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Responses of soil microbial communities to nutrient limitation in the desert-grassland ecological transition zone

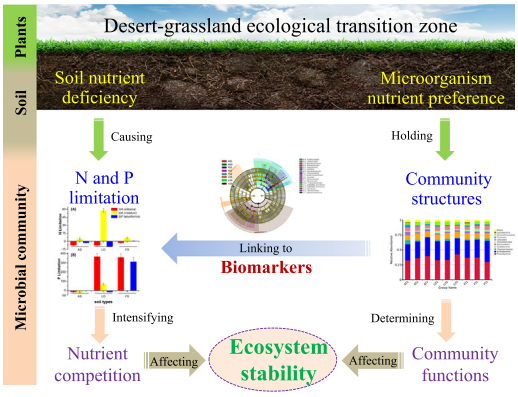
Yongxing Cui [a,b], Linchuan Fang [a,*], Xiaobin Guo [c], Xia Wang [a,b], Yunqiang Wang [d], Pengfei Li [a,b], Yanjiang Zhang [a], Xingchang Zhang [b]

[a] State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling 712100, PR China
[b] State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Yangling 712100, PR China
[c] Agriculture Production and Research Division, Department of Fisheries and Land Resources, Government of Newfoundland and Labrador, Corner Brook, NL A2H 6J8, Canada
[d] State Key Laboratory of Loess and Quaternary Geology, Institute of Earth Environment, Chinese Academy of Sciences, Xi'an, Shaanxi 710061, PR China

HIGHLIGHTS

- Soil microbial metabolisms were strongly limited by N and P in arid regions.
- Key biomarkers shaped microbial metabolic properties and community structures.
- Bacteria and fungus preference for nutrients alleviated the nutrient limitations.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil microorganisms are crucial to indicate ecosystem functions of terrestrial ecosystems. However, the responses of microbial communities to soil nutrient limitation in desert-grassland are still poorly understood. Hence, we investigated soil microbial community structures and metabolic characteristics in a desert-grassland ecological transition zone from the northern Loess Plateau, China, and explored the association of microbial communities with nutrient limitation via high-throughput sequencing. Threshold elemental ratios (TER) indicated that the microbial communities were strongly limited by nitrogen (N) under *A. ordosica* and *P. tabuliformis* communities. The phosphorus (P) limitation of microbial communities was observed in the aeolian sandy soil. The results imply that soil microbial communities had strong nutrient competition for N and P with aboveground vegetation in arid and oligotrophic ecosystems. The LEfSe and linear regression analysis revealed that the microbial taxa of *Micrococcales*, *Micrococcaceae* and *Herpotrichiellaceae* were significantly correlated with microbial N limitation. The *Thermoleophilia* taxa were significantly correlated with microbial P limitation. These biomarkers related to microbial nutrient limitation could be considered as the key microbial taxa to shape microbial communities and functions. Furthermore, N form had different effects on microbial communities, which NH_4^+-N strongly affected bacterial communities, whereas NO_3^--N had a significant influence on fungal communities. The different responses indicate that soil microorganisms had corresponding nutrient preferences for bacterial and fungal

* Corresponding author.
E-mail address: flinc629@hotmail.com (L. Fang).

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communities, which might alleviate the nutrient limitations and environmental stress. This study provided important insights on microbial community structures linking to community functions and on the mechanisms governing microbial N and P limitation in arid land ecosystems.

1. Introduction

Arid land ecosystems are widely considered to be vulnerable and unrecoverable under global climate change. Unlike agroecosystem with a large amount of fertilizers input (Carbonetto et al., 2014), arid natural ecosystems are characterized as continuous accumulation of organic matter and incessant consumption of soil nutrients especially for nitrogen and phosphorus (Craine et al., 2010; Cui et al., 2018). Low nutrient condition in arid ecosystem could result in strong competition of nutrient between microbes and plants (Inselsbacher et al., 2010; Ouyang et al., 2016), which potentially alter underground ecological functions and accelerate vegetation degradation in arid and oligotrophic ecosystems (Craine et al., 2010; Huang et al., 2016). Therefore, to understand microbial metabolism in arid land ecosystems is crucial to illuminate nutrient limitation of microbial communities as well as competition with vegetation.

Soil microbial community structures are powerfully affected by soil properties and environmental factors (Zhou et al., 2002). Such as, land use types (Tian et al., 2013; Zhao et al., 2015), soil fertility (Carbonetto et al., 2014; Zabaloy et al., 2016), and vegetation succession (Nacke et al., 2014; Zhang et al., 2016). Also, in its turn, microbial community structures have great effects on functions and metabolic characteristics (Boyle et al., 2008). Previous studies found that soil microbial communities in ultra-oligotrophic desert ecosystem were co-limited by C and either by N or P (Tapia-Torres et al., 2015). Waring et al. (2014) reported that soil microbial communities were limited by P in tropical soils due to microbial nutrient acquisition was regulated by climatic factors (such as mean annual temperature and precipitation). Our recent research also indicated that the microbial nutrient metabolisms in rhizosphere were co-limited by N and P in the arid area of the northern Loess Plateau (Cui et al., 2018). However, the linkage between microbial community structures and nutrient metabolism is still not well understood.

Normally, soil nutrient availability limits microbial metabolism as well as primary productivity in arid ecosystems (Bünemann et al., 2012; Xu et al., 2015). The nutrients utilized by soil microorganisms are decomposed from soil organic compounds and soil parent material elements (Sinsabaugh et al., 2009, 2015). In arid regions, soil available nutrients are easily fixed by soil particles and ions (Waring et al., 2014; Cui et al., 2018). For example, soil phosphorus can be easily bound with calcium and magnesium as insoluble substance (Cross and Schlesinger, 2001). Thus the decomposition of organic compounds via the extracellular enzyme of heterotrophic microorganisms is the key process of nutrient supply (Waring et al., 2014). Previous studies suggested that soil microbial communities had significantly effect on soil fertility and vegetation (Roszak and Colwell, 1987). Moreover, the processes of microbial nutrient metabolism can simultaneously supply energy and nutrients to aboveground plants (Kowalchuk et al., 2002; Lagerlöf et al., 2014). Thus, to understand the relationships between microbial community structures and nutrient metabolism could provide fundamental insight into these feedback patterns of microbial community and ecosystem function.

Soil microorganisms acclimate to environmental stress by reassigning key resources to nutrient acquisition mechanisms rather than their growth (Schimel et al., 2007; Duan et al., 2018). It has been reported that the ratio of C:N:P in microbial biomass is relatively constrained cross ecosystems compared to it in soil, thus the variations of nutrient ratio could indicate the shifts of nutrient allocation under altering demand (Cleveland and Liptzin, 2007). Ecoenzymatic activities involved in an intersection of ecological stoichiometry theory (EST) with the metabolic theory of ecology (MTE) can help us to understand the energy and nutrient controls on microbial metabolism (Sinsabaugh et al., 2009, 2012). Meanwhile, Threshold Elemental Ratio (TER) can be used to define the shifts of microbial growth between nutrient limitation (represented by N or P, at high C:N or C:P ratios) and energy limitation (represented by C, lower C:N or C:P ratios) (Sterner and Elser, 2002). Hence, the application of those models and methods can assist to reveal microbial metabolic limitation in the ecological frangible regions.

The Loess Plateau is one of the most eroded regions in China and one of the most vulnerable ecosystems in the world (Li et al., 2011). The northern area of Loess Plateau is desert-grassland transition zone (Wen et al., 2007), which is a typical fragile ecosystem (Noymeir, 2003; Pointing and Belnap, 2012). Microbial nutrient limitation affects community metabolism function and soil nutrient cycling, and thus influences the sustainable development of aboveground and underground in ecosystems. Therefore, it is very important to understand the feedbacks of microbial functions to community structures with the mechanisms governing microbial nutrient limitation in the desert-grassland transition zone.

In this study, we hypothesized that: (1) soil microbial nutrient metabolism in desert-grassland transition zone is strongly suffered from nutrient limitation due to soil nutrients (N and P) deficiency and vegetation competition, (2) the key microbial taxa control the microbial nutrient limitation in desert-grassland transition zone. In addition, the effect of environmental factors on microbial community structures in ecological critical zone was also investigated.

2. Materials and methods

2.1. Study site and sampling

This research was carried out in natural grassland and artificial shrubland ecosystems. The sites were located in Jungar Banner of the northern Loess Plateau (latitude 40°10′ to 39°35′ N and longitude 110°35′ to 111°23′ E), China (Fig. S1). The mean annual temperature is 6.7 °C, with a mean minimum temperature in January of −7.6 °C and a mean maximum temperature in August of 36.5 °C. It is arid and semi-arid climate zones and the mean annual precipitation is 390 mm, with over 60% precipitation from July to September. The soil types in this area are Aeolian sandy soil in the northern side, Loess in the eastern side, and Feldspathic sandstone weathered soil in the western side (Calcaric Cambisol, FAO classification). The dominant plant species in this area are natural *Artemisia ordosica*, natural *Agropyron cristatum*, and artificial *Pinu tabuliformis*.

Nine field experiment sites were established in the three soil types with three vegetation communities in August 2016 (Fig. S1). Under each soil type, there were three vegetation communities including the *Artemisia ordosica*, *Agropyron cristatum*, and *Pinus tabuliformis* plant communities. The descriptions of sampling sites were shown in Table 1. Each field experiment site was divided into 3 blocks. Ten quadrats (1 m × 1 m) with the same vegetation community were randomly selected as "W" pattern in each block. Soil cores from the topsoil (0–20 cm) were collected in each quadrat, and then mixed to one composite sample. Each composite sample was divided into three parts for future analysis. The first two parts were placed on ice box and transported to the laboratory within 2 days. The first part was immediately stored at −80 °C for soil genomic DNA extraction. The second part was passed through a 2.0 mm sieve and stored at 4 °C for measurement

Table 1
The geographical features and vegetation composition of the sampling sites (Cui et al., 2018).

Abbreviation	Soil types	Vegetation types	Slope aspect	Slope gradient	Altitude (m)	Main species
AS1	Aeolian sandy soil	*A. ordosica*	E10°N	20°	1291	*A. ordosica*; *L. davurica*; *S. viridis*; *P. sphondylodes*; *A. melilotoides* Pall
AS2		*A. cristatum*	E20°N	18°	1229	*A. cristatum*; *E. humifusa*; *A. scoparia*; *H. altaicus*; *S. viridis*
AS3		*P. tabuliformis*	W17°N	15°	1239	*P. tabuliformis*; *C. chinensis*; *A. scoparia*; *S. nigrum*
LO1	Loess	*A. ordosica*	E15°N	25°	1298	*A. ordosica*; *S. grandis*; *V. amoena; C. endivia*; *C. florida*
LO2		*A. cristatum*	E18°N	28°	1230	*A. cristatum*; *M. suavcolen*; *P. sphondylodes*; *A. melilotoides Pall*; *H. altaicus*
LO3		*P. tabuliformis*	W15°N	20°	1269	*P. tabuliformis*; *C. chinensis*; *S. grandis*; *L. davurica*
FS1	Feldspathic sandstone	*A. ordosica*	E35°N	10°	1243	*A. ordosica*; *L. davurica*; *H. fruticosum*; *P. sativa*
FS2		*A. cristatum*	W25°N	26°	1345	*A. cristatum*; *L. davurica*; *A. frigida*; *B. pilosa*
FS3		*P. tabuliformis*	E15°N	15°	1251	*P. tabuliformis*; *S. grandis*; *A. vestita*; *M. sativa*; *A. scoparia*

of microbial biomass and enzyme activities within 2 weeks. The third part was air-dried for analyzing physicochemical properties.

2.2. Soil properties measurements

About 120 g fresh soil for each sample was oven-dried at 105 °C to constant weight for soil moisture determination using the gravimetric method. Soil bulk density was determined using ring sampler weighing. Soil pH was estimated on a 1:2.5 soil-water (w/v) mixture using a glass electrode meter (InsMark™ IS126, Shanghai, China). The particle composition was analyzed using a laser particle size analyzer (Mastersizer 2000, Malvern, UK). Soil organic carbon (SOC) was analyzed using the standard procedure of dichromate oxidation. Dissolved organic carbon (DOC) were extracted with deionized water (1:4 soil/water ratio), and then filtered through a Millipore 0.45-μm filter (Jones and Willett, 2006). Total nitrogen (TN) was measured by Kjeldahl method (Bremner and Mulvaney, 1982). NO_3^--N and NH_4^+-N were determined using a Seal Auto Analyzer. Total phosphorus (TP) and available phosphorus (Olsen-P) were extracted with H_2SO_4-$HClO_4$ and sodium bicarbonate (Olsen and Sommers, 1982), respectively, and then determined by the molybdenum blue method using an ultraviolet spectrophotometer (Hitachi UV2300).

Microbial biomass for C, N, and P (MBC, MBN, MBP) were analyzed by chloroform fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). To determine of MBC and MBN, fresh soil (25 g oven dry equivalent) was fumigated for 24 h at 25 °C with ethanol-free $CHCl_3$. After fumigant removal, the soil was extracted with 100 ml of 0.5 M K_2SO_4 and shaken for 60 min at 200 rpm on a reciprocal shaker. The non-fumigated 25 g soil sample was extracted with 100 ml 0.5 M K_2SO_4 simultaneously at the time of fumigation commenced. The extracts from fumigated and non-fumigated samples were filtered using Whatman No.42 filter paper and frozen stored at −15 °C prior to analysis. The total organic carbon in the extracts was measured using a Liqui TOCII analyzer (Elementar, Germany). The TN in the extracts was measured using the Kjeldahl method. To measure MBP, fresh soil (10 g oven-dry equivalent) was fumigated for 24 h at 25 °C with ethanol-free $CHCl_3$. After fumigant removal, the soil was extracted with 100 ml of 0.5 M $NaHCO_3$ (pH = 8.5) and shaken for 60 min at 200 rpm a reciprocal shaker. The non-fumigated 10 g soil sample was extracted with 100 ml 0.5 M $NaHCO_3$ simultaneously at the time of fumigation commenced, 10 ml filtrate was added 5 ml Molybdenum antimony reagent then diluted with water to 25 ml. The phosphorus contents were measured by ultraviolet spectrophotometer (Hitachi UV2300) at 700 nm. The experimentally-derived conversion factors were 0.45, 0.54, and 0.40 for MBC, MBN, and MBP, respectively (Joergensen, 1996).

Three potential activities of C-acquiring enzyme (β-1,4-glucosidase, BG), N-acquiring enzymes (β-1,4-*N*-acetylglucosaminidase, NAG) and organic P-acquiring enzyme (alkaline phosphatase, AP) were determined following modified methods (Eivazi and Tabatabai, 1988; Steinweg et al., 2012). β-1,4-glucosidase activity was measured based on paranitrophenol concentration after the hydrolysis reaction. Five grams of fresh soil with 20 ml buffer solution (pH = 6.0) and 5 ml of 25 mM *p*-nitrophenol glucopyranoside was incubated 1 h at 37 °C, and then 5 ml of 0.5 M $CaCl_2$ solution and 20 ml of Tris buffer solution (pH = 12.0) were added and the soil suspension was thoroughly shaken and filtered. The paranitrophenol concentration was then measured at 400 nm with a spectrophotometer (Hitachi UV2300). To measure β-1,4-*N*-acetylglucosaminidase activity, the procedure was the same as the measurement of β-1,4-glucosidase activity, except that the substrate was changed to 4-*N*-acetyl-β-D-glucoside and the incubation time was 2 h. the alkaline phosphatase activity was measured based on phenol concentration. Briefly, 5 g of fresh soil with 10 ml of disodium phenyl phosphate solution and 10 ml of NH_4Cl-NH_4OH buffer solution was incubated for 3 h at 37 °C. The suspension was filtered with 1 ml of potassium ferricyanide and 4-amino antipyrine as the colour developing agent, and the phenol concentration of the filtrate was measured at 578 nm (Hitachi UV2300). Enzyme activities units for β-1,4-glucosidase, β-1,4-*N*-acetylglucosaminidase, and alkaline phosphatase were expressed as nmol paranitrophenol per gram dissolved organic carbon h^{-1}, nmol paranitrophenol per gram dissolved organic carbon h^{-1}, and nmol phenol per gram dissolved organic carbon h^{-1}, respectively.

2.3. DNA extractions and Illumina HiSeq high-throughput sequencing

DNA was extracted from 0.5 g of freeze-dried soil using a FastDNA Spin Kit for Soil (MP Biomedicals, Cleveland, USA) according to the manufacturer's instruction. The DNA extracts were assessed for quality and quantity using an automatic microplate reader (BioTek ELX 800, USA). The integrity of the DNA extracts was confirmed by 1% agarose gel electrophoresis. The 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) primers (Xu et al., 2016) were designed to amplify the V3–V4 hypervariable regions of the bacterial 16S rRNA gene. The 1737F (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and 2043R (5′-GCTGCGTTCTTCATCGATGC-3′) primers (Lu et al., 2013) were designed to amplify the ITS1 regions of fungal ITS rRNA gen. PCR reactions were performed in a thermal cycler (ABI GeneAmp 9700) at a volume of 20 μl and undergone 5 cycling procedure. Successful PCR amplification was verified by 2% agarose gel electrophoresis. The triplicate PCR products were pooled and purified by gel extraction and were quantified using the AxyPrepDNA gel extraction kit (AXYGEN corporation, USA) and the QuantiFluor™-ST blue fluorescence quantitative system (Promega Corporation, USA). Purified PCR products were then mixed at equimolar ratios for sequencing. Sequencing was conducted on an Illumina HiSeq PE150 (Illumina Corporation, USA) by Novogene Bio-pharm Technology Co., Ltd. Approximately 80,000 high-quality sequences per sample with an average length of 413 to 416 bp for bacteria and 236–279 bp for fungus were generated.

Primer sequences were trimmed after the raw sequences were denoised, sorted, and distinguished using the Trimmomatic software platform. The remaining sequences were filtered for redundancy, and all unique sequences for each sample were then clustered into operational taxonomic units (OTUs) at similarities of 97%. The taxonomic identify of representative sequences for each OTU was determined by the bacterial 16S rRNA Silva reference database (http://www.arb-silva.de) and fungal ITS rRNA Unite reference database (http://unite.ut.ee/index.php) using the RDP naïve Bayesian classifier (Pruesse et al., 2007; Xun et al., 2016).

For the high-throughput sequencing data, the indices of community richness (Chao1 and ACE estimators) and community diversity (Shannon index and Simpson index) were calculated. The rarefaction curves were obtained using Mothur (http://www.mothur.org/) and the percentage of taxonomy was designated the relative abundance. Taxonomic alpha diversity was calculated as the estimated richness utilizing the OTU richness and phylogenetic diversity was calculated as Faith's phylogenetic diversity (Faith, 1992). The high-throughput sequencing data are available in the NCBI Sequence Read Archive (SRA) database (Accession numbers SUB3053530 and SUB3057260).

2.4. Stoichiometric homeostasis and threshold elemental ratios

Eq. (1) was used to calculate the degree of community-level microbial C:N and C:P homeostasis (H′) by soil microorganisms (Sterner and Elser, 2002).

$$H' = 1/m \tag{1}$$

In Eq. (1), m is the slope of $\ln C{:}N_R$ (resources) versus $\ln C{:}N_B$ (microbial biomass) or slope of $\ln C{:}P_R$ versus $\ln C{:}P_B$ scatterplot. $H' \gg 1$ represents strong stoichiometric homeostasis, while $H' \approx 1$ represents weak or no homeostasis (Sterner and Elser, 2002).

To connect measured enzymatic activities with EST and MTE, we followed Sinsabaugh et al. (2009) to calculate the TER for C:N and C:P using the following equations:

$$TER_{C:N} = ((BG/NAG)B_{C:N})/n_o \tag{2}$$

$$TER_{C:P} = ((BG/AP)B_{C:P})/p_o \tag{3}$$

where $TER_{C:N}$ and $TER_{C:P}$ are the threshold ratios (dimensionless). BG/NAG is the enzymatic activity ratio for β-1,4-glucosidase and β-1,4-*N*-acetylglucosaminidase, BG/AP is the enzymatic activity ratio for β-1,4-glucosidase and alkaline phosphatase. $B_{C:N}$ and $B_{C:P}$ are the C:N or C:P ratios of the microbial biomass, and n_o and p_o are the dimensionless normalization constants for N and P. $n_o = e^{\text{intercept}}$ in the SMA regressions for ln(BG) vs ln(NAG), $p_o = e^{\text{intercept}}$ in the SMA regressions for ln(BG) vs ln(AP). For a more detailed analysis of the derivation of the equation, see Sinsabaugh et al. (2009).

2.5. Data analysis

Two-way ANOVA was used to examine the effects of vegetation communities and soil types on soil biochemistry properties and microbial communities, and then mean comparisons were performed with Tukey's multiple comparisons test ($P < 0.05$). The most significant factors that shaped soil microbes (bacteria and fungus) were determined by redundancy analysis (RDA) via the Hellinger transferred data of microbial species and the standardized data of environmental factors with Vegan package in R. The relationships between enzymatic activities were analyzed with type II standard major axis (SMA) regression using Smatr package in R. Data were $\log_e$-transformed prior to regression analysis to conform to the conventions of stoichiometric analysis and to normalize variance (Sterner and Elser, 2002). The linear discriminant analysis (LDA) effect size (LEfSe) method (http://huttenhower.sph.harvard.edu/lefse/) was used to detect the potential biomarkers

Table 2
The selected soil physicochemical properties from different sampling sites.

Sampling sites	SOC (g kg^{-1})	DOC (mg kg^{-1})	TN ((g kg^{-1})	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	TP (g kg^{-1})	Olsen-P (mg kg^{-1})	pH	Bulk density (g cm^{-3})	Particle composition (%)		
										≤0.002 mm	0.002–0.02 mm	≥0.02 mm
AS1	4.25 ± 0.26 Cb	68.04 ± 5.66 Aa	0.48 ± 0.01 Cb	8.86 ± 0.24 Bb	3.56 ± 0.31 Aa	0.25 ± 0.04 Ab	6.13 ± 0.31 Aa	8.76 ± 0.03 Aa	1.35 ± 0.05 Ab	4.79 ± 0.43 Ab	6.21 ± 1.18 Ab	89.00 ± 1.61 Aa
AS2	10.25 ± 0.03 Aa	55.29 ± 5.02 Bb	0.60 ± 0.05 Ba	6.64 ± 0.09 Cb	3.06 ± 0.17 Aa	0.39 ± 0.02 Aa	3.15 ± 0.13 Bb	8.77 ± 0.04 Ab	1.26 ± 0.05 Aa	11.28 ± 1.60 Ab	17.1 ± 2.49 Ab	71.61 ± 4.08 Aa
AS3	7,0.52 ± 0.26 Ba	75.65 ± 3.67 Aa	0.69 ± 0.02 Aa	10.99 ± 0.14 Aa	2.17 ± 0.16 Bb	0.32 ± 0.02 Aab	5.42 ± 0.45 Ab	8.67 ± 0.06 Ab	1.36 ± 0.06 Ab	10.45 ± 0.43 Ab	16.2 ± 0.84 Aa	73.35 ± 1.05 Aa
LO1	5.08 ± 0.06 Ca	66.23 ± 0.73 Aab	0.59 ± 0.01 Aa	10.23 ± 0.24 Aa	1.31 ± 0.07 Cb	0.52 ± 0.01 Aa	0.93 ± 0.05 Cb	8.85 ± 0.06 Aa	1.31 ± 0.05 ABb	12.43 ± 1.93 Aa	22.31 ± 3.17 Aa	65.26 ± 5.10 Ab
LO2	6.31 ± 0.13 Bb	67.85 ± 0.78 Aa	0.64 ± 0.01 Aa	7.46 ± 0.09 Ba	2.74 ± 0.05 Ba	0.49 ± 0.01 Aa	5.20 ± 0.44 Ba	8.91 ± 0.05 Aa	1.27 ± 0.05 Ba	13.09 ± 2.35 Ab	25.32 ± 6.17 Aab	61.58 ± 8.51 Aa
LO3	7.38 ± 0.23 Aa	61.67 ± 1.62 Ab	0.62 ± 0.01 Ab	5.84 ± 0.41 Cb	3.71 ± 0.64 Aa	0.45 ± 0.16 Aa	8.39 ± 0.70 Aa	8.85 ± 0.04 Aa	1.42 ± 0.04 Aab	13.29 ± 2.17 Aab	24.37 ± 4.75 Aa	62.33 ± 6.83 Aab
FS1	4.55 ± 0.05 Ab	57.28 ± 3.82 Ab	0.51 ± 0.01 Ab	1.06 ± 0.10 Bc	2.99 ± 0.21 Aa	0.32 ± 0.06 Ab	1.00 ± 0.13 Ab	8.53 ± 0.03 Bb	1.55 ± 0.04 Aa	18.54 ± 2.12 Ba	31.30 ± 4.47 Aa	50.16 ± 4.67 Ab
FS2	4.78 ± 0.18 Ac	49.05 ± 1.76 ABb	0.50 ± 0.01 Ab	2.26 ± 0.10 Ac	2.59 ± 0.13 ABa	0.26 ± 0.05 Ab	0.57 ± 0.05 Ac	8.77 ± 0.07 Ab	1.14 ± 0.03 Ba	30.17 ± 5.31 Aa	34.02 ± 6.85 Aa	35.82 ± 5.77 ABb
FS3	3.75 ± 0.15 Bb	44.06 ± 4.02 Bc	0.50 ± 0.01 Ac	2.03 ± 0.11 Ac	2.12 ± 0.18 Bb	0.21 ± 0.02 Ab	0.77 ± 0.08 Ac	8.59 ± 0.03 Bb	1.52 ± 0.04 Aa	17.66 ± 1.21 Ba	27.29 ± 2.96 Aa	55.05 ± 4.16 Ab

Factors (Df)	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Soil type (2)	685	***	61.3	***	107	***	3048	***	5.1	*	34.4	***	440	***	64.6	***	7.6	**	73.5	***	41.7	***	56.7	***
Vegetation (2)	475	***	7.8	**	42.3	***	97.0	***	1.0	0.38	2.2	0.14	110	***	16.8	***	49.6	***	16.4	***	4.0	*	8.4	**
Soil type × vegetation (4)	294	***	16.1	***	30.7	***	343	***	43.6	***	2.8	0.06	162	***	6.1	**	14.7	***	8.1	***	2.4	0.09	3.5	*

Note: AS: *Aeolian sandy soil*, LO: *Loess*, FS: *Feldspathic sandstone*. Values are means ± standard error ($n = 3$). Different uppercase letters (A, B, and C) indicate that means are significantly different ($P < 0.05$) among different vegetation types (1: *A. ordosica*, 2: *A. cristatum*, 3: *P. tabuliformis*) within a soil type; whereas different lowercase letters (a, b, and c) indicate that means are significantly different ($P < 0.05$) among soil types within a vegetation type.

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

based on a normalized relative abundance matrix. The Kruskal-Wallis test was used to identify the significant differences among different sampling sites and LDA was performed to evaluate the effect size of each feature (Segata et al., 2011). A LDA threshold score of 3.5 and a significant α of 0.05 were used to detect biomarkers by MASS package in R. Furthermore, linear and stepwise regression analysis and bivariate correlation (two-tailed) analyses were performed to explain the correlation among microbial taxa, environmental factors, and threshold elemental ratios (TER).

3. Results

3.1. Soil physicochemical properties and vegetation characteristics

The soils from *A. cristatum* communities displayed higher SOC concentration than those in the *A. ordosica* and *P. tabuliformis* communities except for the loess (Table 2). The aeolian sandy soil with *A. cristatum* communities showed the highest SOC (10.25 g kg^{-1}), whereas the feldspathic sandstone with *P. tabuliformis* communities had the lowest SOC (3.75 g kg^{-1}). TN and TP concentrations had no significant changes under different vegetation types except for that of aeolian sandy soil ($P < 0.05$). The average of mineral nitrogen (NO_3^--N and NH_4^+-N) in the aeolian sandy soil had the greater values than those in the other two soils. The highest values of NO_3^--N and NH_4^+-N were 10.99 mg kg^{-1} in the aeolian sandy soil with *P. tabuliformis* communities and 3.71 mg kg^{-1} in the loess with *P. tabuliformis* communities, respectively. Olsen-P was significantly affected by both soil and vegetation types. The loess with *P. tabuliformis* communities had the highest Olsen-P (8.39 mg kg^{-1}) (Table 2). The particle composition also showed significant variation among sampling sites; the feldspathic sandstone sites had higher contents of clay (≤0.002 mm) and silt particles (0.002–0.02 mm) than the sites of aeolian sandy soil and loess, while the aeolian sandy soil had the highest sand contents (≥0.02 mm). The characteristics of vegetation were reported in our previous study (Cui et al., 2018). Briefly, the biomass was significantly greater in *P. tabuliformis* communities than those in A. *ordosica* and *A. cristatum* communities. The characteristics of vegetation such as coverage, richness and Shannon diversity index had no significant difference among the different sampling sites.

3.2. Microbial biomass and enzymatic activities

Soil microbial biomass was significantly affected by both soil and vegetation types. The vegetation types played more important effect on MBC and MBN, compared with soil types. The *P. tabuliformis* communities had highest MBC, MBN, and MBP in the Aeolian sandy soil and loess types, compared the other two vegetation communities under same soil type. The average of MBC, MBN, and MBP was highest in the loess soils. The highest values of MBC, MBN, and MBP were observed in the loess with *P. tabuliformis* communities, which were 162.9 mg kg^{-1}, 22.7 mg kg^{-1}, and 2.66 mg kg^{-1}, respectively. The feldspathic sandstone with *A. ordosica* communities had the lowest values of MBC (25.63 mg kg^{-1}), MBN (3.69 mg kg^{-1}), and MBP (0.28 mg kg^{-1}) (Table 3).

There were significant main and interaction effects of soil and vegetation types on soil enzyme activities. BG was strongly affected by the interaction effect of soil type and vegetation ($F = 215$, $P < 0.001$). Statistical comparison of soil type and vegetation showed that soil type was the primary factor affecting NAG and AP ($F = 321$ and 398, $P < 0.001$) (Table 3). The loess soil from *A. cristatum* communities had the highest BG activities (85.3 nmol g^{-1} h^{-1}) and the aeolian sandy soil from *A. cristatum* communities had the lowest BG activity (32.1 nmol g^{-1} h^{-1}) (Table 3). The NAG and AP from the feldspathic sandstone sites were lower activities than those from the aeolian sandy and loess sites. The lowest NAG and AP activities (10.0 and 17.7 nmol g^{-1} h^{-1}) were shown in the feldspathic sandstone sites under the *P. tabuliformis* and *A. ordosica* communities, respectively.

3.3. Compositions and structures of microbial communities

The total of 1, 526, 154 and 1, 880, 628 high-quality sequences remained from the complete data set after quality trimming and removal of chimeras (an average of 56, 524 and 69, 653 per sample for bacterial communities and fungal communities, respectively). The total of 8, 593 and 2, 868 OTUs were identified for bacterial communities and fungal communities, respectively. The taxonomic composition of bacterial communities at phylum level was *Proteobacteria* (35.7%), followed by *Actinobacteria* (30.2%), *Acidobacteria* (9.5%), *Chloroflexi* (5.8%), *Gemmatimonadetes* (4.2%), *Thermomicrobia* (3.0%), *Bacteroidetes* (2.8%), *Thaumarchaeota* (2.7%), and *Firmicutes* (2.3%) (Fig. 1A). In terms of fungal communities, the dominant phylum (total 6 phylum) was *Ascomycota* (62.7% on average), followed by *Basidiomycota* (31.4%), *Zygomycota* (4.6%), *Chytridiomycota* (0.8%), *Glomeromycota* (0.2%) (Fig. 1B). The rarefaction curves (Fig. S2) approached saturation at a similarity level of 97% indicated that the volume of sequence data was sufficient and the addition of a large number of reads made a small contribution to the total number of OTUs. The saturation at a similarity level of 97% suggested that these bacterial communities were adequately sampled.

Table 3
The characteristics of soil microbial biomass and enzyme activities in different sampling sites.

Sampling sites	MBC (mg kg^{-1})	MBN (mg kg^{-1})	MBP (mg kg^{-1})	BG (nmol g^{-1} h^{-1})	NAG (nmol g^{-1} h^{-1})	AP (nmol g^{-1} h^{-1})
AS1	34.34 ± 4.21 Cb	5.50 ± 0.51 Ba	0.77 ± 0.05 Ba	54.72 ± 4.15 Ba	12.80 ± 0.55 Bb	31.00 ± 0.90 Bb*
AS2	58.67 ± 5.61 Bb	6.36 ± 0.49 Ba	0.99 ± 0.17 Ba	32.14 ± 1.17 Cb	20.77 ± 0.52 Ab	32.27 ± 1.04 Ba**
AS3	133.8 ± 2.84 Ab	16.08 ± 1.39 Ab	1.76 ± 0.14 Ab	80.39 ± 0.48 Aa	18.17 ± 0.43 Aa	51.92 ± 3.62 Aa
LO1	72.21 ± 6.02 Ca	6.56 ± 0.46 Ba	1.09 ± 0.09 Ba	49.53 ± 3.05 Ba	16.80 ± 0.33 Ba	48.70 ± 0.61 Aa
LO2	96.92 ± 8.03 Ba	8.44 ± 0.42 Ba	1.07 ± 0.15 Ba	85.28 ± 3.43 Aa	27.75 ± 0.44 Aa	33.78 ± 2.33 Ca
LO3	162.9 ± 12.20 Aa	22.7 ± 2.79 Aa	2.66 ± 0.22 Aa	51.65 ± 3.50 Bb	18.52 ± 2.19 Ba	38.85 ± 1.18 Bb
FS1	25.63 ± 2.17 Bb	3.69 ± 0.20 Ba	0.28 ± 0.03 Bb	51.67 ± 0.36 Ba	10.32 ± 0.68 Ab	17.65 ± 0.05 Bc
FS2	66.25 ± 4.85 Ab	8.02 ± 0.69 Aa	0.94 ± 0.05 Aa	78.16 ± 2.63 Aa	10.60 ± 0.69 Ac	24.80 ± 1.37 Ab
FS3	52.70 ± 3.96 Ac	7.91 ± 0.54 Ac	0.56 ± 0.05 Bc	47.35 ± 1.25 Bb	10.03 ± 0.81 Ab	19.47 ± 0.28 Bc

Factors (Df)	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Soil type (2)	227	***	65.1	***	154	***	13.7	***	321	***	398	***
Vegetation (2)	306	***	208	***	142	***	58.9	***	115	***	36.3	***
Soil type × vegetation (4)	51.7	***	36.9	***	50.3	***	251	***	35.7	***	98.6	***

Note: AS: *Aeolian sandy soil*, LO: *Loess*, FS: *Feldspathic sandstone*. MBC: microbial biomass carbon, MBN: microbial biomass nitrogen, MBP: microbial biomass phosphorus, BG: β-1,4-Glucosidase, NAG: β-1,4-*N*-acetylglucosaminidase, AP: Alkaline phosphatase. Values are means ± standard error (n = 3). Different uppercase letters (A, B, and C) indicate that means are significantly different ($P < 0.05$) among different vegetation types (1: *A. ordosica*, 2: *A. cristatum*, 3: *P. tabuliformis*) within a soil type; whereas different lowercase letters (a, b, and c) indicate that means are significantly different ($P < 0.05$) among soil types within a vegetation type.

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

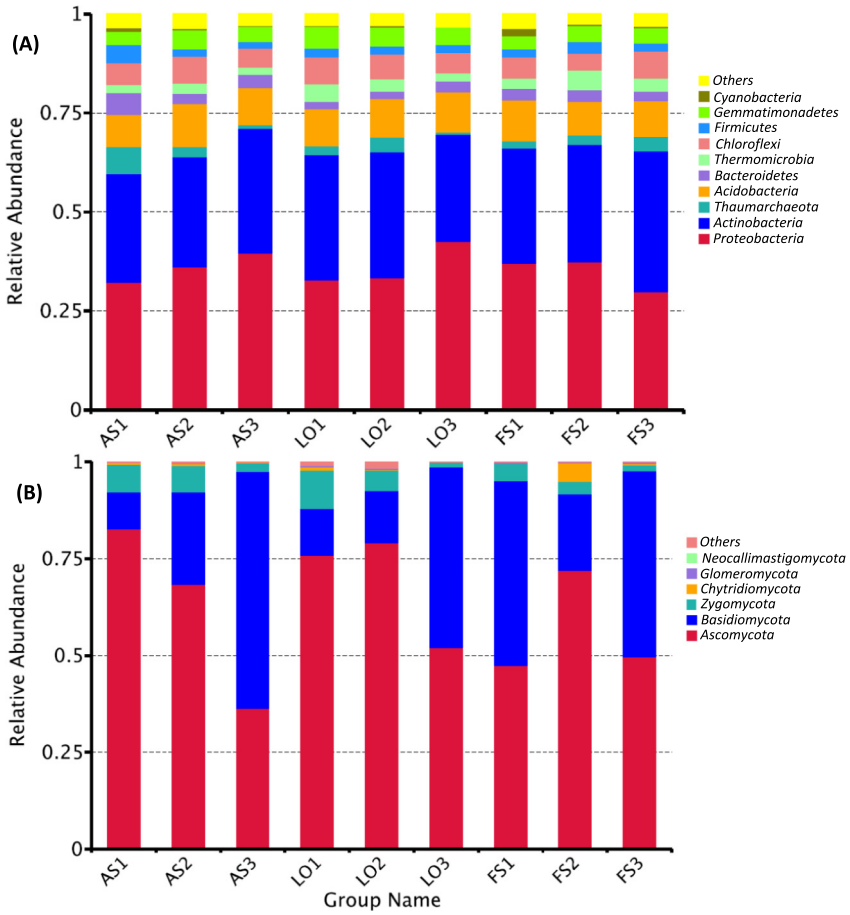

Fig. 1. Relative abundance of soil bacterial communities (A) and fungal communities (B) at the phylum level. Note: AS: Aeolian sandy soil, LO: Loess, FS: Feldspathic sandstone. 1: *A. ordosica*, 2: *A. cristatum*, 3: *P. tabuliformis*.

3.4. Relationships among soil physicochemical properties, plant communities and microbial communities

The RDA showed that variations of soil microbial community structure were shaped by vegetation characteristics and soil physicochemical properties (Fig. 2). The first two axes explained 71.1% of the total variance in the bacterial community structures (Fig. 2A). H_{plant}, NH_4^+-N, plant biomass, TOC, pH, Olsen-P, and TP were significantly correlated with the relative abundance of *Thermomicrobia* and *Actinobacteria*. As shown in Fig. 2B, the first two axes of RDA explained 75.6% of the total

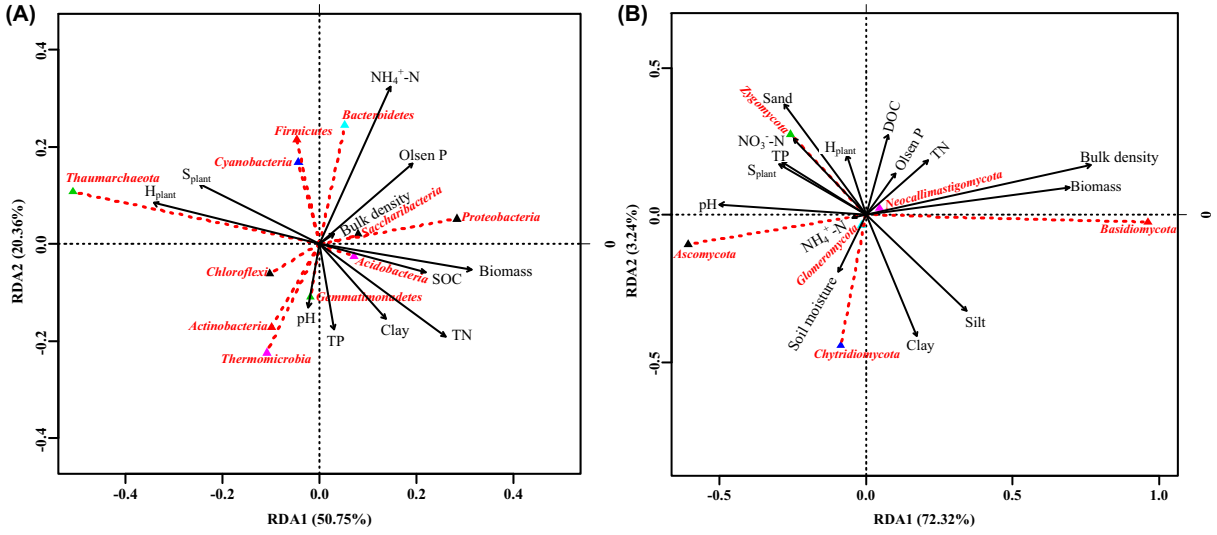

Fig. 2. The redundancy analysis (RDA) used to identify the relationship among the microbial populations (red arrows), vegetation characteristics, and soil properties (black arrows). H_{plant}: Shannon diversity index of plant community, S_{plant}: species richness of plant community, Biomass: plant biomass, SOC: soil organic C, DOC: dissolved organic C, TN: total N, TP: total P, clay: soil clay content (%), silt: soil silt content (%).

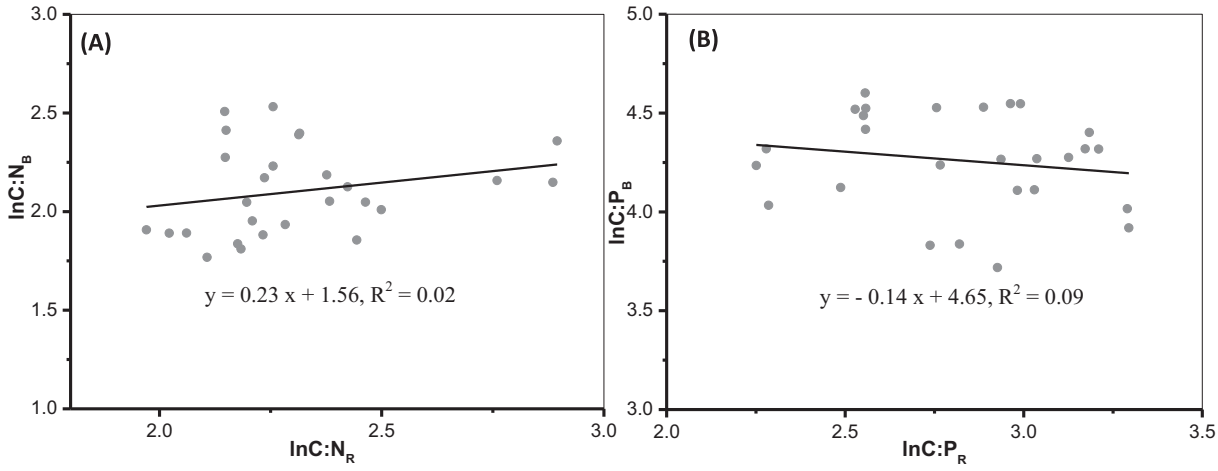

Fig. 3. Soil microbial community homeostasis related with nitrogen (A) and phosphorus (B) acquisition. (A) and (B) date from all sampling sites (27). Indicating soil microbial community strong homeostasis (slopes are not different to zero: (A) $P = 0.243$, (B) $P = 0.219$).

variance in the fungal community structures. NO_3^--N, DOC, pH, bulk density, and plant biomass were significantly correlated with the relative abundance of *Ascomycota* and *Basidiomycota*. According to RDA, soil pH and plant biomass were the common factors affecting both bacterial and fungal community structures.

3.5. Microbial nutrients limitations

The associations between microbial biomass elemental ratios and soil nutrient ratios were used to determine the strength of microbial stoichiometric homeostasis. There were no significant correlations between $lnC:N_R$ and $lnC:N_B$ as well as between $lnC:P_R$ and $lnC:P_B$ ($P > 0.05$), which indicated a strong community-level elemental homeostasis in our study areas (Fig. 3; Tapia-Torres et al., 2015). Based on the microbial C:N:P stoichiometric values generated from the enzymatic data, the estimated $TER_{C:N}$ and $TER_{C:P}$ were significantly different among the different sampling sites ($P < 0.05$). The $TER_{C:N}$ and $TER_{C:P}$ were strongly affected by soil type, vegetation, and their interaction effect (Fig. 4). $TER_{C:N}$ in the soil from *A. cristatum* communities were higher than those in the soils from *A. ordosica* and *P. tabuliformis* communities, while $TER_{C:P}$ in the soils from *A. ordosica* communities were higher than those in the soils from *A. cristatum* and *P. tabuliformis* communities (Fig. 4A, C). The nutrient limitations of microbial community were calculated by soil available nutrient ratios minus corresponding TER value (Fig. 4B, C), which the negative value indicated nutrient limitation in microbial community. The results indicated that the microbial communities in *A. ordosica* and *P. tabuliformis* communities were strongly limited by N (Fig. 4B). As shown in Fig. 4D, the microbial communities in the aeolian sandy soil were strongly limited by P regardless of vegetation type.

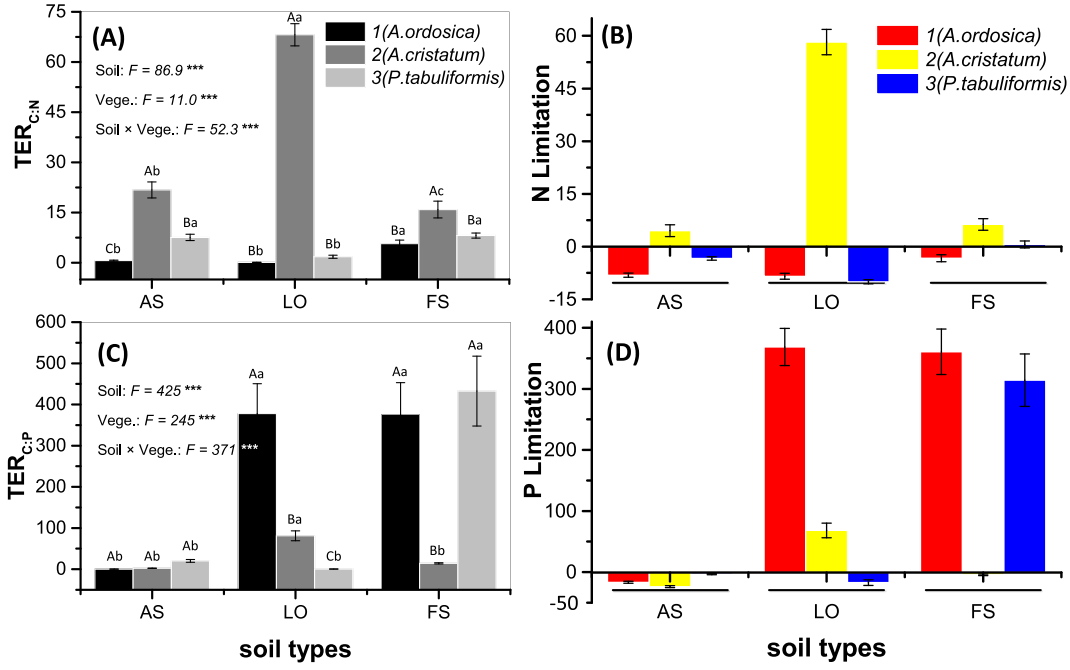

Fig. 4. Threshold Elemental Ratio (TER) C:N and (TER) C:P (A and C, respectively) and N limitation (B) and P limitation (D) of soil microbial community in different sampling sites. AS: *Aeolian sandy soil*, LO: *Loess*, FS: *Feldspathic sandstone*. Different uppercase letters (A, B, and C) indicate that means are significantly different ($P < 0.05$) among different vegetation types (1: *A. ordosica*, 2: *A. cristatum*, 3: *P. tabuliformis*) within a soil type; whereas different lowercase letters (a, b, and c) indicate that means are significantly different ($P < 0.05$) among soil types within a vegetation type. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

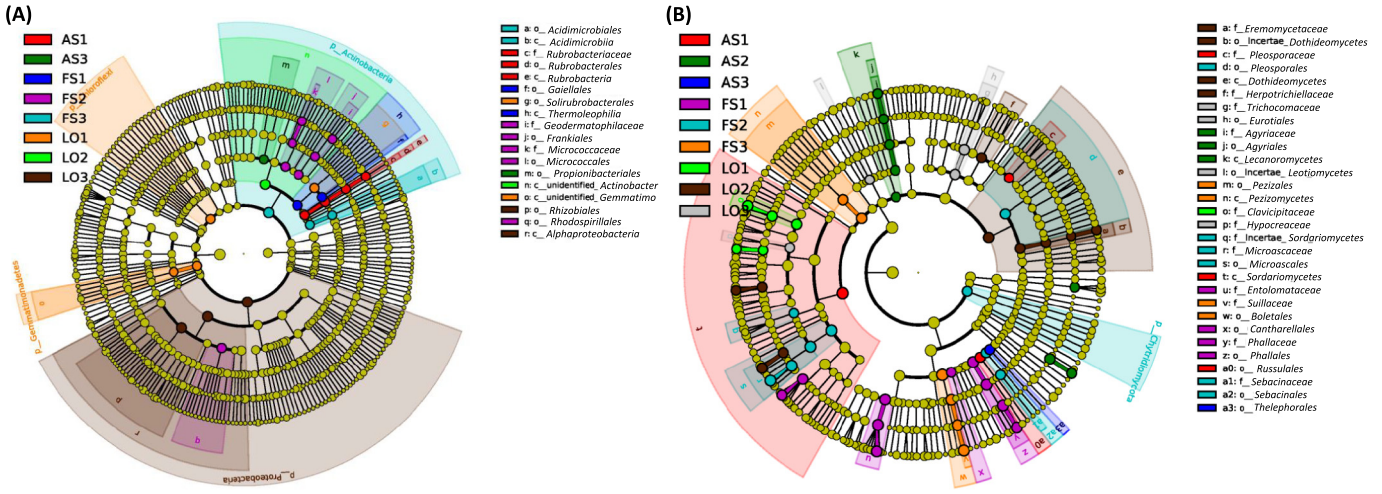

Fig. 5. The cladogram of bacterial communities (A) and fungal communities (B) among different sampling sites. Note: AS: *Aeolian sandy soil*, LO: *Loess*, FS: *Feldspathic sandstone*. 1: *A. ordosica*, 2: *A. cristatum*, 3: *P. tabuliformis*.

3.6. The potential biomarkers among different sampling sites and their connecting with threshold elemental ratios (TER)

The linear discriminant analysis (LDA) effect size (LEfSe) method was performed to determine the classified bacterial and fungal taxa with significant abundance differences among the different sampling sites. As shown in Fig. 5, 25 bacterial clades and 55 fungal clades presented significantly different with a LDA threshold of 3.5 (Fig. S3 and S4), thus those microbial taxa were considered as the potential biomarkers. The regression and correlation analysis were carried out to identify the relationships between key microbial taxa and threshold elemental ratios ($TER_{C:N}$ or $TER_{C:P}$) (Fig. 6, Table S8 and S9). Notably, three identified biomarkers (*Micrococcales*, *Micrococcaceae*, and *Herpotrichiellaceae*) were significantly positively correlated with $TER_{C:N}$ (Fig. 6A, B, C). One identified biomarker (*Thermoleophilia*) was significantly positively correlated with $TER_{C:P}$ (Fig. 6D). These biomarkers could be considered to represent the key microbial taxa shaping microbial community metabolic threshold.

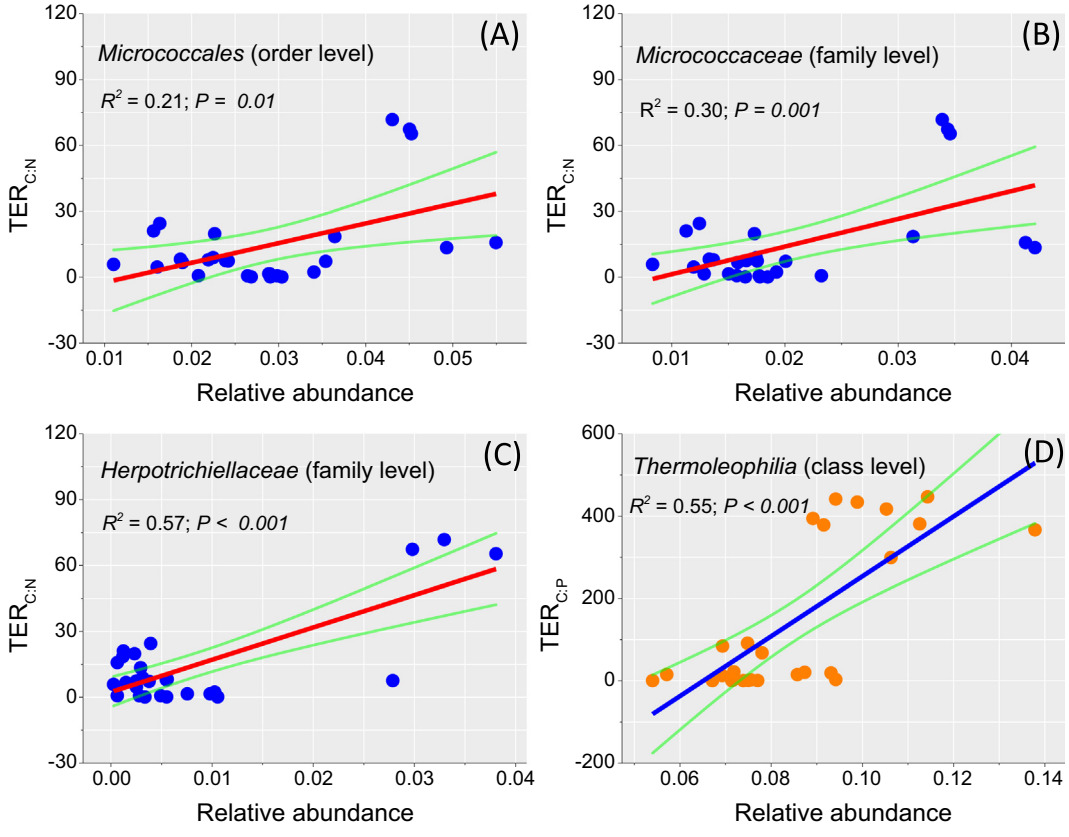

Fig. 6. The correlation analysis of the key microbial taxa (biomarkers) with $TER_{C:N}$ (A, B, C) and $TER_{C:P}$ (D). Solid lines indicate linear model fits between $TER_{C:N}$/$TER_{C:P}$ and the respective biomarkers, while dashed lines are the 95% confidence intervals of these linear models.

4. Discussion

4.1. Nutrient limitation of microbial communities

Microbial communities may be subject to diverse restrictions of soil nutrient under the different environmental conditions (Sinsabaugh et al., 2009). Microbes acquire soil nutrients via secreting enzymes and decomposing organic matter (Cleveland and Liptzin, 2007). In this study, microbial biomass and enzymatic activities showed the significant differences among the different sampling sites (Table 3). Given the limitation of nutrients and water in our research region (Cui et al., 2018), soil microbial communities in the desert-grassland ecological transition zone had strong homeostasis (Fig. 3). Therefore, Microbes could adjust their physiological metabolism to acquire low N and P resources, thereby acclimate to the arid and oligotrophic habitats.

Microbial TER values were used to identify microbial metabolic limitation at the microbial community level in the present study. As shown in Fig. 4, the N and P limitations in microbial communities were observed in the soils from different soil and vegetation types. First of all, soil available N and P were low in our study area, especially in the feldspathic sandstone areas (Table 3). Previous studies also indicated that nutrient availability is generally low in the Loess Plateau because of low primary productivity, high nutrient immobilization by calcium and magnesium, and high nutrient loss by soil erosion (Feng et al., 2013). Thus, low availability of soil nutrient is an important reason leading to microbial nutrient limitations. Additionally, our previous study indicated that microbial nutrient metabolisms in rhizosphere were co-limited by N and P in this area (Cui et al., 2018). The different characteristics of nutrient limitation between rhizosphere and bulk soil could be due to the effect of root systems on soil nutrients such as root exudates as well as the N and P competition between plants and microorganisms (Schimel and Parton, 1986). Microorganisms not only provide nutrients to plants but also compete for nutrients with roots under nutrient deficiency conditions (Inselsbacher et al., 2010; Sinsabaugh and Shah, 2011). The *A. ordosica* communities are gramineous plants with abundant root systems. The *P. tabuliformis* communities had greater aboveground biomass than the other two vegetation communities (Cui et al., 2018). Thus, the more nutrient requirement from these two vegetation communities could aggravate nutrient competition with soil microbial communities. As a result, the greatest microbial N limitation communities occurred in these sites (Fig. 4B). Furthermore, soil water deficiency in our study regions can also limit the transportation and availability of soil nutrients, which would intensify nutrient competition between plants and microbes in micro-environments (Ouyang et al., 2016). The deficiency of soil available nutrients and the nutrient competition between vegetation and microbial communities would also cause ecosystems degradation in the desert-grassland ecological transition zone.

4.2. Microbial nutrient limitation depend on key microbial taxa

Microbial community structures determine community functions and metabolic patterns (Sinsabaugh et al., 2012). The biomarkers led to the differences of microbial community have been identified among different sampling sites, and some identified biomarkers have shown significant correlation with microbial nutrient limitation (Fig. 5, S3 and S4). Our results showed that *Micrococcales*, *Micrococcaceae* and *Herpotrichiellaceae* were positively related to $TER_{C:N}$ (Fig. 6). Moreover, the three microbial taxa have been identified as the biomarkers of the three vegetation communities (Aeolian sandy soil with *P. tabuliformis* community, Feldspathic sandstone with *A. cristatum* community, and Loess soil with *A. cristatum* community, respectively) (Fig. 5A). Meanwhile, the three vegetation communities showed higher $TER_{C:N}$ values than other vegetation types (Fig. 4A), which indicated that *Micrococcales*, *Micrococcaceae* and *Herpotrichiellaceae* had strong effect on microbial N metabolism. Thus these three microbial taxa can be used as the biomarkers of microbial N limitation. In addition, one identified biomarker (*Thermoleophilia*) from the *A. ordosica* community in Feldspathic sandstone was positively related to $TER_{C:P}$ (Fig. 6D), as well as the *A. ordosica* community from Feldspathic sandstone showed relative high $TER_{C:P}$ values (Fig. 4C and 5A), which indicated that the microbial taxa from *Thermoleophilia* had strong effect on microbial P metabolism. Therefore, our results suggest that the biomarkers leading to the variation of microbial community structures could be the key microbial taxa causing microbial N and P limitations.

4.3. Microorganisms alleviating nutrient limitation mechanism

Microbial communities were totally different among the different sampling sites according to the microbial clades (biomarkers) identified via LEfSe analysis (Fig. 5). In the present study, the fungal communities were mainly affected by vegetation characteristics (biomass, S_{plant}, and H_{palnt}) and soil physical properties (Fig. 2). Kyaschenko et al. (2017) suggested that vegetation strongly affected fungal communities, as well as fungus played an important role in regulating plant productivity and soil C storage during forestry rotation cycles. Fungus participated in the first stage of litter decomposition, which could be greatly affected by vegetation types and litter quantity (Pascoal and Cássio, 2004; Osono, 2007). Furthermore, in this study, the structure of bacterial communities were mainly affected by C, N and P resources (Fig. 2), and the availability of nutrient was affected by soil types (Table 2). Due to bacteria have preference to use available nutrients in soil (Smith et al., 2014), soil types could play more important role in regulating the structure of bacterial communities in this study.

The RDA showed that variations of soil microbial community structures were significantly correlated with soil physicochemical properties (Fig. 2). As shown in Fig. 2, the bacterial and fungal communities were both affected by soil pH values. This is consistent with Maspolim et al. (2015) and Nicol et al. (2008) studies which pH was the most important factor affecting microbial community structures and activities. Moreover, the content of NH_4^+-N was significantly correlated with the relative abundances of bacterial communities in our study, whereas NO_3^--N had a significant influence on the fungal communities (Fig. 2). Boyle et al. (2008) found that bacteria are a major sink for mineral NH_4^+-N in forest soils and NH_4^+-N is the main nitrogen source for bacteria via ^{15}N isotope tracing. Bottomley et al. (2012) also observed that soil NH_4^+-N was a dominant environmental factor to affect bacterial community structures. Hence, the bacterial and fungal taxa likely acquire N from different N forms in arid and oligotrophic ecosystems. Additionally, other soil properties such as SOC, DOC, Olsen-P and TP also had different influence on bacterial and fungal community structures (Fig. 2). Therefore, the microbial taxa in the arid and oligotrophic ecosystems have potentially nutrient preferences, and thus adapt the resource deficiency and environmental stress. This conclusion is also supported by the opinions of horizontal transfer of genes and niche differentiations. Rensing et al. (2002) reported that the horizontal transfer of genes is an important adaptive mechanism for microbes to resist environmental pressure, which helps create a new ecological niche that allows the adaptation of soil microbial communities under environmental pressure. In the present study, our results highlighted that the remarkable nutrient preferences of different microbial taxa are important to maintain the stability of microbial community in arid land ecosystems.

5. Conclusions

In this study, we illuminated that the microbial communities were limited by N or P under the different soil types and vegetation in the desert-grassland ecological transition zone. Furthermore, the four biomarkers (such as *Micrococcales*) related to TER could represent the key microbial taxa to shape microbial communities and functions (nutrient limitation). The different responses of bacterial taxa and fungal taxa to soil nutrients demonstrated that soil microorganisms have

distinct nutrient preferences, and thus adapt the resource deficiency and environmental stress in the arid and oligotrophic ecosystems. This study has an insight into the association of microbial nutrient limitations with microbial community structures and how microbial communities respond to nutrient limitations in arid and semi-arid ecosystems.

Acknowledgements

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Appendix A. Supplementary data

Supplementary information provides additional tables showing diversity and richness index of the microbial communities in different sampling sites, correlation and stepwise regression analysis among soil physiochemical properties and microorganism group, comparison of the intercepts and slopes of enzymatic activity relationships for different sampling sites using standardized major axis (Type II) regression, displaying location and map of the sampling sites, rarefaction curve analysis and linear discriminant analysis (LDA). Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.scitotenv.2018.06.033.

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