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The role of biocrusts in nitrogen cycling on the tropical reef islands, South China Sea

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Abstract

Harboring polyextremotolerant microbial topsoil communities, biological soil crusts (biocrusts) occur across various climatic zones, and have been well studied in the terrestrial drylands. However, little is known about the functional metabolic potential of microbial communities involved in the biogeochemical processes during the early succession of biocrusts on the tropical reef islands. We collected 26 biocrusts and bare soil samples from the Xisha Islands and Nansha Islands, and applied a functional gene array (GeoChip 5.0) to reveal nitrogen (N) cycling processes involved in these samples. Both physicochemical measurement and enzyme activity assay were utilized to characterize the soil properties. Results revealed the composition of N-cycling functional genes in biocrusts was distinct from that in bare soil. Additionally, microorganisms in biocrusts showed lower functional potential related to ammonification, denitrification, N assimilation, nitrification, N fixation, and dissimilatory nitrate reduction to ammonium compared to bare soils. Although the abundance of *nifH* gene was lower in biocrusts, nitrogenase activity was significantly higher compared to that in bare soils. Precipitation, soil physicochemical properties (i.e., soil available copper, soil ammonia N and pH) and soil biological properties (i.e., β-glucosidase, fluorescein diacetate hydrolase, alkaline protease, urease, alkaline phosphatase, catalase and chlorophyll *a*) correlated to the N-cycling functional genes structure. Nitrate N and ammonia N were more abundant in biocrusts than bare soil, while pH value was higher in bare soil. Our results suggested biocrusts play an important role in N-cycling in coral sand soil, and will be helpful in understanding the development and ecological functions of biocrusts on tropical reef islands.

Key words: biocrusts, microbial functional structure, metabolic potential, nitrogen cycling, tropical reef islands

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1 Introduction

Biological soil crusts (biocrusts) are polyextremotolerant microbial topsoil communities, consisting of algae, bacteria, archaea, fungi, lichen and mosses in varying proportions that colonize the soil surface and get embedded together within a matrix of extracellular polymeric substance and soil particles to form a surface crust (Belnap et al., 2001, 2016). Biocrusts show different colors, ranging from white through green to black hues, depending on the different successional stages, reflected by the different dominating photoautotrophic organisms, including cyanobacterium-, lichen-, and moss-dominated types (Bowker et al., 2006; Büdel et al., 2009; Weber et al., 2012). As multifunctional communities, biocrusts play important ecological functions in various ecosystems that promote soil formation by increasing its nutrient and water contents (Evans and Johansen, 1999; Belnap, 2006; Pointing and Belnap, 2012), stabilize the soil and reduce soil erosion effectively by producing polysaccharides (Van Den Ancker et al., 1985; Belnap et al., 2001). These functions have a positive influence on seed germination, establishment and performance of plants, and population and behavior of animals (Evans and Johansen, 1999; Belnap et al., 2001; Lan et al., 2015; Guan et al., 2018).

Nitrogen (N) is an essential macronutrient that is initially low in the soil and a vital limiting factor that influences productivity in arid terrestrial and rangeland ecosystems (Hooper and Johnson, 1999; Vitousek et al., 2010). Thus, N supply is one of the most important factors for further development of the initial ecosystem. Diverse N input processes (biological fixation and dust capture) and direct N loss processes (dissolution, vaporisation and erosion) have been found in the biocrusts developed in arid and semiarid ecosystems (Barger et al., 2016). Biological fixation of atmospheric N is an omnipresent biogeochemical transformation in the biological soil crusts. It possibly contributes to the dominant source of N in low-nutrient environments with few symbolic vascular plant N fixers (Evans and Ehleringer, 1993; Evans and Lange, 2001). Furthermore, N fixation by biocrusts leads to N re-

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lease to the surrounding soil in a variety of N forms (inorganic and organic N) and improves the soil nutrient pools, which serves as a food source for plants, animals and other organisms in arid and semiarid ecosystems (Walvoord et al, 2003; Elbert et al., 2012; Darby and Neher, 2016). Studying the functional gene structure regarding N cycling in biocrusts is important to our broader understanding of the role of biocrusts in N input and loss in the soil ecosystem.

Recently, numerous studies have focused on individual N processes (Barger et al., 2005; Johnson et al., 2005), N transfers (Baran et al., 2015), and N budget (Beraldi-Campesi et al., 2009; Brankatschk et al., 2013) in the biocrust ecosystem. Some of them used molecular tools (i.e., quantitative real-time PCR) to measure the abundance of N-cycling functional genes in biocrusts, and to evaluate the genetic potential of the microbial community catalyzing the related N-cycle processes, including *nifH* (N fixation), *chiA* (mineralization as proteolysis and chitinolysis), *amoA* (nitrification), and *nosZ* (denitrification) (Brankatschk et al., 2013; Couradeau et al., 2019). However, the metabolic potential related to global N-cycling has rarely been well evaluated in biocrusts (Liu et al., 2018).

Located in the southernmost part of China, the South China Sea (SCS) contains three archipelagos (i.e., Dongsha Islands, Nansha Islands (NS) and Xisha Islands (XS)), and the predominant structures are coral reefs (Li et al., 2013). Besides, the soil matrix on the island is dominated by coral sand. Due to geographic isolation, climate cycling and ecological amplitude, the tropical reef islands in the SCS are endowed with unique biodiversity (Sivaperuman et al., 2008). However, biocrusts on tropical reef islands have been ignored to date. Here, we aim to present the characterization of N-cycling functional gene compositions of biocrusts and bare soil across NS and XS, and investigate the role of biocrusts in the N retention and balance on tropical reef islands.

2 Materials and methods

2.1 *Sampling and storage*

Samples were collected from four different islands between May and June of 2017, two of them belonging to the NS, and the other two belonging to the XS. Affected by the tropical marine climate, the mean temperatures of the NS and XS were larger than 27°C and 26–27°C, respectively, and their mean annual precipitation were approximately 2 800 mm and 1 500 mm, respectively. The morphology of biocrusts in these islands revealed their characteristics as cyanobacteria-dominated crusts (the early succession of biocrusts). They were located in regions covering from large areas of coral sands without plants to those the interspaced between island plants, or even underneath shrubs. At each sampling site, biocrust samples (top 0–1 cm of biocrusts) and bare soil samples (top 0–1 cm of adjacent soil with no sign of biocrusts) were collected using sterile equipment, including spatulas, medicine spoons, and foil samplers. Among a total of 26 soil samples, 16 and 10 samples were collected from the NS and XS, respectively. Among NS samples, 8 and 8 were originated from biocrusts and bare soil, correspondingly, whereas 7 biocrust and 3 bare soil samples collected from XS.

Bare soil and biocrust samples for studying soil properties (including soil enzyme activities) were collected in sealed plastic bags and stored in the refrigerator (4°C) until further measurements (Miralles et al., 2012; Ghiloufi et al., 2019; Yang et al., 2018). Samples for measuring chlorophyll *a* (Chl *a*) content were collected in sterile sampling opaque bags (EPN-4590, TWIRL'EM, Canada) and stored in the dark in a –20°C freezer. Samples for DNA extraction were collected using sterile equipment, and stored in 15 mL conical centrifuge tubes that were preserved using the LifeGuard^{TM} soil preservation solution (MO BIO Laboratories, USA) according to the manufacturer's instructions; they were transported to the laboratory as soon as possible and stored at –80°C until subsequent processing in the laboratory. Samples for nitrogenase activity tests were collected using a sterile foil sampler (size: 20 cm^2×5 cm) and stored in sealed plastic bags at 4°C until subsequent measurements.

2.2 *Measurement of soil physicochemical and biological properties*

Physicochemical properties of the soil samples, including soil available boron, organic matter, organic carbon, soil available phosphorus, soil exchangeable calcium, soil available kalium, kalium, calcium, soil available zinc, soil available copper, soil available iron, soil available manganese, total water soluble salt, soil total phosphorus, soil available sulphur, soil total N, soil nitrite N, soil nitrate N, soil ammonium N, and soil ammonia N (a mixture of free ammonia and ammonium N), were measured according to the industry measurement standard of China, including agricultural trade standards, forestry industry standards, and national environmental protection standards (Table A1). Soil pH was determined by potentiometry in a 1:2.5 (soil:water, w:w) suspension (Acosta-Martínez et al., 2007) using a pH meter (pH 211, Hanna Instruments, Germany).

For Chl *a* content, 2.0 g samples (dried in a freeze dryer at –80°C) were extracted with 10 mL acetone (80%, v:v) at 4°C for 20 h (Marker and Jinks, 1982; Wellburn, 1994). Chl *a* content of the filtered solution was measured using spectrophotometric methods at 665 nm and 750 nm. Subsequently, 1 mol/L hydrochloric acid was added to the solution for acidification, followed by a final calculation to obtain results with high accuracy (Lorenzen, 1967; Mush, 1980).

Enzyme assays were performed within a month of sample collection. The soil samples were used to analyze enzyme activities involved in N cycling, carbon cycling, phosphorus cycling and peroxide degradation system. Specifically, the activities of soil β-glucosidase, soil lipase, soil fluorescein diacetate hydrolase, soil alkaline protease, soil urease, soil alkaline phosphatase and soil catalase were tested using soil enzyme assay kits (Solarbio LIFE SCIENCE, Beijing Solarbio Science & Technology Co., Ltd., China) following a spectrophotometric method.

2.3 *Measurement of acetylene reduction activity*

Nitrogenase activity, used to represent the rates of N_2 fixation, was estimated as acetylene-ethylene reducing activity (Hardy et al., 1973) in all the collected soil samples. The samples, which had been reactivated by re-wetting, were placed in 100 mL conical flasks and sealed with a rubber stopper. Ten milliliters of air was withdrawn from the flask while adding an equal volume of acetylene and then incubated under laboratory conditions (temperature: 31°C, daylight: 16 h). The amount of ethylene in the headspace samples was determined by gas chromatography (Shanghaifenyi, China) equipped with a flame ionization detector. A standard mixture of 99.9×10^{-6} ethylene in N_2 was used for calibration purposes. Nitrogenase activity was expressed as microgramme level of C_2H_4 per square meter of soil per hour (Belnap, 1996, 2002).

2.4 *DNA extraction and GeoChip analysis*

Total DNA was extracted using the HiPure soil DNA kit

(Magen, China) according to the manufacturer's instructions. Concentration and purity of genomic DNA were determined using a NanoVuePlus spectrophotometer (GE Healthcare, USA). The purified DNA was labeled with the Cy3 dye (GE Healthcare, USA) by random priming (Wang et al., 2014), and then purified by a QIA quick purification kit (Qiagen, USA), and dried in a labconco centrivap concentrator (Labconco, USA) at 50°C for 45 min. The dried and labeled DNA was diluted to the same concentration, incubated at 95°C for 5 min, and maintained at 42°C until hybridization. The labeled DNA was hybridized with GeoChip 5.0 (Zhou et al., 2015) in a hybridization station (MAUI, BioMicro Systems, USA), and scanned by a NimbleGen MS200 scanner (Roche Madison, USA) to obtain the optical signals. The scanned images of the hybridized GeoChip 5.0 were converted and extracted using the Agilent Feature Extraction 11.5 software (Agilent Technologies, USA). Raw data from the Agilent Feature Extraction were submitted to the laboratory's Microarray Data Manager System (http://ieg.ou.edu/microarray/) and analyzed by the following major steps as described previously (He et al., 2010; Yue et al., 2015): (1) spots with a signal to noise ratio less than 2 were removed; (2) if the signal was detected in at least 2/3 of the repeat groups, the probes were considered positive; (3) the normalization of data was done through logarithmic transformation, the average signal intensity of each sample was determined, and intensity of each probe was proportionally enlarged by the mean intensity of the corresponding sample.

2.5 *Statistical analyses*

N-cycling functional gene diversity was estimated using gene number, Shannon index, and Simpson index. Statistical differences between the alpha diversity-related values and functional gene intensities from the different types of soil samples were analyzed by *t*-test (SPSS 18). A significance level of *P*-value less than 0.05 was adopted for all comparisons (He and Wang, 2011). Additionally, permutational multivariate analysis of variance was used to verify the pairwise statistical significance between biocrust and bare soil with vegan package in R. We graphically depicted the multivariate relationships of N-cycling functional gene compositions of different samples based on Bray-Curtis (Steinhaus) distance using non-metric multidimensional scaling (NMDS, vegan package in R) (Kruskal, 1964). The function envfit in vegan package was used to fit environmental vectors onto the ordination.

3 Results

3.1 *Physicochemical and biological soil properties*

To extract the soil properties indicative of the biocrust formation process at the functional gene level, we analyzed 21 physicochemical and 8 biological (soil enzyme and Chl *a* content) soil properties (Tables A2 and A3). Besides, *t*-test was conducted to compare the value of soil pH, total N, ammonia N (a mixture of free ammonia and ammonium N), nitrate N, ammonium N, and nitrite N content (Fig. 1). Compared to that in biocrust (pH: 8.85 ± 0.34), soil pH was significantly higher in bare soil (9.64 ± 0.07), but soil ammonia N and soil nitrate N were significantly lower in bare soil (Fig. 1). Meanwhile, there was no significant change in soil ammonium N and nitrite N contents between bare soil and biocrust samples (Fig. 1). Among soil biological properties, except for soil lipase and soil alkaline protease, the rest showed higher levels of enzymatic activities and biomass in biocrust samples (Tables A2 and A3).

In the present study, compared to that in bare soil ((0.023 ± 0.019) μmol/(m^2·h), we observed significantly higher nitrogenase activity (measured by acetylene reduction assay) in biocrust ((2.390 ± 1.280) μmol/(m^2·h)) (Fig. 2). However, there was no significant difference in nitrogenase activity between NS-Biocrust and XS-Biocrust (Fig. 2).

3.2 *Abundance and diversity of N-cycling genes*

The number of detected genes, Shannon index and Simpson index were measured to understand the composition of N-cycling functional genes on the tropical reef islands (Fig. 3). A total of 37 416 N-cycling functional genes were detected by GeoChip 5.0, and the number of detected N-cycling functional genes ranged from 826 to 2 758 across the samples. A *t*-test analysis showed that the number of detected N-cycling functional genes, and Shannon and Simpson indexes in biocrust were significantly

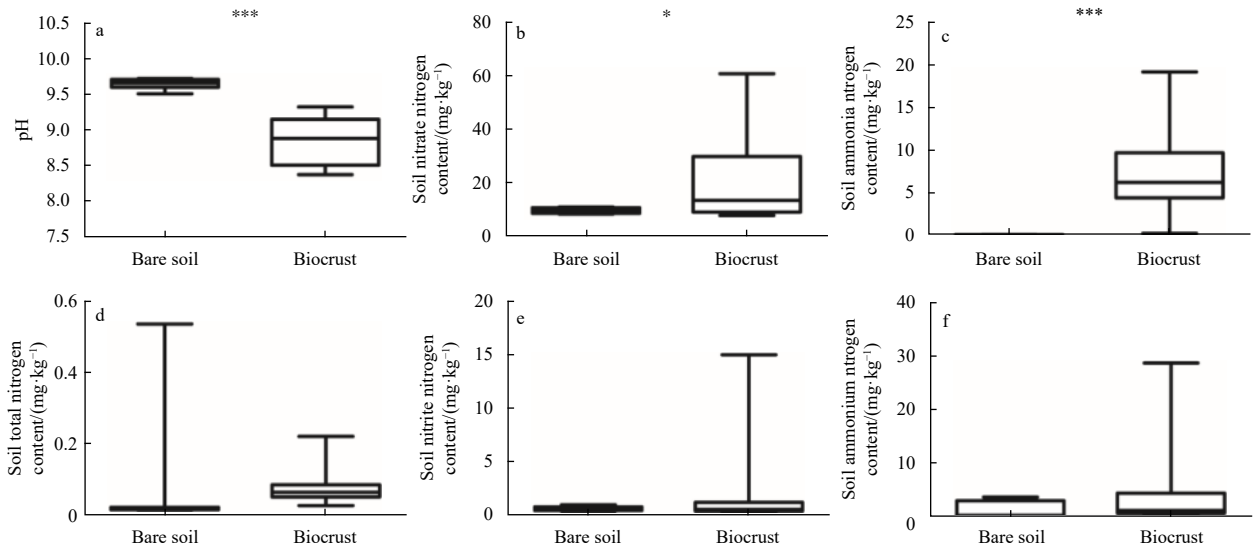

Fig. 1. Alterations of physicochemical soil properties in the biocrusts and bare soil samples are presented in the boxplot. Boxes limit the 25th and 75th percentile with the median presented as lines inside. Error bars present the 1st and 99th percentile. Significant differences: ***P<0.001, *P<0.05.

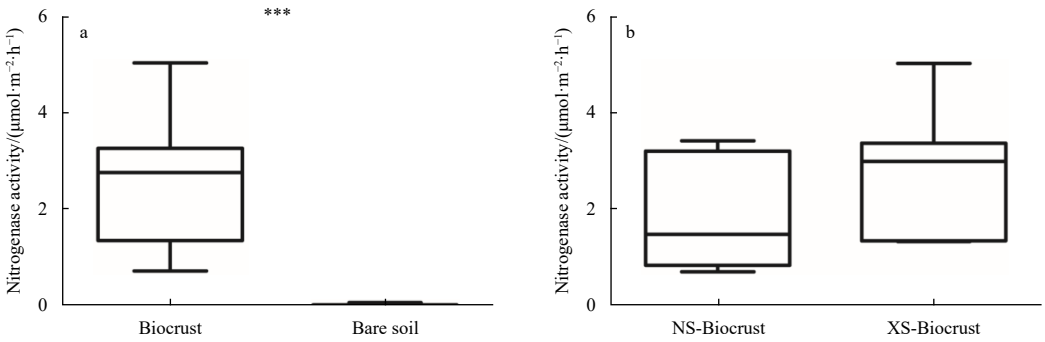

Fig. 2. Alterations of nitrogenase activity in the bare soil and biocrust of samples are presented in the boxplot. Boxes limit the 25th and 75th percentile with the median presented as lines inside. Error bars present the 1st and 99th percentile. Significant differences: ***P<0.001. Abbreviations: Nansha Islands (NS) and Xisha Islands (XS).

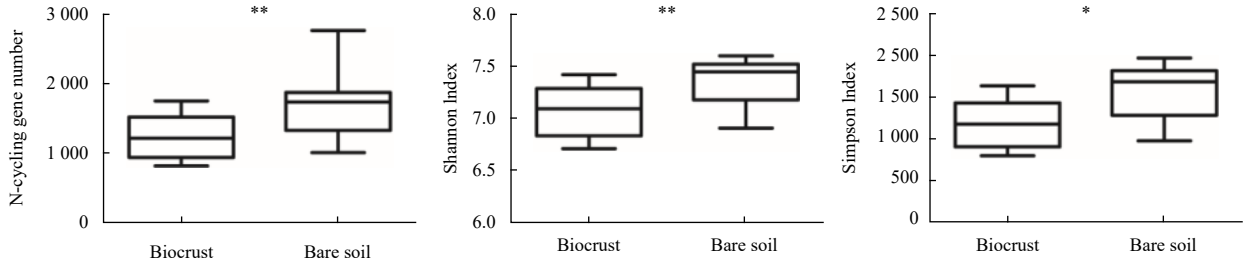

Fig. 3. Alterations of richness and diversity of microbial N-cycling functional genes in the biocrust and bare soil samples are presented in the boxplot. Boxes limit the 25th and 75th percentile with the median presented as lines inside. Error bars present the 1st and 99th percentile. Significant differences: **P<0.01, *P<0.05.

lower than in bare soil (Fig. 3). Higher proportions of common genes were observed in NS-Biocrust vs. XS-Biocrust (81.84%), NS-Bare soil vs. XS-Bare soil (78.52%), and 65.38%–72.88% of the genes were shared between biocrust and bare soil samples.

3.3 *Effects of biocrust formation on N cycle genes structure*

We detected 25 types of N cycle-related genes by GeoChip 5.0, and abundance of the key functional genes involved in all 9 processes, including ammonification, anammox, denitrification, assimilatory N reduction, dissimilatory N reduction, nitrification, N fixation, assimilation and N assimilation (assimilation and N assimilation belong to N-transfer process), was significantly lower in biocrust compared to that in bare soil (Fig. 4a), except for two genes, namely *nirA* and *p450nor* (PR), which were involved in assimilatory N reduction and denitrification, respectively. These results showed that, with biocrust formation, the metabolic potential of N-cycling in soil decreased on the tropical islands. Moreover, the N-cycling functional genes structure in biocrust and bare soil were distinct (F= 6.339, $P_{adjusted}$= 0.045, Table 1, Fig. 5).

The abundance of most N-cycle related functional genes was significantly lower in NS-Biocrust than in XS-Biocrust (Fig. 4b), except for *nirA* (assimilatory N reduction), *hao* (nitrification), PR, *norB* (denitrification), *hzo*, and *hzsA* (anammox). Further, we observed no difference in the N-cycling gene composition between NS and XS in both biocrusts and bare soil (Table 1, Fig. 5).

3.4 *Environmental factors related to N cycle genes in the biocrusts*

NMDS analysis revealed a total of one environmental factor and ten soil properties, including precipitation, soil pH, soil available copper, soil ammonia N, soil β-glucosidase, soil fluorescein diacetate hydrolase, soil alkaline protease, soil urease, soil alkaline phosphatase, soil catalase and Chl *a*, to be significantly correlated with the N-cycling gene compositions (Table 2); 7 properties ($P \leqslant 0.01$) were shown in Fig. 5.

4 Discussion

We have presented the first study on N-cycling functional gene structure of microbial communities on the tropical reef islands, SCS. Notably, in both biocrust and bare soil samples, the abundances of *nirK*, *nirS*, *narG*, and *nosZ* (denitrification), *ureC* (ammonification), *nifH* (N fixation) genes were the highest (Fig. 4b), which suggested the corresponding pathways were dominant in the N-cycling processes. Moreover, we observed N-cycling functional gene composition to be significantly altered as biocrust formation on the islands.

Specifically, the abundance of the most key functional genes involved in denitrification was significantly higher in bare soil than biocrust except for PR (Fig. 4a). Higher abundance of genes involved in denitrification in bare soil might lead to greater nitrate-reducing reactions. Moreover, higher abundance of *nirK*, *nirS*, and *nosZ* genes (Zumft, 1997; Ligi et al., 2014; Orellana et al., 2014) in bare soil might contribute to the higher N_2O and N_2 emission. A previous study demonstrated the alterations of N_2O emission to negatively impact soil nitrate N concentrations (Yang et al., 2014). In our present study, we found the nitrate N content was significantly lower in bare soil than in biocrust, which might be attributed to the higher abundance of denitrification-related genes in the former.

In the present study, we observed that ammonia N content, consisting of free ammonia and ammonium N, was significantly higher in biocrust than in bare soil. Ammonia N was the major

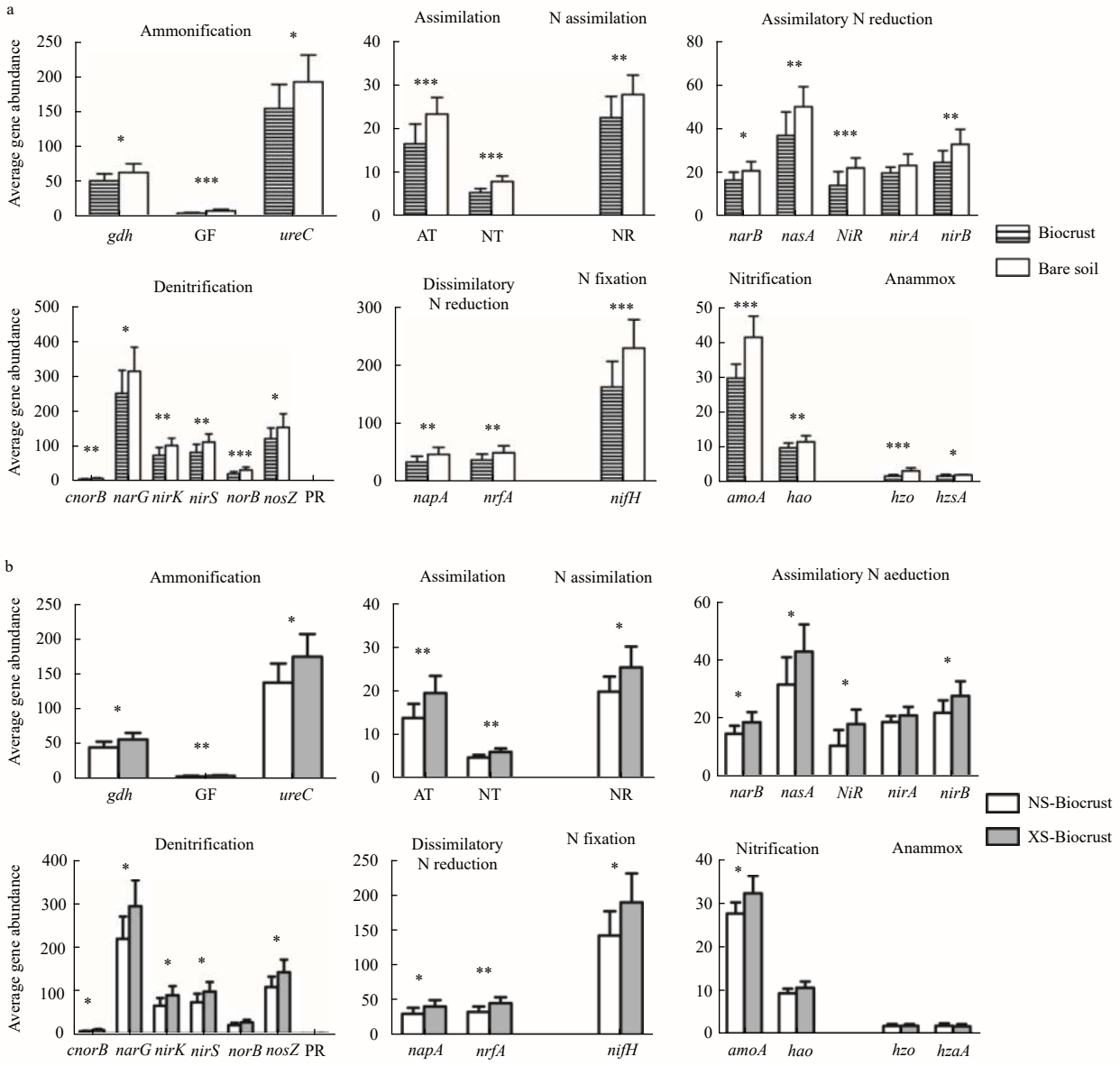

Fig. 4. Average gene abundance of N-cycling functional genes in the biocrust samples. All data are presented as Mean±SD calculated from biological repetition. a. Biocrust compared with bare soil; b. NS-Biocrust compared with XS-Biocrust. Abbreviations: *glnA_fungi* (GF), ammonium transporter (AT), nitrite transporter (NT), nitrate reductase (NR), *p450nor* (PR), Nansha Islands (NS) and Xisha Islands (XS). Significant differences: $^{**}P<0.01$, $^{*}P<0.05$.

Table 1. Permutational multivariate analysis of microbial N-cycling functional genes composition among different soil types

Type	*F*	R^2	*P*	$P_{adjusted}$
Biocrust vs. Bare soil	6.339	0.209	0.003	0.045
NS-Biocrust vs. XS-Biocrust	4.076	0.239	0.028	0.375
NS-Bare soil vs. XS-Bare soil	2.107	0.190	0.094	1.000

Note: Nansha Islands (NS) and Xisha Islands (XS).

product of biological N fixation and assimilation/dissimilation in N-cycling processes. As one of the most important functions in biocrusts, biological N fixation is the dominant source of N in the dryland ecosystem (Barger et al., 2016). Consistently, our result demonstrated that nitrogenase activity was significantly higher in biocrust compared with bare soil. Additionally, higher nitrate concentration (Fig. 1) might contribute to higher ammonia N content in biocrusts (Barger et al., 2016; Kuypers et al., 2018). No significant difference in the ammonium N content was observed between biocrusts and bare soil, which means the free ammonia content was likely significantly higher in biocrusts. Ammonium N has been shown to decrease pH by neutralizing alkaline soil, and elevate free ammonia content in biocrusts (Duan and Xiao, 2000; Barger et al., 2016), which was consistent with our result that pH value was lower in biocrust compared with bare soil (Fig. 1). In addition, a previous study reported that lower soil pH enhanced

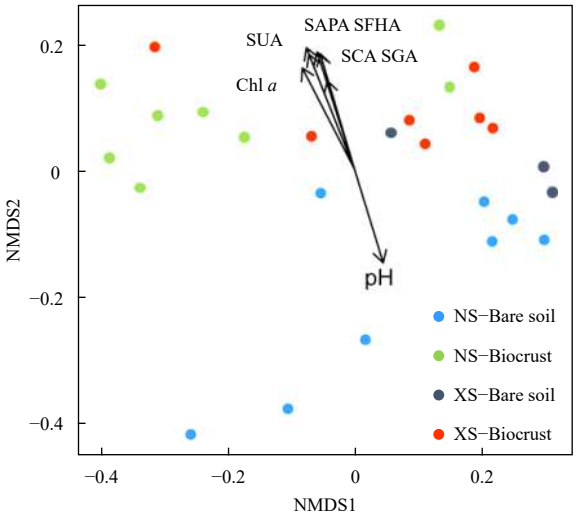

Fig. 5. Dissimilarity of microbial N-cycling functional genes compositions in biocrusts comparing to bare soil samples. Ordination using NMDS is derived from Bray-Curtis dissimilarity and applied to analyze the composition of microbial N-cycling functional genes. Circle shape represents soil samples from NS and XS. Different soil types are color coded. The function envfit from the R vegan package was used to fit environmental vectors onto the ordination (environmental factor significant correlation with NMDS, $P<0.01$). Abbreviations: non-metric multidimensional scaling (NMDS), Nansha Islands (NS), Xisha Islands (XS), soil β-glucosidase (SGA), soil fluorescein diacetate hydrolase (SFHA), soil urease (SUA), soil alkaline phosphatase (SAPA), soil catalase (SCA), and chlorophyll *a* (Chl *a*).

Table 2. Correlations between environmental factors and NMDS axes

	NMDS1	NMDS2	r^2	P (>r)
Pp	–0.532 91	–0.846 17	0.247 0	0.036
pH	**0.290 96**	**–0.956 74**	**0.401 9**	**0.002**
SAC	–0.434 65	0.900 60	0.298 5	0.014
SAN2	–0.011 68	0.999 93	0.272 7	0.032
SCA	**–0.366 42**	**0.930 45**	**0.636 8**	**0.001**
SFHA	**–0.282 77**	**0.959 19**	**0.605 7**	**0.001**
APA	–0.890 96	–0.454 08	0.267 1	0.028
SAPA	**–0.301 46**	**0.953 48**	**0.631 4**	**0.001**
SUA	**–0.365 61**	**0.930 77**	**0.711 0**	**0.001**
SGA	**–0.292 66**	**0.956 22**	**0.355 6**	**0.006**
Chl *a*	**–0.456 15**	**0.889 90**	**0.539 3**	**0.001**

Note: Factors with $P \leqslant 0.01$ are shown in bold type. Abbreviations: non-metric multidimensional scaling (NMDS), precipitation (Pp), soil available copper (SAC), soil ammonia N (SAN2), soil β-glucosidase (SGA), soil fluorescein diacetate hydrolase (SFHA), soil urease (SUA), soil alkaline phosphatase (SAPA), soil catalase (SCA), soil alkaline protease (APA), and chlorophyll *a* (Chl *a*).

the hydrolysis activity of urease, an enzyme converting urea into the ammonia N (Fisher et al., 2017). It agreed with our observation that soil urease activity was higher in biocrust compared to bare soil. Thus, the higher activity of urease in biocrusts probably leads to the accumulation of ammonia N content.

The *nifH* gene encodes a vital structural protein of the nitrogenase enzyme that catalyzes biological N fixation (Zehr and Paerl, 1998). In the present study, we observed nitrogenase activity and ammonia content were significantly higher compared with bare soil (Figs 1 and 2). In contrast, the abundance of *nifH* gene was significantly higher in bare soil (Fig. 4a). These observations are inconsistent with the previous study that N fixation rates were positively correlated with *nifH* gene abundances (Brankatschk et al., 2013). Although nitrogenase catalyzes biological N fixation, its magnitude of activity depends on the energy and availability of electron donors (Dixon and Kahn, 2004). Served as elemental sinks in different ecosystems, biocrusts uptake CO_2 through photosynthesis and provide more energy than bare soil (Belnap et al., 2001). Thus, they might contribute to a higher level of nitrogenase activity in biocrusts. Moreover, according to Warshan et al. (2016), higher N fixation activity should be explained by higher *nifH* gene expression rather than higher *nifH* gene abundance, since not all genes have metabolic activity or present "real" functionality. Further, the biological N fixation process might be regulated by multiple enzymes, but the Geochip analysis based on known genes might lead to the limitation of the data.

In the study, we observed that there was no difference in NS-Biocrust vs. XS-Biocrust and NS-Bare soil vs. XS-Bare soil in the N-cycling functional gene structure. However, compared to XS, the samples collected from NS were dispersally distributed (Fig. 5). This could probably be attributed to the larger spatial distances between the sampling sites in NS. We further investigated the correlation among environmental elements, soil biological properties and the N-cycling functional gene structure in biocrust and bare soil on the tropical reef islands in the SCS (Fig. 5). Yeager et al. (2012) demonstrated that seasonal shifts and variation of precipitation could alter the diazotroph community structure and therefore affect the abundance of *nifH* genes. In this study, we found precipitation was the crucial environmental variable influencing the composition of N-cycling functional genes (Table 2). Soil available copper has been proven to correlate with the N cycle in biocrusts (Ochoa-Hueso and Manrique, 2011; Liu et al., 2012). It was one of the key environmental drivers observed in our study. Furthermore, our results showed that activities of soil β-glucosidase, fluorescein diacetate hydrolase, urease, alkaline phosphatase and catalase were significantly correlated with the microbial N-cycling gene composition and enriched in biocrusts (Fig. 5). In agreement with our observation, Liu et al. (2018) had reported that soil enzyme activities were the significant factors correlated with the microbial functional gene structures. Previous studies have demonstrated that the microbial functional structure was correlated with the microbial community (Liu et al., 2018; Hu et al., 2019; Zhao et al., 2020), and the microbial abundance and composition could impact biocrust enzymatic activities (Bates et al., 2010; Castillo-Monroy et al., 2011). Our results suggest microbial consortia, which take part in N cycling in biocrust, contribute to hydrolytic activities of several kinds of enzymes, such as soil β-glucosidase, fluorescein diacetate hydrolase, urease, alkaline phosphatase and catalase.

In sum, this study first comprehensively investigated the microbial N-cycling functional gene composition in biocrusts on tropical reef islands and observed that it varied significantly with biocrust formation. Abundances of N-cycling related functional genes were lower in biocrusts compared to that in bare soils. However, nitrogenase activity was significantly higher in biocrusts. Further, nitrate N and ammonia N were more abundant in biocrusts. Additionally, climatic factor (i.e., precipitation), soil chemical properties (i.e., soil available copper, soil ammonia N, and pH), and soil biological properties (i.e., β-glucosidase, fluor-

escein diacetate hydrolase, alkaline protease, urease, alkaline phosphatase, catalase and Chl *a*) significantly correlated to the microbial N-cycling functional structures. Our results suggest biocrusts played a vital role in N-cycling in coral sand soil on tropical reef islands. The higher N fixation efficiency, as well as ammonia N and nitrate N contents, in biocrust compared to in bare soil suggested that the N fixed by biocrusts was one of the N input approaches in tropical reef island soil. The role of biocrusts in the N retention and balance in tropical reef islands was worth studying over longer time scales. Given that precipitation was significantly correlated with the N-cycling functional structure observed in the present study, a comprehensive investigation of the impact of rainfall patterns on biocrust communities would be helpful in understanding the development and ecological functions of biocrusts on tropical reef islands in the context of climate changes.

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References

Acosta-Martínez V, Cruz L, Sotomayor-Ramírez D, et al. 2007. Enzyme activities as affected by soil properties and land use in a tropical watershed. Applied Soil Ecology, 35(1): 35–45, doi: 10.1016/j.apsoil.2006.05.012

Baran R, Brodie E L, Mayberry-Lewis J, et al. 2015. Exometabolite niche partitioning among sympatric soil bacteria. Nature Communications, 6: 8289, doi: 10.1038/ncomms9289

Barger N N, Belnap J, Ojima D S, et al. 2005. NO gas loss from biologically crusted soils in Canyonlands National Park, Utah. Biogeochemistry, 75(3): 373–391, doi: 10.1007/s10533-005-1378-9

Barger N N, Weber B, Garcia-Pichel F, et al. 2016. Patterns and controls on nitrogen cycling of biological soil crusts. In: Weber B, Büdel B, Belnap J, eds. Biological Soil Crusts: An Organizing Principle in Drylands. Cham, Switzerland: Springer, 257–285

Bates S T, Nash T H, Sweat K G, et al. 2010. Fungal communities of lichen-dominated biological soil crusts: Diversity, relative microbial biomass, and their relationship to disturbance and crust cover. Journal of Arid Environments, 74(10): 1192–1199

Belnap J. 1996. Soil surface disturbances in cold deserts: effects on nitrogenase activity in cyanobacterial-lichen soil crusts. Biology and Fertility of Soils, 23(4): 362–367, doi: 10.1007/BF00335908

Belnap J. 2002. Nitrogen fixation in biological soil crusts from southeast Utah, USA. Biology and Fertility of Soils, 35(2): 128–135, doi: 10.1007/s00374-002-0452-x

Belnap J. 2006. The potential roles of biological soil crusts in dryland hydrologic cycles. Hydrological Processes, 20(15): 3159–3178, doi: 10.1002/hyp.6325

Belnap J, Büdel B, Lange O L. 2001. Biological soil crusts: characteristics and distribution. In: Belnap J, Lange O L, eds. Biological Soil Crusts: Structure, Function, and Management. Berlin, Heidelberg: Springer, 3–30

Belnap J, Weber B, Büdel B. 2016. Biological soil crusts as an organizing principle in drylands. In: Weber B, Büdel B, Belnap J, eds. Biological Soil Crusts: An Organizing Principle in Drylands. Cham, Switzerland: Springer, 3–13

Beraldi-Campesi H, Hartnett H E, Anbar A, et al. 2009. Effect of biological soil crusts on soil elemental concentrations: implications for biogeochemistry and as traceable biosignatures of ancient life on land. Geobiology, 7(3): 348–359, doi: 10.1111/j.1472-4669.2009.00204.x

Bowker M A, Belnap J, Miller M E. 2006. Spatial modeling of biological soil crusts to support rangeland assessment and monitoring. Rangeland Ecology & Management, 59(5): 519–529

Brankatschk R, Fischer T, Veste M, et al. 2013. Succession of N cycling processes in biological soil crusts on a Central European inland dune. FEMS Microbiology Ecology, 83(1): 149–160, doi: 10.1111/j.1574-6941.2012.01459.x

Büdel B, Darienko T, Deutschewitz K, et al. 2009. Southern African biological soil crusts are ubiquitous and highly diverse in drylands, being restricted by rainfall frequency. Microbial Ecology, 57(2): 229–247, doi: 10.1007/s00248-008-9449-9

Castillo-Monroy A P, Bowker M A, Maestre F T, et al. 2011. Relationships between biological soil crusts, bacterial diversity and abundance, and ecosystem functioning: Insights from a semiarid Mediterranean environment. Journal of Vegetation Science, 22(1): 165–174

Couradeau E, Giraldo-Silva A, De Martini F, et al. 2019. Spatial segregation of the biological soil crust microbiome around its foundational cyanobacterium, *Microcoleus vaginatus*, and the formation of a nitrogen-fixing cyanosphere. Microbiome, 7(1): 55, doi: 10.1186/s40168-019-0661-2

Darby B J, Neher D A. 2016. Microfauna within biological soil crusts. In: Weber B, Büdel B, Belnap J, eds. Biological Soil Crusts: An Organizing Principle in Drylands. Cham: Springer International Publishing, 139–157

Dixon R, Kahn D. 2004. Genetic regulation of biological nitrogen fixation. Nature Reviews Microbiology, 2(8): 621–631, doi: 10.1038/nrmicro954

Duan Zhenghu, Xiao Honglang. 2000. Effects of soil properties on ammonia volatilization. Soil Science and Plant Nutrition, 46(4): 845–852, doi: 10.1080/00380768.2000.10409150

Elbert W, Weber B, Burrows S, et al. 2012. Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. Nature Geoscience, 5(7): 459–462

Evans R D, Ehleringer J R. 1993. A break in the nitrogen cycle in aridlands? Evidence from $\delta^{15}N$ of soils. Oecologia, 94(3): 314–317, doi: 10.1007/BF00317104

Evans R D, Johansen J R. 1999. Microbiotic crusts and ecosystem processes. Critical Reviews in Plant Sciences, 18(2): 183–225, doi: 10.1080/07352689991309199

Evans R D, Lange O L. 2001. Biological soil crusts and ecosystem nitrogen and carbon dynamics. In: Belnap J, Lange O L, eds. Biological Soil Crusts: Structure, Function, and Management. Berlin, Heidelberg: Springer, 263–279

Fisher K A, Yarwood S A, James B R. 2017. Soil urease activity and bacterial *ure*C gene copy numbers: effect of pH. Geoderma, 285: 1–8, doi: 10.1016/j.geoderma.2016.09.012

Ghiloufi W, Seo J, Kim J, et al. 2019. Effects of biological soil crusts on enzyme activities and microbial community in soils of an arid ecosystem. Microbial Ecology, 77(1): 201–216, doi: 10.1007/s00248-018-1219-8

Guan Pingting, Zhang Xiaoke, Yu Jun, et al. 2018. Soil microbial food web channels associated with biological soil crusts in desertification restoration: the carbon flow from microbes to nematodes. Soil Biology and Biochemistry, 116: 82–90, doi: 10.1016/j.soilbio.2017.10.003

Hardy R W F, Burns R C, Holsten R D. 1973. Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biology and Biochemistry, 5(1): 47–81, doi: 10.1016/0038-0717(73)90093-X

He Zhili, Deng Ye, Van Nostrand J D, et al. 2010. GeoChip 3.0 as a high-throughput tool for analyzing microbial community composition, structure and functional activity. The ISME Journal, 4(9): 1167–1179, doi: 10.1038/ismej.2010.46

He Mei, Wang Wenxiong. 2011. Factors affecting the bioaccessibility of methylmercury in several marine fish species. Journal of Ag-

ricultural and Food Chemistry, 59(13): 7155–7162, doi: 10.1021/jf201424g
Hooper D U, Johnson L. 1999. Nitrogen limitation in dryland ecosystems: responses to geographical and temporal variation in precipitation. Biogeochemistry, 46(1): 247–293
Hu Yigang, Zhang Zhishan, Huang Lei, et al. 2019. Shifts in soil microbial community functional gene structure across a 61-year desert revegetation chronosequence. Geoderma, 347(2019): 126–134
Johnson S L, Budinoff C R, Belnap J, et al. 2005. Relevance of ammonium oxidation within biological soil crust communities. Environmental Microbiology, 7(1): 1–12, doi: 10.1111/j.1462-2920.2004.00649.x
Kruskal J B. 1964. Nonmetric multidimensional scaling: a numerical method. Psychometrika, 29(2): 115–129, doi: 10.1007/BF02289694
Kuypers M M, Marchant H K, Kartal B. 2018. The microbial nitrogen-cycling network. Nature Reviews Microbiology, 16(5): 263
Lan Shubin, Wu Li, Zhang Delu, et al. 2015. Analysis of environmental factors determining development and succession in biological soil crusts. Science of the Total Environment, 538: 492–499, doi: 10.1016/j.scitotenv.2015.08.066
Li Jie, Chen Qi, Zhang Si, et al. 2013. Highly heterogeneous bacterial communities associated with the SCS reef corals *Porites lutea*, *Galaxea fascicularis* and *Acropora millepora*. PLoS One, 8(8): e71301, doi: 10.1371/journal.pone.0071301
Ligi T, Truu M, Truu J, et al. 2014. Effects of soil chemical characteristics and water regime on denitrification genes (*nir*S, *nir*K, and *nos*Z) abundances in a created riverine wetland complex. Ecological Engineering, 72: 47–55, doi: 10.1016/j.ecoleng.2013.07.015
Liu Weiqiu, Song Yongsheng, Wang Bin, et al. 2012. Nitrogen fixation in biotic crusts and vascular plant communities on a copper mine tailings. European Journal of Soil Biology, 50: 15–20, doi: 10.1016/j.ejsobi.2011.11.009
Liu Yubin, Zhao Lina, Wang Zengru, et al. 2018. Changes in functional gene structure and metabolic potential of the microbial community in biological soil crusts along a revegetation chronosequence in the Tengger Desert. Soil Biology and Biochemistry, 126: 40–48, doi: 10.1016/j.soilbio.2018.08.012
Lorenzen C J. 1967. Determination of chlorophyll and phaeopigments: spectrophotometric equations. Limnology and Oceanography, 12(2): 343–346, doi: 10.4319/lo.1967.12.2.0343
Marker A F H, Jinks S. 1982. Spectrophotometric analysis of chlorophyll *a* and phaeopigments in acetone, ethanol and methanol. Ergebnisse der Limnologie, 16: 3–7
Miralles I, Domingo F, Cantón Y, et al. 2012. Hydrolase enzyme activities in a successional gradient of biological soil crusts in arid and semi-arid zones. Soil Biology and Biochemistry, 53: 124–132, doi: 10.1016/j.soilbio.2012.05.016
Mush E A. 1980. Comparison of different methods for chlorophyll and phaeopigment determination. Archiv für Hydrobiologie, Beihefte, 14: 14–36
Ochoa-Hueso R, Manrique E. 2011. Effects of nitrogen deposition and soil fertility on cover and physiology of *Cladonia foliacea* (Huds.) Willd., a lichen of biological soil crusts from Mediterranean Spain. Environmental Pollution, 159(2): 449–457, doi: 10.1016/j.envpol.2010.10.021
Orellana L H, Rodriguez-R L M, Higgins S, et al. 2014. Detecting nitrous oxide reductase (NosZ) genes in soil metagenomes: method development and implications for the nitrogen cycle. mBio, 5(3): e01193–14
Pointing S B, Belnap J. 2012. Microbial colonization and controls in dryland systems. Nature Reviews Microbiology, 10(8): 551–562, doi: 10.1038/nrmicro2831
Sivaperuman C, Jaisankar I, Velmurugan A, et al. 2008. Tropical islands: ecosystem and endemism. In: Sivaperuman C, Velmurugan A, Singh A K, et al., eds. Biodiversity and Climate Change Adaptation in Tropical Islands. Amsterdam: Elsevier, 31–52
Van Den Ancker J A M, Jungerius P D, Mur L R. 1985. The role of algae in the stabilization of coastal dune blowouts. Earth Surface Processes and Landforms, 10(2): 189–192, doi: 10.1002/esp.3290100210
Vitousek P M, Porder S, Houlton B Z, et al. 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecological applications, 20(1): 5–15, doi: 10.1890/08-0127.1
Walvoord M A, Phillips F M, Stonestrom D A, et al. 2003. A reservoir of nitrate beneath desert soils. Science, 302(5647): 1021-1024
Wang Chao, Wang Xiaobo, Liu Dongwei, et al. 2014. Aridity threshold in controlling ecosystem nitrogen cycling in arid and semi-arid grasslands. Nature Communications, 5: 4799, doi: 10.1038/ncomms5799
Warshan D, Bay G, Nahar N, et al. 2016. Seasonal variation in *nifH* abundance and expression of cyanobacterial communities associated with boreal feather mosses. The ISME Journal, 10(9): 2198–2208, doi: 10.1038/ismej.2016.17
Weber B, Graf T, Bass M. 2012. Ecophysiological analysis of moss-dominated biological soil crusts and their separate components from the Succulent Karoo, South Africa. Planta, 236(1): 129–139, doi: 10.1007/s00425-012-1595-0
Wellburn A R. 1994. The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Journal of Plant Physiology, 144(3): 307–313, doi: 10.1016/S0176-1617(11)81192-2
Yang Yunfeng, Gao Ying, Wang Shiping, et al. 2014. The microbial gene diversity along an elevation gradient of the Tibetan grassland. The ISME Journal, 8(2): 430–440, doi: 10.1038/ismej.2013.146
Yang Hangyu, Liu Changzhong, Liu Yanmei, et al. 2018. Impact of human trampling on biological soil crusts determined by soil microbial biomass, enzyme activities and nematode communities in a desert ecosystem. European Journal of Soil Biology, 87: 61–71, doi: 10.1016/j.ejsobi.2018.05.005
Yeager C M, Kuske C R, Carney T D, et al. 2012. Response of biological soil crust diazotrophs to season, altered summer precipitation, and year-round increased temperature in an arid grassland of the colorado plateau, USA. Frontiers in Microbiology, 3: 358
Yue Haowei, Wang Mengmeng, Wang Shiping, et al. 2015. The microbe-mediated mechanisms affecting topsoil carbon stock in Tibetan grasslands. The ISME Journal, 9(9): 2012–2020, doi: 10.1038/ismej.2015.19
Zehr J P, Paerl H. 1998. Nitrogen fixation in the marine environment: genetic potential and nitrogenase expression. In: Cooksey K E, ed. Molecular Approaches to the Study of the Ocean. Dordrecht: Springer, 285–301
Zhao Lina, Liu Yubing, Wang Zengru, et al. 2020. Bacteria and fungi differentially contribute to carbon and nitrogen cycles during biological soil crust succession in arid ecosystems. Plant and Soil, 447(1): 379–392
Zhou Jizhong, He Zhili, Yang Yunfeng, et al. 2015. High-throughput metagenomic technologies for complex microbial community analysis: open and closed formats. mBio, 6(1): e02288–14
Zumft W G. 1997. Cell biology and molecular basis of denitrification. Microbiology and Molecular Biology Review, 61(4): 533–616, doi: 10.1128/.61.4.533-616.1997

Appendix:

Table A1. Test method for soil properties and measuring instrument

Soil property	Test method	Measuring instrument
Soil available boron	NY/T 149-1990, Agricultural Trade Standards of PRC	Ultraviolet-visible spectrophotometer, Shimadzu, Japan
Organic matter	NY/T 1121.6-2006, Agricultural Trade Standards of PRC	Digital Bottle-top Burette, Merck, Germany
Organic carbon	HJ 615-2011, National Environmental Protection Standards of PRC	Ultraviolet-visible spectrophotometer, Shimadzu, Japan
Soil available phosphorus	HJ 704-2014, National Environmental Protection Standards of PRC	Ultraviolet-visible spectrophotometer, Shimadzu, Japan
Soil exchangeable calcium	NY/T 1121.13-2006, Agricultural Trade Standards of PRC	Atomic absorption spectrophotometer, Hitachi, Japan
Soil available kalium	NY/T 889-2004, Agricultural Trade Standards of PRC	Flame photometer, INESA, China
Kalium	NY/T 87-1988, Agricultural Trade Standards of PRC	Atomic absorption spectrophotometer, Hitachi, Japan
Calcium	NY/T 296-1995, Agricultural Trade Standards of PRC	Atomic absorption spectrophotometer, Hitachi, Japan
Soil available zinc	NY/T 890-2004, Agricultural Trade Standards of PRC	Atomic absorption spectrophotometer, Hitachi, Japan
Soil available copper	NY/T 890-2004, Agricultural Trade Standards of PRC	Atomic absorption spectrophotometer, Hitachi, Japan
Soil available iron	NY/T 890-2004, Agricultural Trade Standards of PRC	Atomic absorption spectrophotometer, Hitachi, Japan
Soil available manganese	NY/T 890-2004, Agricultural Trade Standards of PRC	Atomic absorption spectrophotometer, Hitachi, Japan
Total water-soluble salt	NY/T 1121.16-2006, Agricultural Trade Standards of PRC	Electronic balance, Sartorius, Germany
Soil total phosphorus	HJ 632-2011, National Environmental Protection Standards of PRC	Ultraviolet-visible spectrophotometer, Shimadzu, Japan
Soil available sulphur	NY/T 1121.14-2006, Agricultural Trade Standards of PRC	Ultraviolet-visible spectrophotometer, Shimadzu, Japan
Soil total nitrogen	NY/T 53-1987, Agricultural Trade Standards of PRC	Ultraviolet-visible spectrophotometer, Shimadzu, Japan
Soil nitrite nitrogen	HJ 634-2012, National Environmental Protection Standards of PRC	Ultraviolet-visible spectrophotometer, Shimadzu, Japan
Soil nitrate nitrogen	HJ 634-2012, National Environmental Protection Standards of PRC	Ultraviolet-visible spectrophotometer, Shimadzu, Japan
Soil ammonium nitrogen	LY/T 1228-2015, Forestry Industry Standards of PRC	Ultraviolet-visible spectrophotometer, Shimadzu, Japan
Soil ammonia nitrogen	HJ 634-2012, National Environmental Protection Standards of PRC	Ultraviolet-visible spectrophotometer, Shimadzu, Japan

Table A2. Soil characteristics of the samples collected from the tropical reef islands, South China Sea

	Sample and type						
	N5.01, BS(NS)	N5.02, BS(NS)	N5.03, BS(NS)	N5.04, BS(NS)	N7.01, BS(NS)	N7.02, BS(NS)	N7.03, BS(NS)
pH	9.61	9.57	9.59	9.67	9.62	9.65	9.71
Soil available boron/(mg·kg^{-1})	0.013	0.012	0.013	0.013	0.028	0.028	0.029
Organic matter/(g·kg^{-1})	8.01	8.05	8.02	7.98	2.89	2.89	2.95
Organic carbon/%	0.61	0.6	0.6	0.62	0.48	0.48	0.49
Soil available phosphorus/(mg·kg^{-1})	17.4	12.1	12.8	12.4	17.8	17.8	18.1
Soil exchangeable calcium/(g·kg^{-1})	46.3	46.1	45.4	47.4	109	108	110
Soil available kalium/(mg·kg^{-1})	21.6	21.7	21.7	21.3	13.8	13.8	13.8
Kalium/(mg·kg^{-1})	706	709	704	704	76.5	76.4	76.3
Calcium/(g·kg^{-1})	285	281	286	287	361	359	363
Soil available zinc/(mg·kg^{-1})	5.51	5.57	5.63	5.33	5.18	5.17	5.2
Soil available copper/(mg·kg^{-1})	0.06	0.06	0.07	0.06	0.006	0	0.016 6
Soil available iron/(mg·kg^{-1})	7.9	7.9	7.8	7.9	2.9	2.9	2.9
Soil available manganese/(mg·kg^{-1})	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Total water soluble salt/(g·kg^{-1})	3.6	3.6	3.9	3.2	2.6	2.9	2.5
Soil total phosphorus/(mg·kg^{-1})	191	195	193	184	156	155	157
Soil available sulphur/(mg·kg^{-1})	9.61	9.68	9.47	9.68	74.5	74.3	74.1
Soil total nitrogen/%	0.018	0.019	0.016	0.018	0.017	0.017	0.017
Soil nitrite nitrogen/(mg·kg^{-1})	0.48	0.53	0.42	0.49	0.78	0.71	0.8

to be continued

Continued from Table A2

	Sample and type						
	N5.01, BS(NS)	N5.02, BS(NS)	N5.03, BS(NS)	N5.04, BS(NS)	N7.01, BS(NS)	N7.02, BS(NS)	N7.03, BS(NS)
Soil nitrate nitrogen/(mg·kg^{-1})	8.77	8.9	8.86	8.54	10.8	10.7	10.9
Soil ammonium nitrogen/(mg·kg^{-1})	3.06	3.06	3.11	3.01	0.27	0.31	0.26
Soil ammonia nitrogen/(mg·kg^{-1})	0.2	0.2	0.2	0.21	0.17	0.16	0.18

	Sample and type						
	N7.04, BS(NS)	YX.01, BS(XS)	YX.02, BS(XS)	ZX.01, BS(XS)	N5.1, BTS(NS)	N5.2, BTS(NS)	N5.3, BTS(NS)
pH	9.5	9.71	9.7	9.68	8.51	9.12	8.88
Soil available boron/(mg·kg^{-1})	0.027	0.037	0.038	0.028	0.042	0.019	0.015
Organic matter/(g·kg^{-1})	2.83	4.11	4.15	74.2	15	9.6	13.2
Organic carbon/%	0.48	0.38	0.37	4.53	0.82	0.48	0.68
Soil available phosphorus/(mg·kg^{-1})	17.4	15.4	15.1	295	76.9	9.2	9.3
Soil exchangeable calcium/(g·kg^{-1})	110	30.8	31.1	37.7	59.2	67.2	38.1
Soil available kalium/(mg·kg^{-1})	13.7	17.8	17.7	21.1	18.2	14.6	8.7
Kalium/(mg·kg^{-1})	76.8	246	251	239	3 300	762	1 700
Calcium/(g·kg^{-1})	361	245	248	224	168	95.7	85.7
Soil available zinc/(mg·kg^{-1})	5.16	5.27	5.1	6.03	10.7	7.26	2.11
Soil available copper/(mg·kg^{-1})	0	0.04	0.04	0.13	0.7	0.17	0.41
Soil available iron/(mg·kg^{-1})	3	2.1	2.2	1.2	8.8	6.8	4.3
Soil available manganese/(mg·kg^{-1})	0.2	0.2	0.2	0.4	1	0.6	0.8
Total water soluble salt/(g·kg^{-1})	2.3	2.4	2.3	1.4	2.4	2.1	2.3
Soil total phosphorus/(mg·kg^{-1})	155	496	496	54 500	351	191	347
Soil available sulphur/(mg·kg^{-1})	75.1	15.3	15.3	13.8	24.6	5.29	8.19
Soil total nitrogen/%	0.018	0.024	0.025	0.536	0.069	0.048	0.063
Soil nitrite nitrogen/(mg·kg^{-1})	0.83	0.98	1.03	0.51	1.48	0.7	0.48
Soil nitrate nitrogen/(mg·kg^{-1})	10.9	9.96	9.77	11.2	60.6	10.3	29.9
Soil ammonium nitrogen/(mg·kg^{-1})	0.26	0.21	0.25	3.73	2.24	0.9	0.8
Soil ammonia nitrogen/(mg·kg^{-1})	0.18	0.3	0.31	0.26	0.45	4.58	4.92

	Sample and type						
	N7.1, BTS(NS)	N7.2, BTS(NS)	N7.3, BTS(NS)	N7.4, BTS(NS)	N7.5, BTS(NS)	YX.1, BTS(XS)	YX.2, BTS(XS)
pH	9.5	9.71	9.7	9.68	8.51	9.12	8.88
Soil available boron/(mg·kg^{-1})	0.027	0.037	0.038	0.028	0.042	0.019	0.015
Organic matter/(g·kg^{-1})	2.83	4.11	4.15	74.2	15	9.6	13.2
Organic carbon/%	0.48	0.38	0.37	4.53	0.82	0.48	0.68
Soil available phosphorus/(mg·kg^{-1})	17.4	15.4	15.1	295	76.9	9.2	9.3
Soil exchangeable calcium/(g·kg^{-1})	110	30.8	31.1	37.7	59.2	67.2	38.1
Soil available kalium/(mg·kg^{-1})	13.7	17.8	17.7	21.1	18.2	14.6	8.7
Kalium/(mg·kg^{-1})	76.8	246	251	239	3 300	762	1 700
Calcium/(g·kg^{-1})	361	245	248	224	168	95.7	85.7
Soil available zinc/(mg·kg^{-1})	5.16	5.27	5.1	6.03	10.7	7.26	2.11
Soil available copper/(mg·kg^{-1})	0	0.04	0.04	0.13	0.7	0.17	0.41
Soil available iron/(mg·kg^{-1})	3	2.1	2.2	1.2	8.8	6.8	4.3
Soil available manganese/(mg·kg^{-1})	0.2	0.2	0.2	0.4	1	0.6	0.8
Total water soluble salt/(g·kg^{-1})	2.3	2.4	2.3	1.4	2.4	2.1	2.3
Soil total phosphorus/(mg·kg^{-1})	155	496	496	54 500	351	191	347
Soil available sulphur/(mg·kg^{-1})	75.1	15.3	15.3	13.8	24.6	5.29	8.19
Soil total nitrogen/%	0.018	0.024	0.025	0.536	0.069	0.048	0.063
Soil nitrite nitrogen/(mg·kg^{-1})	0.83	0.98	1.03	0.51	1.48	0.7	0.48
Soil nitrate nitrogen/(mg·kg^{-1})	10.9	9.96	9.77	11.2	60.6	10.3	29.9
Soil ammonium nitrogen/(mg·kg^{-1})	0.26	0.21	0.25	3.73	2.24	0.9	0.8
Soil ammonia nitrogen/(mg·kg^{-1})	0.18	0.3	0.31	0.26	0.45	4.58	4.92

	Sample and type				
	YX.3, BTS(XS)	YX.4, BTS(XS)	YX.5, BTS(XS)	YX.6, BTS(XS)	ZX.1, BTS(XS)
pH	8.39	8.85	9.3	8.38	9.32
Soil available boron/(mg·kg^{-1})	0.024	0.03	0.134	1.22	0.063

to be continued

Continued from Table A2

	Sample and type				
	YX.3, BTS(XS)	YX.4, BTS(XS)	YX.5, BTS(XS)	YX.6, BTS(XS)	ZX.1, BTS(XS)
Organic matter/(g·kg^{-1})	28.8	22	5.45	15.3	12.7
Organic carbon/%	1.44	1.39	0.51	0.79	0.93
Soil available phosphorus/(mg·kg^{-1})	44.6	26.1	10.6	15	60.5
Soil exchangeable calcium/(g·kg^{-1})	25.9	86	67.4	98	67.4
Soil available kalium/(mg·kg^{-1})	106	32.9	21.6	79.3	16
Kalium/(mg·kg^{-1})	3 300	321	190	1 000	179
Calcium/(g·kg^{-1})	0.96	254	303	292	295
Soil available zinc/(mg·kg^{-1})	4.29	8.05	7.6	7.99	10.9
Soil available copper/(mg·kg^{-1})	0.31	0.31	0.09	0.17	0.11
Soil available iron/(mg·kg^{-1})	0.9	9.1	1.9	2.5	3.3
Soil available manganese/(mg·kg^{-1})	7.5	0.9	0.4	0.7	0.6
Total water soluble salt/(g·kg^{-1})	2.4	2	6.3	10.5	2.5
Soil total phosphorus/(mg·kg^{-1})	1 237	1 110	165	529	759
Soil available sulphur/(mg·kg^{-1})	23.6	23.3	268	3 340	20.1
Soil total nitrogen/%	0.156	0.079	0.029	0.066	0.083
Soil nitrite nitrogen/(mg·kg^{-1})	0.89	0.45	15	1.74	1.26
Soil nitrate nitrogen/(mg·kg^{-1})	9.64	8.05	16.3	50.3	11
Soil ammonium nitrogen/(mg·kg^{-1})	4.44	0.5	28.66	3.81	0.92
Soil ammonia nitrogen/(mg·kg^{-1})	6.54	6.82	19.2	9.81	13.1

Note: N5.1–N5.3 and N7.1–N7.5: biocrust topsoil samples (BTS) from the Nansha Islands (NS); YX.1–YX.6 and ZX.1: BTS from the Xisha Islands (XS); N5.01–N5.04 and N7.01–N7.04: bare soil samples (BS) from the NS; YX.01, YX.02 and ZX.01: BS from the XS.

Table A3. Soil enzyme activities and Chl *a* contents of the samples collected from South China Sea

	SCA/ (μmol·d^{-1}·g^{-1})	SFHA/ (μmol·d^{-1}·g^{-1})	APA/ (μmol·d^{-1}·g^{-1})	SAPA/ (μmol·d^{-1}·g^{-1})	SUA/ (μg·d^{-1}·g^{-1})	SGA/ (μmol·d^{-1}·g^{-1})	SLA/ (μmol·d^{-1}·g^{-1})	Chl *a*/ (μg·g^{-1})
N5.1	52	54.33	126.99	16.01	813.96	1.28	40.77	27.34
N5.2	27.19	37.06	295.03	16.96	715.63	1	38.19	17.9
N5.3	32.32	41.26	292.23	16.86	749.92	0.97	39.78	16.19
N7.1	39.48	49.27	71.76	16.89	851.59	2.32	37.53	9.21
N7.2	45.9	46.01	173.49	16.85	817.3	1.47	35.35	20.12
N7.3	55.47	41.07	100.77	17.25	861.31	1.69	35.15	34.35
N7.4	43.32	52.87	226.97	16.52	811.23	2.28	32.77	18.11
N7.5	49.3	46.74	186.42	16.94	710.47	1.55	38.59	10.17
YX.1	49.31	54.37	39.36	14.49	805.77	1.96	30.66	23.42
YX.2	38.8	29.35	27.48	9.64	816.39	0.94	35.15	13.93
YX.3	44.69	46.23	2.42	19.44	893.17	1.19	37	10.65
YX.4	37.61	55.07	5.45	17.66	844.31	1.54	39.84	10.73
YX.5	18.29	23.85	141.68	7.67	204.25	0.75	37.73	20.08
YX.6	20.16	50.8	84.34	19.05	620.64	0.44	37.53	27.99
ZX.1	31.76	32.33	24.56	16.2	596.66	2.2	34.82	7.01
N5.01	7.22	4.31	75.6	1.44	75.27	0.1	33.17	0
N5.02	7.1	4.18	93.67	1.69	77.09	0.13	35.15	0
N5.03	7.03	4.62	66.05	1.25	78	0.1	33.17	0
N5.04	7.52	4.12	67.1	1.37	70.71	0.07	31.19	0.01
N7.01	5.72	2.81	157.06	0.08	38.85	0.12	33.3	0.04
N7.02	5.52	2.82	184.56	0.06	43.7	0.22	34.36	0.04
N7.03	5.95	2.87	162.88	0.09	35.51	0.08	30	0
N7.04	5.69	2.73	123.73	0.09	37.33	0.05	35.55	0.07
YX.01	6.13	3.1	116.86	1.29	163.58	0.02	38.26	0.05
YX.02	6.4	2.98	109.98	2.13	179.62	0.07	38.03	0.05
ZX.01	50.42	30.93	83.53	18.47	548.41	3.83	35.09	0.05

Note: N5.1–N5.3 and N7.1–7.5: biocrust topsoil samples from the Nansha Islands; YX.1–YX.6 and ZX.1: biocrust topsoil samples from the Xisha Islands; N5.01–N5.04 and N7.01–N7.04: bare soil samples from the Nansha Islands; YX.01, YX.02 and ZX.01: bare soil samples from the Xisha Islands. Abbreviations: soil catalase activity (SCA), soil fluorescein diacetate hydrolase (SFHA), soil alkaline protease activity (APA), soil alkaline phosphatase activity (SAPA), soil urease activity (SUA), soil β-glucosidase activity (SGA), soil lipase activity (SLA), and chlorophyll *a* (Chl *a*).