and abundance

A total of 3553 bacterial OTUs were characterized using a 97% sequence similarity cutoff from roughly 8.6 million high quality sequences. Bacterial diversity level was significantly influenced by the species identity of biocrust as proven by the examined richness of OTU-based observed species and the quantitative Shannon's index ($P < 0.05$, Fig. 2; Fig. S1; Table S3); soils under *X. reptans* had the highest bacterial species richness ($P < 0.01$). Additional N had a negative effect on soil bacterial richness, especially remarkable under *X. reptans* and the bare ground ($P < 0.01$, Table S4). Importantly we found an interactive effect of altered rainfall frequency and N amendment on bacterial richness ($P < 0.05$, Table S3). There is no significant differences of bacterial abundance in the soils under bare ground and the three biocrust species (Fig. 2; Table S3), while biocrusts and rainfall frequency had interactive effects on

Fig. 1. Multifunctionality index in response to altered watering frequency and N addition in bare soil (BS) and soils under different biocrust-forming lichen species: *Diploschistes thunbergianus* (DT), *Psora crystallifera* (PC), and *Xanthoparmelia reptans* (XR). Differences between N amendment and the control for each examined variable are as follows: °, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$. Data are means $\pm$ SE ($n = 5$).

Fig. 2. Soil bacterial abundances and richness in response to added nitrogen (N) and altered watering frequency in the bare soil (BS) and soils under different biocrust-forming lichen species: *Diploschistes thunbergianus* (DT), *Psora crystallifera* (PC) and *Xanthoparmelia reptans* (XR); and bare soil (BS). Letter L, M and H mean low (added water each 12 days), moderate (added water each 6 days) and high watering frequency (added water each 3 days), respectively. Differences between N amendment and the control for each examined variable are as follows: °, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$. Data are means $\pm$ SE ($n = 5$).

bacterial abundance ($P < 0.05$, Table S3). Individually, additional N and increased rainfall frequency had positive effects on soil bacterial abundance under *X. reptans* ($P < 0.01$; Table S4), while the two global change drivers had no overall influence on soil bacterial abundance under *D. thunbergianus* or *P. crystallifera* ($P > 0.05$; Table S4).

3.3. Shifts in bacterial community composition

Most of the bacterial OTUs were classified as *Chloroflexi* (25%), *Actinobacteria* (21%), *Proteobacteria* (18%), *Acidobacteria* (12%), *Planctomycetes* (10%), although their relative abundance varied under the different biocrust species (Fig. S2). We identified the most important bacterial phyla as predictors of multifunctionality based on Random Forest modelling. Our results show that *Chloroflexi*, *Cyanobacteria*, α -*Proteobacteria*, δ -*Proteobacteria* and *Bacteroidetes* were major bacterial predictors of multifunctionality (Fig. S3). Biocrust species significantly affected the relative abundance of all five major bacterial predictors ($P < 0.01$, Fig. 3 and Table S3), especially for *Chloroflexi* with the lowest relative abundance under *X. reptans*. The N addition had overall effects on the relative abundance of δ -*Proteobacteria* and *Bacteroidetes*, and its interaction with rainfall frequency influenced all of the predictors except for α -*Proteobacteria* (Fig. 3 and Table S3). However, the impact patterns of these global change drivers on the predictors varied under different lichens. For instance, added N tended to reduce the relative abundance of *Cyanobacteria*, δ -*Proteobacteria* and *Bacteroidetes* under *P. crystallifera* ($P < 0.10$, Table S4), while no similar negative effect was observed under *D. thunbergianus* and *X. reptans*. We also found an interactive effect of the two global change drivers on all bacterial predictors except α -*Proteobacteria*

Fig. 3. Effects of N amendment and altered rainfall frequencies on the relative abundances of the dominant bacterial predictors of multifunctionality based on the Random Forest analysis. Relative abundances are based on the proportional frequencies of those DNA sequences that could be classified at the phylum level. Differences between N amendment and the control for each examined variable are as follows: °, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$. Data are means $\pm$ SE ($n = 5$). *Diploschistes thunbergianus*, DT; *Psora crystallifera*, PC; *Xanthoparmelia reptans*, XR; and bare soil, BS.

($P < 0.10$, Table S3). Changes in N amendment and rainfall frequency had significant effects on the community structure of the bacterial predictors (PerMANOVA, $P < 0.05$, Fig. S4).

3.4. Direct and indirect impact of identity of biocrust species, microbial communities and global change on multifunctionality

Our SEM explained 60% of the variance in soil multifunctionality, which demonstrates overall positive effects of the three biocrust species on soil multifunctionality (vs bare ground, included as control procedure; $P < 0.01$, Fig. 4a). *D. thunbergianus* and *X. reptans* showed the highest and lowest direct (or total) effect on multifunctionality, respectively (Fig. 4a and b). Interestingly, all

the biocrust species showed indirect effects on multifunctionality via microbial composition, but not richness or total abundance. The bacterial groups involved in these indirect effects seem to be highly dependent on species identity of biocrusts. For instance, *P. crystallifera* and *X. reptans*, but not *D. thunbergianus*, showed positive and negative indirect effects on multifunctionality via the relative abundance of *Bacteroidetes* and *Chloroflexi*, respectively. Interestingly, the relative abundance of δ -Proteobacteria, and α -Proteobacteria showed differential effects on multifunctionality for the different biocrust species: *X. reptans* (positive), *D. thunbergianus* (negative) and *P. crystallifera* (null effect). Only the relative abundance of *Cyanobacteria* showed a constant positive direct effect on multifunctionality for all biocrust species (Fig. 4a).

Fig. 4. (a) Structural equation modelling showing the effects of watering frequency and N amendment on soil microbial community and multifunctionality under *Diploschistes thunbergianus* (DT), *Psora crystallifera* (PC), and *Xanthoparmelia reptans* (XR). Bare ground was selected as our baseline condition, and was not included explicitly in our model. Bacterial community consists of the relative abundance of α -Proteobacteria, δ -Proteobacteria, *Bacteroidetes*, *Cyanobacteria* and *Chloroflexi*, which are the major bacterial predictors of multifunctionality based on Random Forest Analysis. Blue lines and arrows indicate significant. Numbers adjacent to arrows are path directions and coefficients, and width of the arrows is proportional to the strength of path coefficients. Minus represents negative effect of factors on soil multifunctionality. As in other linear models, R^2 denotes the proportion of variance explained and appears under every response variable in the model. Significance levels are as follows: ^a $P < 0.10$, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. (b) Standardized total effects (direct plus indirect effects) derived from the structural equation modelling. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Water frequency and N amendment had indirect negative effects on the multifunctionality through the different bacterial community composition. The added N indirectly influenced soil multifunctionality via *Bacteroidetes* and δ -*Proteobacteria*, while altered water frequency had a negative effect via *Cyanobacteria*. However, those global change impacts on soil multifunctionality could be mitigated by the biocrust-forming lichens in this order: *X. reptans* > *P. crystallifera* > *D. thunbergianus*, as the total positive effects from these lichens on multifunctionality was higher (*D. thunbergianus* and *P. crystallifera*) and much higher (*X. reptans*) than any negative/positive impact of altered water frequency and N amendment on multifunctionality (Fig. 4b).

4. Discussion

4.1. Biocrust-forming lichens indirectly drive multifunctionality via microbial communities

Our results demonstrate that all three biocrust-forming lichens enhanced soil multifunctionality in this study, and that the positive effects of identity of biocrust species can differentially (*X. reptans* > *P. crystallifera* > *D. thunbergianus*) buffer the global change impacts on multifunctionality. These findings are in agreement with the growing idea that biocrusts promote the resistance and stability of ecosystem-multifunctionality in response to global change (Reed et al., 2012; Maestre et al., 2013; Delgado-Baquerizo et al., 2016c), but further indicate that the identity of biocrusts needs to be considered to properly assess their role in regulating the responses of multifunctionality to global change.

In this case, *X. reptans* had higher direct and indirect (via microbial composition) positive effects on soil multifunctionality, compared with *D. thunbergianus* and *P. crystallifera*. Identity of biocrusts could influence the responses of multifunctionality to global change in several ways including through indirect control via microbial communities (studied here), lichen traits and/or soil properties. A large body of literature has demonstrated key roles of microbial communities in regulating belowground ecosystem functions and services (Bardgett and van der Putten, 2014; Wagg et al., 2014; Jing et al., 2015; Delgado-Baquerizo et al., 2016b). Here, we included bacterial richness, abundance and community composition of the major bacterial predictors to explore their relative contributions to soil multifunctionality. Bacterial richness and abundance had little effect on soil multifunctionality in the present study, while the bacterial community composition (major

predictors of multifunctionality) linked to the aboveground biocrust species plays crucial roles in driving ecosystem multifunctionality (Fig. 4a and b). Correlation of these dominant predictors with each single function also demonstrates the significance of the bacterial predictors in controlling soil functions (Table 1). These findings further support the accumulating evidence that soil community composition could be a key factor regulating ecosystem functioning (Bell et al., 2005; Wagg et al., 2014). The positive effects of *Bacteroidetes* and *Cyanobacteria* on soil multifunctionality suggest that these phyla dominated communities linked with biocrusts, may enhance ecosystem functions and services. On the contrary, *Chloroflexi* could impair ecosystem processes in drylands. Shifts in bacterial community composition driven by aboveground biocrust species could be particularly significant for ecosystem multifunctionality in dryland ecosystems (Delgado-Baquerizo et al., 2016c).

The results from this study showed overall positive effects of the bacterial community on the soil multifunctionality involved in nutrient cycling and greenhouse gas flux regulation, which could be elucidated by similar positive trends for most of the single variables associated with cycling of C and N. Interestingly, we found only negative correlations of *Chloroflexi* with most of examined functional variables (e.g. CO₂ emission), agreeing with a previous study suggesting the potential role of *Chloroflexi* in CO₂ fixation (Hug et al., 2013). However, we found the lowest relative abundance of *Chloroflexi* under *X. reptans*, and this may partially explain the highest positive effect of *X. reptans* on soil multifunctionality. *Chloroflexi* are believed to have multiple adaptations to harsh environments and were negatively correlated to soil C in drylands (Battistuzzi and Hedges, 2009; Maestre et al., 2015a). These observations probably explain the relatively high abundance of *Chloroflexi* under *D. thunbergianus* where soils had relatively low nutrient status as reported previously (Liu et al., 2016). These findings further emphasize the great importance of microbial communities on ecosystem processes such as the cycles of C and N (Anderson and Domsch, 1989; Jiao et al., 2010; Kraft et al., 2014). However it should be noted that little effect was observed on the activity of phosphatase which is an important indicator of soil P cycling. These observations are in line with previous studies suggesting lower sensitivity of P cycling to global change than the turnover of C and N due to the dissolution of P from soil minerals (Walker and Syers, 1976; McGill and Cole, 1981; Delgado-Baquerizo et al., 2016c). The different impact patterns of the three biocrust species on soil multifunctionality could also be a consequence of different soil properties related to their species identity of biocrust-

Table 1
Correlation coefficients (Spearman's ρ) between single function parameter and bacterial community composition, abundance and richness of observed species. AN, available nitrogen; PNR, potential nitrification rate; MBN, microbial biomass nitrogen; DOC, dissolved organic carbon; CO₂, CH₄ and N₂O represent their flux rates, respectively; BG, CB, NAG, PHOX, XYL, AG and LAP represent the activity of β -D-glucopyranoside, β -D-cellulose, β -Xylosidase, α -glucosidase, L-Leucine-7-amido-4-methylcoumarin hydrochloride, phosphate, and N-acetyl- β -glucosaminidase, respectively. P values below 0.05 are in bold.

	Abundance	Richness	Chloroflexi	Cyanobacteria	Alphaproteobacteria	Deltaproteobacteria	Bacteroidetes
AN	0.83	0.22*	-0.40**	0.28**	0.38**	0.08	0.28*
N ₂ O	0.06	0.18*	-0.16	0.30**	0.14	0.13	0.20*
PNR	-0.09	0.53**	-0.59**	0.08	0.63**	0.51**	0.24**
MBN	-0.16	0.48**	-0.46**	0.24**	0.33**	0.41**	0.50**
DOC	-0.05	0.35**	-0.43**	0.23*	0.42**	0.34**	0.33**
CO ₂	0.04	0.37**	-0.40**	0.19*	0.23*	0.31**	0.39**
CH ₄	0.07	-0.16	0.17	-0.15	-0.07	-0.09	-0.14
BG	0.09	0.46**	-0.47**	0.32**	0.31**	0.42**	0.53**
CB	-0.01	0.37**	-0.37**	0.46**	0.32**	0.28**	0.46**
NAG	0.02	0.11	-0.27**	0.36**	0.33**	0.09	0.29**
PHOS	0.17	-0.05	0.09	0.02	-0.10	-0.06	0.07
XYL	0.07	0.26**	-0.39**	0.34**	0.36**	0.20*	0.34**
AG	-0.01	0.17	-0.16	0.28**	0.11	0.10	0.26**
LAP	0.04	0.27**	-0.31**	0.20*	0.19*	0.26**	0.27**
MBC	-0.19*	0.50**	-0.57**	0.34**	0.47**	0.42**	0.60**

forming lichens (Bowker and Belnap, 2008; Maestre et al., 2012a; Liu et al., 2016). For example, soils under *X. reptans* had the highest availability of nutrient before our experiment (Table S1), which may increase the diversity and activities of the microbial community (Fig. S1; Fig. 4). Differentially, *D. thunbergianus* thalli are firmly attached to the soil (with relatively low nutrient), which may produce local anaerobic conditions (Kidron et al., 2015), influencing the microbial communities involved in ecosystem multifunctionality. The thallus of *P. crystallifera* is also highly attached to the soil, albeit they usually form smaller patches than *D. thunbergianus* and thus may promote more similar conditions to *X. reptans* (Liu et al., 2016). Consequently, the differences in biocrust species result in altered soil properties and microbial community compositions, which will in turn influence ecosystem multifunctionality in drylands.

4.2. Relative importance of biocrust species, microbial communities and global change in controlling multifunctionality

Our results suggest that all three biocrusts, their microbial communities and global change had effects on soil multifunctionality directly or indirectly, though the patterns and direction were varied under different conditions (Fig. 4a and b). The three biocrust species enhanced soil multifunctionality compared to bare ground, while altered rainfall frequency and N amendment had negative indirect impacts on multifunctionality via bacterial communities. Contemporary global change in this study, for example N addition, altered the relative dominance of the main bacterial community taxa (Fig. 3; Fig. S3); our results suggest that the major bacterial predictors of multifunctionality such as *δ-Proteobacteria* and *Bacteroidetes* are vulnerable to N addition, which are important for C and N cycling, as well as CO₂ fluxes according to our results (Table 1). Furthermore, interactions between altered water frequency and added N influenced most of the bacterial predictors, which could have important impacts on soil multifunctionality. Our observations further suggest negative effects of global change on soil microbial communities (Ramirez et al., 2010, 2012; Zeglin et al., 2013; Martins et al., 2015), which are closely related to ecosystem functioning.

Most important to our research topic, the identity of biocrusts and their associated microbial communities play an essential role in regulating the responses of multifunctionality to global change. Of special interest was the positive effect of some biocrust species on soil multifunctionality were considerably larger than the negative effects from the global change drivers through bacterial community (Fig. 4a and b), hence suggesting that maintaining key biocrust species is essential to offset negative effects of global change on ecosystem functioning. This is especially remarkable under *X. reptans*, as our estimate of the effect strength was greater than any other factor influencing soil multifunctionality. Consequently, these positive effects from biocrusts on multifunctionality may offset any impacts from the global change drivers on ecosystem functioning. These observations are further supported by a recent filed study suggesting biocrust-forming mosses mitigate global change impacts (increasing aridity) on soil multifunctionality in drylands (Delgado-Baquerizo et al., 2016c). Thus, biocrust-forming lichens play important roles in buffering global change impacts on ecosystem multiple functions. We argue that biocrusts may be an important shelter protecting ecosystem functions from global change impacts in dryland ecosystems.

Correlations between bacterial richness with many specialized functions further demonstrate the crucial roles of microbial diversity on ecosystem processes (Bell et al., 2005; Peter et al., 2011; Wagg et al., 2014; Delgado-Baquerizo et al., 2016b). Our results indicate the negative effect of added N on bacterial richness despite

the positive effect on soil bacterial abundance (Fig. 1, Table S3). These contrary effects on the bacterial community are similar to those observed in previous studies (Fog, 1988; Sinsabaugh et al., 2015). This suggests that additional N has a negative effect on to some microbial species, though probably benefits the microbes involved in N cycling that can utilize freshly spiked N as a substrate. Consequently, amended N may enhance microbial abundance while reducing microbial diversity (species richness), thereby influencing ecosystem functioning. These results also indicate that enhanced rainfall frequency reduced the richness under bare ground without N addition. No similar effects under the three biocrusts were observed suggesting that biocrusts might enhance the resistance of bacterial diversity to the disturbance of altered rainfall frequency. Biocrusts may provide better habitats for microbial communities compared with bare soils, buffering global change impacts on the ecosystem (Delgado-Baquerizo et al., 2016c). Our results are in line with previously reported studies demonstrating the important roles of biocrusts in enhancing ecosystem resistance (Delgado-Baquerizo et al., 2013; Sancho et al., 2014; Escolar et al., 2015). These observations also suggest that biocrust-forming lichens may play crucial roles in relieving negative effects of global change drivers on soil multifunctionality via bacterial communities in drylands. Given important role of fungal communities in regulating soil functions (Tedersoo et al., 2014; Wagg et al., 2014), positive effect of microbial diversity on soil multifunctionality in drylands may have been strengthen (Delgado-Baquerizo et al., 2016b). However, differences in biocrust species could modulate the response of multifunctionality to global change in drylands.

5. Conclusions

Taken together, our findings provide evidence that identity of biocrust species and their associated microbial communities regulate the impact of N addition and altered rainfall frequency on soil multifunctionality. We emphasize the lichen-dominated biocrusts enhanced soil multifunctionality, compared with bare soils. Bacterial communities are important drivers of soil multifunctionality, and shifts in community composition related to aboveground biocrust species may have significant functional consequences in dryland ecosystems; this is also an important mechanism by which biocrusts regulate ecosystem services and delivery. Protecting biocrust species is therefore of paramount importance for buffering global change impacts on microbially-driven ecosystem multifunctionality in dryland ecosystems. Owing to limitations of the microcosm study, however, further field studies with natural conditions will strengthen our understanding of biocrust regulations on the response of ecosystem multiple functions to global change.

Acknowledgements

We gratefully acknowledge Raul Ochoa-Hueso for his assistance during field sampling and David Eldridge for help identifying biocrust species. We thank Catriona A. Macdonald and Yui Osanai for their help in chemical analysis and measurement of greenhouse gas, and the NGS team from Western Sydney University for their support with the Illumina platform for MiSeq sequencing. We also thank Jasmine Grinyer for revising the English of this manuscript. This work was financially supported by UWS-CAS bilateral agreement and the Australian Research Council project DP13010484. M.D.-B. also acknowledge support from the Marie Skłodowska-Curie Actions of the Horizon 2020 Framework Programme H2020-MSCA-IF-2016 under REA grant agreement n° 702057. The authors declare no competing financial interests.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2016.12.003>.

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