Science of the Total Environment 874 (2023) 162504

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Soil extracellular enzyme stoichiometry reflects microbial metabolic limitations in different desert types of northwestern China

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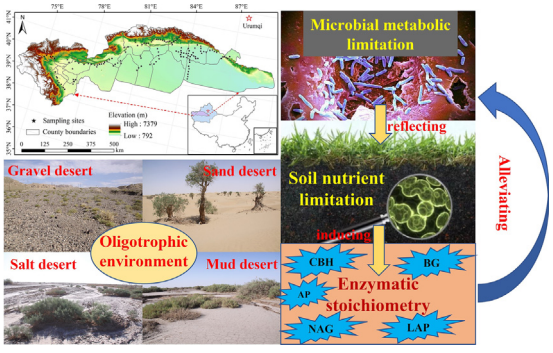
HIGHLIGHTS

- C-, N-, and P-acquiring enzyme activities were 1:1.1:0.9 for all desert types.
- Soil microbial metabolism was co-limited by C and N.
- C and N limitations were strongest in the gravel and salt deserts.
- Climatic factors determined the spatial pattern of soil microbial limitations.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Wei Shi

Keywords:
Extracellular enzyme activity
Enzymatic stoichiometry
Microbial limitation
Microbial biomass
Desert

ABSTRACT

Soil extracellular enzyme activity (EEA) stoichiometry reflects the dynamic balance between microorganism metabolic demands for resources and nutrient availability. However, variations in metabolic limitations and their driving factors in arid desert areas with oligotrophic environments remain poorly understood. In this study, we investigated sites in different desert types in western China and measured the activities of two C-acquiring enzymes (β-1,4-glucosidase and β-D-cellobiohydrolase), two N-acquiring enzymes (β-1,4-*N*-acetylglucosaminidase and L-leucine aminopeptidase), and one organic-P-acquiring enzyme (alkaline phosphatase) to quantify and compare the metabolic limitations of soil microorganisms based on their EEA stoichiometry. The ratios of log-transformed C-, N-, and P-acquiring enzyme activities for all deserts combined were 1:1.1:0.9, which is close to the hypothetical global mean EEA stoichiometry (1:1:1). We quantified the microbial nutrient limitation by means of vector analysis using the proportional EEAs, and found that microbial metabolism was co-limited by soil C and N. For different desert types, the microbial N limitation increased in the following order: gravel desert < sand desert < mud desert < salt desert. Overall, the study area's climate explained the largest proportion of the variation in the microbial limitation (17.9 %), followed by soil abiotic factors (6.6 %) and biological factors (5.1 %). Our results confirmed that the EEA stoichiometry method can be used in microbial resource ecology research in a range of desert types, and that the soil microorganisms maintained community-level nutrient element homeostasis by adjusting enzyme production to increase uptake of scarce nutrients even in extremely oligotrophic environments such as deserts.

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http://dx.doi.org/10.1016/j.scitotenv.2023.162504
Received 2 December 2022; Received in revised form 22 February 2023; Accepted 23 February 2023
Available online 28 February 2023

1. Introduction

Soil microorganisms are an essential component of the soil ecosystem, where they contribute to the decomposition of soil organic matter, the formation of humus, and the transformation and cycling of soil nutrients (Leff et al., 2015). Due to their need to maintain elemental homeostasis (Sinsabaugh et al., 2009), any imbalance between the supply and demand for soil resources constrains metabolism. Nutrient limitation not only affects the microbial metabolic rate, but also affects the resource allocation among microbial metabolic processes. For example, it affects the retention of carbon (C) in the soil by changing the utilization efficiency of C (Sinsabaugh et al., 2012; Soong et al., 2020). As a result, microbial metabolic limitation is a key factor that controls the C cycle in terrestrial ecosystems. In recent years, soil microbial processes, which are currently seen as a key "black box" in the C cycle of terrestrial ecosystems, have attracted more and more attention. Therefore, microbial nutrient limitation has been widely studied (Sinsabaugh et al., 2009; Mooshammer et al., 2014; Sinsabaugh and Follstad Shah, 2012; Moorhead et al., 2016; Sinsabaugh et al., 2017; Cui et al., 2019a, 2019b; Deng et al., 2019; Yang et al., 2020). However, these studies reached inconsistent conclusions about limitations and key driving factors. This demonstrates how the underlying causes of soil microbial nutrient limitation are spatially heterogeneous, temporally dynamic, and scale-dependent. Therefore, it is crucial to clarify the rules and driving mechanisms that govern soil microbial nutrient limitations in typical ecosystems at a regional scale.

Microorganisms release extracellular enzymes to obtain energy and nutrients (Sinsabaugh et al., 2008). Since these enzymes are produced by cell metabolism and in response to nutrient availability in the environment, extracellular enzyme activity (EEA) represents a major link between ecological metabolic theory and ecological stoichiometry theory (Allen and Gillooly, 2009; Sinsabaugh et al., 2009). Utilizing EEA ratios and stoichiometric invariance (i.e., the need for elements to be present at certain relative levels to sustain metabolism), soil EEA stoichiometry has been used to predict the availability of nutrients in the environment and the metabolic activity of microorganisms (Sinsabaugh et al., 2009; Moorhead et al., 2013, 2016). For example, soil microbial metabolism was limited by P in highly weathered tropical ecosystems (Xu et al., 2017; Mori et al., 2018), but P limitation was also found in temperate forest ecosystems (Jing et al., 2020; Cui et al., 2020). Yang et al. (2020) confirmed the shift from P- to N-limitation of microorganisms with grassland restoration based on soil extracellular enzyme stoichiometry. Rosinger et al. (2019) found that soil microorganisms in subtropical steppe were not only limited by P, but also limited by C and N. Therefore, microorganisms in a single ecosystem may be limited by different nutrients, and microorganisms in different ecosystems may also be limited by the same nutrient. Although there is an increasing number of studies on microbial metabolic limitation at regional and ecosystem scales (Xu et al., 2017; Rosinger et al., 2019; Cui et al., 2019a; Cui et al. 2020; DeForest and Moorhead, 2020), the inconsistencies of these findings urgently require us to determine the characteristics and key drivers of microbial metabolic limitation in greater detail at larger scales.

Sinsabaugh et al. (2008) noted that the synthesis of enzymes that specifically target the main sources of C, N, and P reflects the metabolic and stoichiometric requirements of soil microbes. The main extracellular enzymes include the C-acquiring enzymes, β-1,4-glucosidase (BG, which is involved in cellulose degradation) and β-D-cellobiohydrolase (CBH, which is involved in the biodegradation of cellulose); N-acquiring enzymes, β-1,4-*N*-acetylglucosaminidase (NAG, which is involved in chitin or peptidoglycan degradation) and leucine aminopeptidase (LAP, which is involved in the degradation of proteins and peptide substrates); and P-acquiring enzymes, alkaline phosphatase (AP, which is involved in the degradation of organic phosphorus) (Sinsabaugh et al., 2008; Burns et al., 2013; Cenini et al., 2016; Mori, 2020). At a global scale, the average ratio of these enzyme activities to acquire C, N, and P in terrestrial soil and freshwater sediments is close to 1:1:1 (Sinsabaugh et al., 2009). At an ecosystem scale, Xu et al. (2017) investigated the North–South Transect of forest ecosystem in eastern China and found that the activities of soil BG and NAG were higher in temperate forests than in subtropical and tropical forests, whereas soil AP activity showed the opposite trend; however, the soil C:N:P acquisition enzyme activity ratio was consistently close to 1:1:1.

In contrast, many recent studies have found that the ecological enzyme stoichiometry varies greatly between different ecosystem types. Waring et al. (2014) found that the soil BG:AP and NAG:AP ratios in tropical forests were significantly lower than those in temperate ecosystems, and that climate factors (average annual temperature and rainfall) and soil acidification profoundly affected the patterns of microbial enzyme activities in the tropical forest ecosystems. Peng and Wang's (2016) study also found that the *C:N* (0.47) and *C:P* (0.18) ratios for enzyme activity to acquire these nutrients were lower in a temperate grassland than in a tropical forest soil (1.83 and 0.21, respectively). Soil total C, N, and P contents, soil soluble nutrient contents, and microbial biomass were the key factors that influenced EEA and stoichiometry. Xu et al. (2017) also found that soil EEA stoichiometry was jointly affected by climate and soil pH.

Clearly, the spatial pattern of microbial metabolic limitation is affected by multiple environmental factors. Previous studies have shown that soil nutrient contents, pH, moisture content, and vegetation type, which vary spatially, are important factors that influence microbial metabolic limitation at a community level (Cui et al., 2018; Chen et al., 2019). In addition, the soil's clay content varies between soil types and is a main factor that constrains microbial metabolism (Cui et al., 2019b). Climate factors such as mean annual temperature (*MAT*) and mean annual precipitation (*MAP*) are dominant factors that constrain soil microbial metabolism at regional and continental scales (Xu et al., 2017; Cui et al., 2019a). Other studies have also reported that microbial metabolic limitations at a large spatial scale are influenced by both soil properties and/or climate (Jing et al., 2020; Zhou et al., 2020). For example, Chen et al. (2017) suggested that regional EEA variation was explained by soil properties in karst regions of southern China. These studies show that the factors that control microbial metabolic restriction are diverse and complex. The inconsistencies between these findings require us to examine the spatial patterns of soil microbial metabolic limitations and key drivers in greater detail and with attention to spatial scales.

Aridlands (defined as sites with 500 mm mean annual precipitation; Noy-Meir, 1973) cover more than one-third of the Earth's continental surface, making them the world's most extensive terrestrial biome (Pointing and Belnap, 2012). Carbon storage estimates for aridland regions show that they account for 36 % of total carbon storage globally (Campbell et al., 2008). Poulter et al. (2014) found that the global carbon sink anomaly was driven by growth of semi-arid vegetation in the Southern Hemisphere. Furthermore, Ahlström et al. (2015) found that the trend and interannual variation of global carbon sinks are mainly dominated by semi-arid ecosystems. Thus, the aridlands play a critical role in the global biogeochemical cycle and human society. Desert is one of the typical landforms in aridlands, according to the geographical definition, a desert is "an arid area with little precipitation (less than 250 mm per year) and minimal vegetation cover, thus limiting human activity". The ecological definition of desert is "xerophytic, strong xerophytic low woody plants, including semi-trees, shrubs, semi-shrubs and small semi-shrubs composed of sparse non-canopy community" (Zhou and Shen, 2013). Deserts are a widespread ecosystem type and are home to diverse populations of plants and animals that have evolved to survive the desert's harsh conditions. Desert ecosystems have limited water resources, so the decomposition of soil organic matter (SOM) is slower than in environments with greater moisture availability (Burke et al., 1998). As a result, desert ecosystems are typically characterized as a stressful environment with low energy and nutrient availability for soil microorganisms (Schimel et al., 2007). In particular, N and P availability frequently limit primary productivity as well as microbial activity (López-Lozano et al., 2012). However, recent studies have identified alternative mechanisms that challenge the idea that all soil processes in aridlands are proximately water-limited, and highlighted the significance of photodegradation of aboveground litter and the overriding importance of spatial heterogeneity as a modulator of biotic responses to water

availability (Vanderbilt et al., 2008; Austin, 2011). In addition, Tapia-Torres et al. (2015) reported that although soil microbial communities in desert grassland had very low enzyme activity, they followed the global EEA stoichiometry pattern (logarithmic C:N:P scaling ratios ~1:1:1). However, desert is a diverse landform combination in aridland, and the EEA, EEA stoichiometry and microbial metabolic limitation between different desert types are rarely studied.

To improve our understanding of soil microbial metabolism in arid ecosystems, we designed the present study to answer the following questions: First, does the soil EEA stoichiometry in severely oligotrophic desert habitats exhibit the 1:1:1 pattern that is common to most terrestrial ecosystems around the world? Second, is soil microbial metabolism constrained in the dry desert soils by more than moisture? Third, what are the main factors that control microbial metabolism limitations across different desert ecosystems at a regional scale?

Based on these questions, our research objectives were: (1) to evaluate microbial homeostasis in different desert types by measuring soil organic nutrients, microbial biomass, and soil EEA, and quantify the C:N:P ratios of soils, microorganisms, and associated EEA stoichiometry; (2) to quantify the proportional allocation of soil extracellular enzyme activity and use these data to infer the nature and magnitude of the limitations to microbial metabolism; and (3) to determine environmental controls on the spatial pattern of soil microbial metabolic limitations in deserts. We hypothesized that the dominant constraints would differ among desert types but that the overall EEA stoichiometry would remain close to the global average of 1:1:1 for enzymes that acquire soil C, N, and P.

2. Materials and methods

2.1. Study area and sampling

We manually collected samples of the surface soil to a depth of 10 cm at 129 sites in northwestern China. The samples were collected from July to August 2021 between 38.41°N and 42.41°N and between 75.03°E and 86.76°E (Fig. 1). The sampling sites included four desert types: those with gravel, sand, salt, and mud surfaces (Fig. 2). Table 1 summarizes the classification criteria for these desert types. From the 1960 to 2010s, *MAT* ranged from 2.4 °C to 12.4 °C and *MAP* ranged from 32 mm to 360 mm (Fig. S1). The elevation of the sampling sites ranged from 867 m to 2087 m above sea level.

At each site, we first established three representative 10 m × 10 m plots, and removed any vegetation and surface debris. We then collected five core samples to a depth of 10 cm using a 5-cm-diameter soil auger: one sample in each of the four corners of the plot and one at the center of each plot. The 15 cores at each site were combined to form a single composite sample. At the same time, we took three replicate measurements for soil temperature (*ST*) and the volumetric water content (*VWC*) at a depth of 10 cm at the center of each plot using a time-domain reflectometer (Spectrum Technologies, Haltom, TX, USA). All samples were sieved through a 2-mm screen to remove roots and other debris. We then separated the samples into two subsamples: one was air-dried and stored at room temperature before determining the soil physical and chemical properties, and the other was stored at 4 °C in a portable refrigerator during field sampling and was used to measure soil microbial biomass and EEAs. In addition, we collected three undisturbed soil cores at the center of each plot to a depth of 10 cm using a soil bulk density sampler with a stainless steel cylinder (100 cm^3 volume).

2.2. Soil physical and chemical properties

We determined soil organic carbon (*SOC*) using the Walkley-Black method (Nelson and Sommers, 1982), total N (*TN*) using the Kjeldahl method (Bremner, 1996), and total P (*TP*) using the Olsen method (Bremner, 1996; Olsen and Sommers, 1982). We measured soil pH and electrical conductivity (*EC*) at soil:water ratios of 1:2.5 *v*/v and 1:5 v/v using pH and *EC* meters, respectively (PHS-3C, Shanghai Puchun Measurement Instruments, Shanghai, China). We dried three undisturbed soil cores from each site for 24 h at 105 °C to determine the bulk density (*BD*), which was then averaged as *BD* at each site (Blake and Hartge, 1986).

We determined the grain-size composition to define the soil texture using an MS2000 laser particle size analyzer (Malvern Panalytical, Malvern, U.K.). We divided the soil particle sizes into sand (50 to 2000 μm), silt (2 to 50 μm), and clay (0.01 to 2 μm) (USDA, 1951). We also calculated the volumetric fraction (%) occupied by coarse fragments > 2 mm (the gravel content) using the method proposed by Cools and De Vos (2010). We measured the C, N, and P contents of the microbial biomass using the chloroform fumigation–extraction method (Brookes et al., 1982, 1985; Vance et al., 1987).

2.3. Measurement of soil enzyme activity

We measured the activities of the soil C-acquiring enzymes (BG and CBH), N-acquiring enzymes (NAG and LAP), and P-acquiring enzyme (AP) using standard fluorometric techniques with the highly fluorescent compounds 7-amino-4 methylcoumarin and 4-methylumbelliferone (Saiya-Cork et al., 2002; Sinsabaugh et al., 2008, 2009; Steinweg et al.,

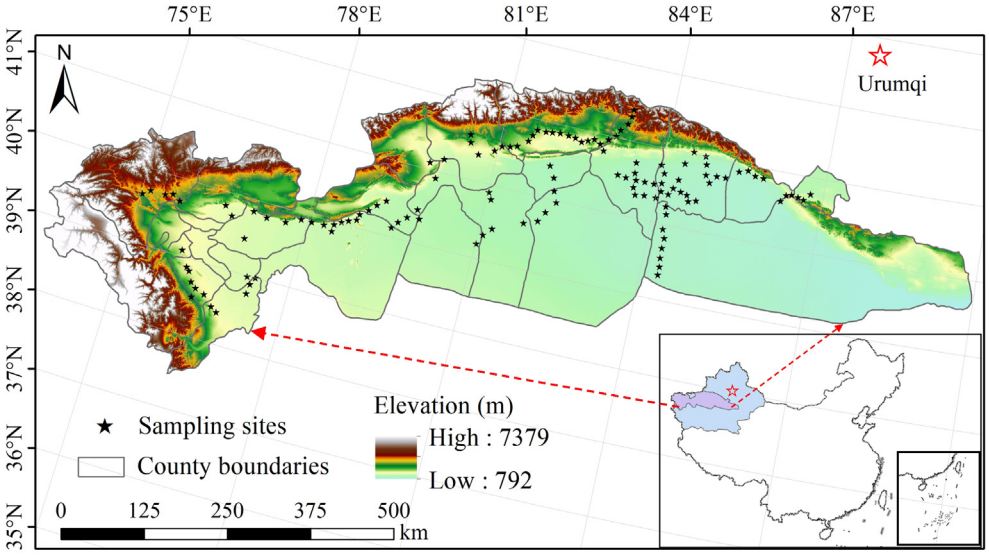

Fig. 1. Location of the 129 sampling sites and of the study area.

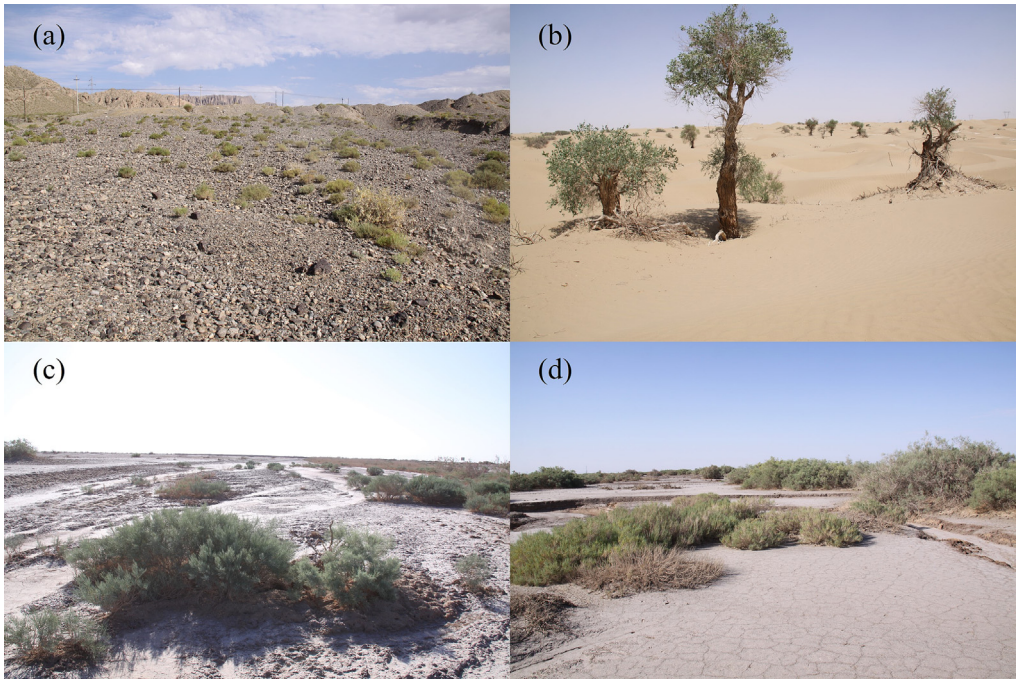

Fig. 2. Four desert landscape types in arid northwestern China: (a) gravel desert (gobi), (b) sand desert, (c) salt desert, (d) mud desert.

2012). To express EEA stoichiometry, we computed the ratios of the C-, N-, and P-acquiring enzymes based on the enzymatic activity, which was measured in units of nmol activity g^{-1} dry soil h^{-1} (Sinsabaugh et al., 2008):

$$\text{C : N EEA ratio} = \ln\ (BG + CBH)/\ln\ (LAP + NAG) \quad (1)$$

$$\text{C : P EEA ratio} = \ln\ (BG + CBH)/\ln\ (AP) \quad (2)$$

$$\text{N : P EEA ratio} = \ln\ (LAP + NAG)/\ln\ (AP) \quad (3)$$

2.4. Microbial metabolic limitation

Soil microbial metabolic limitation can be quantified by means of vector analysis of the untransformed EEA proportions (Moorhead et al., 2016), the vector length and vector angle were calculated as follows:

$$\textit{Vector length} = \left(x^2 + y^2\right)^{0.5} \quad (4)$$

$$\textit{Vector angle} = \arctan 2\ (x, y) \quad (5)$$

where $x = (BG + CBH)/(BG + CBH + AP)$, y is $(BG + CBH)/(BG + CBH + NAG + LAP)$, and arctan2 represents the two-argument arctan function. The longer the vector, the greater the C limitation, and N or P limitation is indicated by a vector angle (respectively) less than or >45°, respectively. The P limitation increases as the angle increases, whereas the N limitation increases as the angle decreases (Moorhead et al., 2016).

2.5. Environmental variables

We collected data on 15 environmental variables that represented the climate, topography, and vegetation, and used these data to identify regional-scale factors that control the spatial pattern of soil microbial metabolic limitations across the study area. We obtained data on *MAT*, *MAP*, mean annual air pressure (*MAAP*), mean annual ground temperature at a depth of 10 cm (*MAGT*), mean annual wind speed (*MAWS*), and annual evaporation (*AE*) values. We obtained these data with a spatial resolution of 1 km from the China Meteorological Elements datasets (https://www.resdc.cn/DOI/DOI.aspx?DOIID=96) (Xu, 2017) in 2021, which was generated using the ANUSPLIN software (Liu et al., 2008) based on daily meteorological element observation data from >2400 stations across China from 1960 to 2021.

For topography, we obtained each sample site's elevation from a digital elevation model created using data from the Shuttle Radar Topography Mission (https://www2.jpl.nasa.gov/srtm/), and calculated the slope at each site using the spatial analysis module of version 10.3 of ArcGIS (https://www.esri.com/).

Table 1
The classification system for the four arid desert types in this study: gravel desert, sand desert, salt desert, and mud desert (Ji, 2001).

Types	Landscape description
Gravel desert	The gravel desert is distributed in a piedmont alluvial fan, in steep terrain, and the soil is composed primarily of gravel and coarse sand. The groundwater depth is mostly 20–50 m but sometimes deeper. The vegetation is extremely sparse, with cover of <1 %. The main plants are *Ephedra sinica* Stapf and *Reaumuria soongorica.* (Pall) Maxim., and *Halogeton glomeratus* (M. Bieb.) C. A. Mey.
Sand desert	The sandy desert's soil texture is primarily fine sand. It is found in areas with fixed, semi-fixed, or mobile dunes. Fixed and semi-fixed dunes are found at the periphery of the desert, and mobile dunes are found in the hinterland. Fixed and semi-fixed dunes have higher vegetation cover (30 %–60 %), and mobile dunes have almost no vegetation. The main plants are *Alhagi sparsifolia* Shap., *Populus euphratica* Oliv., and *Calligonum caput-medusae* Schrenk.
Salt desert	This desert type is most common in modern low and flat alluvial plains, ancient alluvial plains, in-river basins, and the shores of saltwater lakes (mostly dried). These places gather a lot of surface water, but the salinity and groundwater table are both high because the groundwater is in a closed-flow state. This leads to the accumulation of surface salt contents above 150 g kg^{-1}. The vegetation is dominated by sparse thickets of saline-tolerant vegetation such as *Haloxylon ammodendron* (C. A. Mey.) Bunge, *Kalidium foliatum* (Pall.) Moq., *Halostachys caspica* (Bieb.) C. A. Mey., *Tamarix chinensis* Lour., and *Alhagi sparsifolia* Shap.
Mud desert	This desert is spread across ancient alluvial plains and high river terraces. The top of the groundwater table is generally below 10 m deep. The soil texture is primarily fine particles, and the salt content is low. The soil is dry, the ground is bare, and only a few shrubs survive (e.g., *Tamarix chinensis* Lour).

For vegetation cover, we derived the normalized-difference vegetation index (*NDVI*) for each site from the annual maximum *NDVI* dataset for China in 2021 with a spatial resolution of 30 m (https://www.resdc.cn/DOI/DOI.aspx?DOIID=68) (Xu, 2022). This dataset is based on the Google Earth Engine remote-sensing cloud-computing platform, which uses data from Landsat 5 and 8 remote sensing images to calculate the maximum annual *NDVI* dataset.

We extracted the data for all environmental variables for our sampling sites using the Extract Multi Values to Points function of the Spatial Analyst geoprocessing tool in version 10.3 of ArcMap (http://www.esri.com), which extracts cell values from one or more rasters to a point feature class. Table 2 summarizes the climatic, geographical, vegetation, and soil characteristics for the different desert types.

2.6. Statistical analysis

Before performing our statistical analysis, we checked for heterogeneity of variance and, if necessary, log-transformed or standardized the data (using *z* scores). We used one-way ANOVA to test for significant differences in soil enzyme activities and enzymatic stoichiometry among the different desert types, and if the result was significant, we used least-significant-difference (LSD) tests for multiple comparisons to identify significant differences between desert types ($P < 0.05$). We used Pearson's correlation (*r*) to assess relationships between environmental factors and soil EEA. We used the variance inflation factor (*VIF*) and tolerance, which are two widely used and closely related statistics, to diagnose collinearity in the multiple regression, and used stepwise regression to eliminate factors with high multicollinearity. We then applied redundancy analysis (RDA), a constrained ordination method developed by van den Wollenberg (1977), to determine the relative contributions of the environmental factors to the variance in soil EEA. This analysis was carried out using version 5.0 of the Canoco software (http://www.canoco5.com/). We used a total of 1000 Monte-Carlo permutations to examine the significance of the eigenvalues for the first canonical axis and all other axes combined.

3. Results

3.1. Content and ratios of soil C, N, and P and microbial biomass C, N, and P

The highest *SOC*, *TN*, *TP*, *C:N*, *C:P* and *N:P* were found in salt deserts, whereas the lowest values of these variables were found in gravel deserts; however, the difference between these sites and the other sites was only significant for *SOC* and *C:N* (Table 3). Otherwise, there was no significant difference in *TN*, *N:P*, soil microbial biomass C, N, and P (*MBC*, *MBN*, and *MBP*, respectively), *MBC:MBN*, and *MBC:MBP* among the desert types, but the *MBN:MBP* was significantly higher in sand deserts than in the other desert types.

3.2. Soil EEA and EEA stoichiometry in the different desert types

Fig. 3 illustrates differences in enzyme activity among the desert types. In gravel deserts, BG + CBH, AP, and the ratio of *EEA C* to *N* were significantly higher than in one or more of the other deserts. In sand deserts, the ratios of *EEA C* to *N* were significantly higher than in salt deserts. In salt deserts, *AP* and the ratio of *EEA C* to *N* were significantly lower than in gravel deserts and the ratio of *EEA N* to *P* was significantly higher than in the gravel and sand deserts. The sum of *NAG* + *LAP* and the ratio of *EEA C* to *P* did not differ significantly among the deserts. The activities of the C-acquiring enzymes (BG + CBH) decreased in the following order: gravel desert (685.41 ± 106.00 nmol g^{-1} h^{-1}, mean ± SE) > mud desert (256.75 ± 37.77 nmol g^{-1} h^{-1}) > sand desert (173.62 ± 27.79 nmol g^{-1} h^{-1}) > salt desert (106.76 ± 14.39 nmol g^{-1} h^{-1}). The P-acquiring enzyme (AP) followed the same pattern being lowest in salt desert (72.22 ± 12.48 nmol g^{-1} h^{-1}) and highest in gravel desert (182.70 ± 19.01 nmol g^{-1} h^{-1}). The enzyme C:N ratio, calculated by ln (BG + CBH)/ln (NAG + LAP), was highest in gravel deserts (1.00 ± 0.02) and lowest in salt deserts (0.73 ± 0.03). The enzyme C:N ratio in gravel deserts was approximately equal to 1, but it was <1 in the other desert types. In contrast, the enzyme N:P ratio calculated by ln (NAG + LAP)/ln (AP) was highest in salt deserts (2.26 ± 0.72) and lowest in gravel deserts (1.26 ± 0.03). There was no significant difference in the enzyme C:P ratio calculated by ln (BG + CBH)/ln (AP) between desert types. Thus, the enzyme N:P ratio and the enzyme C:P ratio were both >1.

3.3. Soil microbial metabolic limitations in the different desert types

To measure microbial nutrient limitation, we performed vector analysis on the untransformed proportional activities using the vector length (microbial C limitation; Fig. 4a) and vector angle (soil microbial N or P limitation; Fig. 4b). The microbial C limitation was significantly higher in gravel deserts than in the other desert types, which did not differ significantly. The vector angle was <45° for all desert types, indicating that all were experiencing N limitation, although the magnitude of the limitation differed significantly among the desert types. Because the N limitation

Table 2
Summary of the climatic, geographical, vegetation, and soil characteristics for the four desert types in northwestern China. Values are means ± SE Abbreviations: *AE*, annual evaporation; *BD*, bulk density; *Clay*, clay content; *EC*, electric conductivity; *Gravel*, gravel content; *MAAP*, mean annual air pressure; *MAGT*, mean annual ground temperature; *MAP*, mean annual precipitation, *MAT*, mean annual temperature; *MAWS*, mean annual wind speed; *NDVI*, the normalized-difference vegetation index; *Sand*, sand content; *Silt*, silt content; *ST*, soil temperature; *VWC*, volumetric water content.

Factors	Gravel desert (n = 58)	Sand desert (n = 29)	Salt desert (n = 24)	Mud desert (n = 18)
Clay (% *w*/w)	6.83 ± 0.40b	5.39 ± 0.85b	10.54 ± 1.16a	10.88 ± 1.30a
Silt (% w/w)	36.25 ± 2.06b	32.63 ± 4.54b	50.91 ± 4.69a	53.87 ± 5.77a
Sand (% w/w)	56.93 ± 2.37a	61.98 ± 5.36a	38.55 ± 5.67b	35.25 ± 6.83b
Gravel (% w/w)	26.82 ± 2.81a	0.00	0.25 ± 0.21b	0.88 ± 0.53b
VWC (% w/w)	7.07 ± 0.37bc	4.82 ± 0.45c	13.75 ± 2.05a	9.05 ± 1.37b
ST (°C)	36.17 ± 0.80b	42.01 ± 1.25a	39.95 ± 1.27a	40.42 ± 1.26a
BD (g cm^{-3})	1.53 ± 0.03a	1.25 ± 0.04b	1.18 ± 0.05b	1.20 ± 0.05b
pH	9.35 ± 0.07a	9.17 ± 0.10ab	8.93 ± 0.07b	9.06 ± 0.11b
EC (mS cm^{-1})	9.45 ± 3.00a	7.67 ± 1.91a	13.37 ± 1.59a	10.27 ± 1.89a
NDVI	0.113 ± 0.01bc	0.107 ± 0.01c	0.15 ± 0.01a	0.14 ± 0.01ab
Elevation (m)	1297.12 ± 36.27a	951.60 ± 12.49b	971.52 ± 18.35b	976.58 ± 17.51b
Slope (°)	2.40 ± 0.30a	0.14 ± 0.03b	0.10 ± 0.02b	0.37 ± 0.16b
MAP (mm)	125.33 ± 9.59a	43.17 ± 3.34b	39.93 ± 3.33b	45.63 ± 5.41b
MAT (°C)	9.74 ± 0.31b	12.24 ± 0.14a	12.17 ± 0.12a	12.01 ± 0.22a
MAAP (hPa)	862.72 ± 4.09b	902.44 ± 1.67a	901.36 ± 2.47a	899.27 ± 2.50a
MAGT (°C)	13.52 ± 0.30b	15.88 ± 0.14a	15.85 ± 0.12a	15.71 ± 0.22a
AE (mm)	895.92 ± 14.50b	976.38 ± 13.71a	972.39 ± 20.41a	966.96 ± 21.67a
MAWS (m s^{-1})	1.72 ± 0.05a	1.87 ± 0.07a	1.83 ± 0.08a	1.78 ± 0.10a

Table 3
Differences in soil properties among the four types of arid desert in northwestern China.

Soil properties[a]	Gravel desert	Sand desert	Salt desert	Mud desert
SOC (g kg^{-1})	3.48 ± 0.53c	4.78 ± 0.72bc	9.88 ± 1.12a	6.76 ± 0.96b
TN (g kg^{-1})	0.31 ± 0.06a	0.35 ± 0.05a	0.46 ± 0.07a	0.39 ± 0.04a
TP (g kg^{-1})	0.43 ± 0.02b	0.51 ± 0.02a	0.50 ± 0.02ab	0.51 ± 0.03a
C:N	13.84 ± 1.20b	13.65 ± 0.71b	25.18 ± 2.89a	19.14 ± 3.52b
C:P	8.10 ± 0.92c	8.88 ± 1.19c	20.87 ± 2.94a	15.23 ± 3.32b
N:P	0.67 ± 0.09a	0.65 ± 0.08a	0.91 ± 0.11a	0.76 ± 0.06a
MBC (mg kg^{-1})	102.25 ± 15.33a	116.17 ± 18.57a	94.44 ± 14.90a	141.88 ± 30.35a
MBN (mg kg^{-1})	9.69 ± 0.87a	12.88 ± 0.90a	11.31 ± 1.08a	10.70 ± 1.32a
MBP (mg kg^{-1})	3.60 ± 0.23a	2.87 ± 0.35a	3.45 ± 0.26a	3.48 ± 0.45a
MBC:MBN	10.59 ± 1.00a	9.67 ± 1.47a	9.02 ± 1.09a	13.25 ± 2.20a
MBC:MBP	34.27 ± 5.61a	58.65 ± 17.13a	29.84 ± 4.87a	46.52 ± 8.84a
MBN:MBP	3.33 ± 0.39b	6.05 ± 0.78a	3.75 ± 0.47b	3.74 ± 0.60b

[a] Variables: C:N, C:P, and N:P indicate the ratios of *SOC* to *TN*, *SOC* to *TP*, and *TN* to *TP*, respectively; *MBC*, *MBN*, and *MBP* indicate soil microbial biomass C, N, and P, respectively; *SOC*, soil organic carbon; *TN*, soil total nitrogen; *TP*, soil total phosphorus. Values of a parameter followed by different letters differ significantly among different desert types (ANOVA followed by LSD test; $p < 0.05$).

increases as the angle decreases, the strength of the microbial N limitation increased in the following order: gravel desert (33.68 ± 1.22°) < sand desert (29.98 ± 1.67°) < mud desert (28.03 ± 1.65°) < salt desert (17.03 ± 1.81°). Similarly, almost all study sites for the different desert types appeared on the lower right side of a graph of the ratios of C to N enzyme activities versus the ratios of C to P enzyme activities (Fig. 5); that is, the angles were mostly <45°, which supports our conclusion based on Fig. 4, namely that soil microbial activity was primarily limited by N rather than by P in the arid deserts of our study area in western China.

3.4. Relationships between soil EEA, EEA stoichiometry, microbial metabolic limitations, and environmental factors

Table 4 summarizes the correlations between the enzyme variables and the soil abiotic and biotic factors, vegetation and climate factors, and topographic factors for all desert types combined. The enzyme C:P and N:P ratios were not significantly correlated with any of these environmental factors. Among the soil abiotic factors, pH and gravel content were positively correlated with C-acquiring enzymes. *SOC*, *TN*, *C:P*, *N:P*, and silt content were positively correlated with the N-acquiring enzymes. There was a significant positive correlation between *TN*, *N:P*, and gravel content and the P-acquiring enzyme. The gravel content was positively correlated with carbon limitation (vector length) and N or P limitation (vector angle). The N limitation (vector angle) was significantly positively correlated with pH and both sand and gravel contents, and negatively correlated with *SOC*, *C:P*, clay content, *VWC*, and *ST*. The enzyme C:N ratio was significantly positively correlated with *BD*, pH, and gravel content but significantly negatively correlated with *SOC*, *C:N*, *C:P*, and *ST*. Biological factors had significant positive effects on the P-acquiring enzyme and vector angle. It is worth noting that almost all climatic and topographic factors significantly affected soil EEA and soil microbial metabolic limitations, although the directions of these correlations (positive or negative) differed among factors. Soil EEA, enzyme C:N, and soil microbial metabolic limitation were significantly positively correlated with *MAP*, elevation, and slope, but negatively

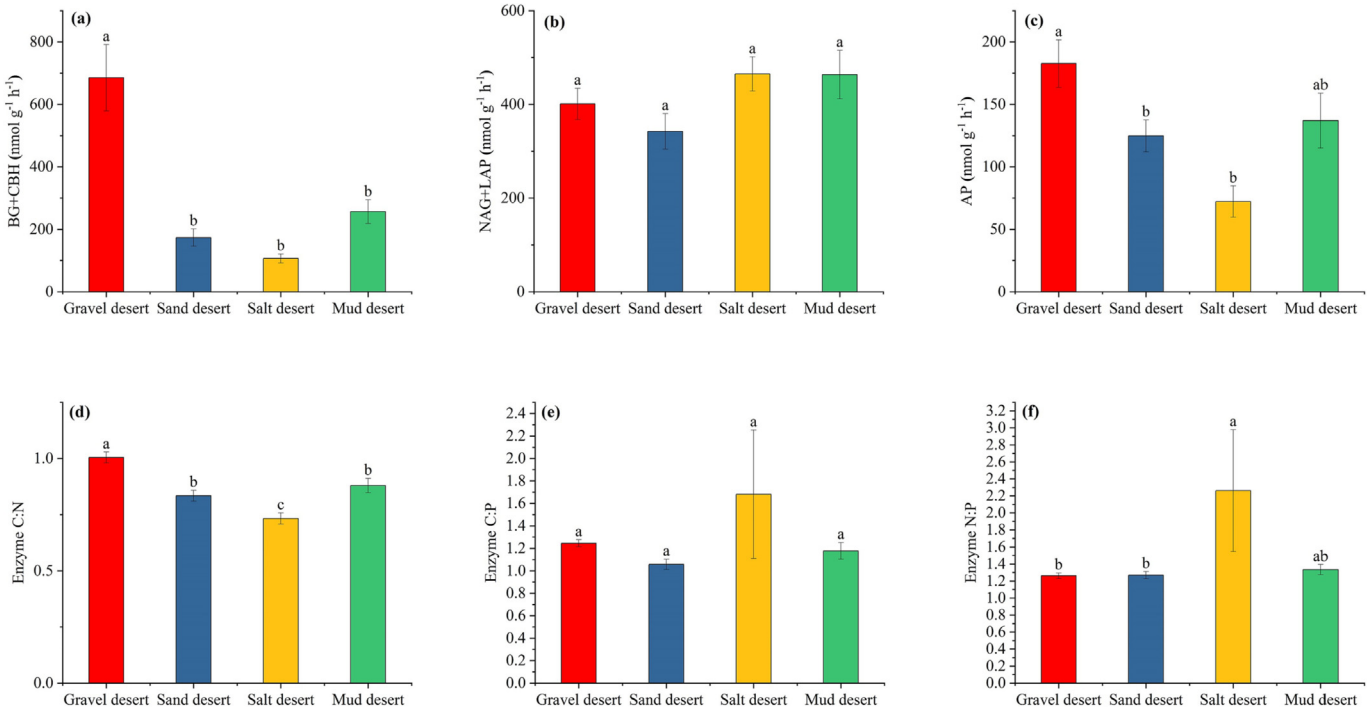

Fig. 3. Differences of soil enzymatic activity and enzymatic stoichiometry among the four types of arid desert: (a) C-acquiring enzymes (β-1,4-glucosidase and β-D-cellobiohydrolase [BG + CBH]); (b) N-acquiring enzymes (β-1,4-*N*-acetylglucosaminidase and L-leucine aminopeptidase [NAG + LAP]); (c) P-acquiring enzyme (alkaline phosphatase [AP]); and the logarithmic ratios (d) (BG + CBH)/(NAG + LAP); (e) (BG + CBH)/AP; and (f) (NAG + LAP)/AP. Values are mean ± SE. Values of a parameter followed by different letters differ significantly among the desert types (ANOVA followed by LSD test; $p < 0.05$).

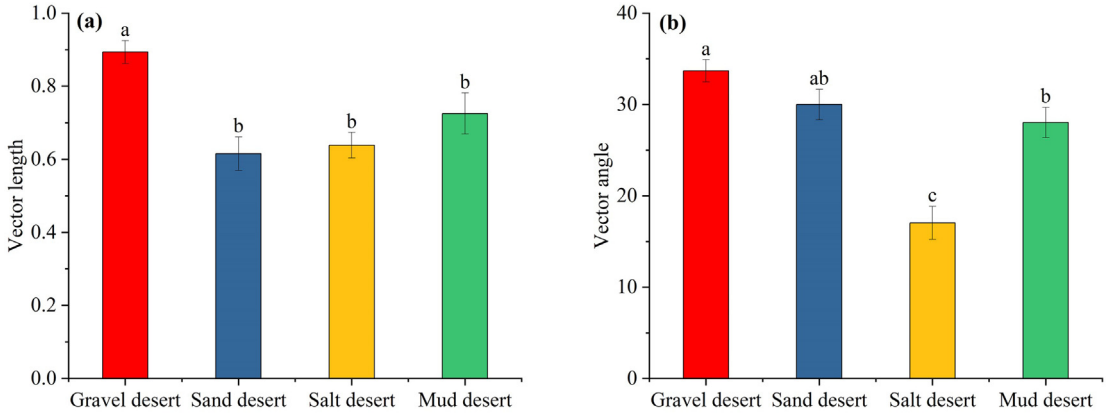

Fig. 4. Differences in the (a) soil microbial C limitation (greater vector lengths represent greater C limitation) and (b) soil microbial N or P limitation (angles less than and >45°, respectively) between the different types of arid desert. Values of a parameter followed by different lowercase letters differ significantly among the desert types (ANOVA followed by LSD test; $p < 0.05$).

correlated with *MAT*, *MAAP*, and mean annual ground temperature (*MAGT*).

4. Discussion

4.1. Soil EEA stoichiometry for the different desert types

Soil enzyme activities are closely linked to the primary productivity of ecosystems, and generally have a substantial impact on nutrient cycling, soil structure, and soil function (Raiesi and Salek-Gilani, 2018). In our study, the soil EEA values (Fig. 3) were among the lowest quantified levels reported to date (Sinsabaugh et al., 2008; Abdalla and Langer, 2009; Hortal et al., 2013; Cenini et al., 2016; Xu et al., 2017; Deng et al., 2019; Guan et al., 2022). This likely reflects the study area's extreme aridity, low plant productivity and oligotrophic soils. Nonetheless, the ratios of the log-transformed C-, N- and P-acquiring enzymes were 1:1.1:0.9 in the present study for data from all deserts combined, which generally agreed with the 1:1:1 ratio obtained for other ecosystems around the world (Sinsabaugh et al., 2008). The enzyme *C:N* and *C:P* ratios in this study were 0.90 and 1.16 and were also similar to the results (1.09 and 1.16, respectively) for soil extracellular enzymes in a global study (Sinsabaugh et al., 2009).

Although the overall EEA in the desert ecosystems that we studied is low, the patterns of relative C, N and P acquisition follow the same patterns as in other desert ecosystems (Tapia-Torres et al., 2015). However, there seems to be a generally higher N demand and lower P demand than

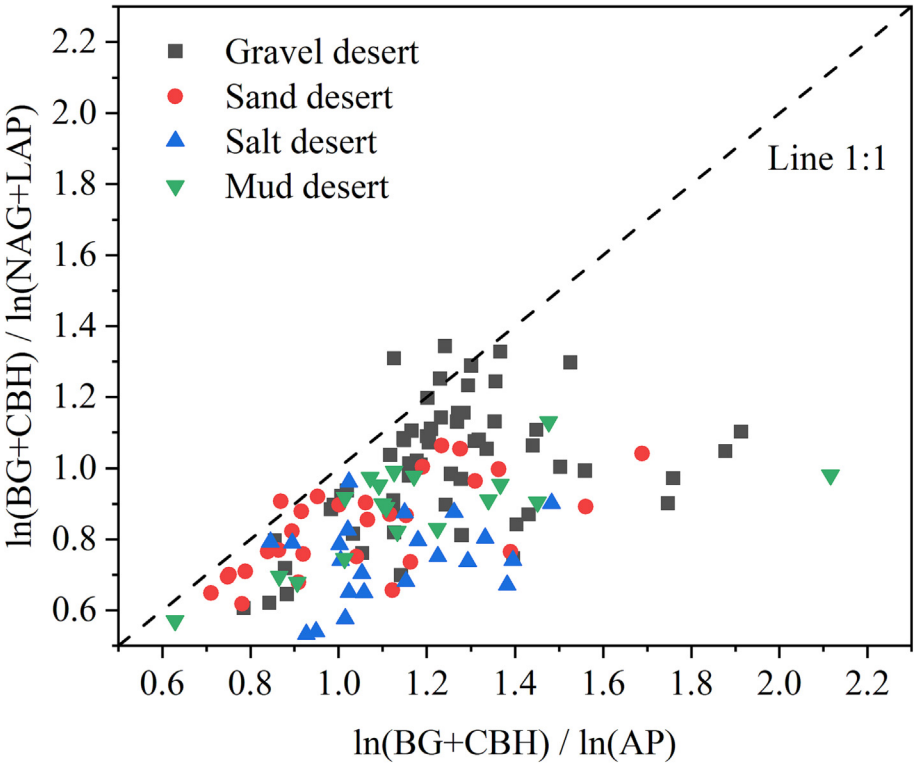

Fig. 5. The pattern of microbial resource limitation illustrated by a scatterplot of soil enzymatic stoichiometries: AP, alkaline phosphatase; BG, β-1,4-glucosidase; CBH, β-D-cellobiohydrolase; LAP, L-leucine aminopeptidase; NAG, β-1,4-*N*-acetylglucosaminidase. Soil microbial C limitation is represented by vector length, with greater length representing greater limitation (Fig. 4a). Vector angles represent soil microbial N or P limitation: angles <45° represent N limitation, and angles >45° represent P limitation. The P limitation increases as the angle increases, whereas the N limitation increases as the angle decreases.

Table 4
Correlations (Pearson's *r*) between soil extracellular enzyme activities, enzymatic stoichiometry, microbial metabolic limitations, and (a) the soil abiotic factors and (b) the biotic factors, vegetation and climate factors, and topographic factors. Significance: *, $P < 0.05$; **, $P < 0.01$. Abbreviations: *AE*, annual evaporation; *BD*, bulk density; *C:N*, *C:P*, and *N:P* indicate the ratios of *SOC* to *TN*, *SOC* to *TP*, and *TN* to *TP*, respectively; EC, electrical conductivity; *MAAP*, mean annual air pressure; *MAGT*, mean annual ground temperature; *MAP*, mean annual precipitation; *MAWS*, mean annual wind speed; *MBC*, *MBN*, and *MBP* indicate soil microbial biomass C, N, and P, respectively; *MBCN*, *MBCP*, and *MBNP* represent the microbial enzyme activity ratios for *C:N*, *C:P*, and *N:P* enzymes, respectively; *NDVI*, the normalized-difference vegetation index; *MAT*, mean annual temperature, *SOC*, soil organic carbon; *ST*, soil temperature; *TN*, soil total nitrogen; *TP*, soil total phosphorus; *VWC*, volumetric water content.

a)	Soil abiotic factors														
	SOC	*TN*	*TP*	*C:N*	*C:P*	*N:P*	*BD*	pH	*EC*	Clay content	Silt content	Sand content	Gravel content	*VWC*	*ST*
C enzymes	−0.11	0.08	−0.03	−0.19*	−0.12	0.07	0.21*	0.29**	−0.09	−0.04	0.04	−0.02	0.35**	−0.05	−0.17*
N enzymes	0.35**	0.42**	0.09	−0.04	0.28**	0.40**	−0.10	0.02	−0.03	0.13	0.21*	−0.20*	−0.05	0.03	−0.07
P enzyme	0.03	0.22*	0.05	−0.15	−0.002	0.20*	0.08	0.17	−0.10	−0.12	0.00	0.02	0.24**	−0.18*	−0.18*
Enzyme *C:N*	−0.24**	−0.04	−0.02	−0.18*	−0.22*	−0.05	0.20*	0.20*	−0.07	−0.08	−0.04	0.05	0.39**	−0.16	−0.22*
Enzyme *C:P*	−0.03	−0.06	0.01	0.07	−0.03	−0.07	−0.04	−0.02	0.02	−0.09	−0.12	0.12	−0.01	−0.03	0.03
Enzyme *N:P*	0.01	−0.04	0.02	0.10	0.01	−0.05	−0.07	−0.06	0.04	−0.07	−0.11	0.11	−0.08	0.01	0.07
Length	−0.17*	−0.02	−0.02	−0.13	−0.17	−0.03	0.18*	0.15	−0.06	0.05	0.03	−0.04	0.34**	−0.01	−0.17
Angle	−0.27**	−0.08	−0.04	−0.16	−0.23**	−0.10	0.16	0.19*	−0.09	−0.25**	−0.17	0.18*	0.30**	−0.30**	−0.21*

b)	Biotic factors							Vegetation and climate factors						Topographic factors	
	MBC	*MBN*	*MBP*	*MBCN*	*MBCP*	*MBNP*	*NDVI*	*MAP*	*MAT*	*MAAP*	*MAGT*	*AE*	*MAWS*	Elevation	Slope
C enzymes	0.22*	0.12	0.23**	0.16	0.07	−0.05	−0.03	0.53**	−0.48**	−0.51**	−0.45**	−0.21*	0.15	0.52**	0.27**
N enzymes	0.20*	0.15	0.17	0.12	0.04	−0.06	0.32**	0.19*	−0.21*	−0.14	−0.20*	0.07	0.24**	0.16	0.20*
P enzymes	0.36**	0.19*	0.23**	0.32**	0.22*	0.07	0.09	0.55**	−0.48**	−0.47**	−0.46**	−0.13	0.28**	0.49**	0.31**
Enzyme *C:N*	0.18*	0.07	0.16	0.17	0.11	0.00	−0.06	0.52**	−0.51**	−0.48**	−0.49**	−0.18*	0.07	0.49**	0.34**
Enzyme *C:P*	−0.09	−0.17	−0.08	0.06	−0.07	−0.12	−0.02	0.01	0.06	−0.04	0.07	−0.15	−0.15	0.03	−0.01
Enzyme *N:P*	−0.12	−0.17	−0.10	0.03	−0.08	−0.12	0.00	−0.07	0.14	0.04	0.15	−0.11	−0.15	−0.05	−0.08
Length	0.04	−0.03	0.12	0.08	−0.01	−0.11	0.00	0.42**	−0.41**	−0.42**	−0.40**	−0.19*	−0.01	0.42**	0.30**
Angle	0.25**	0.09	0.10	0.23**	0.23**	0.13	−0.16	0.42**	−0.41**	−0.38**	−0.40**	−0.13	0.08	0.38**	0.27**

average. This is consistent with earlier studies that reported low N content in desert soils (Gallardo and Schlesinger, 1992). In addition, it is worth noting that the relatively low *N:P* in the present study (Table 3), is speculated to result from low leaching losses of P combined with high volatilization losses of N (Gallardo and Schlesinger 1992). When soil N is limited in arid deserts, the reduced availability of N causes microorganisms to increase the production of enzymes that obtain N, resulting in an enzyme *C:N* ratio of <1. The C, N, and P acquisition strategies based on soil extracellular enzymes should adapt to changes of the substrate and nutrient supply (Peng and Wang, 2016). Among the four desert types we studied, the gravel and salt deserts had both the highest and lowest C- and P-acquisition enzyme activities, respectively, suggesting that the gravel desert had the highest level of microbial metabolism, whereas the salt desert had the lowest. The enzyme *C:N* also was highest in the gravel desert and lowest in the salt desert, indicating that N limitation was strongest in the salt desert and weakest in the gravel desert. The enzyme *N:P* in the salt desert was significantly higher than in the other desert types, again implying a stronger N limitation in the salt deserts.

4.2. Microbial metabolic limitations revealed by vector analysis

We quantified the metabolic limitation of soil microorganisms by vector analysis using the vector length to illustrate C limitation and the vector angle to evaluate N or P limitation. The vector length never exceeded 1, but was significantly higher in the gravel desert than in the other desert types (Fig. 4a), showing little indication of overall C limitation in these deserts but indicating that C limitation was strongest in the gravel desert where SOC was lowest. In addition, the vector angles for all desert types were <45° (Fig. 4b), indicating a pervasive limitation by N. Also, the overall enzyme *C:N* ratio (0.90) was <1 and the enzyme *N:P* ratio (1.29) was >1, which supports our suggestion that soil microorganisms are primarily limited by N in these ecosystems, although N-acquiring enzyme activities also provide C to microorganisms (Sinsabaugh and Follstad Shah, 2012, Mori et al., 2018; Mori, 2020).

The finding that soil microbial metabolism in these deserts was primarily limited by N rather than by P was consistent with studies of the Tibetan Plateau (Kou et al., 2020), the karst areas of southern China (Guan et al., 2022), and terrestrial ecosystems on a global scale (LeBauer and Treseder, 2008; Meyerholt et al., 2020). In contrast, other studies have suggested that soil microorganisms were limited by P in natural grasslands on China's Loess Plateau (Xiao et al., 2020) and across a range of Chinese forests (Cui et al., 2022). Also, previous studies suggested that microbial metabolic limitations are more co-limited by C and P than by N in karst and non-karst forests (Chen et al., 2018; Zheng et al., 2020). These differences may be mainly caused by the differences in climate, vegetation, and the soils of the desert ecosystems in our study compared to other ecosystems.

In addition, soil *N:P* ratios (0.65 to 0.91) and microbial biomass *N:P* (ranging 3.33 to 6.05) in our study (Table 3) were lower than global soil *N:P* values (17:1) and global microbial biomass *N:P* (6) (Xu et al., 2013), indicating a higher constraint on N by soil microorganisms in deserts. The desert's lower soil *N:P* can be explained as follows: First, some nitrogen compounds, such NO_2, N_2O, NO, and NH_3, are dissolved by raindrops and reach the soil in precipitation, but the low rainfall in arid areas results in reduced nitrogen input from this source. Second, in arid desert areas, nitrogen-fixing plants, including legumes, algae, lichens, mosses, and ferns (Zahran, 1999; Sprent and Parsons, 2000), were rare. This would have greatly reduced inputs to the soil by plant N fixation. In contrast, the natural weathering of rocks is a primary source of phosphorus (Ren et al., 2017). The lack of water in deserts curtails leaching losses, soils in our study area should have a relatively high P concentration. The angles in the vector analysis in the present study generally support this belief (i.e., that N limitation was stronger).

4.3. Factors determining microbial metabolic limitations in arid deserts

The proportion of the variance explained by means of RDA can be used to summarize the factors that influence microbial metabolic limitations (i.e., the vector length and angle; Table 5). For the whole desert area in this study, the cumulative percentage of the variance explained by factors for axes 1 and 2 totaled 41.5 %. The climatic factors explained the largest proportion of the variation in microbial limitation (17.9 %), followed by soil abiotic factors (6.6 %) and biological factors (5.1 %), with no contribution from topographic factors. This indicates that soil microbial metabolism

Table 5
Proportions of total variation of microbial metabolic limitation explained by the soil abiotic factors, biotic factors, climatic factors, and topographic factors in the redundancy analysis. Significance of the eigenvalues for the first canonical axis and for all axes combined were tested by Monte Carlo analysis with 1000 permutations, and yielded $P < 0.05$ for both simulations.

Desert type	Proportion of variance explained (%)				
	Soil abiotic factors	Biotic factors	Climatic factors	Topographic factors	Total variance
Gravel desert	0.0	10.8	11.5	0.0	56.8
Sand desert	7.1	0.0	8.3	11.1	99.4
Salt desert	16.6	0.0	4.3	0.0	100.0
Mud desert	57.6	8.4	0.0	0.0	100.0
Whole area (all desert types)	6.6	5.1	17.9	0.0	41.5

was affected by climate, soil abiotic factors, and biological factors, but that climate, especially *MAP*, was most important. However, there were differences in the main factors that controlled microbial metabolism in different desert ecosystems. Soil abiotic factors were the most important factors for the mud and salt deserts, whereas topographic factors were the most important factors for sand deserts, and the gravel desert was mainly affected by climatic and biological factors.

Microbial metabolic C and N limitations were significantly positively correlated with *MAP*, but negatively correlated with *MAT*, *MAAP*, and *MAGT* (Table 4). This was because *MAP* determines soil moisture content and thereby directly regulates soil nutrient status as well as microbial metabolism and function (Xiang et al., 2008; Werner and Egbert, 2009). Low soil moisture content degrades soil aggregates, resulting in the exposure of new mineral surfaces and previously protected organic matter (Denef et al., 2001). When soil is re-moistened, its physical structure is further altered by expansion. The resulting increased soil surface area and release of previously protected organic matter bound in the aggregates will improve soil nutrient availability and promote microbial metabolism (Mvan et al., 1993; Goebel et al., 2007). Importantly, Wang et al. (2022) highlighted the larger importance of rewetting of dry soils on microbial communities, as compared to decreased precipitation, with potential for changes in the soil N cycling. This gives us a research implication that the response of soil microbial metabolism to precipitation reduction and rewetting events in arid ecosystems needs further study.

At the ecosystem level, precipitation will also affect primary productivity and the return of plant residues to the soil, thus affecting soil nutrient availability and microbial metabolism (Cregger et al., 2012; Jia et al., 2014; Ru et al., 2018). For example, when precipitation increases, photosynthesis increases and vegetation can provide more C for microorganisms (through litter and root exudates). This can stimulate microbial demand for N, which may increase N limitation and explain the significant negative correlation between *SOC* and N limitation (vector angle) in our study (Table 4).

The spatial variation in microbial metabolic limitations was closely related to the geographical distribution of the desert types. For example, gravel deserts were mainly found at higher elevations and on large slopes (Table 2), where the precipitation is highest and the temperature is lowest. Because gravel deserts had the highest gravel content and their *SOC* content was significantly lower than in other desert types, the corresponding C limitation was the greatest (Fig. 4). *MAT* can influence microbial nutrient limitation through its effects on soil respiration and enzyme activity (Zhou et al., 2013). Extracellular enzymes are very temperature-sensitive, and since different enzymes have different optimal temperatures, temperature changes will affect substrate utilization by microorganisms (Bornscheuer et al., 2002). Microorganisms are also sensitive to temperature, and changes in temperature will affect the microbial community composition, thereby affecting community metabolism and functioning (Schimel and Parton, 1986; Yang et al., 2015). Therefore, the influence of climate factors (particularly *MAT* and *MAP*) on microbial metabolism is coupled and complex, and can directly affect microbial metabolism by affecting both microbial activity and the community composition, but can also indirectly affect microbial metabolism by affecting soil properties, microclimate, and the vegetation community and productivity.

Soil nutrients mainly come from soil minerals and the decomposition of plant residues (Mary et al., 1996; Cui et al., 2019b), but their bioavailability also depends on the microbial community and soil environment (Kuske et al., 2002). In this study, *SOC* and *TN* were positively correlated with the N-acquiring enzyme activity (Table 4), such that N limitation increased significantly (the vector angle decreased) with increasing *SOC*. This was because soil with a higher *SOC* content has stronger water-holding capacity and higher available carbon, both of which may provide more favorable conditions for microbial growth and enzyme production (Keeler et al., 2009). In almost all sample sites in our study, soil microorganisms were limited by N rather than P (Fig. 5), which resulted in microorganisms allocating relatively more investment (C compounds) to obtain N. Especially in places with high *SOC* content (thus, higher levels of energy substances), microorganisms produce more N-acquiring enzymes, to help mitigate soil N limitation of microbial metabolism. Similarly, a higher soil *C:P* ratio is likely to mean a greater limitation by P availability. Since *TN*, *C:P* and *N:P* were all significantly positively correlated with *SOC*, this means that *TN*, *C:P* and *N:P* also have significant positive effects on N-acquiring enzymes. Indeed, in the deserts we studied, *SOC* and *TN* both had a significant positive effect on N-acquiring enzymes (Table 4), but the N limitation to microbial metabolism was controlled by *SOC*.

In addition, we found that the gravel content was positively correlated with C- and P-acquiring enzymes, which suggests that gravel maybe served as a type of surface mulch, retaining humidity and surface moisture film. Soil *VWC* had a significant positive effect on the intensity of the N limitation, because when soil water content increased, plant growth would have increased, and the competition for N between plants and microorganisms would have been stronger, which would have exacerbated microbial N limitation (Püschel et al., 2016).

The characteristics of biomass affect its influence on the soil and reflects its microbial community. In our study, *MBC* and *MBP* significantly positively affected both C- and P-acquiring enzymes. Liang et al. (2017) found that the regulation of soil microorganisms on C cycling included both ex vivo modification and in vivo turnover. The former refers to the processes in which soil microorganisms decompose or transform macromolecular plant-derived carbon substrates in the soil by secreting extracellular enzymes and transporting plant-derived residues by directly assimilating small plant-source carbon substrate molecules into their biomass and then adding microbial-source organic C, N, and P to the soil through the formation and accumulation of dead microbial residues. In our study, the metabolism of soil microorganisms was strongly limited by N availability, so soil microorganisms would mainly supplement their N source through ex vivo modification (i.e., by increasing production of N-acquiring enzymes). In addition, when soil water content was low, the soil microbial biomass C:N ratio would have increased and fungal biomass would have tended to dominate (Ahmed et al., 2019), because fungi have lower nutrient requirements than bacteria and show higher carbon-use efficiency in poor-quality substrates (Keiblinger et al., 2010; McGuire et al., 2010). As a result, high *MBCN* reduced the N limitation (Table 4).

5. Conclusions

We confirmed our hypotheses that the dominant constraints on microbial metabolism differed among the desert types but that the overall EEA stoichiometry remained close to the global average of 1:1:1 for the activities of enzymes that acquire soil C, N, and P. In the arid desert ecosystems that we studied, we found the lowest soil EEA levels reported to date. However, the ratios of the log-transformed C-, N-, and P-acquiring enzymes were 1:1.1:0.9 for all desert types combined, which compared with the global mean (1:1:1). We also quantified microbial nutrient limitations by means of vector analysis based on proportional EEA stoichiometry, confirming

that soil microbial metabolism was co-limited by C and N, and suggesting a stronger N than C limitation although both were stronger than apparent P limitations. The C and N limitations of microbial metabolism were strongest in the gravel desert and salt desert, respectively, because the SOM content in gravel desert was significantly lower than other desert types, which aggravated the C limitation of microbial metabolism. In addition, the salt desert can not only inhibit the N absorption by microorganisms, but also promote the volatilization of amino N. For all deserts combined, climatic factors had the strongest influence on the spatial pattern of soil microbial limitations, followed by soil abiotic factors and then biological factors. However, this varied among desert types. For example, soil abiotic factors were the most important factors for the mud and salt deserts, whereas topographic factors were the most important factors for sand deserts, biotic factors were as strong as climatic factors in gravel deserts.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.162504.

CRediT authorship contribution statement

XW and YL conceived and designed the experiments. XW, YL, LW, and YD performed the sample collection. YC and WC performed the laboratory analysis. XW and BY analyzed the data and wrote the paper. All authors have read and approved the paper before submission.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

This research was supported by the Second Tibetan Plateau Scientific Expedition and Research (STEP) program (Grant No. 2019QZKK0305) and by the National Natural Science Foundation of China (grants 31971466 and 32001214).

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