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# Ecoenzymatic stoichiometry and microbial nutrient limitation in rhizosphere soil in the arid area of the northern Loess Plateau, China

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ABSTRACT

Arid ecosystems are characterized as having stressful conditions of low energy and nutrient availability for soil microorganisms and vegetation. The rhizosphere serves as the one of most active microorganism habitats, however, the general understanding of the ecoenzymatic stoichiometry (exoenzymes) and microbial nutrient acquisition in rhizosphere soil is limited. Here, we investigated the vegetation communities and determined the soil physicochemical properties, microbial biomass, and enzymatic activities in rhizosphere under different vegetation and soil types in the arid area of the northern Loess Plateau. Type II standard major axis (SMA) regression analysis showed that the plants played a more important role than soil properties in determining ecoenzymatic stoichiometry. Linear regression analysis displayed a microbial stoichiometric homeostasis (community-level) in rhizosphere. The Threshold Elemental Ratio (TER) revealed that the microbial nutrient metabolisms of rhizosphere were co-limited by N and P in the *A. ordosica* and *A. cristatum* communities of loess, and *A. cristatum* communities of feldspathic sandstone weathered soil. Binding spatial ordination analysis (RDA and CCA) demonstrated that soil physical properties (e.g., soil moisture, silt and clay contents) have more contribution to ecoenzymatic stoichiometry than the other investigated soil parameters, whereas soil nutrients (e.g., total organic carbon, nitrogen, and phosphorus) predominantly controlled microbial nutrient ratios. Therefore, the ecoenzymatic stoichiometry in rhizosphere is greatly regulated by plants and soil physical properties. The microbial N and P are co-limited under Gramineae plant in loess and feldspathic sandstone weathered soil regions. Meanwhile, the microbial nutrient limitation is mainly affected by soil nutrient supply. These findings could be crucial for illuminating rhizosphere microbial metabolism and revealing the nutrient cycling of root-soil interface under arid and oligotrophic ecosystems.

## 1. Introduction

The Loess Plateau is one of the most eroded regions in China and has some of the most vulnerable ecological systems in the world (Li et al., 2011). The northern region of Loess Plateau is a prairie desert transition zone (Wen et al., 2007) and is a typical dryland (Noymeir, 2003; Pointing and Belnap, 2012). Estimates of carbon storage for dryland regions indicate that they possess 36% of the total carbon storage worldwide (Campbell et al., 2008). The main vegetation types in the Loess Plateau are desert grasslands, which represent an important pool (8%) of global carbon (C) reservoirs. In desert grasslands, the major inputs of soil organic matter (SOM) are derived from underground biomass (root systems), rather than aerial biomass. The underground biomass also provides the principal source of soil nitrogen and phosphorus to the aerial biomass (Sims, 1978). Therefore, soil nutrient turnover and its availability in rhizosphere soils are critical for plant survival and ecosystem stabilization in ecological critical zones.

Nutrient turnover is mainly driven by microorganisms through SOM decomposition, but arid ecosystems are usually characterized by low energy and nutrient availability for soil microorganisms (Schimel et al., 2007). Due to the low water availability of these regions, the decomposition efficiency is slower than that of humid regions (Burke et al.,

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1998), thus soil nutrient availability often limits both primary productivity and microbial growth (Bünemann et al., 2012; Xu et al., 2015). For example, soil phosphorus derived from plant residues can easily form an insoluble substance with calcium and magnesium. Thus, organic and occluded P become the dominant forms in the soil (Cross and Schlesinger, 2001). The decomposition of soil organic compounds can provide energy to microorganisms, making microbial nutrient acquisition especially relevant to soil carbon processing in dryland regions, which renders the transformation and metabolism of microorganisms in the soil crucial for the improvement of nutrient availability.

The transformation of SOM is mainly conducted by the ecoenzymes of heterotrophic microorganisms that cleave organic molecules to allow the assimilation of C, N, and P (Waring et al., 2014). Ecoenzyme biosynthesis responds to environmental signals such as nutrient availability, but they can also be released into the soil via microbial cell lysis. Several ecoenzymes have been identified as useful indicators of nutrient deficiency and microbial nutrient demand, since they are major drivers of C and nutrient turnover in different ecosystems. β-1,4-glucosidase (BG), β-1,4-N-acetylglucosaminidase (NAG), and acid or alkaline phosphatase (AP) can serve as indicators of energy (C) demand, N demand, and P demand, respectively (Schimel and Weintraub, 2003), since they catalyze terminal reactions that produce assimilable molecules containing C, N, and P from high weight molecular organic compounds (Sinsabaugh et al., 2009).

The rhizosphere soil is the most active microorganism habitat with very high ecoenzymatic activities (Gartner et al., 2012). The cycling of nutrients between the soil, microbes, and plants of the rhizosphere is mediated by enzymes that are produced to depolymerize organic substrates (Sterner and Elser, 2002b; Bell et al., 2013). Studies have shown that ecoenzymes are not only produced by soil microorganisms, but also by plant root cells (Dakora and Phillips, 2002; Sinsabaugh, 2010). Nutrient cycles such as organic matter decomposition and N mineralization can be altered by the presence of plant roots (Cheng et al., 2003). Roots also affect the activity and composition of soil microbial communities through altering soil physical properties during plant growth (Bird et al., 2011). Therefore, understanding the ecoenzymatic stoichiometry and the pattern of nutrient turnover involving microbes in rhizosphere soil is vitally important to achieve a better picture of soil nutrients cycling and availability in the ecological critical zone.

Soil microorganisms acclimate to stress by reassigning key resources to nutrient acquisition mechanisms, rather than growth (Schimel et al., 2007). While it has also been reported that the ratio of C:N:P in microbial biomass is relatively conserved across ecosystems compared to the ratio in the soil, the microbial biomass ratio could indicate how allocation shifts alter nutrient demand (Cleveland and Liptzin, 2007). According to Sinsabaugh et al. (2009), ecoenzyme activities are involved in an intersection of Ecological Stoichiometry Theory (EST) with the Metabolic Theory of Ecology (MTE), the combination of which can improve our understanding of energy and nutrient controls on microbial metabolism (Sinsabaugh et al., 2012). This intersection can be illuminated via the Threshold Elemental Ratio (TER), which defines the element ratios at which growth shifts between nutrient limitation (represented by N and P, at high C:N or C:P) and energy (represented by C, lower C:N or C:P) (Sterner and Elser, 2002a). Additionally, under EST, organisms can be characterized with respect to the strength of their stoichiometric homeostasis. When the stoichiometric composition of the organism does not vary with changes in resource stoichiometry, it is considered to be strictly homeostatic (Sterner and Elser, 2002a). Therefore, the application of those methods and models can assist to identify microbial metabolic limitation in the ecological critical zone.

In the present research, we hypothesized that: (1) rhizosphere ecoenzymatic stoichiometry is greatly affected by plant species because of the different root systems and their correspondingly physiological processes; and (2) microbial nutrient acquisition in rhizosphere is limited by N or/and P rather than by C due to the nutrients (N or/and P) competition between roots and microbes. Specially, factors shaping the ecoenzymatic stoichiometry and microbial nutrient limitation in rhizosphere were also investigated. Therefore, we studied the ecoenzymatic stoichiometry related to C, N, and P cycling, identified microbial nutrient limitation in the rhizosphere soil in the arid area of the northern Loess Plateau, China.

## 2. Materials and methods

### 2.1. Study site and sampling

This research was carried out in natural grassland and shrubland ecosystems. The sites were located in Zhun Geer county of the northern region of the Loess Plateau (latitude 40° 10′ to 39° 35′ N and longitude 110° 35′ to 111° 23′ E), China (Fig. S1). The mean annual temperature of this region 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 has arid and semi-arid climate zones and the mean annual precipitation is 390 mm, with over 60% falling between July and September. The dominant plant communities in the three sections are *Artemisia ordosica*, *Agropyron cristatum*, and *Pinu tabuliformis*.

Three soil types were selected as the field experiment areas (Fig. S1), which are aeolian sandy soil on the northern side, loess on the eastern side, and feldspathic sandstone weathered soil on the western side (Calcaric Cambisol, FAO classification). There were three sampling sites from each experiment area that include the *Artemisia ordosica*, *Agropyron cristatum*, and *Pinus tabuliformis* plant communities. The descriptions of each sampling site were shown in Table 1. Three 100 m × 100 m plots were established at each sampling site in August 2016. Five 1 m × 1 m (grass community) and 5 m × 5 m (shrub community) quadrats were randomly established in each plot for measuring the characteristics of the vegetation. Plant coverage, aboveground biomass, and maximum/mean height were separately measured for each species in each quadrat. The Shannon index of plant community ($H_{plant}$) was calculated (Tscherko et al., 2004) and the number of species was used to estimate the richness ($S_{plant}$).

**Table 1**
The geographical features of the sampling sites.

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

Soil samples were collected from each quadrat. Five randomly selected plants of each species were removed from their corresponding quadrat. The soil strongly adhering to the roots and collected within the space exploited by the roots was considered to be rhizosphere soil (Garcia et al., 2005). Each sample was divided into two parts, which one part was air-dried for analyzing physicochemical properties and another part was immediately passed through a 2-mm sieve and stored at 4 °C for the microbial biomass and enzyme activity analysis within two weeks. Meanwhile, bulk soil samples were also collected from each quadrat for the measurement of soil bulk density and soil moisture.

### 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. The 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 (Master-sizer 2000, Malvern, UK). In detail, about 2.5 g air-dried soil with 25 ml 10% $H_2O_2$ was boiled to remove SOM, and then 25 ml 10% HCl was added to remove carbonate. Afterwards, 25 ml distilled water was added and sank suspension for 48 h, and then removed supernatant liquid and injected 25 ml $(NaPO_3)_6$ (dispersant). After 5 min shaking solution was analyzed by laser particle size analyzer. Soil organic matter was analyzed using dichromate oxidation method; about 0.600 g air-dried soil with 5 ml 0.8 M $K_2Cr_2O_7$ and 5 ml $H_2SO_4$ was digested for 5 min at 170˜180 °C, and then was titrated by 0.2 M $FeSO_4$. Dissolved organic carbon was extracted with deionized water after shaking for 45 min 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). In detail, about 0.700 g air-dried soil with 1.85 g mixed catalyst ($K_2SO_4$: $CuSO_4$: Se = 100:10:1) and 5 ml $H_2SO_4$ was digested for 45 min at 385 °C, and then was titrated by 0.02 M HCl. $NO_3^-$-N and $NH_4^+$-N were measured using a Seal Auto Analyzer. Total phosphorus (TP) was determined by melt-molybdenum, antimony and scandium colorimetry, Olsen method was used to determine available phosphorus forms (Olsen-P) for plants (Olsen and Sommers, 1982). For determination of TP, 0.25 g air-dried soil with 2 ml $HClO_4$ and 3 ml $H_2SO_4$ was digested for 60 min at 120˜130 °C, and then diluted with water to 50 ml. After the overnight stratification of digestion liquid, 5 ml supernatant liquid was added with 5 ml Molybdenum antimony reagent and then added water to 50 ml. The solution was measured by ultraviolet spectrophotometer (Hitachi UV2300) at 700 nm. For determination of Olsen-P, 2.500 g air-dried soil with 50 ml 0.5 M $NaHCO_4$ and one spoon of non-phosphorus active carbon was shaken for 30 min at 25 °C, filtered, and then 10 ml filtrate was added with 5 ml Molybdenum antimony reagent and diluted with water to 25 ml. The solution was measured by ultraviolet spectrophotometer at 700 nm.

Microbial biomass for C, N, and P ($C_{mic}$, $N_{mic}$, $P_{mic}$) were analyzed by chloroform fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). To determine of $C_{mic}$ and $N_{mic}$, 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 $P_{mic}$, 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 $C_{mic}$, $N_{mic}$, and $P_{mic}$, respectively (Joergensen, 1996).

### 2.3. Enzyme activity assays

Three potential activities of C-acquiring enzyme (BG), N-acquiring enzyme (NAG), and organic P-acquiring enzyme (AP) were determined following modified methods (Tabatabai and Bremner, 1969; Eivazi and Tabatabai, 1988; Steinweg et al., 2012). β-1,4-glucosidase activity was measured based on the paranitrophenol concentration after the hydrolysis reaction. A total of 5 g of fresh soil with 20 ml buffer solution (pH = 6.0) and 5 ml of 25 mM *p*-nitrophenol glucopyranoside was incubated for 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). In order to measure β-1,4-N-acetylglucosaminidase activity, the procedure was the same as the β-1,4-glucosidase activity measure, 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 were incubated for 3 h at 37 °C. The suspension was filtered with 1 ml of potassium ferricyanide and 4-amino antipyrine as the color-developing agent, and the phenol concentration of the filtrate was measured at 578 nm (Hitachi UV2300). Enzyme activity 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.

### 2.4. Data analysis

Two-way ANOVA was used to examine the effects of vegetation communities and soil types on soil biochemistry and ecoenzymatic parameters, and then mean comparisons were performed with Tukey's multiple comparisons test ($p < 0.05$) using the R software package v.3.3.2. Data were $\log_e$-transformed prior to regression analysis to adhere to the conventions of stoichiometric analysis and to normalize variance (Sterner and Elser, 2002a). After that, relationships between ecoenzymatic activities were calculated with type II standard major axis (SMA) regression using the Smatr package in R. Furthermore, redundancy analysis (RDA) and canonical correspondence analysis (CCA) were used after the enzyme activity data underwent Hellinger transformation and environmental factor data was standardized, in an effort to determine the most significant factors that shaped soil ecoenzyme activities and ecoenzymatic stoichiometry using the Vegan package in R.

### 2.5. Stoichiometric homeostasis and threshold elemental ratio

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

$$H' = 1/m \quad (1)$$

In equation (1), $m$ is the slope of $\log_e C{:}N_R$ (resources) versus $\log_e C{:}N_B$ (microbial biomass) or slope of $\log_e C{:}P_R$ versus $\log_e C{:}P_B$ scatterplot. $H' \gg 1$ represents strong stoichiometric homeostasis, while H'

**Table 2**
The rhizosphere soil nutrients, microbial biomass and ecoenzyme activities quantified from different sampling sites.

| Parameters | Soil types | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Aeolian sandy soil | | | Loess | | | Feldspathic sandstone weathered soil | | |
| | *A. ordosica* | *A. cristatum* | *P. tabuliformis* | *A. ordosica* | *A. cristatum* | *P. tabuliformis* | *A. ordosica* | *A. cristatum* | *P. tabuliformis* |
| DOC (mg $kg^{-1}$)[a] | 35.03 ± 4.13Aa | 44.56 ± 2.81Ab | 42.12 ± 0.93Aa | 38.35 ± 3.30Aa | 39.61 ± 0.85Ab | 41.86 ± 0.42Aa | 44.98 ± 7.40Ba | 69.83 ± 6.98Aa | 38.19 ± 4.07Ba |
| AN (mg $kg^{-1}$)[b] | 5.71 ± 0.29Aa | 5.10 ± 0.13Ba | 2.30 ± 0.16Cb | 4.90 ± 0.06Ab | 5.15 ± 0.13Aa | 2.72 ± 0.11Bb | 4.50 ± 0.31Ab | 4.24 ± 0.19ABb | 3.82 ± 0.10Ba |
| Olsen-P (mg $kg^{-1}$)[c] | 3.05 ± 0.29Aa | 2.54 ± 0.13Ba | 2.98 ± 0.21Aa | 1.80 ± 0.08Bb | 2.46 ± 0.11Aa | 2.35 ± 0.09Ab | 1.41 ± 0.05Bb | 2.26 ± 0.14Aa | 1.47 ± 0.13Bc |
| $C_{mic}$ (mg $kg^{-1}$)[d] | 31.51 ± 4.03Aa | 27.50 ± 3.50Ab | 36.79 ± 3.43Ab | 28.06 ± 3.11Ca | 38.87 ± 3.74Ba | 73.99 ± 4.04Aa | 28.04 ± 2.12Ba | 26.64 ± 2.20Bb | 38.96 ± 2.28Ab |
| $N_{mic}$ (mg $kg^{-1}$)[e] | 3.77 ± 0.40Aa | 3.10 ± 0.32Ab | 4.24 ± 0.41Ab | 3.57 ± 0.25Ba | 3.54 ± 0.21Bab | 8.98 ± 0.73Aa | 3.74 ± 0.38Ba | 4.86 ± 0.54ABa | 5.38 ± 0.41Ab |
| $P_{mic}$ (mg $kg^{-1}$)[f] | 0.44 ± 0.04Aa | 0.25 ± 0.02Aa | 0.53 ± 0.05Ab | 0.56 ± 0.04Ba | 0.50.042Ba | 1.20 ± 0.14Aa | 0.32 ± 0.03Aa | 0.51 ± 0.03Aa | 0.42 ± 0.03Ab |
| BG (nmol $g^{-1}$ $h^{-1}$)[g] | 20.42 ± 0.42Ab | 19.95 ± 1.07Ab | 17.11 ± 0.90Aa | 16.10 ± 1.18Ab | 19.26 ± 1.06Ab | 20.24 ± 0.91Aa | 26.94 ± 0.62Ba | 39.25 ± 1.81Aa | 18.38 ± 0.31Ca |
| NAG (nmol $g^{-1}$ $h^{-1}$)[h] | 8.53 ± 0.50Ba | 15.07 ± 0.96Aa | 10.87 ± 0.37Ba | 7.18 ± 0.63Ba | 12.57 ± 0.33Aa | 8.60 ± 0.55Ba | 5.83 ± 0.55Ba | 14.05 ± 0.30Aa | 10.87 ± 0.38Aa |
| AP (nmol $g^{-1}$ $h^{-1}$)[i] | 13.40 ± 0.54ABa | 16.62 ± 0.40Aa | 11.18 ± 0.43Ba | 8.63 ± 0.33Ab | 8.43 ± 0.32Ab | 11.15 ± 0.51Aa | 9.10 ± 0.09Bb | 14.83 ± 1.50Aa | 8.98 ± 0.56Ba |
| DOC:AN | 6.15 ± 0.84Ba | 8.73 ± 0.34Bb | 18.38 ± 0.88Aa | 7.84 ± 0.76Ba | 7.69 ± 0.31Bb | 15.40 ± 0.50Aa | 9.96 ± 1.14Ba | 16.62 ± 0.76Aa | 9.98 ± 0.87Bb |
| DOC: Olsen-P | 11.64 ± 1.31Ac | 17.58 ± 1.43Ab | 14.20 ± 1.05Ab | 21.31 ± 1.00Ab | 16.14 ± 0.73Ab | 17.84 ± 0.63Ab | 31.95 ± 1.64Aa | 30.69 ± 1.82Aa | 26.08 ± 1.33Aa |
| AN: Olsen-P | 1.88 ± 0.22Ac | 2.01 ± 0.10Aa | 0.77 ± 0.06Bb | 2.73 ± 0.15Ab | 2.10 ± 0.14Ba | 1.16 ± 0.02Cb | 3.20 ± 0.12Aa | 1.88 ± 0.19Ca | 2.61 ± 0.26Ba |
| C: $N_{mic}$ | 8.48 ± 0.49Aa | 8.97 ± 0.22Ab | 8.72 ± 1.01Aa | 7.87 ± 0.46Ba | 10.99 ± 0.42Aa | 8.26 ± 0.24Ba | 7.51 ± 0.19Aa | 5.47 ± 0.21Bc | 7.28 ± 0.14ABa |
| C: $P_{mic}$ | 73.09 ± 11.6Bab | 113.58 ± 7.7Aa | 69.96 ± 1.6Ba | 50.81 ± 9.7Ab | 74.85 ± 1.7Ab | 63.72 ± 6.7Aa | 89.12 ± 3.4ABa | 52.98 ± 4.1Bb | 93.54 ± 2.4Aa |
| N: $P_{mic}$ | 8.80 ± 0.95Aab | 12.75 ± 0.64Aa | 8.16 ± 0.92Aa | 6.46 ± 0.95Ab | 6.82 ± 0.20Ab | 7.76 ± 0.71Aa | 11.90 ± 0.37Aa | 9.65 ± 1.06Aab | 12.86 ± 0.12Aa |
| BG:NAG | 2.43 ± 0.44Ab | 1.32 ± 0.08Ab | 1.60 ± 0.32Aa | 2.29 ± 0.47Ab | 1.53 ± 0.13Aab | 2.36 ± 0.15Aa | 4.79 ± 1.09Aa | 2.80 ± 0.30Ba | 1.70 ± 0.26Ba |
| BG:AP | 1.52 ± 0.09Ab | 1.21 ± 0.24Ab | 1.54 ± 0.20Aa | 1.92 ± 0.48Ab | 2.32 ± 0.54Aa | 1.81 ± 0.07Aa | 3.00 ± 0.33Aa | 2.65 ± 0.09ABa | 2.06 ± 0.11Ba |
| NAG:AP | 0.64 ± 0.10Aa | 0.91 ± 0.14Ab | 0.97 ± 0.10Aa | 0.85 ± 0.22Ba | 1.51 ± 0.28Aa | 0.77 ± 0.07Ba | 0.66 ± 0.04Ba | 0.95 ± 0.12ABb | 1.23 ± 0.14Aa |

**Note:** Values followed horizontally by a different uppercase letter (A, B, and C) indicate that means are significantly different ($P < 0.05$) among different vegetation types (*A. ordosica*, *A. cristatum*, and *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.

[a] Dissolved organic carbon.
[b] Mineral nitrogen.
[c] Olsen phosphorus.
[d] Microbial biomass carbon.
[e] Microbial biomass nitrogen.
[f] Microbial biomass phosphorus.
[g] β-1,4-Glucosidase.
[h] β-1,4-N-acetylglucosaminidase.
[i] Alkaline phosphatase.

**Table 3**
Summary statistics (F statistic and probability level) of a two-way ANOVA on the effects of soil types and vegetation communities on soil nutrients properties and microbial biomass nutrients.

| Parameters | Source of variation | | |
|---|---|---|---|
| | Vegetation | Soil types | Soil types × vegetation |
| DOC[a] | 8.67 (0.002) | 7.86 (0.004) | 6.03 (0.003) |
| AN[b] | 360 (< 0.001) | 2.24 (0.136) ns | 56.0 (< 0.001) |
| Olsen-P[c] | 11.0 (< 0.001) | 129 (< 0.001) | 22.4 (< 0.001) |
| $C_{mic}$[d] | 92.9 (< 0.001) | 55.9 (< 0.001) | 29.3 (< 0.001) |
| $N_{mic}$[e] | 55.5 (< 0.001) | 19.4 (< 0.001) | 22.8 (< 0.001) |
| $P_{mic}$[f] | 24.4 (< 0.001) | 37.3 (< 0.001) | 14.0 (< 0.001) |
| BG[g] | 29.5(< 0.001) | 58.0 (< 0.001) | 24.3 (< 0.001) |
| NAG[h] | 70.0 (< 0.001) | 6.53 (0.007) | 2.01 (0.136)[ns] |
| AP[i] | 16.9 (< 0.001) | 29.3 (< 0.001) | 12.8 (< 0.001) |
| DOC:AN | 32.6 (< 0.001) | 2.64 (0.1)[ns] | 21.3 (< 0.001) |
| DOC: Olsen-P | 1.67 (0.215) [ns] | 64.5 (< 0.001) | 3.62 (0.025) |
| AN: Olsen-P | 108 (< 0.001) | 93.3 (< 0.001) | 38.4 (< 0.001) |
| C: $N_{mic}$ | 1.49 (0.251)[ns] | 30.9 (< 0.001) | 13.4 (< 0.001) |
| C: $P_{mic}$ | 1.22 (0.320)[ns] | 7.14 (0.005) | 10.5 (< 0.001) |
| N: $P_{mic}$ | 0.37 (0.697)[ns] | 14.4 (< 0.001) | 4.22 (0.014) |
| BG:NAG | 23.4 (< 0.001) | 20.5 (< 0.001) | 9.55 (< 0.001) |
| BG:AP | 3.36 (0.058)[ns] | 34.6 (< 0.001) | 4.19 (0.014) |
| NAG:AP | 11.7 (< 0.001) | 2.84 (0.085) | 6.66 (0.002) |

**Note:** *P* values are in parenthesis, ns = not significant.
[a] Dissolved organic carbon.
[b] Mineral nitrogen.
[c] Olsen phosphorus.
[d] Microbial biomass carbon.
[e] Microbial biomass nitrogen.
[f] Microbial biomass phosphorus.
[g] β-1,4-Glucosidase.
[h] β-1,4-N-acetylglucosaminidase.
[i] Alkaline phosphatase.

≈ 1 represents weak or no homeostasis (Sterner and Elser, 2002a).

In order to connect the measured ecoenzymatic activities with EST and MTE, we followed the method published by Sinsabaugh et al. (2009), to calculate the TER for C:N and C:P using the following equations:

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

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

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

## 3. Results

### 3.1. Vegetation characteristics and soil physiochemical properties

There were no significant differences between the vegetation community coverage and Shannon diversity index of the plant community ($H_{plant}$) among the sampling sites. The biomass was significantly higher in *P. tabuliformis* communities than those in *A. ordosica* and *A. cristatum* communities. The vegetation of *A. ordosica* communities had the maximum species richness ($S_{plant}$) (15 ± 1). In a comparison of the different soil types, the biomass and $S_{plant}$ from aeolian sandy soil had the highest values (Table S1). Overall, the characteristics of the vegetation among the different sampling sites were similar except for the vegetation biomass.

In terms of soil physicochemical properties (Table S2), soils from *A. cristatum* communities displayed higher SOC contents (1.5 times) than those from *A. ordosica* and *P. tabuliformis* communities except for the loess soil. In all sampling sites, the highest SOC (4.27 ± 0.27 g $kg^{-1}$) was observed in the feldspathic sandstone weathered soil with *A. cristatum* communities, while the feldspathic sandstone weathered soil with *A. ordosica* communities had the lowest SOC (2.50 ± 0.11 g $kg^{-1}$). The TN in feldspathic sandstone weathered soil with *A. cristatum* communities was significantly higher (0.52 ± 0.04 g $kg^{-1}$) than those in the aeolian sandy and loess soils under same vegetation. Also, it was significantly greater than those in the other vegetation with same soil type ($P < 0.05$) (Table S2). TP was varied among vegetation and soil types, the loess with *A. ordosica* and *A. cristatum* communities showed the highest TP contents (0.50 ± 0.01 g $kg^{-1}$) (Table S2). Soil pH showed alkaline soil in the sampling sites, with the highest value (9.07 ± 0.02) in the loess soil with *A. cristatum* communities and the lowest value (8.55 ± 0.03) in the feldspathic sandstone weathered soil with *A. ordosica* communities. Soil from *A. cristatum* communities had lower bulk density than those from *A. ordosica* and *P. tabuliformis* communities. The highest (1.63 ± 0.04 g $cm^{-3}$) and lowest (1.23 ± 0.05 g $cm^{-3}$) bulk density occurred in the feldspathic sandstone weathered soil with *P. tabuliformis* communities and the loess with *A. cristatum* communities, respectively. For particle composition, the feldspathic sandstone weathered soil had 1.7 times higher clay contents (≤0.002 mm) and 1.4 times higher silt particles (0.002–0.02 mm) than aeolian sandy soil and loess, while the aeolian sandy soil had the highest sand contents (≥0.02 mm).

### 3.2. Soil available nutrients and microbial biomass nutrients

The soil from *A. cristatum* communities showed 1.5 times higher DOC contents than those from *A. ordosica* and *P. tabuliformis* communities in the feldspathic sandstone weathered soil site. The lowest (35.03 ± 4.13 mg $kg^{-1}$) and the highest (69.83 ± 6.98 mg $kg^{-1}$) DOC contents occurred in the aeolian sandy soil with *A. ordosica* communities and the feldspathic sandstone weathered soil with *A. cristatum* communities, respectively (Tables 2 and 3). Generally, the soils from feldspathic sandstone weathered soil site had higher DOC contents compared to the other soil types. Mineral nitrogen (AN) contents were significantly different among the three vegetation types ($P < 0.05$), the highest value (5.71 ± 0.29 mg $kg^{-1}$) was found in the aeolian sandy soil with *A. ordosica* communities (Tables 2 and 3). Regardless of the vegetation, the aeolian sandy soil had higher Olsen-P contents (range from 2.54 ± 0.13 to 3.05 ± 0.29 mg $kg^{-1}$) compared with the other two soil types (Tables 2 and 3). Meanwhile, the ratios of DOC:AN, DOC:Olsen-P, and AN:Olsen-P were varied significantly among all soil and vegetation types. The feldspathic sandstone weathered soils had higher DOC:Olsen-P than the other two soil types.

Soils from *P. tabuliformis* communities had significantly greater $C_{mic}$ than the soils from *A. ordosica* and *A. cristatum* at the loess and feldspathic sandstone weathered soil sites. Additionally, the values of soil $N_{mic}$ and $P_{mic}$ were significantly higher in *P. tabuliformis* communities than the other vegetation at the loess sites ($P < 0.05$) (Tables 2 and 3). The loess with *A. cristatum* communities had significantly greater C:$N_{mic}$ ratio than those from *A. ordosica and P. tabuliformis* communities, whereas the C: $N_{mic}$ ratio in the soil from *A. cristatum* communities had significantly lower than those from the other vegetation at the feldspathic sandstone weathered soil site. In contrast, N:$P_{mic}$ ratios in aeolian sandy soil were higher than in other two soil types. The highest C:$P_{mic}$ ratio (114 ± 7.7) was observed in the aeolian sandy soil with *A. cristatum* communities, which was significantly greater than those from the other vegetation communities at the same soil site. However, at the feldspathic sandstone weathered soil site, the soil from *A. cristatum* communities had significantly lower C:$P_{mic}$ ratio than those from *P. tabuliformis* communities. Although there were no significant differences in N:$P_{mic}$ ratios among the vegetation communities under each

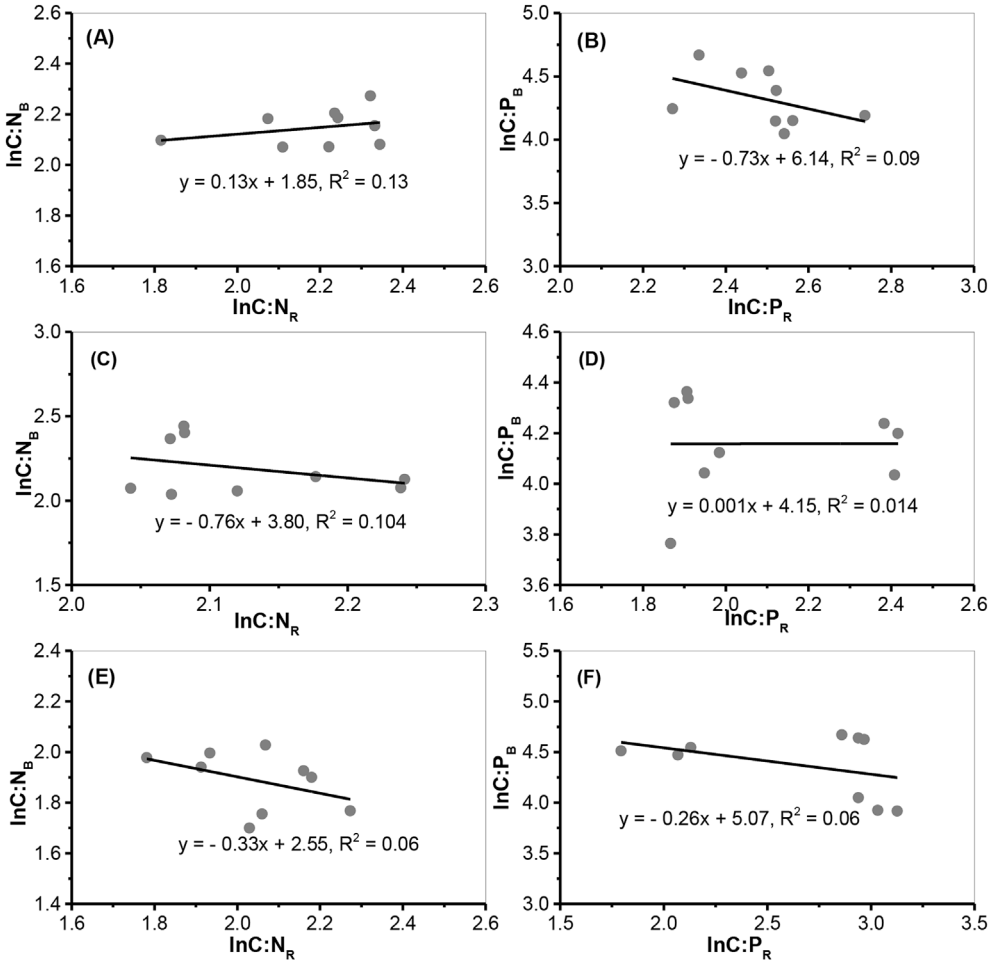


**Fig. 1.** Soil microbial community homeostasis related to nitrogen (N: panels on left) and phosphorus (P: panels on right) acquisition. (A) and (B) data from aeolian sandy soil, (C) and (D) data from loess, (E) and (F) data from feldspathic sandstone weathered soil (slopes are not different from zero, (A) $P = 0.186$, (B) $P = 0.229$, (C) $P = 0.215$, (D) $P = 0.395$, (E) $P = 0.264$, (F) $P = 0.264$).

soil types, the loess soils had lower N:$P_{mic}$ ratios than the other soil types regardless of the vegetation (Tables 2 and 3).

### 3.3. Ecoenzymatic stoichiometry

β-1,4-glucosidase (BG) activity was significantly different among the soil types as well as among the vegetation communities. The feldspathic sandstone weathered soil with *A. cristatum* communities showed the highest activity (39.25 ± 1.81 nmol $g^{-1}$ $h^{-1}$), while the loess soil with *A. ordosica* communities had the lowest activity (16.1 ± 1.18 nmol $g^{-1}$ $h^{-1}$) (Tables 2 and 3). β-1,4-N-acetylglucosaminidase (NAG) was significantly different among the vegetation types ($P < 0.05$). The NAG activity in the aeolian sandy soil with *A. cristatum* communities had the highest value (15.07 ± 0.96 nmol $g^{-1}$ $h^{-1}$), while the lowest value (5.83 ± 0.55 nmol $g^{-1}$ $h^{-1}$) showed in the feldspathic sandstone weathered soil with *A. ordosica* communities. Alkaline phosphatase (AP) activity was significantly different among all sampling sites. The highest AP activity (16.62 ± 0.40 nmol $g^{-1}$ $h^{-1}$) showed in the aeolian sandy soil with *A. cristatum* communities, while the lowest value (8.43 ± 0.32 nmol $g^{-1}$ $h^{-1}$) displayed in the loess with *A. cristatum* communities ($P < 0.05$) (Tables 2 and 3).

The highest ratios for BG:NAG (4.79 ± 1.09) and BG:AP (3.0 ± 0.33) occurred in the feldspathic sandstone weathered soil with *A. ordosica* communities (Table 2). The ratios of BG:NAG and BG:AP in the aeolian sandy soil were the lowest values(1.32 ± 0.08 and 1.21 ± 0.24 nmol $g^{-1}$ $h^{-1}$) (Table 2). The ratios were of significantly affected by the vegetation types. The highest NAG:AP ratio (1.51 ± 0.28 nmol $g^{-1}$ $h^{-1}$) was in the loess with *A. cristatum* communities ($P < 0.05$) (Tables 2 and 3).

The analysis of model II regressions showed significant differences among soil types ($P < 0.05$). The slopes of regression analysis represented the effect degree of vegetation or soil types on enzymatic stoichiometry. The slopes are more deviating from 1, which indicated the more effect of vegetation or soil on ln(BG) vs ln(NAG) or ln(BG) vs ln(AP) (Sinsabaugh et al., 2009; Tapia-Torres et al., 2015). At the aeolian sandy soil site, the soil from *P. tabuliformis* communities had the steepest slopes for ln(BG) vs. ln(AP) and ln(NAG) vs. ln(AP) (1.39 and 1.00, respectively), and soil from *A. cristatum* communities had the steepest slope for ln(BG) vs. ln(NAG) (1.44) (Table S3). Similarly, soil from *P. tabuliformis* communities in loess had the steepest slopes for ln (BG) vs. ln(NAG), ln(BG) vs. ln(AP), and ln(NAG) vs. ln(AP) (0.72, −1.12, and 1.56, respectively) (Table S4). In contrast, soil from *A. ordosica* communities in feldspathic sandstone weathered soil had the steepest slopes for ln(BG) vs. ln(NAG), ln(BG) vs. ln(AP), and ln(NAG) vs. ln(AP) (0.22, 2.36, and 10.99, respectively) (Table S5). Compared with the other soil types, feldspathic sandstone weathered soil had the steepest slopes for ln(BG) vs. ln(NAG), ln(BG) vs. ln(AP), and ln(NAG) vs. ln(AP) (0.73, 1.26, and 1.72, respectively) (Table S6). When the data were analyzed for the three vegetation types, *A. cristatum* communities had the steepest slopes for ln(BG) vs. ln(NAG) and ln(BG) vs. ln(AP) (3.61 and 1.17, respectively), and soil from *A. ordosica* communities had the steepest slope for ln(NAG) vs. ln(AP) (1.12) (Table S7). Those results indicated that the slopes of ecoenzymatic stoichiometry of rhizosphere soils for organic N and organic P acquisition both scale with C acquisition are significantly different from 1. However, previous studies indicated that the slopes of ecoenzymatic stoichiometry in bulk soil are close to 1 under different ecosystems on a global

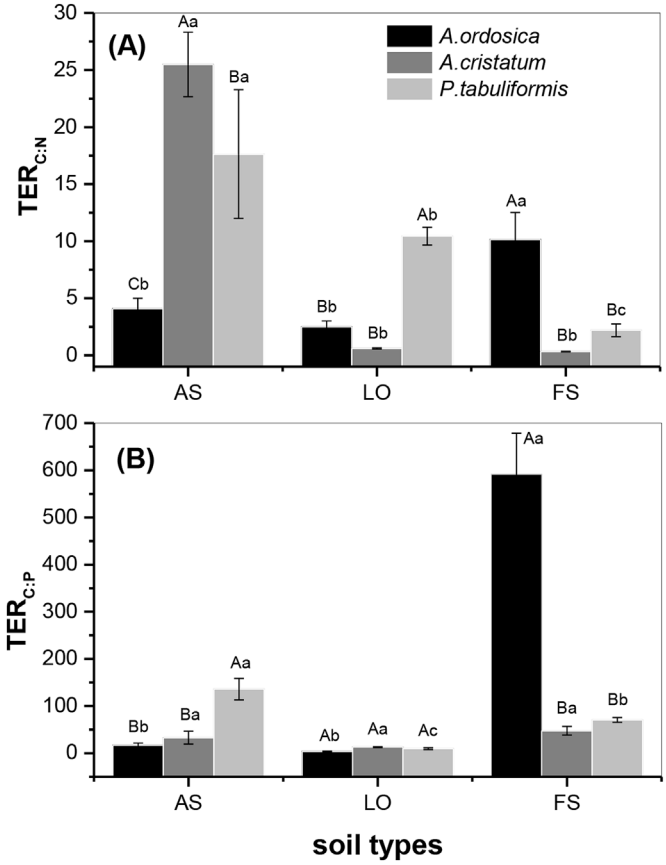


**Fig. 2.** Threshold Elemental Ratio $(TER)_{C:N}$ (A) and $(TER)_{C:P}$ (B) of soil microbial community from different soil sites. AS: aeolian sandy soil, LO: loess, FS: feldspathic sandstone weathered soil. Different uppercase letters (A, B, and C) indicate that means are significantly different ($P < 0.05$) among different vegetation types (*A. ordosica*, *A. cristatum*, and *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.

scale (Sinsabaugh et al., 2009; Tapia-Torres et al., 2015). Therefore, our results suggest ecoenzymatic stoichiometry of rhizosphere could be greatly affected by vegetation.

### 3.4. Stoichiometric homeostasis and threshold elemental ratios

In order to test the strength of stoichiometric homeostasis, we analyzed the associations between microbial biomass elemental ratios and nutrient ratios in soil resources. When all the data were analyzed together for the three soil types, 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 the three soil types (Fig. 1).

Based on the microbial biomass C:N:P stoichiometric values and parameters generated from the enzymatic data, the estimated $TER_{C:N}$ values in the aeolian sandy soil are significantly higher than those in loess and feldspathic sandstone weathered soil except for the soils from *A. ordosica* communities. The highest $TER_{C:N}$ ($25.5 \pm 0.82$) is in the aeolian sandy soil with *A. cristatum* communities (Fig. 2A). In the feldspathic sandstone weathered soils, both $TER_{C:N}$ and $TER_{C:P}$ in the soils from *A. ordosica* communities were significantly greater than those in the soils from the other two vegetation communities. There were no significant differences in $TER_{C:P}$ from *A. cristatum* communities among soil types. However, the $TER_{C:P}$ in the feldspathic sandstone weathered soil with *A. ordosica* communities was significantly greater than those in the other soils with same vegetation, whereas the $TER_{C:P}$ in the aeolian sandy soil with *P. tabuliformis* communities was significantly greater than those in the other soils with same vegetation($P < 0.05$) (Fig. 2B).

### 3.5. Relationships among ecoenzymatic stoichiometry, microbial nutrients, plant communities, and soil properties

The canonical correspondence analysis showed that variations in soil enzyme activities were well accounted (77.72%) by vegetation characteristics and soil physicochemical properties (Fig. 3). It showed strongly positive relationship between NAG and SOC as well as strongly negative relationship between BG and TP. Meanwhile, strongly negative relationships between $C_{mic}$ and TN, and between $P_{mic}$ and soil moisture were observed. Furthermore, the redundancy analysis identified that the soil physicochemical properties explained most of the variation (74.24%) in soil ecoenzymatic stoichiometry and microbial nutrient ratios (Fig. 4). The ratio of $C:N_{mic}$ was strongly positive correlated with TP and pH. The ratios of BG:NAG and BG:AP were positively correlated with soil moisture.

## 4. Discussion

### 4.1. The characteristics of ecoenzymatic stoichiometry in the rhizosphere

Previous studies have shown that the slopes of ecoenzymatic regressions can significantly change in various habitats (e.g., terrestrial soils vs. lotic and lentic sediments) (Sinsabaugh et al., 2009, 2012; Tapia-Torres et al., 2015; Peng and Wang, 2016). In the same habitat, C acquisition enzymes were shown to have similar scaling relationships as N and P acquisition enzymes (Tapia-Torres et al., 2015). However, our results showed significant change in the slopes of the ecoenzymatic stoichiometry regressions among different soil and vegetation types (Table S3 ~ S7). It should be noted that the feldspathic sandstone weathered soil displayed the maximal slopes of regression (Table S6), which indicated that ecoenzymatic stoichiometry might be greatly affected by the physicochemical properties in this soil type. Due to the high clay particle (20.4–32.1%) and montmorillonite content in the feldspathic sandstone weathered soil, the strong ability of adsorbing nutrients and heavy metals may also absorb the ecoenzymes and affect the ecoenzymatic stoichiometry in the present study (Ijagbemi et al., 2009; Zhen et al., 2015).

In terms of vegetation types, the maximal slopes were found in the soils from *A. cristatum* communities (Table S7). Most of previous studies were conducted in bulk soil, which could avoid or weaken the direct and indirect effects of plant roots on soil enzymes, such as microbial community function and expression of enzymes (Blagodatskaya et al., 2009; Razavi et al., 2016). However, our results showed that the N and P enzymes had very dissimilar scaling relationships compared with previous studies (Tapia-Torres et al., 2015; Peng and Wang, 2016), which suggested that the characteristic ecoenzymatic stoichiometry in rhizosphere soil was apparently different from bulk soil. Soil enzymes are mainly produced by soil microbes and plant root systems (Bohlen et al., 2008; Gianfreda, 2014), while different soil microbial communities have similar patterns of ecoenzymatic allocation to nutrient acquisition in different soil conditions (Sinsabaugh et al., 2009; Tapia-Torres et al., 2015). Moreover, soil microbes are capable of producing both acid and alkaline phosphatases, but plant root can only produce acid phosphatase (Juma and Tabatabai, 1988; Nannipieri et al., 2011). The alkaline phosphatase activity in rhizosphere is significantly different from bulk soil because of effect of roots on alkaline phosphatase activity (Spohn et al., 2013, 2015). Therefore, the differences in ecoenzymatic stoichiometry in our study may be attributable to the involvement of root systems.

In particular, the C vs. N and C vs. P enzyme regressions in *A. cristatum* communities were shown to have the maximum slopes (Table S7), which indicated that N and P-related enzyme activities were strongly affected by the root systems of *A. cristatum* communities. Previous studies showed that root systems could extremely effect

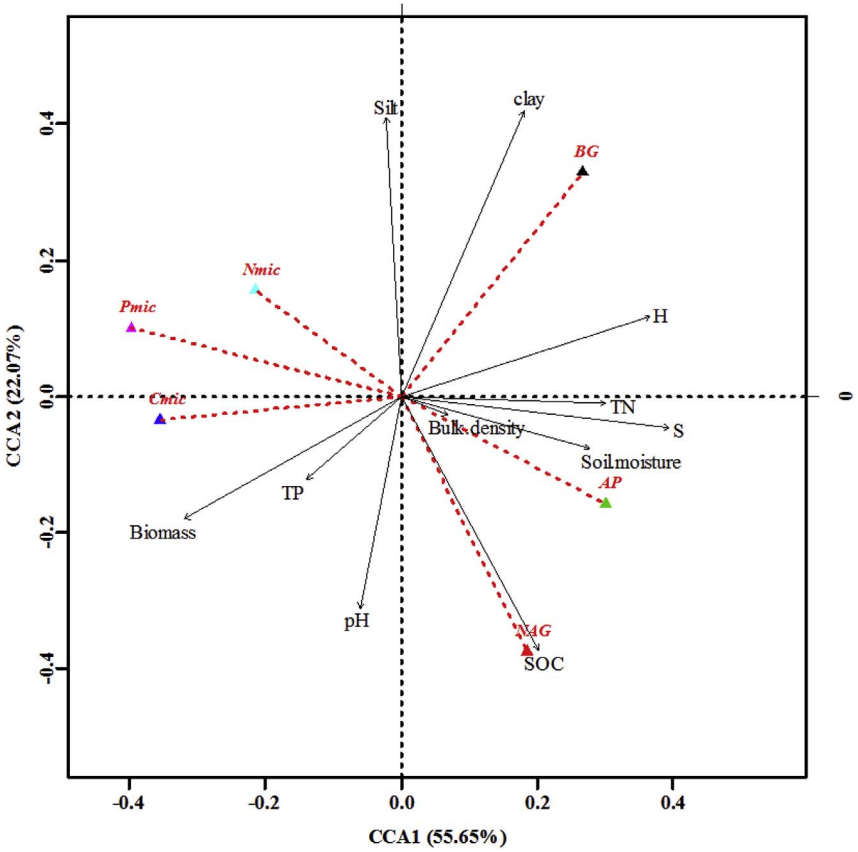


**Fig. 3.** The canonical correspondence analysis (CCA) used to identify the relationships between soil enzymes, microbial nutrients, vegetation characteristics, and soil properties. H: Shannon diversity index of the plant community, S: species richness of the plant community, Biomass: plant biomass, SOC: soil organic carbon, TN: total nitrogen, TP: total phosphorus, clay: soil clay content (%), silt: soil silt content (%), BG: β-1,4-Glucosidase, NAG: β-1,4-N-acetylglucosaminidase, AP: alkaline phosphatase, $C_{mic}$; Microbial biomass carbon, $N_{mic}$: Microbial biomass nitrogen, $P_{mic}$: Microbial biomass phosphorus.

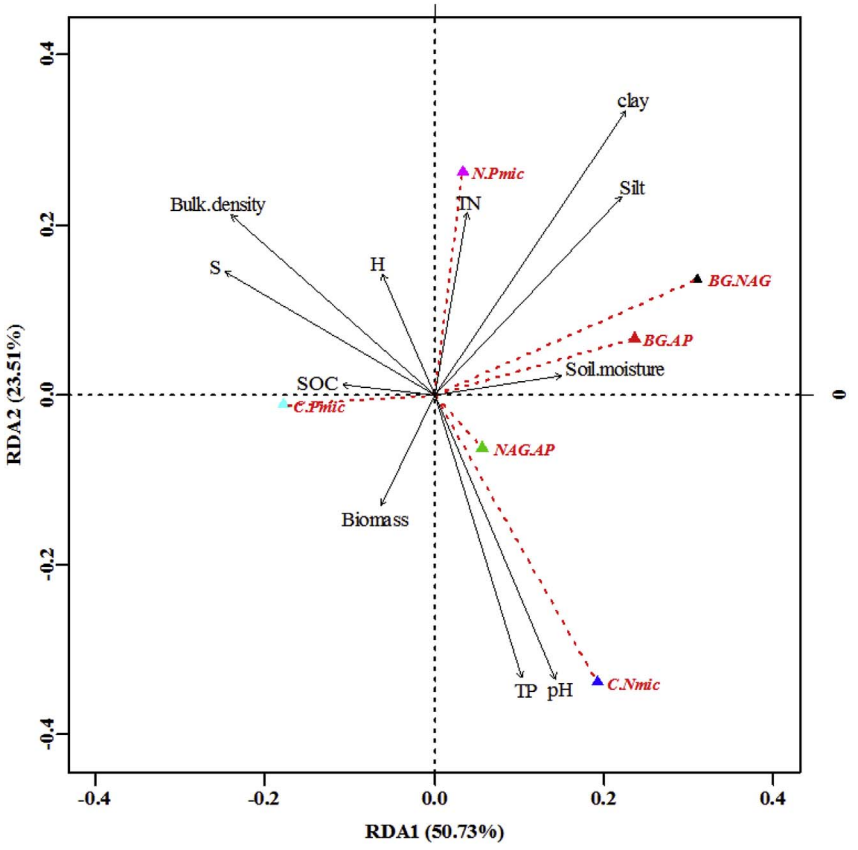


**Fig. 4.** The redundancy analysis (RDA) used to identify the relationship between the soil enzyme ratios, microbial nutrients ratios, vegetation characteristics, and soil properties. H: Shannon diversity index of the plant community, S: species richness of the plant community, Biomass: plant biomass, SOC: soil organic carbon, TN: total nitrogen, TP: total phosphorus, clay: soil clay content (%), silt: soil silt content (%), BG: β-1,4-Glucosidase, NAG: β-1,4-N-acetylglucosaminidase, AP: alkaline phosphatase, $C_{mic}$; Microbial biomass carbon, $N_{mic}$: Microbial biomass nitrogen, $P_{mic}$: Microbial biomass phosphorus.

microbial composition and expression of enzymes (Blagodatskaya et al., 2009; Razavi et al., 2016). Also, Dakora and Phillips (2002) suggested that root physiology and exudate constituents are the mediators of mineral acquisition in low-nutrient environments. Moreover, the gramineous plants (e.g., *A. cristatum*) usually have abundant fibrous root systems that are the most active parts of plant metabolism. The results in our study showed that the soils from *A. cristatum* communities had higher NAG and AP than other soils (Table 2), which may be caused from the roots of gramineous plants investing more extracellular enzymes to access their own required nutrients (Bell et al., 2013). Previous studies also showed that the root-produced ecoenzymes can enter the soil after root death (Matthiasc et al., 2007). The dead roots could change the levels of C, N, and P-cycled enzymes, which may lead to the differences of ecoenzymatic stoichiometry (Aerts et al., 1992; Spohn and Kuzyakov, 2014; Duyen and Razavi, 2016). Therefore, the vegetation play an important role in determining the soil ecoenzyme stoichiometry through direct effect of root systems (secreting exoenzyme) and indirect effect of root systems (affecting rhizosphere microbial community). The soil ecoenzyme stoichiometry exhibited distinct responses to different plant species. The communities of gramineous plants exhibited a greater effect on ecoenzyme stoichiometry compared with other vegetation communities, which suggested that the gramineous plants could have a greater potential ability to acquire soil nutrients in arid and oligotrophic ecosystems.

Compared with the effects of root system, the slopes of ecoenzymatic regressions had less variation among the three soil types (Table S6). Previous studies reported that the soil ecoenzymatic stoichiometry followed global patterns even in different soil types and climate conditions (Sinsabaugh et al., 2009; Waring et al., 2014; Tapia-Torres et al., 2015), which indicated that soil types had less impact on ecoenzymatic stoichiometry. Generally, soil conditions act on biochemical cycles by indirectly affecting plants and microbes. Enzyme activities may be varied with soil types, but the ecoenzymatic stoichiometry usually exhibits a consistent pattern around the world (Sinsabaugh et al., 2009). Interestingly, the feldspathic sandstone weathered soil displayed the greatest slopes (Table S6). Compared with aeolian sandy soil and loess, our previous research indicated that the feldspathic sandstone weathered soil had the higher clay particle (20.4–32.1%) and montmorillonite contents (Zhen et al., 2015). Those properties contributed to the soil enzyme stability, but the effect is discrepant on different kind of enzymes (Allison, 2006). Furthermore, the soil pH in feldspathic sandstone weathered soil was the lowest in our study. Sinsabaugh et al. (2008) suggested that soil pH had direct effects on the activity of the extracelluar enzymes, and it lead to enzymes having their own preferred pH. Therefore, those physicochemical properties affect the ecoenzymatic stoichiometry through affecting enzyme activities, which lead to feldspathic sandstone weathered soil exhibited different ecoenzymatic stoichiometry compared with aeolian sandy soil and loess.

Overall, rhizosphere as key microzone that build up a connection between plant and microbial communities are of crucial in regulating ecoenzymatic stoichiometry and nutrient cycling (Luster et al., 2009). In order to further clarify the effects of root systems and soil on ecoenzymes, therefore, more studies of the ecoenzymatic stoichiometry for rhizospheres and bulk soil under different vegetation and soil types are needed.

### 4.2. Limitation of microbial nutrients in the rhizosphere

Microbes are the major fabricants of soil ecoenzymes (Cleveland and Liptzin, 2007), especially in arid and oligotrophic ecosystems (e.g., the northern Loess Plateau), and their transformation to soil nutrients is critical for the maintenance of natural vegetation communities (Schimel and Parton, 1986; Bell et al., 2014; Peng and Wang, 2016). Despite conditions of strong nutrients and water limitation, our result still indicated that the soil microbial communities from rhizosphere soil have strong homeostasis (Fig. 1). Microbes can adjust their physiological metabolism to require low N and P resources, thereby acclimating to arid and oligotrophic habitats, and these physiological adjustments can be reflected in the degree of ecoenzyme expression (Sinsabaugh et al., 2009; Tapia-Torres et al., 2015). Generally, ecoenzyme expression is related to the quality of available organic matter and nutrient demands of the microbial biomass (Sinsabaugh et al., 2009; Sinsabaugh et al., 2015). Based on nutrient stoichiometry, the microbes of those sampling sites exhibited different nutrient limitation patterns. Microbes in the loess soil with *A. ordosica* and *A. cristatum* communities, and the feldspathic sandstone weathered soil with *A. cristatum* communities exhibited lower $B_{C:N}/R_{C:N}$ ratios (6.51, 9.17, and 13.67, respectively). This indicated that the N limitation of soil microbial community occurred in the loess with *A. ordosica* and *A. cristatum* communities (the most widely distributed soil in the Loess Plateau), and in the feldspathic sandstone weathered soil with *A. cristatum* communities (high clay and montmorillonite contents).

A similar pattern was observed for P limitation. Soil total P and Olsen-P showed low levels in our sampling sites (Tables S2 and 2). In addition, phosphorus may be strongly bound by calcium and magnesium ions in alkaline soils (Liu et al., 2013; Perroni et al., 2014), thus the soil phosphorus represent a critical nutrient constraint in the Loess Plateau. But its role in limiting microbial activity might vary in different sampling sites. Soil microbes in the loess soils with *A. ordosica* and *A. cristatum* communities, and the feldspathic sandstone weathered soil with *A. cristatum* communities exhibited lower $B_{C:P}/R_{C:P}$ ratios (5.01, 7.80, and 16.42, respectively), ($B_{C:P}$ is the C:P ratio of microbial biomass and $R_{C:P}$ is the C:P ratio of labile organic matter.) which indicated that P was limited in the microbial community from those soils. Meanwhile, previous studies showed that microbial nutrient ratios and ecoenzyme ratios had a coupling relationship (Sinsabaugh et al., 2015; Ayuso et al., 2017). However, our results showed that the variations of microbial nutrient ratios are apparently inconsistent with ecoenzyme ratios (Table 2). This might be explained by our above-mentioned conclusions that the ecoenzymatic stoichiometry of rhizosphere soil is greatly affected by root systems.

To better understand microbial metabolic limitations, ecoenzymatic data and elemental composition data were jointly analyzed to estimate microbial TER values at microbial community level in this arid and oligotrophic region. The site-specific contrasts were exhibited. Regarding to soil types, the lower values of estimated $TER_{C:N}$ and $TER_{C:P}$ occurred in the loess and feldspathic sandstone weathered soils, respectively. In different vegetation types, the lower values of estimated $TER_{C:N}$ and $TER_{C:P}$ were displayed in the soils from *A. ordosica* and *A. cristatum* communities, respectively (Fig. 2). The lower $TER_{C:N}$ and $TER_{C:P}$ were observed in the sampling sites with N and P limitation, which likely reflect metabolic shifts the soil microbial community that modulate their sensitivity to nutrient limitation (Tapia-Torres et al., 2015; Xu et al., 2017). The conclusions are consistent with those on microbial and soil nutrient ratios above. Hence, we concluded that in the arid and oligotrophic Loess Plateau, the microbial nutrient metabolism was simultaneously limited by nitrogen (N) and phosphorus (P) in the loess with *A. ordosica* and *A. cristatum* communities, and in the feldspathic sandstone weathered soil with *A. cristatum* communities.

### 4.3. Factors affecting ecoenzymatic stoichiometry and microbial nutrients

In order to more definitively identify the factors that affect ecoenzymatic stoichiometry and microbial nutrients, we carried out space ordination analysis (RDA and CCA). The results showed that the variations in soil enzyme activities were best accounted by vegetation characteristics and soil physicochemical properties (Fig. 3). The $H_{plant}$, $S_{plant}$, plant biomass, SOC, soil moisture, and clay contents are the important factors to NAG, AP, and BG. These conclusions are consistent with previous studies, which demonstrated that variation in soil ecoenzymatic activities was best explained by vegetation (Bowles et al.,

2014; Kivlin and Treseder, 2014; Peng and Wang, 2016). However, microbial nutrients ($C_{mic}$, $N_{mic}$ and $P_{mic}$) had weak relationship with vegetation characteristics and soil physicochemical properties (Fig. 3). These results indicated that enzyme activities of rhizosphere soil were greatly affected by above-ground vegetation and soil physicochemical properties, whereas these effects were small to microbial nutrients.

Furthermore, the soil physicochemical properties and vegetation characteristics accounted for the variation in soil ecoenzymatic stoichiometry (Fig. 4). The silt and clay contents were the important factors for BG:NAG and BG:AP. As mentioned above, the soil enzymes absorbed by soil particles and minerals could result in the variation of the ecoenzymatic ratios. Soil moisture was also a major factor affecting the three ecoenzymatic stoichiometry ratios. Soil water content determines whether plants roots can maintain growth and acquire soil nutrients (Manzoni et al., 2012). Soil microbial activity and function also largely depend on the soil water content (Romanowicz et al., 2016). Therefore, the influence of soil moisture on ecoenzymatic stoichiometry ratios could be caused by the sensitive response (the production of enzymes) of plant root systems and microbes to soil available water. Soil physicochemical properties might largely impact soil ecoenzymatic stoichiometry through altering the concentrations of available substrate and soil C, N, and P stoichiometry (Peng and Wang, 2016), implying that soil ecoenzymatic stoichiometry is largely controlled by edaphic factors. The microbial nutrient ratios, however, were mainly affected by soil nutrients (Fig. 4). The SOC, TN, and TP were the most important factors to $C_{mic}$: $P_{mic}$, $N_{mic}$: $P_{mic}$, and $C_{mic}$: $N_{mic}$, respectively. Those soil nutrients could affect microorganism nutrient acquisitions and alter the microbial nutrient ratios (Waring et al., 2014; Tapia-Torres et al., 2015). Therefore, the results suggested that the soil physical properties are the most influential factors on ecoenzymatic stoichiometry, while soil nutrient availability is the most influential factor on microbial nutrient ratios.

## 5. Conclusions

Our results provided a direct evidence to illustrate the characteristics of rhizosphere ecoenzymatic stoichiometry. The plants had a greater effect than soil on soil ecoenzymatic stoichiometry. The microbial communities broadly existed in stoichiometric homeostasis in different types of rhizosphere soil. The new insights of this study are that, other than the soil nutrients that were previous reported, soil physical properties and vegetation characteristics at the community-level are one of the most important factors affecting rhizosphere ecoenzymatic stoichiometry and the acquisitions of microbial nutrients. More importantly, this research also clearly proved that rhizosphere microbial nutrient metabolisms are co-limited by N and P in the loess soil with *A. ordosica* and *A. cristatum* communities, and in the feldspathic sandstone weathered soil with *A. cristatum* communities. In summary, our study provided insight into the ecoenzymatic stoichiometry and limitations of microbial nutrients in the grassland-desert transition zone, and highlighted the coupling limitation relationship of microbial metabolisms by nitrogen and phosphorus. These findings may prove vital in our understanding of microbial metabolic limitation and nutrient cycling in arid and oligotrophic ecosystems.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.09.025.

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