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Bacterial contributions of bio-crusts and litter crusts to nutrient cycling in the Mu Us Sandy Land

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ABSTRACT

Desertification has become an important issue for the sustainable development of human society at global scale and has led to the changes in soil properties and vegetation cover. Biocrusts and litter crusts play roles in improving the soil microhabitat of sandy ecosystems. Soil microbial communities mediate ecosystem functions in various ecosystems, e.g., soil biogeochemical processes. However, limited information is available about how the underlying processes of bio-crusts/litter crusts restoration are driven by soil bacterial communities in sandy land. Here, we investigated the changes in soil bacteria from three groups (sandy land, bio-crusts, and litter crusts) and three soil layers (0-2 cm, 2-5 cm, 5-10 cm) with nine replicates each collected in July 2019 utilized high-throughput pyrosequencing of the V4-V5 rRNA gene region. Most soil nutrients (SOM, AP, AK, and TN) and enzyme activities (BG and DHA) had differences among the three groups and three soil layers. OTU richness and diversity of bacteria were positively correlated with most soil variables. The constructed co-occurrence networks between soil variables and bacterial communities, and within bacterial communities showed that bacterial taxa had closer relationships with all soil variables in crusts than sandy land and varied among the three sand groups (sandy land, bio-crusts, and litter crusts). The result showed that the composition of bacterial community was regulated mainly by soil variables and crust types. Compared with sandy land, more predictors in nutrient cycling were found in crust types. They played major roles in nutrient cycling in desert ecosystem restoration on the basis of random forest modeling. Our findings indicate some bacterial taxa may played the predominant roles in connecting with soil variables and other bacterial taxa across crusts types, and litter crusts and bio-crusts drive the nutrient cycling by mediating the restoration of bacterial taxa in sandy ecosystems.

Keywords:

Bacterial communities; Bio-crusts; Litter crusts; Nutrient cycling; Sandy land restoration

1. Introduction

Land desertification poses a great threat to all types of ecosystems, it can damage ecosystem's basic functions and services to sustain life, causes the losses in soil nutrients, the decline in soil potential productivity, and the reduction in vegetation (D'Odorico et al., 2013). Arid and semiarid areas are among the most susceptible to land desertification, however, they covered approximately one-third of the earth's land and have been expanding rapidly, this is due to climate change and human activities, such as overcultivation, overgrazing, and urbanization (Asner et al., 2003; Sivakumar et al., 2005)(Gao et al., 2017). With the increase of the world population and deterioration of living environment, desertification has becoming one of a major issue for the in human societies at global scale(D'Odorico et al., 2013). For instance, according to The Desertification and Sanditification Sate of China, China had a desert area of 2.6 million square kilometers, and another 1.7 million square kilometers of sandy area in 2014, which covers about 27.2% and 17.9% of the country's land, respectively (State Forestry Administration, 2015). The Mu Us Sandy Land, which is located in central north of China, is the region with high risks of desertification in arid Asia (Wang et al., 2017). In 1999, the Grain for Green Program was launched by Chinese government with aims to halt soil erosion and improve the ecological environment (i.e. the losses of soil fertility and the decrease of vegetation coverage). It is the largest ongoing revegetation project in China and also one of the largest conservation projects in the world. This project converted croplands into grasslands or shrubs and increased vegetation coverage from 31.6 % in 1999 to 59.6 % in 2013 on the Loess Plateau (Chen et al., 2015b; Uchida et al., 2005).

Meanwhile, apart from the "Grain for Green" Program, several other initiatives have been carried out to restore soil fertility and alter the sand surface to control desertification in sand areas, such as mechanical sand barriers (Bo et al., 2015) and afforestation (Zeng et al., 2008). These initiatives enhanced development of bio-crusts and litter crusts on the Loess Plateau, either directly or as a result of a general improvement of environmental conditions. Better environmental conditions, including appropriate humidity and temperature, promote the development of bio-crusts and litter crusts in the Mu Us Sandy Land (Liu et al., 2019b).

Biological soil crusts (bio-crusts), which are composed of cyanobacteria, lichens, mosses, fungi, and other nonvascular photoautotrophs, are typical for dryland ecosystems worldwide and represent an essential functional component of the pedosphere. Bio-crusts can stabilize soil, increase soil fertility, impact hydrologic cycles, alter soil organic matter content, and provide a home for belowground organisms (Reed et al., 2019; Torres-Cruz et al., 2018). Litter crusts are defined as the cohesiveness of the soil surface shaped by litter and soil and forms a hard shell by the mixing of sand and litter organisms in the wind - water erosion crisscross zone Bio-crusts and litter crusts play crucial roles in improving microhabitat conditions, forming soil organic matter, affecting hydrological processes, and soil bacterial communities in sandy lands during restoration (Jia et al., 2018; Leloup et al., 2018; Liu et al., 2019b).

In this context, soil variables are most important factors to impact microbial communities, such as soil pH, soil texture and, available nutrients (Chen et al., 2015a; Fierer and Jackson, 2006). On the contrary, microbial communities are important indicators of rehabilitated ecosystems (Banning et al., 2011) and drive the Earth’s biogeochemical cycles (Falkowski et al., 2008). Soil bacterial communities represent the greatest biodiversity reservoir and greatly affect ecosystem functions and services (Falkowski et al., 2008; Wagg et al., 2014). An enhanced appreciation of the connection

between environment and microbial ecology, in the last decade, has led to many studies focused on the distribution of soil microbial communities (Karimi et al., 2018), the influence of microbial diversity on plant community (Jiao et al., 2019) and multifunctionality of terrestrial ecosystem (Delgado-Baquerizo et al., 2016; Falkowski et al., 2008; Jiao et al., 2019; Karimi et al., 2018). However, limited information is available about the response of bacterial communities on soil variables in natural desert ecosystems. Moreover, each microbe may play a different functional role in complex microbial ecosystem (Li et al., 2019). Experimental evidence suggested that the bacterial communities at phylum level are similar in the two sample types. The relative abundance of several genera has considerably differences at the genus level (Jakobsen et al., 2019). However, the foundational role of the bacterial genera in regulating key ecosystem processes (i.e. nutrient cycling) of litter crusts and bio-crusts in the sandy ecosystem is lacking. Thus, we must expand our insight into the functions of the microorganisms, particularly bacteria genera, in the bio-crusts and litter crusts in the restoring sand ecosystem.

The present study aims to (1) elucidate the variations in soil quality and bacterial communities coupled with the soil quality of bio-crusts and litter crusts, (2) explore the correlations between the soil bacterial taxa and soil variables and the bacterial taxa among themselves in the bio-crusts and litter crusts networks, (3) identify the contributions of the annotated bacterial taxa to nutrient cycling during ecosystem restoration. To achieve these aims, we used high-throughput pyrosequencing of the V4-V5 rRNA gene region to compare the variations of bacterial communities in relation to soil variables, their co-occurrence networks, and their contributions to the soil functioning (nutrient cycling) in bio-crusts and litter crusts of restoring sandy ecosystems.

2. Materials and methods

2.1. Study sites and sample collection

The study was carried out in the eastern part of the Mu Us Sandy Land ecosystem (110°21’ -110°23’E, 38°46’-38°51’N; 1080-1270 m Altitude), located in Shenmu County at the northern of Shanxi Province, China. This region, as the most arid area in Asia, is one of the largest dune areas in the north of China (Wang et al., 2017). Psammophytic shrubs and herbaceous plants are mainly dominant plant species in this study site (Jia et al., 2018). Accordding to our previous studies, bio-crusts and litter crusts, as two major contributors, covered about 40% and 30% of the Mu Us Sandy Land, respectively (Jia et al., 2018). To compare the effects of bio-crusts and litter crusts on the surface microhabitats of the sandy land, three sites (sandy land, bio-crusts, litter crusts) with similar environmental conditions in terms of underlying subsoil, microtopography, and soil hydrology, were selected and the distance between them was above 500 m apart. Sand samples were collected in July 2019 from sampling sites covered by bio-crusts and litter crusts, respectively. After removing the litter horizon, nine replicate sites were randomly selected above 10 m apart and three sand layers (0-2 cm, 2-5 cm, 5-10 cm). Each replicate was mixed with five sand cores by a zigzag pattern (Liu et al., 2019a). In total, 81 soil samples = 3 sites (sandy land, bio-crusts, litter crusts) × 3 depths (0-2 cm, 2-5 cm, 5-10 cm) × 9 replicates were obtained, and all the sand samples were taken to the laboratory on ice within 24 h. A small part of each sample (~2 g) for the DNA analysis was transported to the company (Novogene, Beijing, China) on ice. Another part was sieved (~2 mm) for the analysis of soil properties. The rest was stored at −80 °C.

2.2. Sand characters and enzyme activities

Sand organic matter (SOM) were measured by potassium dichromate colorimetric method (Nelson and Sommers, 1982); total phosphorus (TP), and available phosphorus (AP) were

134 determined by molybdenum anti-colorimetric method (Olsen and Sommers, 1982); ; total nitrogen
135 (TN) was determined with the Kjeldahl method (Bremner and Mulvaney, 1996); total potassium
136 (TK), and available potassium (AK) were determined with Flame photometry as described
137 previously (Page et al., 1982). β-glucosidase (BG), dehydrogenase (DHA), Urease (UA), alkaline
138 phosphatase (ALP), and acid phosphatase (ACP) were measured by the methods described in
139 Tabatabai, 1994 (Tabatabai, 1994; Taylor et al., 2002). Briefly, BG activity was determined as the
140 amount of p-nitrophenol (PNP) released; ALP and ACP were determined by p-nitrophenol (PNP)
141 released at pH 11 and 6.5, respectively. UA was measured by the determination of ammonia
142 released. DHA was determined as the amount of the TPF released (Taylor et al., 2002). All the
143 samples were determined using three replicates. The selected parameters reflect either resource
144 pools (SOM, TN, TP, AP, TK) in biogeochemical cycles or important processes regulating
145 availability of these pools (BG, DHA, UA, ALP, ACP). Such as, the important ecosystem processes
146 related to the cycling of carbon (SOM, BG and DHA), nitrogen (TN and UA), phosphorus (AP, TP,
147 ALP, and ACP), and potassium (AK and TK) (Jiao et al., 2019; Jing et al., 2015).
148
149 **2.3. DNA extraction and 16S rRNA gene sequencing**
150 Genomic DNA from each sand sample was extracted from 1g sand using the OMEGA soil
151 DNA Kit (Omega Bio-Tek, Inc., Norcross, GA, USA) based on the manufacturer's instructions. To
152 assess the bacterial communities, the V4–V5 region of the bacterial 16S rRNA gene was amplified
153 with the primers: 515F (5’ – GTGCCAGCMGCCGCGGTAA – 3’) / 907R (5’ –
154 CCGTCAATTCCTTTGAGTTT – 3’). The purified PCR products were pooled and sequenced on
155 the Illumina HiSeq (300~bp paired-end reads) platform (Novogene, Beijing, China).
156

157 **2.4. Data analysis**

158 The acquired sequences were processed to remove low - quality sequences using the QIIME
159 pipeline (Caporaso et al., 2011). The remaining sequences were classified into operational
160 taxonomic units (OTUs) with 97% sequence similarity by the UPARSE pipeline (Edgar, 2013).
161 OTU richness, Chao1 index, Shannon index, ACE index, and Simpson index were calculated by the
162 OTU table in QIIME (Caporaso et al., 2010).The changes in soil nutrients, enzyme activities, and
163 bacterial communities, as well as the relative abundance of the microbial phyla among three soil
164 groups (sandy land, bio-crusts, and litter crusts) and three soil layers (0-2 cm, 2-5 cm, and 5-10 cm),
165 were conducted based on one-way ANOVAs with Tukey's tests by GraphPad Prism version 8.0.2
166 (GraphPad Inc. San Diego, CA, USA). The normality of data and the equality of variance were
167 tested. If the data did not meet normality or homogeneity, non-parametric Kruskal-Wallis analyses
168 were used. Redundancy analysis (RDA) was performed to visualize the influence of soil variables
169 on bacterial community composition in R package "vegan" (Oksanen et al., 2013).

170 The co-occurrence networks were constructed for bio-crusts and litter crusts based on
171 significant correlations between bacterial genera and all of the soil nutrients (Pearson's correlation,
172 p.thres = 0.05, r.thres = 0.6), and among the bacterial genera themselves (Pearson's correlation,
173 p.thres = 0.05, r.thres = 0.8), which were visualized by R packages 'igraph' (Hartmann et al., 2015;
174 Qian et al., 2018). In the co-occurrence networks, each node represents one bacterial genus and
175 each edge represents a significant correlation between two nodes. A set of metrics: number of edges
176 (Num. edges), average. degree, average. path. length, diameter, and modularity were calculated to
177 estimate network topological features. Num. edges represent the number of edges. To identify the
178 major statistically significant bacterial predictors for sand nutrient cycling, a Random Forest (RF)
179 modeling was performed with the forest (5,000 trees) using the "random Forest" package (Archer,

2016). The model significance was computed by the R package "A3" (Fortmann-Roe, 2013). A total of 35 classified microbial phyla and 143 annotated genera from 6 predictors at the phylum level were selected in the Random Forest modeling. Percentage increases in the mean squared error (MSE) was used to estimate the importance of variables. All statistical analyses were performed by GraphPad Prism 8.02 or R software (v3.6.3; https://www.r-project.org/).

3. Results

3.1. Variation in sand nutrients and enzyme activities

Soil nutrients and enzyme activities typically differed among three soil groups and three sand layers, most of which were significant differences. Litter crusts had significantly higher SOM, TN, AP, AK, BG, DHA, UA, and ACP than those in the sandy land and the bio-crusts in surface soil (0-2 cm and 2-5 cm); TK, SOM, TK, AK, TN, ALP, ACP were significantly higher in the bio-crusts than those in the sandy land in the surface soil (0-2 cm and 2-5 cm). SOM, AP, AK, BG, and DHA were highest in the topsoil (0-2 cm) of the bio-crusts and litter crusts. (Fig. 1). These results indicated that both litter crusts and bio-crusts improved the sand soil quality compared with sandy land, and litter crusts had more significant improvement in sandy soil quality than bio-crusts, especially, in the surface of the sand layer. The statistical information listed in Table S1.

3.2. Variation in sandy bacterial community

In total, 6,865,683 high-quality sequences were classified into 14560 operational taxonomic units (OTUs) after the 97% sequence similarity cutoff across 81 sand samples (Table S2). The OTU richness in biocrust and the litter crusts were significantly greater than this in the sandy land in the surface soil (0-2 cm and 2-5 cm) (Fig. 2B and Table S3). Bacterial OTU were primarily classified into the 66 microbial phyla, and the most dominant bacterial phyla were the phyla Proteobacteria

(28.2%), Actinobacteria (23,86%), Acidobacteria (18.92%) (Fig. 2A, and Table S4). 39.57% OUT were classified into the 707 genera, the most dominant identified genera were Sphingomonas (2.4%) and Nocardioides (2.2%), respectively belonging to the Proteobacteria, and Actinobacteria phyla (Table S5). For most of the bacterial phyla and genera, relative abundance of the phyla or genera bacteria significantly different among three sand groups ($P > 0.05$) (Table S4, Table S5). The Shannon index of bacterial community diversity in the litter crusts and bio-crusts were significantly higher than this in sandy land in the surface soil (0-2 cm and 2-5 cm). The bacterial communities clearly differed among three soil groups (Fig. 2E, Fig. 2F, Fig. 2H and Table S3).

3.3. Co-occurrence patterns of soil bacterial communities

Redundancy analysis (RDA) was performed to investigate the influence of soil variables on bacterial community among three sand groups and three sand layers. We found that soil variables well explain dynamic changes of bacterial communities at the phylum level or genus level. However, the correlations are not alike between the level of bacterial phylum and genus (Fig. 3 and Table S6). For instance, AK was the most important variable for litter crusts bacterial community at genus level, whereas, SOM, AP, TN, DHA, GB, and UA were more important at the genus than at the phylum level (Fig. 3 and Table S6). Furthermore, the co-occurrence networks were constructed for three sand groups to investigate the correlations between the bacterial genera and soil nutrients (Fig. 4A and Table S7). The network structure was distinct, the bio-crusts network had more edges (34) than sandy land network (33) and litter crusts network had more than two times (2.71) as many edges as found in bio-crusts. There is the highest average degree in the litter crusts and higher average degree in the bio-crusts network than these in the sandy land network. These showed that most soil nutrients had closer relationships with bacterial genera in the litter crusts than in the

bio-crusts and sandy land.

Given the interactions between bacterial taxa, we constructed co-occurrence networks to explore the interactions between the bacterial genera with each other in three sand groups, respectively (Fig. 4B and Table S8). Diverse topological characteristics were observed. The modularity indices were 0.8810.587, and 0.892 in the sandy land, bio-crusts, and litter crusts network, respectively. These values (The modularity indices > 0.4 show that the network has a modular structure) mean these networks had a modular structure. The highest average degree in the sandy land network than these in the litter crusts and bio-crusts networks. There were 975 edges and 94.26% positive correlations identified as co-occurrences in the bio-crusts and 1,787 edges and 99.89% positive correlations in the litter crusts. In sum, bacterial co-occurrence patterns were distinctly different between the bio-crusts and litter crusts.

3.4. The potential contributions of bacterial taxa to sand nutrient cycling

The contributions of bacterial communities to sand functionings were evaluated by Random Forest (RF) modeling. We uncovered the potential major bacterial drivers of sand nutrient cycling in the crust types by RF analysis, including 35 microbial phyla. We discovered that 9 bacteria phyla were the most important predictors to nutrient cycling in the bio-crusts and litter crusts (Fig. 5A). Planctomycetes， Cyanobacteria， Armatimonadetes， Rokubacteria， Nitrospirae， Latescibacteria， Deinococcus-Thermus were predictors in bio-crusts and Actinobacteria, Chloroflexi, Planctomycetes, Cyanobacteria, Armatimonadetes， Rokubacteria, Latescibacteria, and Deinococcus-Thermus were predictors in litter crusts. Furtherly, 143 annotated genera from the Actinobacteria, Planctomycetes, Chloroflexi, Armatimonadetes, Deinococcus-Thermus, Nitrospirae were selected to identify the major predictors at the genus level (Table S5). Compare to sandy land,

more predictors were found in the bio-crusts and litter crusts. We observed 22 and 20 predictors to nutrient cycling in bio-crusts and litter crusts, respectively (Fig. 6). *Blastococcus*, *Couchioplanes*, *Crossiella*, *Geodermatophilus*, *Actinoplanes*, *Parviterribacter*, *Marmoricola*, *Rhizocola*, *Tepidisphaera*, and *Fimbriiglobus* are the same predictors to the nutrient cycling between the bio-crusts and litter crusts. Most of the predictors were distinct between bio-crusts and litter crusts not only at the phylum level but also at the genus level and the particular bacterial consortium made important contributions to soil functionings.

4. Discussion

4.1 Influence of crusts on sand characters and bacterial communities

Bio-crusts and litter crusts improved sand surface microhabitats, including soil properties and hydrological processes, and caused the development of soil fertility (Ferrenberg et al., 2018; Liu et al., 2019b). Our study showed that most sand nutrients, enzyme activities, and the diversity of soil bacteria communities increased markedly during the development of bio-crusts and litter crusts. This finding indicated that these crusts have a positive effect on sandy ecosystem restoration.

In this study, litter crusts enhanced most sand nutrients and enzyme activities compared with bio-crusts. This result is consistent with previous study that litter crusts significantly increase soil organic matter than those in bio-crusts (Liu et al., 2019b). This condition can be apparently and partially due to many substrates for decomposition provided from the litter crusts, thereby elucidating the improvement of soil quality in sandy litter crusts. Soil quality determined the nature of vegetation series and the achievement of ecological restoration (Putten, W.H 2013). Previous

studies indicated that the diversity of bacteria typically increases with ecosystem restoration. The richness and diversity of bacterial communities in the litter crusts and bio-crusts were significantly higher than in the sandy land. This condition is attributed to the opportunities for the interactions of different species among themselves are provided by the improved soil quality (Liu et al., 2019a). These crusts can provide favorable environment for vegetation species formation via improving soil surface microhabitats of sandy land in the wind-water erosion crisscross region. Moreover, most soil nutrients contents and enzyme activities were greatest in topsoil, as shown in previous results (Liu et al., 2018). These differences in soil properties may have an influence on the soil microorganisms. The diversity of bacteria was higher in the deep layer (5-10 cm) than in the other layers in sandy land. This finding is inconsistent with studies that the diversity of bacterial communities commonly decreases with increasing soil depth (Jiao et al., 2018a). This result may be due to the specific environmental conditions of sandy land, including the high air temperature, low soil humidity, and abundant solar radiation in surface soil (Liu et al., 2018).

4.2 Influence of litter crusts and bio-crusts on co-occurrence networks

Many studies have reported that the co-occurrence patterns of complex ecological interactions that form bacterial communities can demonstrate the interactions of soil variables and bacterial taxa. These patterns are generally used to evaluate the information on community interactions in natural habitats. In our co-occurrence networks, the interactions of soil variables and bacterial genera were complicated in the litter crusts network. This result may be related to the accumulation of soil nutrients and enzyme activities that contributed to the bacterial community activity. The bio-crusts and litter crusts networks were significantly different at the genus level, which may be due to their heterogeneity in response to the soil properties and different habitats.

Soil microbes may be related to the soil properties and among themselves through various

294 mechanisms (Ma et al., 2016). In this study, we found that the bacterial networks were distinct
295 among the three sample groups. Bacterial genera had more connections with each other in the litter
296 crusts network (num. edges = 1787) than in the bio-crusts network (num. edges = 975). More
297 positive correlations identified as co-occurrences were found in the litter crusts (99.89%) than in the
298 bio-crusts (94.26%). Dominant positive correlations illustrate that most bacterial genera may share
299 similar ecological niches or synergistically operate in the litter crusts environment, which is
300 consistent with other microbial networks (Aschenbrenner et al., 2017; Zhang et al., 2018). The
301 bacterial taxa enriched in the litter crusts might benefit from sufficient soil nutrients, thereby
302 enabling them to take up the leading ecological niches in the interaction network. In litter crusts
303 network, the most important genera, including *Romboutsia*, *Paeniclostridium*, and *Mogibacterium*
304 belong to the phyla Firmicutes. The lowest relative abundance of these genera and the most
305 important roles were observed. Our study showed that the relative abundance of bacterial taxa is not
306 directly related to ecosystem function, consistent with sulfate reducer *Desulfosporosinus* with the
307 low (0.006%) abundance managed the majority of soil SO4 reduction (Pester et al., 2010). It is
308 known that members of the Firmicutes have ability to degrade cellulose in the litter. But, the highest
309 relative abundance of the Firmicutes were observed in the sandy land probably because the much
310 phenotypic variation of its members enables these organisms to live in various environments
311 (Lawson et al., 1993), and many members with spore-forming ability are able to endure harsh
312 environmental conditions (Zhuang et al., 2010). However, negative correlations (5.74%), which
313 show co-exclusion between the two bacterial genera, were rarer than positive ones in the bio-crusts
314 network. The number of negative links was higher than in the litter crusts network, probablydue to a
315 more competitive connection between bacterial genera in the bio-crusts. More negative correlations
316 were found between Gaiella and other genera, for instance, Gaiella and Hydrocarboniphaga,

317 Microvirga, or Belnapia. Gemmatimonas, Hydrocarboniphaga, Microvirga, and Belnapia were the
318 important genera in the bio-crust network. Compared with the sandy land, the highest relative
319 abundance of the Gaiella and lower abundance of the Hydrocarboniphaga, Microvirga, or Belnapia
320 were found in the bio-crusts. Gaiella is chemoorganotrophic and had the ability to utilize organic
321 acids, amino acids, and some sugars as single carbon sources but not utilize hydrocarbons as carbon
322 (Albuquerque et al., 2018). Hydrocarboniphaga, Microvirga, or Belnapia belong to the
323 Proteobacteria, these members were facultative and aerobic bacteria and can utilize various organic
324 substrates (Slezak et al., 2017). Most organisms contain or produce small amounts of hydrocarbon,
325 such as, the fermentation of many bacteria in the soil and the decomposition of plants.
326 Hydrocarboniphaga active in hydrocarbon degradation (Palleroni et al., 2004) and Microvirga can
327 degrade some hydrocarbon (i.e. Tween 20, D-sorbitol, adonitol, and alphaiso-leucine) as carbon and
328 nitrogen sources (Veyisoglu et al., 2016). This observation may be because the accumulation of
329 hydrocarbons has an opposite effect on the growth of Gaiella and Hydrocarboniphaga or Gaiella
330 and Microvirga in bio-crusts. These results may indicate the preferences of specific bacteria for soil
331 crust types and substrates.

332 **4.3 Bacterial community predictors of sandy nutrient cycling**

333 Plant and microbial diversity drive terrestrial ecosystem multifunctionality
334 (Delgado-Baquerizo et al., 2016). Recent research provides evidence that microbial communities
335 play pivotal roles in driving soil nutrient cycling in reforested ecosystems (Jiao et al., 2018a). Our
336 results showed that the members of the predictors varied with the crust types in sandy land.
337 Nitrospirae was the important and unique predictor to the nutrient cycling in the bio-crusts likely
338 due to its diverse metabolism. Most of its genera are aerobic chemolithotrophs, including nitrifiers,
339 dissimilatory sulfate reducers, and magnetotactic forms (Garrity and Holt, 2001). Nitrospirae is an

extensive nitrite-oxidizing bacterial taxa and plays a major role in the soil nitrogen cycle. In our study, Nitrospirae showed the highest relative abundance in the bio-crusts, and had a sight higher content of TN and UA compared with in sandy land, which participate in nitrogen cycle. This result showed that the nitrogen-cycling bacterial group was crucial in the development of the bio-crusts, and the soil conditions in the bio-crusts were enhanced with long-term crust restoration in the sandy land. Actinobacteria and Chloroflexi are the important and unique predictors to the nutrient cycling in the litter crusts. This condition is probably because Actinobacteria, as an excellent indicator of soil biological activity, metabolize cellulose, lignin, and other complex polymers, mediate the decomposition of organic matter in ecosystems, and influence the nutrient cycling in the soil (Kirby, 2005). The relative abundance of Actinobacteria accounted for the high proportion across the three groups with their capacity to colonize bare soil (Suela Silva et al., 2013). However, their decrease with the increase in soil nutrients agrees with the study that Actinobacteridae are more abundant in patches without vegetation than in shrubs (Hortal et al., 2013). Actinobacteria play a beneficial role in the soil by providing protection against abiotic stresses and enhancing plant nutrition acquisition (Shi et al., 2019).Chloroflexi can offer energy through photosynthesis, degradation of plant-derived compounds, and organic matter decomposition (Wang et al., 2018). Previous study showed that it was negatively correlated with TN and organic carbon in the litter crusts (Lozano et al., 2014) and the relative abundance of Chloroflexi decreased with the development of soil (Brown and Jumpponen, 2014). These findings are consistent with our study that showed the lowest relative abundance of Chloroflexi and the highest content of TN and organic matter, and its relative abundance is indirectly correlated with soil nutrients and enzyme activities. Actinobacteria and Chloroflexi are pivotal in predicting the cycling of sand nutrients under the litter crusts. Our experimental results reveal the distinct contributions of bacterial taxa to soil functions

(multi-nutrient cycling) in bio-crusts and litter crusts in sandy lands.

Increasing attentions in manipulating host-microbiome interactions by adding bacteria in a range of systems should focus on a fine scale to analyze the relationships between the microbial populations and soil functioning under natural conditions. However, most studies have focused on the microbial indicator at the phylum level or class level of bacteria. In our study, we aimed to determine the predictors of nutrient cycling at the genus level. Our results indicated that approximately 31% of predictors (10) were the same between the bio-crusts and litter crusts at genus level, and they were more connected with other bacterial genera in the co-occurrence networks. Hence, they may affect the soil ecosystem functioning by contributing to nutrient cycling in the crust types of ecosystem restoration. The unique bacterial predictors' groups in the bio-crusts or litter crusts with these bacterial consortiums can play important roles in nutrient cycling in different habitats. This finding is consistent with the study of distinct microbial communities that can exhibit distinct responses in different habitats (Wagner et al., 2016). The number of bacterial predictors were more in the crusts than in the sandy land. This result suggested the importance of soil bacterial communities in impacting ecosystem functioning (multiple nutrient cycling) during the development of the bio-crusts and litter crusts in sandy land.Our results showed particular bacterial consortium play important roles in predicting soil nutrient cycling in sandy ecosystem restoration. In a microbial ecosystem, the identification of the key microbial populations is often associated with the occurrence and abundance of species in local habitat (Mei et al., 2016). Therefore, the important drivers at the genus level with litter crusts and bio-crusts contribute to the applications of the key microbial driver in ecosystem restoration. These observations indicate bacteria participate in the biogeochemical cycling of multi-nutrients in the litter crusts and bio-crusts and the importance of investigating distinct responses contributed to sand nutrient cycling

386 in the sandy ecosystem restoration. These studies enrich our knowledge on crusts and bacterial
387 communities in restoring sandy land.

388 **5. Conclusions**

389 Soil bacterial communities represent important variables for predicting nutrients cycling of
390 restoration trajectories, thereby affecting belowground ecological restoration. In this study, the
391 bacterial communities showed increased diversity and varied composition and structure in the crust
392 types compared with the sandy land. The diversity and OUT richness were positively correlated
393 with soil nutrients (except TP) in surface soil (0-2 cm and 2-5 cm). Litter crusts network had closer
394 relationships between the soil bacterial taxa and soil nutrients and more positive correlations among
395 themselves than in the bio-crusts network. The bacterial drivers play the most important roles in
396 mediating sand nutrient cycling in the crust types of ecosystem restoration. These findings increase
397 our understanding of the complex interactions between bacterial communities and crust types
398 during the ecosystem recovery. The distinct response strategies of particular bacterial groups at the
399 genus level can be important for the comprehensive understanding of the belowground microcosms
400 with litter crusts and bio-crusts in the surface sand. Our study provides a new perspective that the
401 exploration of the specific functions of particular bacterial consortiums in nutrient cycling is crucial
402 to their applications in pivotal ecosystem functioning. Future work should be conducted to isolate
403 the most important drivers of the bacterial taxa. Bacterial inoculants may promote soil bacterial
404 functioning in nutrient cycling and may be potentially implemented as an approach for increasing
405 soil fertility in degraded lands or agricultural lands.

406

407 **Supporting Information**

408 Appendix A: Table S1, S2, S3, S4, S5, S6, S7 and S8

Declaration of Competing Interest

The authors declare no competing financial interests.

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Authors' contributions

G.L.W conceived the idea and designed the study; X.L., Y.L. and L.Z. performed the experiment and collected the data; X.L. analyzed the data; X.L. and G.L.W led the writing of the manuscript with the help of R.Y. All authors contributed critically to the draft and gave final approval for publication.

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646 **Figure legends**

647

648 **Figure 1**. Variation in soil properties and enzyme activities (n=9) among three soil groups (sandy
649 land, biocrusts, and litter crusts) and three soil layers (0-2 cm, 2-5 cm, and 5-10 cm). Different
650 lowercase letters indicate significant difference among three soil group in the same soil layer ($p < 0.05$), and different uppercase letters indicate significant difference among three soil layers in the
652 same soil group ($p < 0.05$). Error bars indicate standard deviation.

653

654 **Figure 2**. Variation in the microbial communities among three sand groups (sandy land, biocrusts,
655 and litter crusts). (A) Relative abundances of the microbial taxa annotated (>1% of total community)
656 at the phylum level; Difference in OTU richness (B), Chao1 index (C), Shannon index (D) of the
657 microbial community (n=9) among three soil groups (sandy land, biocrusts, and litter crusts) and
658 three soil layers (0-2 cm, 2-5 cm, and 5-10 cm). Difference in OTU richness (E), Chao1 index (F),
659 Shannon index (H) of the microbial community (n=27) among three soil groups. Color of blue, red,
660 green represent sandy land, biocrusts, and litter crusts, respectively. Different lowercase letters
661 indicate significant difference among three soil group in the same soil layer ($p < 0.05$), and different
662 uppercase letters indicate significant difference among three soil layers in the same soil group ($p < 0.05$). Error bars indicate standard deviation.

664 **Figure 3.** Redundancy analysis (RDA) for identifying the influence of soil nutrients and enzyme
665 activities on bacterial community composition at the phylum level (A) or the genus level (B) .
666 Dashed ellipses represent nine treatments; Arrows represent the soil variables associated with
667 bacterial community composition.

668

669 **Figure 4**.

670 Co-occurrence networks of soil bacterial communities in the sandy land, biocrusts, and litter crusts.
671 The color of nodes represent bacterial genera and soil variables (red nodes represent soil variables,
672 the other color nodes represent bacterial genera). (A) The correlations between the soil variables
673 and bacterial taxa. (B) The correlations among the bacterial taxa themselves. Red edges represent
674 positive correlation, blue edges represent the negative correlation.

675 SOM, sand organic matter; TK, total potassium; AK, available potassium; TP, total phosphorus; AP, available
676 phosphorus; TN, total nitrogen; UA, urease activity; GB, β-glucosidase activity; DHA, dehydrogenase activity;
677 ALP, alkaline phosphatase activity; ACP, acid phosphatase activity.

678

679 **Figure 5.** Random forest (RF) shows all annotated microbial drivers at the phyla level for sand
680 nutrient cycling in sandy land, biocrusts, and litter crusts, respectively. MSE is the mean square
681 error. MSE% values represent the importance of these predictors. Higher MSE% values mean more
682 important predictors. The significance of the model was estimated by the R package "A3". *, $P <$
683 0.05; **, $P < 0.01$.

684

685 **Figure 6**. Random forest (RF) shows all potential drivers (MSE% values > 5%) of the phyla
686 Actinobacteria, Planctomycetes, Chloroflexi, Armatimonadetes, Deinococcus-Thermus, Nitrospirae
687 at the genus level for sand nutrient cycling in the sandy land, biocrusts and litter crusts, respectively.
688 Color of orange, red and green represent the sandy land, biocrusts and litter crusts, respectively.
689 MSE is the mean square error. MSE% values represent the importance of these predictors. Higher
690 MSE% values mean more important predictors. The significance of the model was estimated by the
691 R package "A3". *, $P < 0.05$; **, $P < 0.01$.

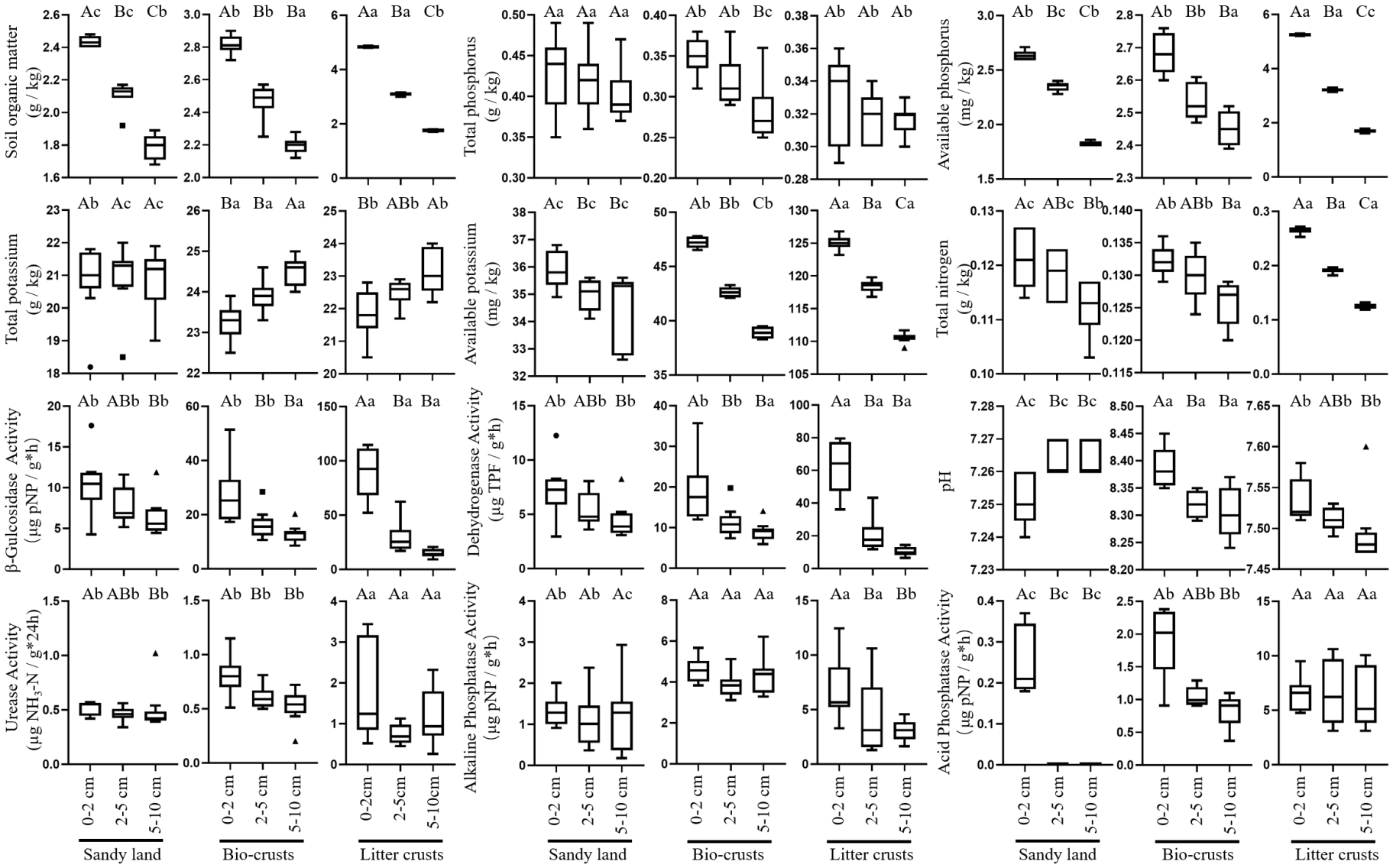
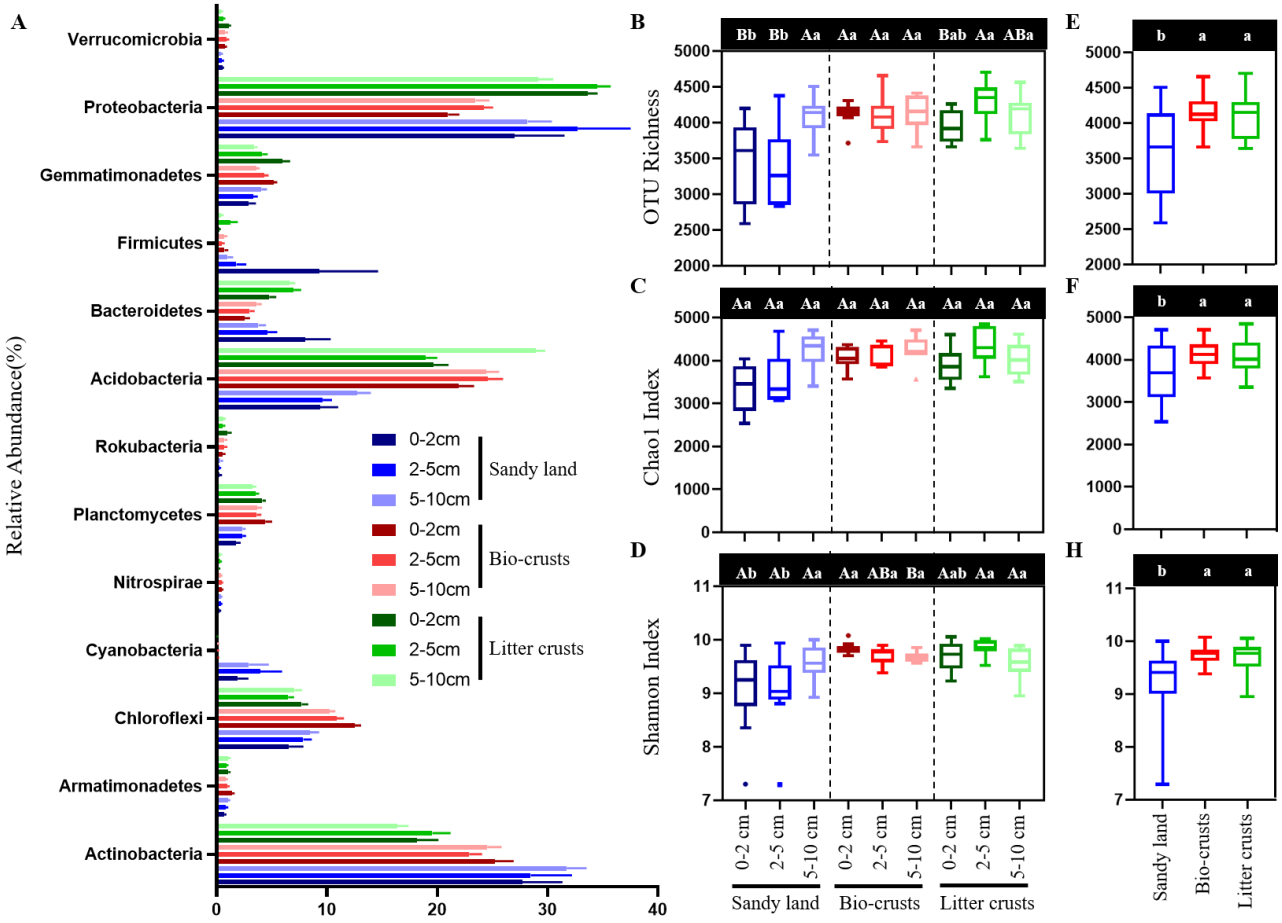
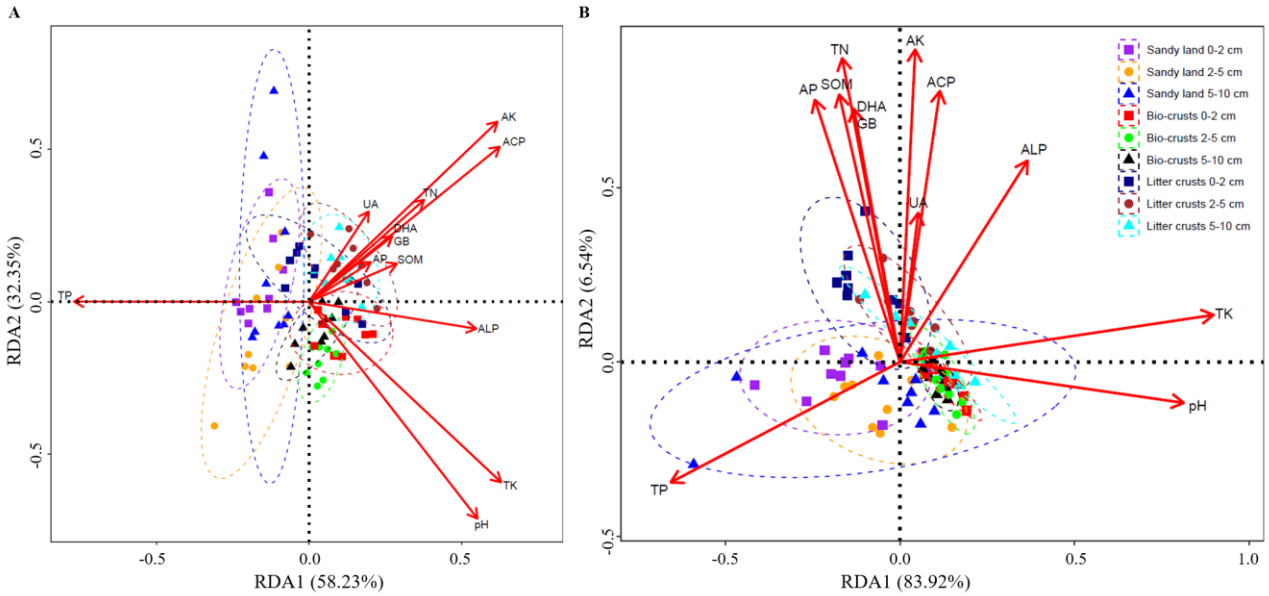

Soil organic matter (g / kg)
Total phosphorus (g / kg)
Available phosphorus (mg / kg)
Total potassium (g / kg)
Available potassium (mg / kg)
Total nitrogen (g / kg)
β-Gulcosidase Activity (μg pNP / g*h)
Dehydrogenase Activity (μg TPF / g*h)
pH
Urease Activity (μg NH3-N / g*24h)
Alkaline Phosphatase Activity (μg pNP / g*h)
Acid Phosphatase Activity (μg pNP / g*h)
0-2 cm
2-5 cm
5-10 cm
0-2cm
2-5cm
5-10cm
Sandy land
Bio-crusts
Litter crusts

Figure 1



694

695 Figure 2



696

697 Figure 3

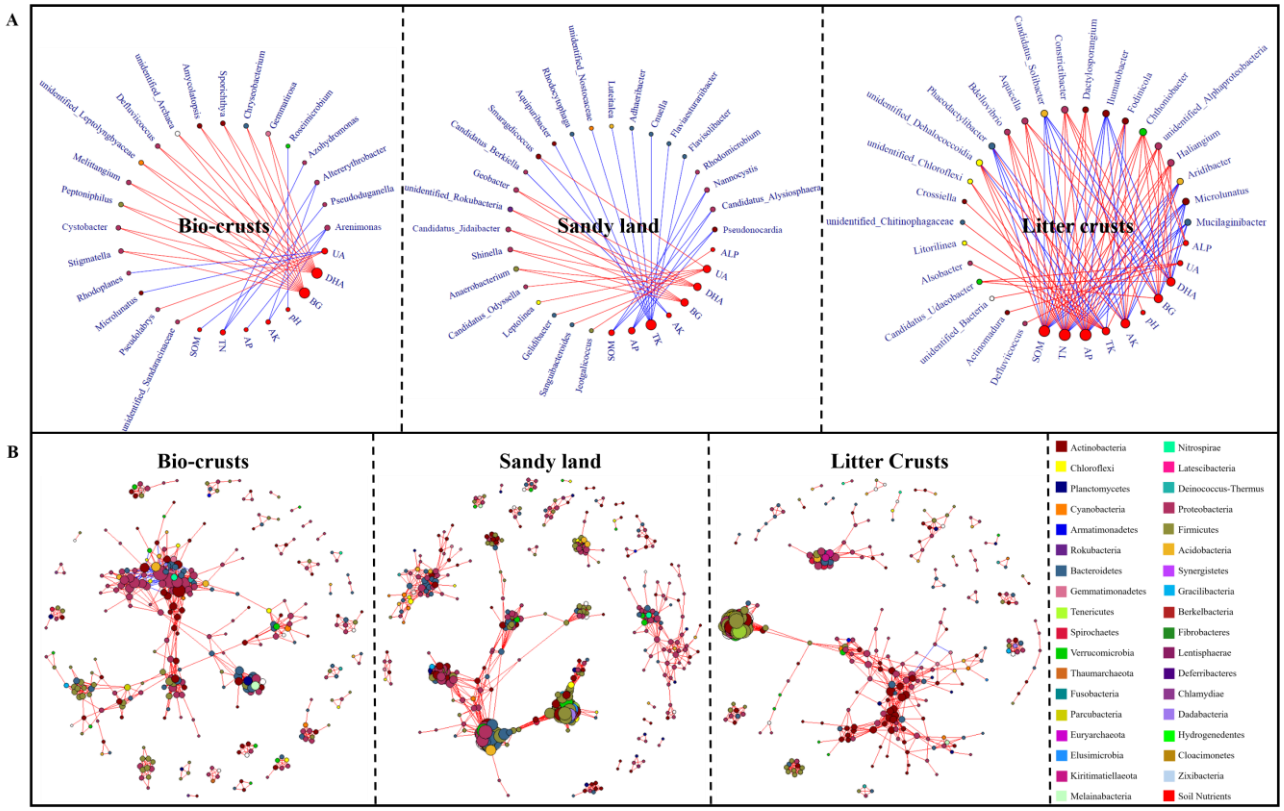

A
Bio-crusts
Sandy land
Litter crusts
B
Bio-crusts
Sandy land
Litter Crusts
Actinobacteria
Chloroflexi
Planctomycetes
Cyanobacteria
Armatimonadetes
Rokubacteria
Bacteroidetes
Gemmatimonadetes
Tenericutes
Spirochaetes
Verrucomicrobia
Thaumarchaeota
Fusobacteria
Parcubacteria
Euryarchaeota
Elusimicrobia
Kiritimatiellaeota
Melainabacteria
Nitrospirae
Latescibacteria
Deinococcus-Thermus
Proteobacteria
Firmicutes
Acidobacteria
Synergistetes
Gracilibacteria
Berkelbacteria
Fibrobacteres
Lentisphaerae
Deferribacteres
Chlamydiae
Dadabacteria
Hydrogenedentes
Cloacimonetes
Zixibacteria
Soil Nutrients

Figure 4

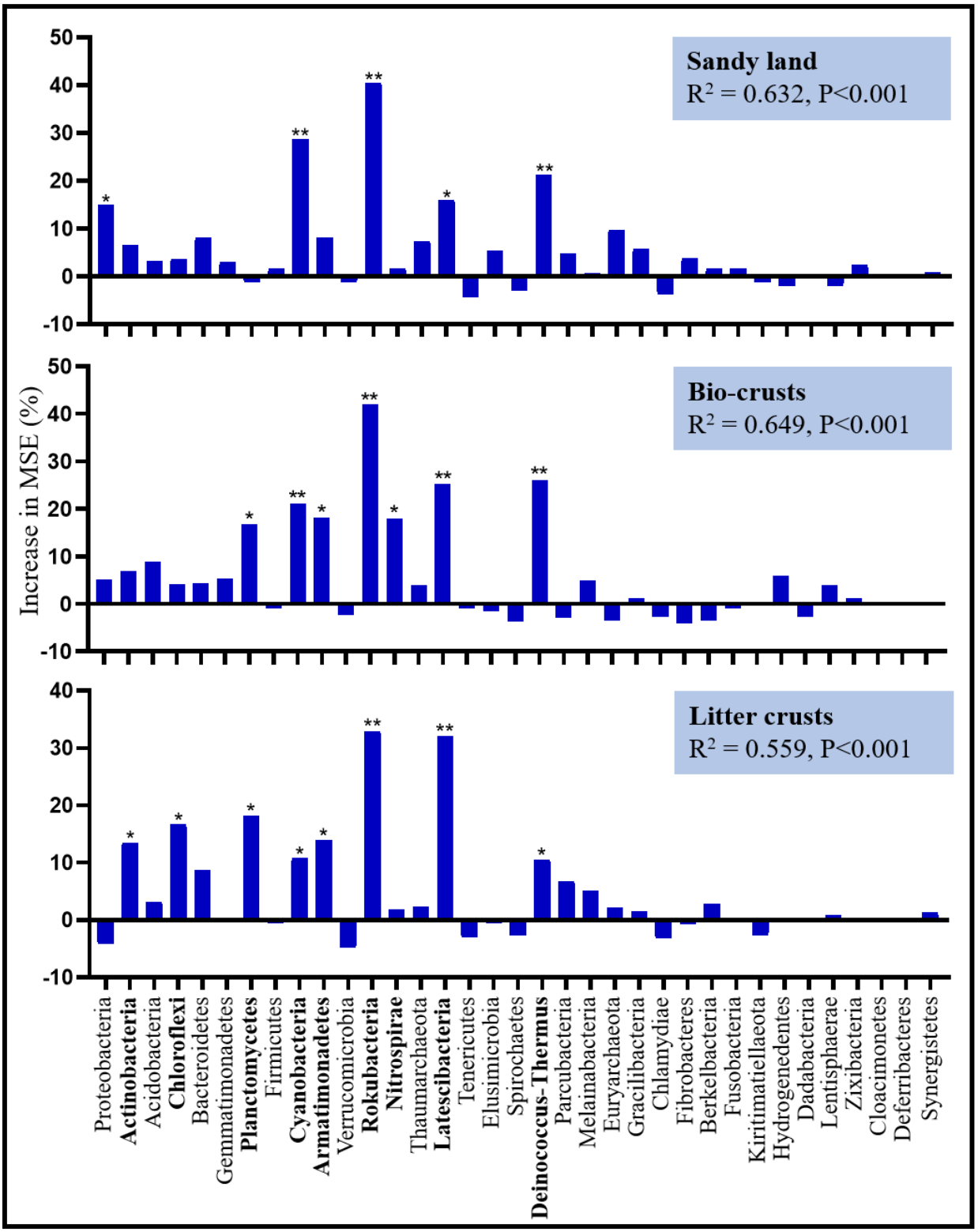

Sandy land
R² = 0.632, P<0.001
Bio-crusts
R² = 0.649, P<0.001
Litter crusts
R² = 0.559, P<0.001
Increase in MSE (%)
Proteobacteria
Actinobacteria
Acidobacteria
Chloroflexi
Bacteroidetes
Gemmatimonadetes
Planctomycetes
Firmicutes
Cyanobacteria
Armatimonadetes
Verrucomicrobia
Rokubacteria
Nitrospirae
Thaumarchaeota
Latescibacteria
Tenericutes
Elusimicrobia
Spirochaetes
Deinococcus-Thermus
Parcubacteria
Melainabacteria
Euryarchaeota
Gracilibacteria
Chlamydiae
Fibrobacteres
Berkelbacteria
Fusobacteria
Kiritimatiellaeota
Hydrogenedentes
Dadabacteria
Lentisphaerae
Zixibacteria
Cloacimonetes
Deferribacteres
Synergistetes

Figure 5

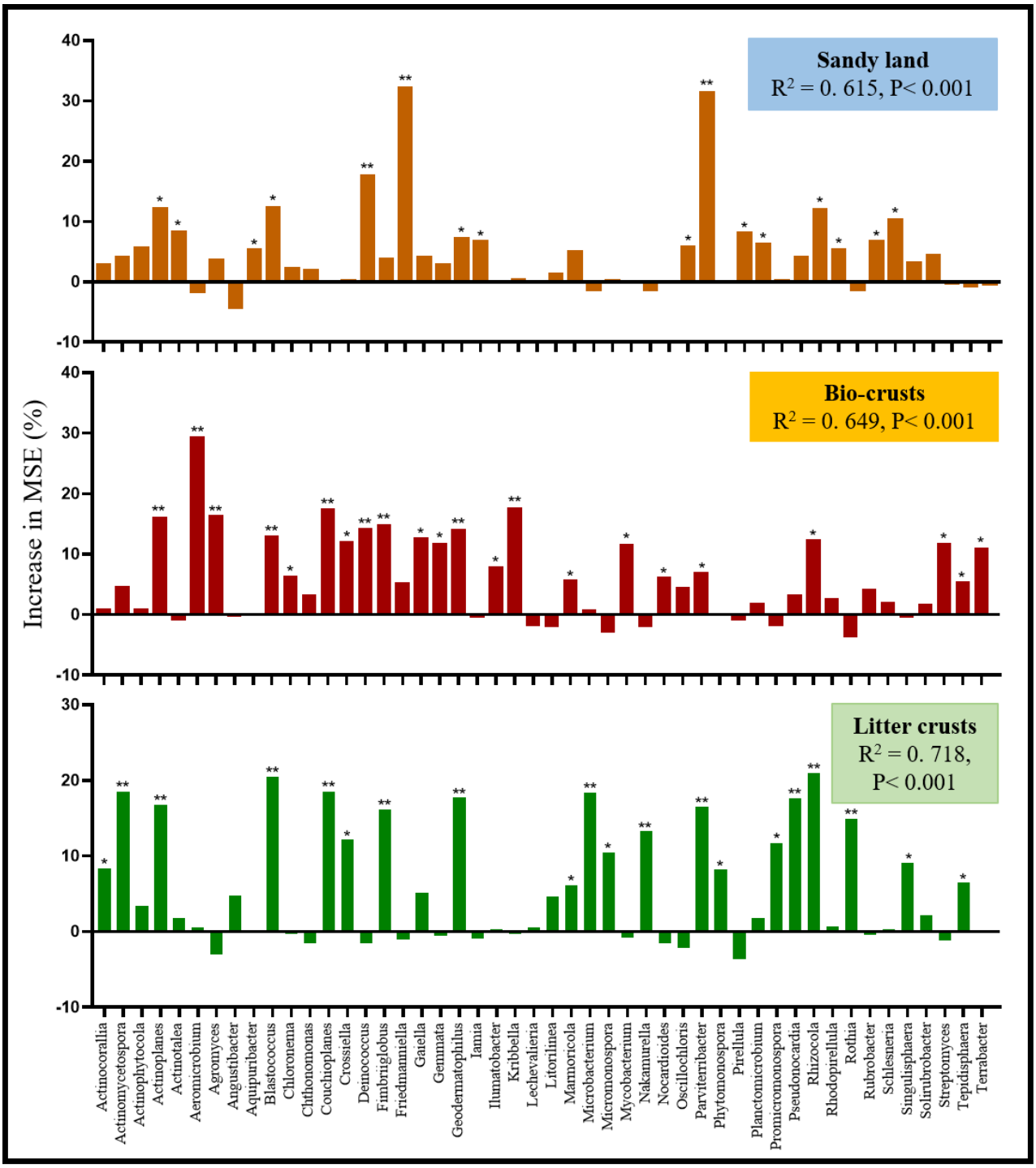

Sandy land
R² = 0. 615, P< 0.001
Bio-crusts
R² = 0. 649, P< 0.001
Litter crusts
R² = 0. 718,
P< 0.001
Increase in MSE (%)
40
30
20
10
0
-10
Actinocorallia
Actinomycetospora
Actinophytocola
Actinoplanes
Actinotalea
Aeromicrobium
Agromyces
Angustibacter
Aquipuribacter
Blastococcus
Chloronema
Chthonomonas
Couchioplanes
Crossiella
Demococcus
Fimbriglobus
Friedmanniella
Gaiella
Gemmata
Geodermatophilus
Iamia
Ilumatobacter
Kribbella
Lechevalieria
Litorilinea
Marmoricola
Microbacterium
Micromonospora
Mycobacterium
Nakamurella
Nocardioides
Oscillochloris
Parviterribacter
Phytomonospora
Pirellula
Planctomicrobium
Promicromonospora
Pseudonocardia
Rhizocola
Rhodopirellula
Rothia
Rubrobacter
Schlesneria
Singulisphaera
Solirubrobacter
Streptomyces
Tepidisphaera
Terrabacter

Figure 6