Multiple diversity facets of crucial microbial groups in biological soil crusts promote soil multifunctionality

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Running title

Multifaceted biodiversity and functioning

Abstracts

Aim: Microbial diversity is one of the most important factors for maintaining the performance of multiple functions in soils (multifunctionality). However, existing studies typically consider taxonomic richness or the Shannon index at the community level, whilst the revealed associations between diversity and functioning are not highly consistent. In this study, we disentangled the underlying linkages between crucial functional groups and soil multifunctionality in a real-world ecosystem.

Location: Twenty-four sites in the central of Tibetan Plateau (~100,000 km^2), the roof of our planet.

Time period: Present.

Major taxa studied: Phototrophic and diazotrophic bacteria (mainly cyanobacteria).

Methods: We conducted a high throughput sequencing of carbon- and nitrogen-fixing bacterial functional groups from biological soil crusts, and evaluated multiple diversity facets (*i.e.*, richness, evenness, and phylogeny-related trait dissimilarity), together with seven crucial variables of soil functioning to calculate multifunctionality. The relations between multifaceted diversity and abundance with individual functions and multifunctionality were validated by a set of solid statistical analyses.

Results: We found that the integrated biodiversity index obtained by combining three diversity components, was a stronger predictor of ecosystem multifunctionality than

taxonomic richness. Moreover, the divergent performance of different diversity facets determined the idiosyncratic effect of each functional group on the multifunctionality. Namely, richness was the dominant factor for diazotrophs to maximize multifunctionality, whereas phylogenetic dissimilarity was the most important one for phototrophs.

Main conclusions: our study demonstrated that multiple diversity facets should be considered when trying to disentangle the linkages between biodiversity and ecosystem functioning. The heterogeneity among the focal functional groups derived from the significant differentiation of the extent of multifunctional redundancy. We speculated that the multifaceted diversity pattern depicts the response ability of crucial functional groups by which biocrusts stabilize multifunctionality under environmental perturbation. Our results provided a perspective to bridge the gap between taxonomic and trait-based approaches for elucidating the biodiversity-ecosystem function relationship, and could ultimately help to boost the practices of dryland management against global change.

Keywords

Biocrusts, Biodiversity, Cyanobacteria, Diversity-function relationships, Ecosystem functions, Functional redundancy, Tibetan Plateau

1. Introduction

The era of global change is characterized by a rapid reduction in biodiversity (Cadotte et al., 2008; Maestre et al., 2016). A growing body of evidence highlights that biodiversity does not merely respond to environmental variability but also shapes ecosystem properties at various spatio-temporal scales (Cardinale et al., 2012; Carey, 2016; Hector et al., 1999; Loreau et al., 2001). The potential for biodiversity loss to impair the delivery of ecosystem functions and services has motived researchers to better understand the underlying linkages between biodiversity and ecosystem functioning (Allan et al., 2015; Cernansky, 2017; Tilman et al., 1996; Zavaleta et al., 2010). The emerging consensus seems to be that higher levels of biodiversity are required to maintain the simultaneous performance of multiple ecosystem functions ('multifunctionality') (Bowker et al., 2013; Byrnes et al., 2014; Hector and Bagchi, 2007).

As one of the most complex habitats on Earth, soil contains an immense diversity of microorganisms that play a crucial role on the stability and resilience of terrestrial ecosystems (Bastida et al., 2016; Delgado-Baquerizo et al., 2017; Wagg et al., 2014). However, the biodiversity of this mega-diverse community remain largely unknown (Bender et al., 2016; Carey, 2016). We are just beginning to test how soil microbial diversity explicitly responds to external perturbation and elucidate the mechanisms by which it drives soil multifunctionality (Baveye et al., 2016; Delgado-Baquerizo et al., 2016b; Jing et al., 2015; Mori et al., 2016). Emerging studies, mainly conducted on the metric of taxonomic diversity at the community level, suggest that microbial diversity is of key importance on ecosystem functioning, even

67 simultaneously accounting for spatial and environmental drivers (Castillo-Monroy et al.,
68 2011; Delgado-Baquerizo et al., 2017; Mori et al., 2016; Soliveres et al., 2016). However, the
69 general relationship between microbial diversity and multifunctionality is not highly
70 consistent among different taxonomic groups (Bradford et al., 2014). For instance, studying
71 soil richness of the Tibetan Plateau it was observed that, despite positive relations of bacteria
72 and microfauna with multifunctionality, some other groups (archaea and fungi) were not
73 significantly associated (Jing et al., 2015). These contrasting patterns make it difficult to
74 translate this knowledge about diversity-functioning relations into practical applications for
75 ecosystem management (Bradford et al., 2014; Mori et al., 2013).
76 Furthermore, traditional taxonomic measures may not adequately capture the features of
77 biodiversity that are most correlated with ecosystem functioning (Bender et al., 2016;
78 Cernansky, 2017; Mori et al., 2013). The alternative view focuses on functional groups
79 according to trait-based categories, rather than on the entire community (Krause et al., 2014).
80 Because of inherent association (*i.e.*, loss of any functional groups will likely result in loss of
81 certain functioning) (Mori et al., 2013), relating particular ecological processes to the
82 diversity of special functionally coherent groups could facilitate our understanding on the
83 linkages between microbial diversity and multifunctionality (Krause et al., 2014). However, it
84 is also noteworthy that sustaining more species of every functional groups in an unspecified
85 manner might not in practice be rational and realizable (Bender et al., 2016). Furthermore,
86 current biodiversity loss is non-random and probably causes crucial losses of species
87 belonging to special functional groups that contribute more to multifunctionality (Selmants et

al., 2012). Hence, the most pivotal ecological processes of an ecosystem need to be identified and prioritized, in order to enable realistic decisions on which functional groups are of particular concern for management actions (Mori et al., 2013). Such a perspective could especially bridge the gap between taxonomy- and trait-based approaches to benefit the efforts on sustaining soil multifunctionality that microbial diversity provides (Krause et al., 2014; Mori et al., 2013).

According to the insurance hypothesis (Yachi and Loreau, 1999), an assembly of species with similar functional traits should idiosyncratically respond to external fluctuations, otherwise even slight environmental changes may cause the collapse of species responsible for a particular function (Duffy et al., 2005; Mori et al., 2013). In light of the above, both the number and the variation among species within crucial functional groups are essential to sufficiently depict the 'response-ability' of functional groups against disturbances (Bender et al., 2016; Cadotte et al., 2008; Maestre et al., 2012a; Soliveres et al., 2016). It means that multifaceted diversity pattern and microbial abundance could ensure functional compensation and enable the ecosystem to maintain multifunctionality under environmental fluctuations (functional redundancy) (Elmqvist et al., 2003). Actually, high functional redundancy of microbes has been widely assumed in natural assemblages, whilst the extent to which it occurs is still debated (Delgado-Baquerizo et al., 2016a; Nielsen et al., 2011; Roger et al., 2016), partly due to its large variability among different functional groups (Schimel and Schaeffer, 2012). Therefore, it is of practical importance to quantify the degree of multifunctional redundancy in order to disentangle the underlying mechanisms by which

109 microbial diversity promotes multifunctionality (see **Supplementary Fig. 1**) (Mori et al.,
110 2016).

111 To address the above concerns, we used biological soil crusts (biocrusts) as a model
112 system (Bowker et al., 2010). Biocrusts occur in various biomes and carry out vital ecosystem
113 functions especially in harsh arid habitats, where severe abiotic conditions significantly
114 constrain the growth of higher plants (**Supplementary Fig. 2**) (Belnap, 2003; Bowker, 2007).
115 Importantly, biocrusts account for 7% of net primary production and nearly half of biological
116 nitrogen fixation on the global terrestrial habitats (Belnap and Lange, 2003; Elbert et al.,
117 2012). Given the obvious carbon- and nitrogen-limited nature of arid soils (Chu et al., 2016;
118 Dobson et al., 1997; Pointing and Belnap, 2012), we focused on two crucial functional
119 groups, photoautotrophic and nitrogen-fixing bacteria. C/N-fixation by phototrophs and
120 diazotrophs supports the heterotrophic assemblages from all domains in biocrusts and further
121 facilitates the potential establishment of higher plants (Belnap, 2002; Pointing and Belnap,
122 2012).

123 Coupling with a regional survey on the central Tibetan Plateau, 'the roof of our planet'
124 (**Supplementary Fig. 3**), we examined the relationships between multiple diversity facets and
125 total abundances of phototrophs and diazotrophs and soil multifunctionality. The composition
126 of the focal functional groups was determined by using the high-throughput amplicon
127 sequencing. Three basic α-diversity metrics, as the model-based estimation of species
128 richness, evenness, and phylogeny-related trait dissimilarity, were quantified. In addition,
129 seven key variables of soil functions were measured to calculate multifunctionality, which

reflect the ecological performances of biocrusts on metabolic potential, soil fertility, primary productivity, carbon sequestration, and climate resistance. We hypothesized that diversity patterns of phototrophs and diazotrophs in biocrusts positively relate to multifunctionality, while the underlying linkages rely on the multiplicative impact of multiple diversity facets and ultimately depend on the extent of multifunctional redundancy in different functional groups. Given the unique role of each diversity facet and their potential interactive effects (Maestre et al., 2012a; Mori et al., 2013), we expect that multifaceted diversity explains more variance of soil multifunctionality, rather than focusing on any single ones. We further suppose that the approach used here will broaden our understanding of how an assembly of microbial species operates on multifunctionality and help to boost our practice on ecological management in drylands.

2. Materials and Methods

2.1 Study sites and sampling

The study sites are located in the largest and highest plateau on Earth, the Qinghai-Tibetan Plateau (Chen et al., 2013). We collected crust samples from 24 sites (20×20 m) in different arid areas of the region (>100 000 km^2) during the warm and wet season, in 2015 (**Supplementary Fig. 3**). In each site, five plots (50×50 cm) were randomly selected in the open field between the perennial herbs and apart from the nearest shrubs (if present). Upper biocrust cores (0~2 cm) were collected in triplicate with a 70-mm Ø ring knife in each plot, and cores from the same site were bulked and homogenized. Samples were air-dried to a constant weight in a short time, packed in paper bags, and immediately stored in a portable

refrigerator. After the field survey, samples were transferred to the lab and further ground by using mortar and pestle and sieved through a 2-mm mesh to remove gravel. Subsamples were stored at -20℃ until laboratory measurements.

We obtained the climate data from an online database 'WorldClim'. Gridded climate datasets (1 km^2 resolution) were compiled with monthly mean, minimum and maximum surface temperature and precipitation records (Hijmans et al., 2005). For the temperature pattern, we first conducted a principal component analysis (PCA) to reduce the dimensionality of the data of annual mean temperature, as well as mean temperatures of the wettest, warmest and coldest quarters. The primary component value (PC1) was retained to summarize local temperature patterns, which explained 69% of the total variance. The PC1 accounted for 48%, 68%, 95%, and 66% of the variance in four temperature variables, respectively. The PC1 value of the precipitation dataset was also calculated (explained 84% of variance), which accounted for 97%, 67%, and 89% of the variance in annual precipitation, precipitation seasonality and precipitation of the warmest quarter. In addition, Elevation data was collected by a handheld GPS device in the field.

2.2 Laboratory measurement procedures

To evaluate the abundances of target functional groups, qPCR was employed to measure the absolute copy numbers of the identification genes. For phototrophs, the group-special primers CYA359 (5'-ggg gaa tyt tcc gca atg gg-3') and CYA781 (A: 5'-gac tac tgg ggt atc taa tcc cat t-3'; B: 5'-gac tac agg ggt atc taa tcc ctt t-3') were used to obtain 16S rRNA gene segments (Nübel et al., 1997). For diazotrophs, the primers nifH-F (5'-aaa ggy ggw atc ggy aar tcc acc

ac-3') and nifH-R (5'-ttg tts gcs gcr tac ats gcc atc at-3') were used to amplify the segment of nitrogenase reductase gene *nif*H (Rosch et al., 2002). The compositions of functional groups were assessed by using a high-throughput sequencing method on an Illumina MiSeq PE300 platform (**Supplemental Information S2**). MiSeq sequencing datasets are available in the National Center for Biotechnology Information (NCBI) under the accession numbers SRR5576302 and SRR5576303.

Soil variables of each site were measured, including total nitrogen (TN), total phosphorus (TP), soil pH, dissolved ionic concentrations, total organic carbon (TOC), water-holding capacity (WHC), and chlorophyll contents (Chl) (**Supplementary Tables 1** and **2**). To measure soil pH, TN and TP, subsamples were first suspended in centrifuge tubes with 1M KCl (1:5 ratio, w/w), then vortexed for 1 min and shaken for 30 min at room temperature. TN and TP were determined using classic colorimetric methods (Sparks, 1996). For soil pH, after a 60 min standing of the extract, the clear supernatant was filtered through a 0.45 μm Millipore filter and measured using a pH meter (Sartorius Intec, Germany). To measure dissolved anionic and cationic concentrations (NO_3^-, NO_2^-, PO_4^{3-}, Cl^- and NH_4^+, Na^+, Mg^{2+}, Ca^{2+}, respectively), subsamples were extracted with 0.5M K_2SO_4 (1:5 w/w) by vortexing for 5 min and then shaking for 90 min. The extracts were 10^{-1} diluted after filtering and analyzed on a Dionex ICS-1600 ion chromatography system (Thermo-Fisher Scientific, USA). Soil salinity was calculated as the sum of Cl^-, Na^+, Mg^{2+} and Ca^{2+} concentrations. To analyze TOC, subsamples were pretreated with an excess of 3M HCl to exclude the impact of inorganic carbonates and were freeze-dried overnight. The analysis was performed on a Vario

193 TOC/TN_b Select analyzer (Elementar, Germany). The WHC was measured gravimetrically, by
194 saturating 5 g of air-dried sample with a known amount of water and then left to drain in a
195 perforated centrifugal tube until the last drop of water had drained. The WHC was calculated
196 as the percent weight of water absorbed per gram of soil. Chlorophyll *a* and *b* contents were
197 calculated after extracting with 90% ethanol in dark at 4℃ for 24 h and determining
198 spectrophotometrically.

199 *2.3 Estimating biodiversity and individual ecosystem functions*

200 Three metrics of biodiversity were measured, including richness, evenness and phylogenetic
201 dissimilarity. More specifically, the abundance coverage-based estimator (ACE) was
202 quantified using EstimateS v9.1.0 (http://purl.oclc.org/estimates) based on the relative
203 abundance of species, as a proxy of richness (Chao et al., 2000). Pielou's evenness (J_{sw}) was
204 then calculated. To account for phylogenetic dissimilarity, mean pairwise phylogenetic
205 distance (MPD) among all pairs of phototrophic or diazotrophic species, respectively, were
206 calculated, (**Supplemental Information S2**). In brief, the consensus phylogenetic trees
207 incorporated with relative abundance data of species were used to compute abundance-
208 weighted MPD value of each site. The calculation was performed in the *R* package 'picante'
209 with 999 randomizations and 1000 iterations of null models to obtain a standardized effect
210 size (SES) of MPD (Kembel et al., 2010). In addition, PCA was applied on ACE, J_{sw}, and
211 MPD to gain an integrated standardized index 'MultiDiver', which represented the pattern of
212 biodiversity and explained 56% and 53% of the variance in two functional groups,
213 respectively. A noteworthy issue when evaluating the overall diversity is the fractional overlap

of species that belong to both functional groups (*e.g.*, some of *Nostoc* spp. and *Scytonema* spp.). This may introduce overestimating of species richness if adding the ACE indices of functional groups together. To avoid this problem, we conducted this study mainly under a framework of parallel comparative analyses checking separately different functional groups. Even when integrated indices of overall diversity were needed in the to compare their explanatory power with each other (see below), they were obtained by using PCA with covariance matrix rather than simple summing or averaging approaches. We believed that the issue of species overlapping would not invalidate our results.

We identified seven key ecosystem functions biocrusts as **i.** belowground biomass (BelowBio, nucleic acid contents per gram), **ii.** TN, **iii.** TP, **iv.** soil available nutrients for plants (AN, standardized sum of NH_4^+, NO_3^-, NO_2^-, and PO_4^{3-} concentrations), **v.** potential primary productivity (Chl, chlorophyll contents per gram), **vi.** TOC, and **vii.** WHC. They are often used as indicators to evaluate the primary succession of biocrusts, and represent the status of soil ecosystem under ambient stresses that induce quick loss of soil functioning (Bowker, 2007; Hu and Liu, 2003). Despite a wide debate on whether stock variables are appropriate to indicate the functional level of ecosystems, we believe that the choice of surrogates for ecosystem functions are context-dependent according to the characteristics of different ecosystem types. For biocrusts in the arid environments, the static indicators depicting soil properties could be at least equally crucial for the functionality than the measures of metabolic activities (Mallen‐Cooper et al., 2019). Furthermore, our selected function variables are to a large extent determined by the target C/N-fixation bacteria which

play a key role in the formation and development of biocrusts (Belnap and Lange, 2003).

The aforementioned variables were normalized to a unified comparable scale ranging from 0 to1 (min-max standardization) (Soliveres et al., 2016). To address multicollinearity problems, Spearman's rank correlation coefficients (ρ) of all pairs of predictors were measured (**Supplementary Tables 3** and **4**). Those highly correlated predictors ($|\rho|>0.60$) were prevented from being included in the same regression model. Because we tried to focus on the effect of biotic predictors, the residuals of diversity predictor and functional variables could correct them for the co-varying environmental variability of climate and soils, whereby the biodiversity-ecosystem function relationship would be segregated from potential confounding factors (Soliveres et al., 2016). Hence, the residuals were calculated by fitting multiple regression models using standardized temperature/precipitation PC1 values, elevation, soil pH, and soil salinity as explanatory variables, and only environment-corrected residual data was retained for further analyses.

2.4 Evaluating the relations between biodiversity and soil multifunctionality

We conducted an analysis of multiple thresholds approach in the *R* package 'multifunc' to evaluate the relations between diversity and multifunctionality (Byrnes et al., 2014). The multifunctionality level was measured as the number of individual functions that quantitatively exceeded a critical percentage of the maximum observed value of each function, and along with a continuous gradient of thresholds from 1% to 99% with intervals of 1%. A high number of functions above a threshold for multiple different threshold values indicates a high level of multifunctionality. Since the outliers may introduce bias on the

maximum observed value across the sites, we calculated it as the average of the top three sites (Bradford et al., 2014). The biodiversity-functioning relationship was evaluated by fitting a series of general linear models (GLMs) with integrated (**Fig. 1**) and individual biotic predictors (**Supplementary Fig. 4**), respectively. We also fitted GLMs with synthetic ACE richness of two functional groups, which is the common metric of biodiversity (Jing et al., 2015; Loreau et al., 2001; Maestre et al., 2016; Soliveres et al., 2016), to compare its explanatory power with that of MultiDiver index. As an often used metric, Shannon index was highly consistent with ACE richness and/or Pielou's evenness in our datasets ($|\rho|>0.60$, **Supplementary Table 4**), thus it was not analyzed in this study.

Three particular multifunctionality metrics were highlighted to identify key parameters (**Table 1**), as **i.** the minimum/maximum diversity-independent multifunctionality level (M_{min}/M_{max}), which indicated the boundary of effects that arose due to diversity from those that would exist by chance; **ii.** the diversity-maximized multifunctionality level (M_{mde}); **iii.** the realized maximum effect of diversity (R_{mde}, *i.e.*, slope); and their corresponding threshold values (T_{min}, T_{max}, and T_{mde}) (Byrnes et al., 2014). To verify the possible false conclusion arising from the arbitrary selection of ecosystem functions, we further conducted a multiple thresholds analysis by removing one most highly related functioning variable with multifunctionality at each time. The result showed that the association pattern did not change significantly (**Supplementary Fig. 5**). In addition, we also used an averaging approach to calculate soil multifunctionality level. The standardized Z-scores of seven measured ecosystem functions were averaged to obtain an multifunctionality index (Maestre et al.,

277 2012b). We then fitted linear models with environmental factors alongside uncorrected
278 diversity predictors (**Supplementary Fig. 6**).
279 Given the importance of identifying the responses of individual ecosystem functions to
280 supplement the quantifying of the overall response of multifunctionality, we used multiple
281 linear models to evaluate the relationships between biotic predictors and seven individual
282 ecosystem functions. This might supply additional information for understanding the possible
283 trade-offs among different ecosystem functions (Allan et al., 2015). To implement it, we
284 separated the highly correlated predictors (*i.e.*, multicollinearity problem) into two paired
285 models for each function variable (**Supplementary Table 4**). Then, model averaging was
286 applied based on adjusted R^2 values of two models, and the incorporated slope of each
287 predictor was used to estimate its relation with individual ecosystem functions. For each
288 model, we selected the most parsimonious one by using a complete subset regression method
289 in the *R* package 'leaps' to test every candidate combination of predictors, and further
290 simplified models by removing nonsignificant predictor according to *F*-ratio tests
291 (significance $P_r(>|t|) > 0.05$).
292 Furthermore, we applied piecewise structural equation models (pSEM) to estimate the
293 connection network and additive effects of biotic predictors on soil multifunctionality in the *R*
294 package 'piecewiseSEM' (Lefcheck and Freckleton, 2016). Because the individual function
295 variables were highly correlated with each other (*i.e.*, multicollinearity, **Supplementary Fig.**
296 **7**), we used PCA to gain a synthetic averaging index in advance to represent the level of
297 multiple functions, which accounted for 63% of total variance in seven function variables.

298 This is an alternative option for pSEM, which is unable to invoke a latent variable, to handle
299 the problem of highly related variables. We constructed two independent sets of models for
300 phototrophic and diazotrophic predictors, respectively. Shipley's test of *d*-separation was used
301 to check whether any paths were missing from *a priori* schematic diagram (**Supplementary**
302 **Fig. 8**). AIC was also used to estimate the goodness-of-fit and to compare nested models.
303 Standardized coefficients of each component model were reported along with the statistical
304 parameters for the global fit test, such as Fisher's *C* statistic, *p*-value, R^2, AIC, as well as AICc
305 (Shipley, 2013). The results of SEM were cross-checked with the abovementioned approaches
306 that we used, and different approaches gave very similar patterns (**Supplementary Fig. 9**).

307 *2.5 Determining species functional importance on soil multifunctionality*

308 We quantified the multifunctional importance of two functional groups at the species level,
309 relying on randomization tests in 'Impact', a Fortran 95 program (Gotelli et al., 2011). We
310 excluded those species that occurred only once or twice in the meta-community of the region.
311 Because of abundance-dependent properties of soil microbes on functioning (Peter et al.,
312 2010), the species-specific values of multifunctional importance were calculated as the
313 Spearman's rank correlation coefficient between the relative abundance of target species and
314 the number of functions which equaled to or exceeded a critical threshold across all sites. We
315 used randomization (999 permutations) to reassign observed values in different sites and
316 repeated the null modeling for all species. The SES of multifunctional importance value was
317 then measured based on the null model. This index quantified the extent to which an observed
318 metric deviated from the distribution of metrics generated by a stochastic simulation (Gotelli

and McCabe, 2002). If $|SES_i|$ of species *i* is greater than 1.96 (Z-value of the critical threshold of 95% confidence interval), the value of functional importance approximately falls into the 5% tail of a normal distribution, which means that species *i* has significantly positive or negative association with soil multifunctionality. We calculated SES values in a series of thresholds of function variables from 1% to 99% with an interval of 5%. The mean values of SES at multiple thresholds were evaluated by simple *t*-tests, respectively (n=21), and were then checked on the difference between functional groups by a paired-samples *t*-test with a two-tailed significance criterion of 0.05. We also conducted null modeling on multifunctional importance by calculating multifunctionality values via averaging the standardized Z-scores of individual functions. In addition, the patterns of SESs derived from the matrix of abundance data were compared with those by using the presence-absence matrix. Results exhibited high consistency and therefore only the former were shown (**Supplementary Fig. 10**).

3. Results

3.1 Relations of microbial diversity and soil multifunctionality

As predicted, microbial diversity of overall functional groups exhibited an unambiguously strong positive relationship with multifunctionality in biocrusts (**Fig. 1a, e**). This combined association resulted significant from a low threshold of 20% (T_{min}), peaking at a very high threshold of 96% (T_{mde}) with an effect size of roughly 3.08 functions added (R_{mde}) between the lowest and the highest diversity levels (**Table 1**). As the strongest relation, overall MultiDiver achieved 44% of the maximum possible effect size on multifunctionality.

However, in spite of the synthetic richness of two functional groups that still presented a positive relation with multifunctionality (**Fig. 1d, h**), the linkage was moderate (thresholds between 50%~67%) and much weaker than overall MultiDiver. It could only reach 25% of the maximum possible effect. Between functional groups, diazotrophic diversity was positively correlated with multifunctionality (**Fig. 1c, g**) while phototrophic diversity showed no significant effect (**Fig. 1b, f**). These results remained when we conducted ordinary least squares regression with environment-uncorrected raw data instead of residuals (**Supplementary Fig. 6**). When MultiDiver was disaggregated to inspect individual diversity facets, different functional groups exhibited distinct patterns with soil multifunctionality (**Supplementary Fig. 4**). For example, although phototrophic diversity as integrating was unrelated to multifunctionality, its MPD showed a strong positive association (T_{min}=26%, T_{mde}=95%, and R_{mde}=3.37). In contrast, albeit all three diversity facets of diazotrophs were predominant factors to promote multifunctionality to a high level, its evenness and MPD were linked only to multifunctionality over a moderate range of thresholds (approx. 25%~75%) with a low effect size around 1.6~2.0 (R_{mde}). We found a strong positive relationship between diazotrophic richness and multifunctionality (T_{min}=23%, T_{mde}=97%, and R_{mde}=3.05). However, despite that total abundance is often supposed to play a main role in biogeochemical processes (Bardgett and van der Putten, 2014; Winfree et al., 2015), moderately negative associations were observed between microbial abundances of both functional groups and soil multifunctionality (**Supplementary Fig. 4**).

3.2 Effects of diversity and abundance on individual ecosystem functions

Biotic predictors of two functional groups explained a large proportion of variance in different ecosystem functions (mean adjusted R^2=0.64, **Fig. 2**). Their associations with various ecosystem functions were considerably strong as that of climatic and ambient factors (**Supplementary Fig. 11**). The most parsimonious models included 1.86±0.35 predictors across all functions (mean±s.e.m.). The diazotrophic richness was a widely positive predictor explaining the variance in the strength of diversity-individual function relations, whilst MPDs were positively linked with BelowBio, TN, Chl, and TOC (**Fig. 2a**). There were only two functions, TP and WHC, which were negatively correlated to the increase of diazotrophic richness or phototrophic MPD, respectively (p<0.05). Most of the findings remained when we cross-checked by using environmental-uncorrected raw data (**Supplementary Fig. 11**). Despite that diazotrophic abundance consistently showed negative relations with most individual functions (p<0.05), both phototrophic and diazotrophic abundances were significant positive explanatory variables for the belowground biomass. Furthermore, we did not detect obvious trade-offs between the effects of diversity facets and/or abundances, or among individual ecosystem functions (**Supplementary Fig. 7**).

3.3 Connection network of diversity and abundance on soil multifunctionality

Piecewise SEM was conducted in an independent set of models for each functional group separately (**Fig. 3**). We did not fit the interaction of biotic predictors between two functional groups despite the fact that soil microorganisms to some extent complement each other in supporting ecosystem functions (van der Heijden et al., 2016), because this would require a larger dataset and would determine a complicated network which might be difficult to

interpret. Overall, two best-fitting models had Fisher's *C* statistic $p>0.05$ with AICc values of 53.39 and 34.85 for phototroph and diazotroph, respectively. The result demonstrated that phototrophic MPD exhibited the highest positive direct link with soil multifunctionality (standardized coefficient $\beta=0.76$). Meanwhile, it connected with multifunctionality indirectly via phototrophic richness ($\beta=0.56$), but phototrophic richness *per se* showed an equal negative direct relationship with multifunctionality ($\beta=-0.52$). Besides, phototrophic evenness was negatively and directly linked with multifunctionality ($\beta=-0.33$). These direct and indirect associations of phototrophs occurred simultaneously and offset mutually. It resulted in a neutral effect of phototrophic diversity on multifunctionality as an integral factor (net coefficient $\beta=-0.09$), which was also observed by using a multiple thresholds approach. In contrast, the relationship between phototrophic abundance and multifunctionality was weaker and nonsignificant ($\beta=-0.27$). For diazotrophs, only richness showed a significant positive relation with multifunctionality ($\beta=0.49$), and it contributed to the generally beneficial effect of diazotrophic diversity as a whole on soil multifunctionality.

3.4 Species functional importance

The standardized functional importance of phototrophs exhibited that the abundance of 7.66±0.52% (mean±s.e.m. across thresholds) of species significantly increased with the enhancement of soil multifunctionality than the null expectation, while 19.02±0.90% of species were negatively related, and another 73.32±1.23% were not functionally important ($p>0.05$) (**Fig. 4a, c**). For diazotrophs, the abundance of 10.32±1.02% of species were positively MORE ? correlated with the multifunctionality level than expected by chance,

while only 1.77±0.64% of species, which were observed rarely at high threshold levels, were negatively associated with multifunctionality,. Most diazotrophic species (87.91±1.25%) were not functionally more important than the null expectation (p>0.05, **Fig. 4b, d**). The proportion of phototrophs playing significant functional roles was over two-fold greater than that of diazotrophs, while the proportion of phototrophs with positive association was lower. Furthermore, there was a significant difference between the functional importance values of phototrophic and diazotrophic species (t =-12.92, df=20, p<0.001), which implied that the extent of multifunctional redundancy between phototrophs and diazotrophs in biocrusts was differentiated. In addition, the standardized functional importance calculated by averaging approach supported the findings that more proportion of diazotrophs had no significant associations between their abundance and the multifunctionality level, rather than phototrophs (**Fig. 4e, f**). That was, 50 phototrophic species exhibited positive relations and 159 species were negatively correlated, while only nine diazotrophic species were positively and 11 species were negatively related to multifunctionality (p<0.05).

4. Discussion

4.1 Differentiated effects of diversity facets on soil functionality

In our study, after separating the co-varying influence of abiotic factors, we observed a clear increase of soil multifunctionality with multifaceted diversity of the target functional groups. This positive relationship appeared to reach saturation at a very high level of threshold (96%), suggesting that maintaining phototrophic and diazotrophic diversity in biocrusts is important to maintain soil multifunctionality. In contrast, the synthetic richness of two functional groups

could just sustain soil multifunctionality moderately with a relatively weak effect (50%~67%). The result for richness here is somewhat consistent with previous studies which often claimed that, while diversity is crucial for multifunctionality in a given considered suite of ecosystem functions, high species richness could not guarantee a grand slam of all functions to gain their maxima with any levels of biodiversity (Byrnes et al., 2014; Zavaleta et al., 2010). In these cases, the moderate effect of richness on multifunctionality is frequently attributed to potential trade-offs among different individual functions (Byrnes et al., 2014; Mori et al., 2016). For instance, in an agricultural ecosystem, increased crop yield (primary productivity) often results in declining soil formation (soil fertility) (Allan et al., 2015). An enhanced plant-available nutrient level (soil fertility), in a forest ecosystem, is very likely to associate with an increased nutrient leaching (climate resistance) (Mori et al., 2016). However, in our study, we did not detect obvious trade-offs among functions (**Supplementary Fig. 7**). Therefore, we inferred that it is alternatively possible that the moderate relationship of species richness with multifunctionality arises from the inherent susceptibility of various ecosystem functions responding to different diversity facets. Indeed, according to multiple linear regression analysis, we verified that phototrophic richness has nonsignificant association with most individual functions, while phototrophic MPD, as a proxy for phylogeny-related trait dissimilarity, exhibits strong power to predict the variance in various ecosystem functions.

A previous study proposed to identify ecosystem functioning into phylogenetically and physiologically narrow and broad processes carried out by special constrained and a wide

range of microorganisms, respectively (Schimel and Schaeffer, 2012). Regardless of the cumulative effect by richness increasing (Bender et al., 2016), if every species performs the same process identically, it doesn't matter which one is more important, and *vice versa* (Schimel and Schaeffer, 2012). In other words, the richness *per se* is possibly crucial for some 'broad' ecosystem functions performed by common species, but for the other 'narrow' functions (*e.g.*, ammonia oxidation), the presence and properties of certain key species seem to be more meaningful (Bender et al., 2016; Heemsbergen et al., 2004). The results demonstrated that the multifaceted diversity indices explained more than 64% of the variance in ecosystem functions, while ACE richness accounted for less than 39% (Adjusted R^2). Hence, validating the role of microbial diversity needs to incorporate multiple facets to assess their combined influence, otherwise microbial diversity as a driver of multifunctionality would be underestimated.

4.2 Cascading network of biodiversity and functioning

For multiple facets of diversity, we found an apparent imbalance between the respective effects of phototroph and diazotroph on soil multifunctionality. That is, diazotrophic diversity dominates and determines the overall association with multifunctionality, while multifunctionality is relatively independent of phototrophic diversity. However, the results of pSEM suggest that this result may partly arise from a statistical fallacy (Simpson's paradox) whereby aggregated data likely conceal underlying links (Bradford et al., 2014). The effects of disaggregated phototrophic richness, evenness and MPD offset each other and collectively lead to a neutral relationship. Actually, phototrophic MPD is the strongest predictor among all

466 biotic drivers of both functional groups on multifunctionality (β=0.76), followed by
467 diazotrophic richness (β=0.49).
468 Abundance-weighted MPD represents the extent of evolutionary relatedness among
469 individuals of each functional group (Cadotte et al., 2010). Evolutionary relatedness
470 presumably reflects the integrated phenotypic difference among taxa and ultimately decides
471 inter-/intra-species interaction (Alexandrou et al., 2015; Cadotte et al., 2010). Thus, following
472 from the competition-relatedness hypothesis (Cahill et al., 2008), larger MPD indicates
473 increased trait dissimilarity and a broader niche breadth, and gives rise to resource
474 specialization and reduced competition within individuals of the functional groups (*i.e.*, more
475 taxa co-occurring) (Ashton et al., 2010). We indeed found that phototrophic MPD positively
476 impacts on its richness. However, we further detected that phototrophic richness and evenness
477 are jointly related to soil multifunctionality negatively, whilst MPD is negatively related to
478 abundance. It means that enhanced multifunctionality is associated with fewer dominant
479 phototrophs, and the overwhelming majority of the rest might be dormant or inactive with
480 lower abundances. This predictable tendency of phototrophic species to be more convergent
481 along with the improvement of multifunctionality was verified by our analysis on species
482 functional importance. We observed that a relative larger fraction of phototrophic species has
483 significant negative associations between their abundances and the multifunctionality level,
484 nevertheless, the prevalence of a few key phototrophs is adequate to sustain soil
485 multifunctionality of biocrusts. In fact, previous studies have proven that mature biocrusts are
486 often dominated by a limited suite of cyanobacterial species (*e.g.*, *Microcoleus* spp.) (Freeman

et al., 2009; Garcia-Pichel et al., 2013). The trade-off between phototrophic richness and MPD is consistent with Chesson's framework that species coexistence is driven by the interaction of niche differences and relative fitness differences, which are two types of species differences (Chesson, 2000; Mayfield and Levine, 2010). Although larger MPD leads to greater niche differences which favors phototrophs when they drop to low densities, relative fitness difference could drive a few species to dominate. In this context, the species pool of phototrophs with high trait dissimilarity serve as redundancy to ensure the maxima of soil multifunctionality against the fluctuation of environmental conditions during a long-term period (*i.e.*, insurance effect), rather than by the cumulative effect of richness.

In contrast, we found that diazotrophic MPD has no significant effect on its richness, abundance, as well as multifunctionality. Nonetheless, it is possible that the focal molecular marker for diazotrophs, in reality, contains little detectable phylogenetic signal on their trait dissimilarity. However, given the relatively fewer members of diazotrophs than phototrophs, the stochastic effect of diazotrophic richness is likely to provide direct benefits for multifunctionality (Bender et al., 2016). As a proof, we demonstrated that, in contrast to phototrophs, the majority of diazotrophs are multi-functionally nonsignificant. It indicates a relatively low redundancy of diazotroph that the number of species, namely its richness, is more important to maintain multifunctionality (Mori et al., 2016). Our results clearly exhibited the different extents of functional redundancy between phototrophs and diazotrophs. It partly explains the distinctive influencing patterns of multiple diversity facets between different functional groups on soil multifunctionality.

4.3 'Minority report' of the narrow functional groups on soil multifunctionality

Most importantly, it should be noted that our study sheds light on the subsets of a microbial community characterized by particular 'narrow' functioning (C- and N-fixation), rather than the whole community. This is to tprovide deeper insights on the mechanisms of diversity effects on multifunctionality. The intra-group diversity pattern is beyond the significance of whether keystone species are present, which was previously described as a straightforward change in community composition (Wagg et al., 2014). The results imply that multifaceted diversity patterns within crucial functional groups play an important role on ensuring the response variability of biocrusts to stabilize multifunctionality against environmental perturbation (Elmqvist et al., 2003; Mori et al., 2013). Meanwhile, ecological processes performed by microbes are largely density-dependent (Yang et al., 2014). Some ecologists argue that species loss will not impair ecosystem functioning due to expected community-wide increases in average population abundance (density compensation), according to the mass-ratio hypothesis (Gonzalez and Loreau, 2009; Grime, 1998). It would be predicted that the lack of diversity effect on functioning could tend to be more observable within the functional group because it is comprised of functionally similar species. However, our results of different models demonstrate that density compensation does not seem to be the ubiquitous mechanism by which multifunctionality is maintained in biocrusts. The abundance-weighted multifaceted diversity exhibits stronger explanatory power on the variance of multifunctionality than the abundance. Furthermore, our supplementary results show that certain relatively less dominant taxonomic groups in the functional groups, such as

529 Synechococcales and Rhodospirillales, contribute disproportionately more on explaining the
530 variance in ecosystem functioning than Oscillatoriales or Nostocales, which are the most
531 abundant orders (**Supplementary Fig. 12** and **Table 5**). It provides the opposite evidence on
532 the mass-ratio hypothesis.

533 **5. Conclusions**

534 In conclusion, our study demonstrated that multiple diversity facets should be considered
535 when trying to disentangle the linkages between biodiversity and ecosystem functioning.
536 Given that multifaceted diversity pattern within C/N fixing functional groups is a strong
537 predictor of the variance in ecosystem functions/multifunctionality, it could be used as a
538 proxy of the respond variability of biocrusts against external stresses. The results may help
539 bridging the gap between taxonomic and functional approaches in the biodiversity-ecosystem
540 function relationship studies. Since different diversity facets have idiosyncratic performance
541 in different functional groups, they determine divergent influencing patterns on soil
542 multifunctionality. This shift is attributed to the significant differentiation of the extent of
543 multifunctional redundancy between phototrophs and diazotrophs. Nevertheless, as our study
544 focused on the particular functional groups in biocrusts, further studies should aim to verify
545 the results under different habitats characteristics, as well as in other key functional groups of
546 distinct ecosystems, which could ultimately help to improve the practices of dryland
547 management against global change.

548 **Data Accessibility Statement**

549 MiSeq sequencing data sets are available in the Sequence Read Archive of National Center

for Biotechnology Information (NCBI-SRA, https://www.ncbi.nlm.nih.gov/sra/docs/) under accession numbers SRR5576302 and SRR5576303. The climatic data are available from http://www.worldclim.org/.

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Table 1 Summary of multiple thresholds approach for elucidating the relations of microbial diversity with soil multifunctionality.

Predictors†	Critical Thresholds	Achieving multifunctionality levels	Estimate	Std. Error	*t*-value	Significant P_r (>|*t*|)
MultiDiver	T_{min}: 0.20	M_{min}: 6.92	0.383	0.185	2.075	0.049
	T_{max}: 0.99	M_{max}: 2.05	2.770	1.136	2.439	0.023
	T_{mde}: 0.96	M_{mde}: 2.65	3.075	1.016	3.026	0.006
Phototroph‡	-	-	-	-	-	n.s.
Diazotroph	T_{min}: 0.19	M_{min}: 7.23	0.451	0.216	2.090	0.048
	T_{max}: 0.99	M_{max}: 2.41	3.383	1.330	2.544	0.019
	T_{mde}: 0.95	M_{mde}: 3.06	3.641	1.158	3.143	0.005
Richness	T_{min}: 0.50	M_{min}: 4.31	1.229	0.546	2.252	0.035
	T_{max}: 0.67	M_{max}: 3.26	1.745	0.788	2.214	0.037
	T_{mde}: 0.66	M_{mde}: 3.26	1.745	0.788	2.214	0.037

† synthetic single indices are calculated as the primary component values (PC1) by PCA, and the statistical parameters are obtained from the general linear models (GLMs), see details in **Fig. 1**; ‡ under the criterion of 0.95 confidence interval, phototrophic diversity in biocrusts shows no significant association with multifunctionality through the whole range of thresholds; $T_{min/max}$: the lowest or highest threshold where biodiversity affects multifunctionality significantly; T_{mde}: the threshold where biodiversity has its strongest effect on multifunctionality; $M_{min/max/mde}$: the number of functions achieving at the thresholds $T_{min/max/mde}$.

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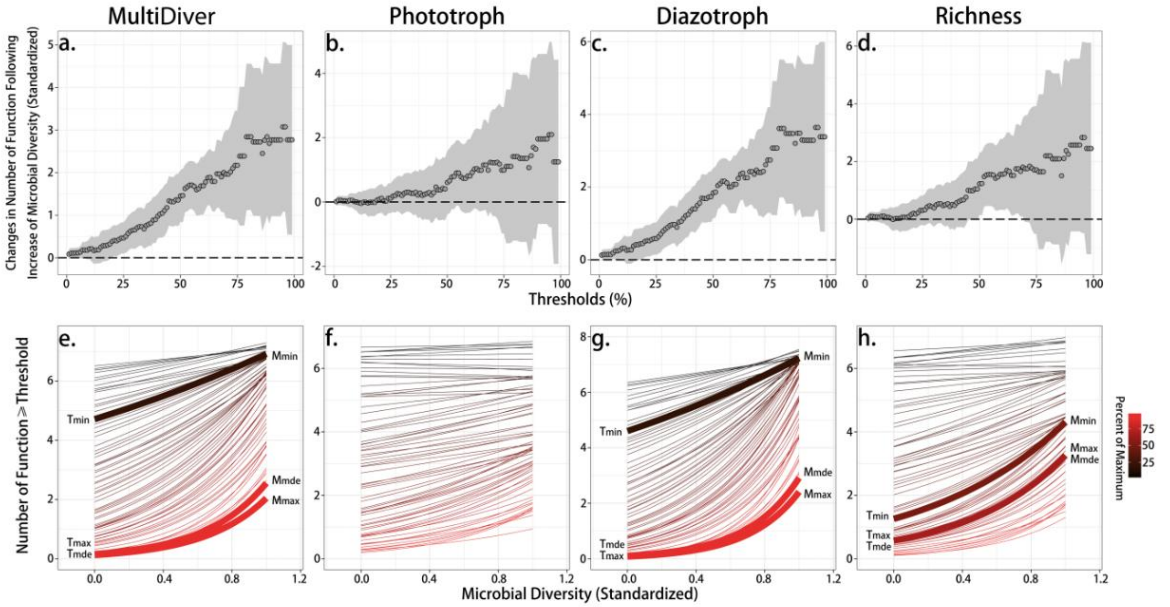
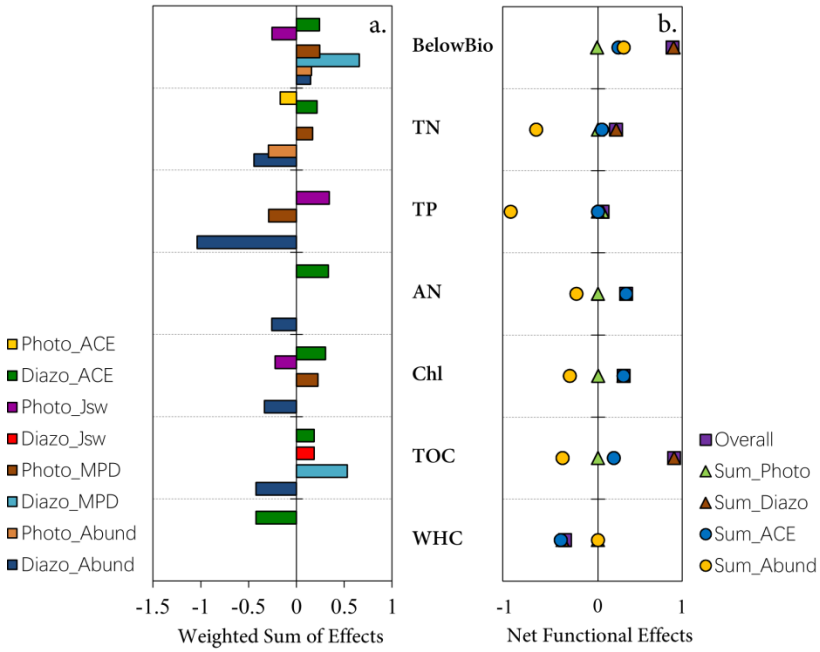


Figure 1 Diversity effects along with a threshold range of soil multifunctionality. MultiDiver calculated by multiple metrics of diversity (*i.e.*, PC1 value of ACE richness, Pielou's evenness, and phylogenetic dissimilarity (MPD) of both functional groups) (a, e), synthetic phototrophic (b, f) and diazotrophic (c, g) diversity, and synthetic richness of functional groups (PC1 value) (d, h) are evaluated, respectively. The upper panels (a-d) exhibit the changes in numbers of ecosystem functions reaching a percentage threshold of the maximum observed functioning variable following one standardized unit increment of microbial diversity at multiple threshold levels. Hollow points are the fitted values of regressions and grey shading indicates 95% confidence intervals. The lower panels (e-h) show the regression lines between diversity indices and the number of ecosystem functions above a threshold for multiple threshold values. Colors of lines indicate the thresholds from low (black) to high (red). $M_{min/max/mde}$ and their corresponding thresholds $T_{min/max/mde}$ are shown when the diversity effects are significantly different from zero (*i.e.*, the grey confidence interval area does not overlap the dashed line).



c.

EFs		ACE	J_{sw}	MPD	Abund	adjusted R^2
BelowBio	F1					0.79
	F2					0.82
TN	F1					0.75
	F2					0.75
TP	F1					na.
	F2					0.70
AN	F1					0.36
	F2					0.23
Chl	F1					0.61
	F2					0.74
TOC	F1					0.38
	F2					0.76
WHC	F1					0.36
	F2					na.
	sum	- +	- +	+ ++	- --	

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Figure 2 Relations of multiple biotic predictors with individual ecosystem functions. Standardized effects of diversity facets and abundances of C/N fixing functional groups on each functioning variable (a), as well as the respective net effect sizes of all diversity facets (Overall), phototrophic/diazotrophic diversity facets (Sum_Photo/Sum_Diazo), and the sum of richness/abundances (Sum_ACE/Sum_Abund) are shown (b). The effect size of each predictor is the sum of estimates from the pair models (Fit 1: EF ~ Photo_ACE + Diazo_ACE + Photo_J_{sw} + Diazo_J_{sw} + Diazo_MPD + Photo_Abund; and Fit 2: EF ~ Photo_J_{sw} + Diazo_J_{sw} + Photo_MPD + Diazo_MPD + Diazo_Abund). Variance explained after accounting for the influence of predictor number (adjusted R^2) and standardized slopes of predictors included in the pair models for each functioning variable are shown (c). Colors indicate the values of slopes from low (blue) to high (red).

‘+’ and ‘-’ indicate that the sum of the effect size of each predictor through individual ecosystem functions is positive or negative, respectively. ‘na.’ means that no predictor has a significant association in the most parsimonious model. ACE: ACE richness; J_{sw}: Pielou’s evenness; MPD: mean pairwise distance; Abund: abundance; BelowBio: belowground biomass; TN: total nitrogen; TP: total phosphorus; AN: available nutrients; Chl: chlorophyll content; TOC: total organic carbon; WHC: water-holding capacity. ‘Photo’ and ‘Diazo’ in the figure indicate phototroph and diazotroph, respectively.

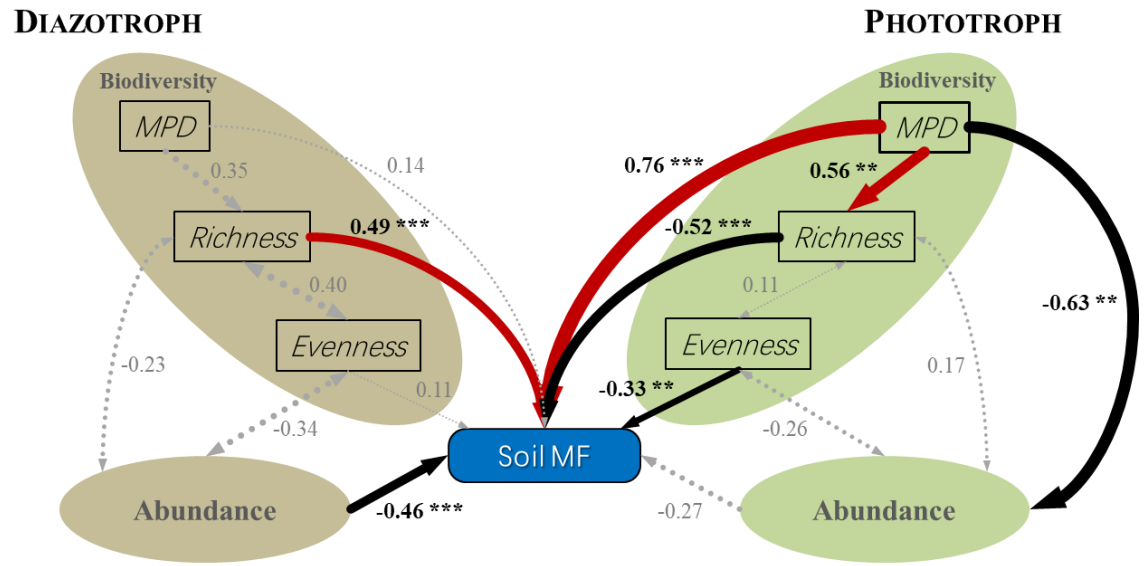

Figure 3 Piecewise structural equation modeling of biotic predictors (diversity and abundance) and soil multifunctionality. The connection network represents the relations among ACE richness, Pielou's evenness, phylogenetic dissimilarity (MPD), and microbial abundances of phototrophs and diazotrophs, and their individual influences on multifunctionality. Solid red arrows indicate significant positive paths, solid black arrows indicate significant negative paths, and dotted grey arrows represent nonsignificant paths (** p<0.01, *** p<0.001). Numbers adjacent to arrows are the path standardized coefficients, and the width of arrows is proportional to the values of path coefficients. The statistical parameters for the global fit test (Fisher's C statistic, p-value, R^2, AIC, and AICc) are also shown.

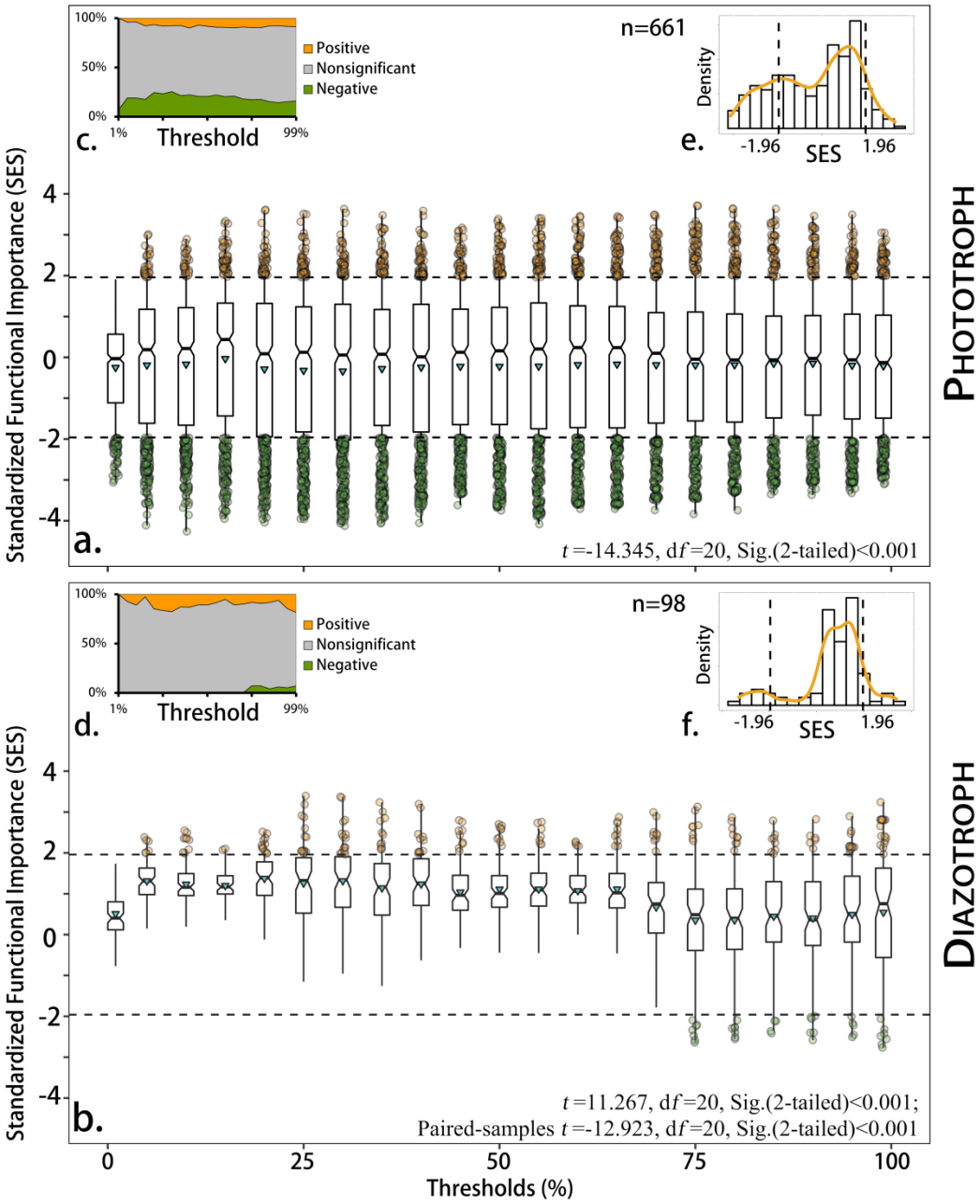

Figure 4 Functional importance of species to maintain multiple ecosystem functions simultaneously. Standardized functional importance (SFI) of each phototrophic (a) and diazotrophic (b) species are calculated based on multiple thresholds approach. Boxplots show the distribution of SFI at every 5% threshold levels from 1% to 99%. The upper/lower quartiles, medians and average values of SFIs are shown. Black dashed lines indicate the 95% confidence intervals of SFI values. Red and green hollow points represent species with significantly positive and negative associations with soil multifunctionality, respectively. Their percent proportions through the thresholds are shown (c, d). The

histograms exhibit the distribution of species' SFI values of phototrophs (e) and diazotrophs (f) based on the averaging approach. Orange lines indicate kernel density curves of SFI distributions. The statistical parameters of simple/paired-samples *t*-tests are shown.

(Supplementary material can be found on-line in the Supporting Information section as an open source)