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Soil extracellular enzymes characteristics and their controlling factors along the elevation gradient in Qinghai-Tibet Plateau, China

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ABSTRACT

Soil extracellular enzyme activities (EEAs) and ecoenzymatic stoichiometry (EES) play an essential role in soil nutrient cycling and organic matter decomposition. Understanding EEAs and EES variation patterns and their influencing factors could offer direct information about the soil structure, function, and soil response to anthropogenic disturbances and climate change. This issue is noteworthy, especially in high-altitude areas where climate change is imminent and vegetation is diversified. This study measured different soil EEAs and EES characteristics and explored their key controlling factors along nine altitudes ranging from 2500 m to over 5200 m in the Qinghai-Tibet Plateau of western China. We also analyzed the effects of plant microhabitats on soil EEAs and EES. The results showed that most soil EEAs and EES had significant variability in spatial characteristics, and enzymatic activity increased with altitude. Compared to the soil nutrient distribution which also increased with altitude, this same change trend of soil EEAs and soil nutrients was inconsistent with the resource allocation theory. Microorganisms might mediate the effects of environmental factors on soil EEAs by altering the enzyme production efficiency. Specific soil EEAs (EEAs/g SOC), like soil enzyme carbon: phosphorus ratios (E_{CP}), and nitrogen: phosphorus ratios (E_{NP}), showed an opposing trend in variation, which decreased with increasing altitude. Plant microhabitats significantly promoted soil EEAs due to the accumulation of soil nutrients (carbon, nitrogen and phosphorus). Soil EEAs and EES's spatial variability was mainly determined by edaphic factors, accounting for >70.24 % and 55.67 % of latitudinal variations, respectively. Generally, carbon and nitrogen limitations were substantial in this area and gradually alleviated with increasing altitude. This study provided a data support for ecological protection of the Qinghai-Tibet Plateau based on the spatial variation of soil EEAs and nutrient limitation.

1. Introduction

Soil extracellular enzyme activities (EEAs) and ecoenzymatic stoichiometry (EES) are crucial indicators of soil nutrient limitations widely used to reveal soil ecosystem's nutritional status and the microbial resource limitations (Sinsabaugh et al., 2009; Mori, 2020). Macroecological studies have shown that the most widely measured EEAs have a similar stoichiometry for all microbial communities. EES corresponding to soil C, N, and P requirements reflects the biogeochemical balance between metabolic requirements and nutrient utilization under environmental changes (Adamczyk et al., 2014). Soil EEAs and EES have been widely used to explore microbe's nutrient cycling and resource constraints in terrestrial ecosystems (Fujita et al., 2019; Zhou et al., 2020). EEAs data sets extending to continental and global scales make it possible to compare EEAs patterns to large-scale biogeochemical trends and evaluate models that link EES to metabolic and stoichiometric theories of ecology (Sinsabaugh and Follstad Shah, 2012). Unfortunately, few studies (Cao et al., 2021) have used EEAs and EES to determine nutrient resource limitation in the soil at high altitude area.

The dynamics of soil EEAs and EES are acknowledged as being comprehensively regulated by abiotic (e.g., climate and soil properties) and biotic (e.g., plant nutrient and vegetation types) factors from local to global scales (He et al., 2020; Zhou et al., 2020). Several recent studies performed at different climate zones and within different ecosystems have revealed that the critical driving factors to the responses of soil EEAs and EES are the soil nutrient content or pH value (Truong et al.,

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2019; Xu et al., 2020). However, the effects of individual factors on soil EEAs and EES are varied in different ecosystems and different spaces. For instance, soil EEAs might increase (Peng and Wang, 2016; Zhu et al., 2020), decrease (Fatemi et al., 2016), or have no apparent changes (Jing et al., 2017) with increased soil nutrients. Soil EES has been substantially negatively associated with the soil total and available C:N:P stoichiometry (Zhu et al., 2020), and in other studies, a positive or a weak association was also observed (Sinsabaugh et al., 2008; Zhou et al., 2020; Qiu et al., 2021). The relationship between soil β-glucosidase activity and soil pH has also varied from nonsignificant (Sinsabaugh et al., 2008) and positive (Xu et al., 2017) in humid regions to negative in other experiments in arid and semi-arid areas (Peng and Wang, 2016; Zuo et al., 2018). Moreover, soil temperature (Zheng et al., 2020), soil moisture (Chen et al., 2019), bulk density (Xiao et al., 2020), soil particle size (Zuo et al., 2018), microbial biomass and community structure (Xiao et al., 2020; Yang et al., 2020) has also been observed to have different effects on soil EEAs and EES. Generally, the study results in different experimental areas suggest that the factors regulating soil EEAs and EES might be ecosystem-specific or enzyme-specific, but the potential mechanisms of variation in soil EEAs and EES have not been elucidated (Liu et al., 2021). Identifying the key controlling factors of soil EEAs and EES is necessary to promote specific ecosystem services by manipulating soil EEAs (Burns et al., 2013). Any generalizations of the abiotic and biotic controls on soil EEAs and EES across different spaces and ecosystem types is primarily constrained by the fact that most previous studies were either conducted at single sample sites or by meta-analyses (Sinsabaugh et al., 2008; Zheng et al., 2018). Large-scale experimental studies on this topic are currently lacking at high altitude areas, which creates a knowledge gap in our understanding of the spatial-temporal dynamics and controls of soil EEAs and EES.

The Qinghai-Tibet Plateau of China is known as "the roof of the world" and "the third pole", with an average elevation of 4500 m (Sun et al., 2012). The unique geomorphology and altitude gradient variation characteristics provide a space for the intersection of soil EEAs, EES, and environmental factors within the different gradients. Alpine meadows are the dominant ecosystem type, with alpine steppes and deserts occurring at lower altitudes in this area (Wang et al., 2014). In recent years, gradual climate change and overgrazing induced ecosystem degradation has created a vegetative cover that is discontinuous or fragmented on the major part of plateau. The fragmented vegetative cover is caused by disturbance agents such as overgrazing, drought, and water erosion, which are expected to worsen in the face of global climate change (Li et al., 2008; Wu et al., 2017). Despite its importance, little is known about the effects of altitude and vegetation microsites on critical ecosystem functions in this area.

This study investigated soil EEAs and EES along an altitude gradient from the south to the north of the Qinghai-Tibet Plateau in China that captured a wide range of altitude, microclimate, soil type, and vegetation in this area (Fig. 1). The area allowed us to study the variations within-site and between-sites. Our study aimed to answer the following questions: in a high altitude Qinghai-Tibet plateau area, (i) what are the distribution characteristics of soil EEAs and EES at different altitudes, (ii) how do vegetation patches and open microsites affect soil EEAs and EES, (iii) what are the main factors affecting soil EEAs and EES variations, and (iv) what is the relative importance of vegetation types, altitude, climate, and soil physicochemical variables in creating these variations. Specifically, we hypothesize that (i) soil EEAs decrease with increases in elevation, (ii) vegetation microsites change the distribution characteristics of soil EEAs, (iii) the key drivers of soil EEAs differ among elevations and between vegetation microsites. The observations made in this study could be constructive for understanding the biogeochemical limits of the environmental soil carbon reserves and nutrient cycles in this ecosystem.

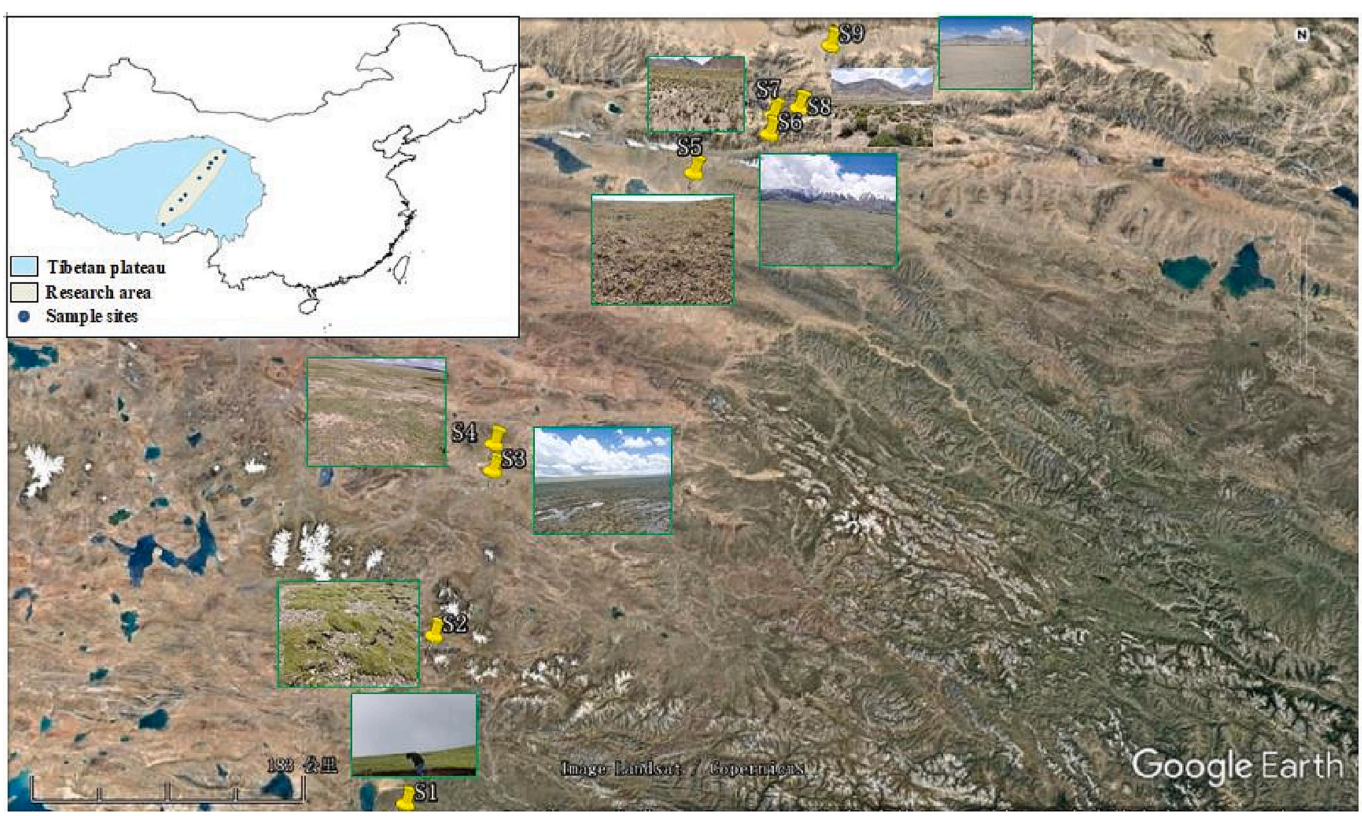

Fig. 1. The geographical location and sample sites of the study area.

2. Materials and methods

2.1. Study sites

The study area was located in the eastern Qinghai-Tibet Plateau of China, changing in altitude from 2500 m to 5500 m. We established nine representative sites from south to north along an altitudinal gradient (latitude 31.90°–36.24° N, longitude 91.71°–94.78° E) (Fig. 1). The mean annual temperature (MAT) ranged from −3.67 °C (south) to 5.63 °C (north) and the mean annual precipitation (MAP) from 90.83 millimeters (mm) (south) to 439.68 mm (north). The ecotones in these regions include alpine meadows, alpine steppes, temperate shrub deserts, and the Gobi desert. The main soil types present were alpine meadow soil, alpine steppe soil, and desert soil (Table 1).

The identified plant species were *Kobresia pygmaea* (C. B. Clarke) C. B. Clarke, *Potentilla saundersiana* Royle var. *caespitosa* (Lehm.) Wolf, *Androsace tapete* Maxim., *Pedicularis oederi* Vahl. *Pedicularis oederi* Vahl subsp. *oederi var. sinensis* (Maxim.) Hurus., *Oxygraphis glacialis* (Fisch) Bunpe, *Potentilla bifurca* L., *Pomatosace filicula* Maxim., *Euphorbia altotibetica* O. Pauls, *Dimorphostemon glandulosus* (Kar. et Kir.) Golubk in alpine meadow. For alpine steppe, *Carex moorcroftii* Falc.ex Boott, *Agropyron cristatum* (L.) Gaertn., *Stipa purpurea* Griseb., *Achnatherum splendens* (Trin.) Nevski, *Kobresia pygmaea* C. B. Clarke, *Thermopsis lanceolata* R. Br., Oxytropis, *Potentilla parvifolia* Fisch. ap Lehm., *Oxytropis aciphylla* Ledeb were identified plant species. The identified plant species were *Ceratoides latens* (J. F. Gmel.) Reveal et Holmgren, *Salsola passerina* Bunge, *Salsola laricifolia* Turcz. ex Litv., *Limonium aureum* (L.) Hill, *Lepidium apetalum* Willd. in shrub desert.

Longitude, latitude, and altitude data were measured using a handheld GPS (UniStrong G138, China). MAT, MAP, and normalized difference vegetation index (NDVI) were interpolated using Kriging interpolation (1 km × 1 km resolution) in ArcGIS 10.2 (https://www.esri.com) based on the data from the Resource and Environment Sciences and Data Center of China (http://www.resdc.cn).

2.2. Soil sampling and processing

Three 1 m × 1 m plots for the two most contrasting surface types (plant patch versus bare land) were selected at each site, respectively. Three replicated plant patch plots were separated by at least 10 m and each of these had similar slopes and aspects. The bare land plots were located at the open space adjacent to the plant patch plots. Soil, excluding surface plants and litter, was collected from depths of 0 to 10 centimeters (cm) in July 2019 and assayed to measure the potential activity of extracellular soil enzymes (EEAs). After visible roots, stones, and soil fauna were removed from the soil samples, half of every soil sample was passed through a 2 mm mesh sieve, transported in an icebox to the laboratory as soon as possible, and then stored at 4 °C until enzymatic analysis, prevent microbial activity and minimizing changes to the enzymatic community during storage. The another half of each soil sample was air-dried and stored at room temperature to analyze soil physical and chemical properties.

2.3. Soil analysis

Soil pH was measured using a PHS-3G digital pH meter (PB-10 pH meter, Sartorius, Gottingen, Germany) in samples containing a 1:5 ratio of fresh soil to water. The electrode method was used to determine electric conductivity (EC) (conductivity meter HJ 802–2016). The total carbon (TC), total nitrogen (TN), total phosphorus (TP), dissolved organic nitrogen (DON), ammonia (NH_4^+-N), nitrate-nitrogen (NO_3^--N), and available phosphorus (AP) contents were measured using colorimetric analysis in a discrete auto-analyzer (Smart Chem 450, AMS, France). Soil organic carbon (SOC) was measured using the potassium dichromate oxidation-external heating method (oil bath). Mechanical composition (Clay, Silt, Sand) of the soil was measured using MS-S lighter scattering apparatus (Malvern Instruments Ltd., Malvern, UK) in the soil laboratory of the Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences.

2.4. Soil EEAs assay and calculation

The soil EEAs measured were carbon (C)-acquiring enzymes: β-1,4-glucosidase (BG), Cellobiohyrolase (CBH), β-Xylosidase (XS), nitrogen (N)-acquiring enzymes: β-1,4-*N*-acetylglucosaminidase (NAG), L-leucine aminopeptidase (LAP), Urease (UR), phosphorous (P)-acquiring enzymes: acid phosphatase (ACP), alkaline phosphatase (ALP), and polyphenol oxidase (POX). Standard fluorometric protocols for soil enzyme measurement were obtained and used from previous studies (German et al., 2011; Wallenius et al., 2011). Briefly, 1 g dry mass of fresh soil was homogenized and 125 ml of deionized water was used as buffer to extract soil enzymes. The total potential activity was determined by adding 200 ul of soil suspension and 50 ul of fluorescent substrate solution for each enzyme to 96-well microplates. Soil enzyme ratio of C:N, C:P, N:P (E_{CN}, E_{CP}, and E_{NP}, respectively) and soil carbon quality index (CQI) were calculated using the following formulas (Sinsabaugh et al., 2008):

$$E_{CN} = lnBG/ln(NAG + LAP)$$

$$E_{CP} = lnBG/lnACP$$

$$E_{NP} = ln(NAG + LAP)/lnACP$$

$$\mathrm{CQI} = \mathrm{lnPOX}/(\mathrm{lnPOX} + \mathrm{lnBG})$$

The vector length (Vector L) and angle (Vector A) of soil EES were calculated using the following formulas (Hill et al., 2014):

$$\text{Vector L} = \left\{ [lnBG/ln(NAG + LAP)]^2 + [lnBG/lnACP]^2 \right\}^{1/2}$$

$$\text{Vector A} = \text{Degrees}\left\{ ATAN2\left[\frac{lnBG}{lnACP}, \frac{lnBG}{ln(NAG + LAP)}\right] \right\}$$

Vector L indicates soil microorganism carbon restriction degree. Vector A represents soil microorganism nitrogen and phosphorus restriction degree; if the value deviates from 45°, it indicates that the soil

Table 1
General information of sampling sites.

Sample plot	Altitude (m)	Longitude and latitude (°)	Plant type	NDVI	MAP (mm)	MAT (°C)	Soil type
S1	4650.60	31.90 N, 91.71 E	Alpine Kobresia meadow	0.57	439.68	0.03	Alpine meadow soil
S2	5202.09	32.88 N, 91.92 E	Alpine Kobresia and forb meadow	0.43	476.88	−3.67	Alpine meadow soil
S3	4561.68	33.83 N, 92.33 E	Alpine Kobresia and forb meadow	0.18	366.89	−1.56	Alpine meadow soil
S4	4598.75	33.98 N, 92.34 E	Alpine grass and carex steppe	0.31	362.02	−1.94	Alpine steppe soil
S5	4524.25	35.52 N, 93.77 E	Alpine grass and carex steppe	0.17	301.26	−2.64	Alpine steppe soil
S6	4079.21	35.74 N, 94.32 E	Alpine Kobresia and forb meadow	0.16	243.19	−0.28	Alpine meadow soil
S7	3800.32	35.85 N, 94.35 E	Alpine grass and carex steppe	0.10	206.74	1.00	Alpine steppe soil
S8	3556.44	35.88 N, 94.53 E	Temperate shrub desert	0.14	169.49	2.69	Desert soil
S9	2955.91	36.24 N, 94.78 E	Gobi desert	0.04	90.83	5.63	Desert soil

NDVI, normalized difference vegetation index; MAP, mean annual precipitation; MAT, mean annual temperature.

sample is restricted by nitrogen or phosphorus. Furthermore, phosphorus restriction is stronger if the value is >45°, and nitrogen restriction is stronger if the value is <45° (Moorhead et al., 2016).

2.5. Statistical analysis

All statistical analyses were performed in SPSS v. 21.0 (IBMCorp., Armonk, NY, USA). Data were tested for normality (ShapiroeWilks), and log transformed when necessary to meet assumptions of normality prior to analysis. The effects of altitude and vegetation microhabitat on soil EEAs and EES were evaluated by ANOVA. The significance of the differences in soil EEAs among the different altitudes and between the different plant microhabitats were tested by Tukey's honestly significant difference (HSD) test when the one-way ANOVA results were significant at $p = 0.05$. Figures were drawn using Origin v. 9.0 (OriginLab Corp., Northampton, MA, USA). Redundancy analysis (RDA) was applied to find the main drivers influencing soil EEAs and EES variation. Variation partitioning analysis (VPA) was conducted to assess the relative contribution of abiotic and biotic factors. RDA and VPA analyses were conducted using the 'vegan' package of r 3.5.3 platform (R Development Core Team, 2019).

3. Results

3.1. Spatial distribution of soil EEAs and EES (between-site variation)

The soil EEAs showed distinct variation ($p < 0.05$) among study sites except for CBH and POX (Fig. 2). BG, XS, NAG, ACP, and ALP were significantly higher at the highest altitude than other sites, while LAP and UR at the lowest altitude were significantly lower than other values ($p < 0.05$). The altitudinal patterns of soil-specific EEAs and soil EEAs were opposite. BG and NAG increased significantly along the altitude. The specific CBH, NAG, and LAP significantly decreased with altitude (Fig. 3).

The values of E_{CN} were between 1.04 and 1.79, and varied irregularly at different altitude sites (Fig. 4). The E_{CP} and E_{NP} values were 1.21–11.33 and 0.86–7.38, respectively. The mean ratio of soil EES (E_{CNP}) was approximately 1:0.46:0.29 in the study area. E_{CP}, $E_{NP,}$ and CQI decreased significantly with increasing altitude. The change of Vector L was not noticeable, while Vector A significantly increased with increasing altitude ($p < 0.05$).

3.2. Plant effect on soil EEAs and EES (within-site variation)

Three soil EEAs closely related to C, N, P cycling (BG, NAG, and ACP), and POX were selected to study the effect of vegetation microsites on key soil EEAs (Fig. 5). There was a strong microsite effect on the four

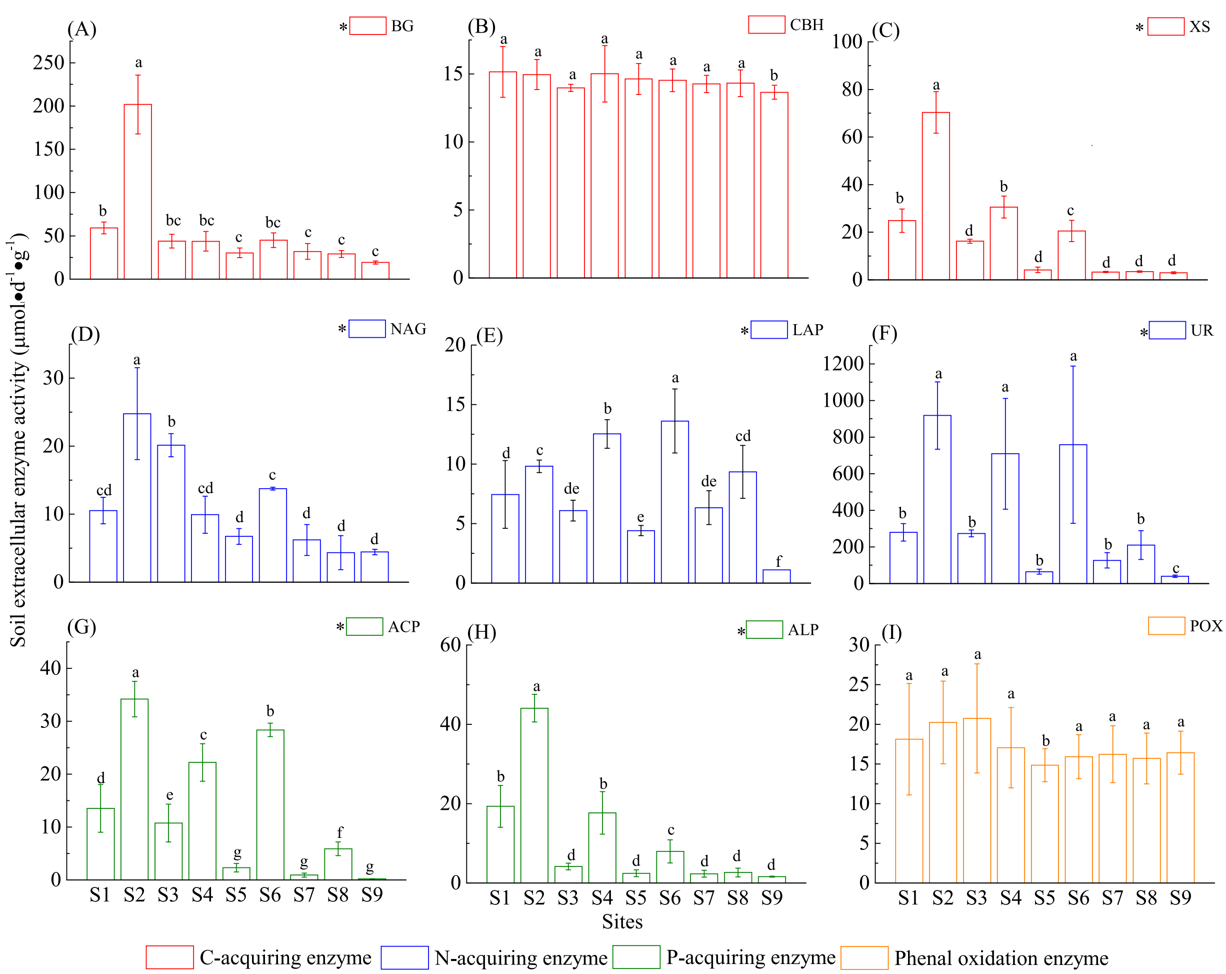

Fig. 2. Spatial variations in soil extracelluar enzyme activity (EEAs). (A) β-1,4-glucosidase, BG; (B) Cellobiohyrolase, CBH; (C) β-xylosidase, XS; (D) β-1,4-*N*-acetylglucosaminidase, NAG; (E) L-eucine aminopeptidase, LAP; (F) Urease, UR; (G) Acid phosphatase, ACP; (H) Alkaline phosphatase, ALP; (I) Phenol oxidase, POX. Lowercases are results of ANOVA, different letters indicate significant ($P < 0.05$) differences at different sites. All values are expressed as the mean ± stdev (n = 6). Asterisks show significant differences among sites (significance level = 0.05).

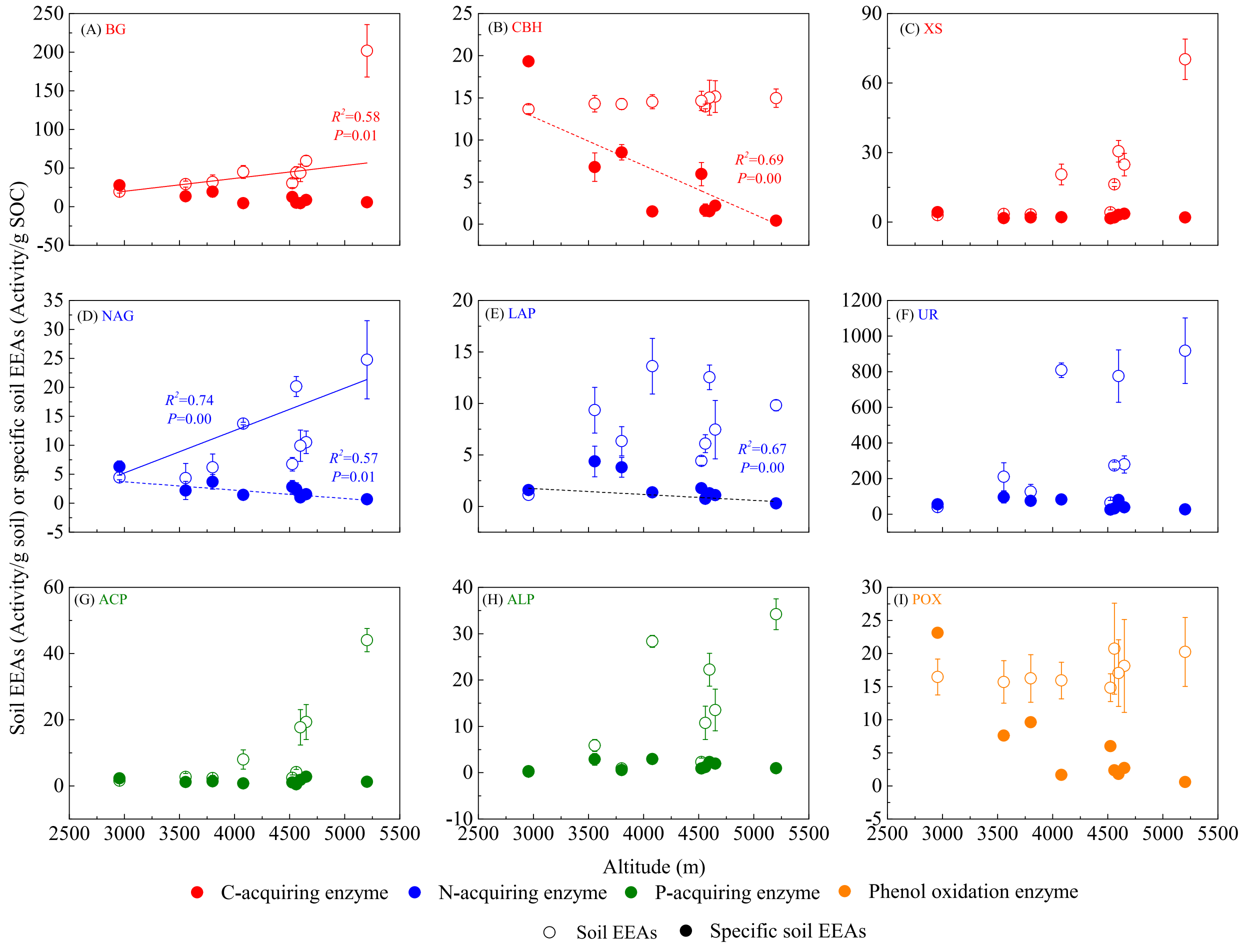

Fig. 3. Altitude patterns of soil extracellular enzyme activity (EEAs) and specific soil EEAs (normalized by soil organic carbon [SOC]). (A) *β*-1,4-glucosidase, BG; (B) Cellobiohyrolase, CBH; (C) *β*-xylosidase, XS; (D) *β*-1,4-*N*-acetylglucosaminidase, NAG; (E) L-eucine aminopeptidase, LAP; (F) Urease, UR; (G) Acid phosphatase, ACP; (H) Alkaline phosphatase, ALP; (I) Phenol oxidase, POX. The activities value is expressed as the mean ± stdev.

selected soil EEAs, with higher values in plant patches than in adjacent bare areas ($p < 0.05$). This trend was observed at all of the study sites, and the difference between microsites was higher in the case of POX and lowered for ACP.

The difference between plant patches and bare on soil EES were not significant ($p > 0.05$) (Fig. 6). The E_{CN} values for plant patches were between 1.24 and 1.84, which being a little higher than the values in bare areas (0.96–1.54). The E_{CP} values in plant patches (1.46–7.86) were slightly higher than those in bare areas (1.26–4.58). The E_{NP} values were similar in plant patches and bare areas found at most sample sites except for the lowest altitude site, where the E_{NP} value was higher in plant patches than in bare areas. The mean ratio of soil EES (E_{CNP}) was about 1:0.68:0.43 and 1:0.88:0.49 in plant patches and bare areas respectively. CQI and Vector A were higher in bare areas than plant patches, while the values of Vector L were marginally higher in plant patches.

3.3. Combined effects of abiotic and biotic factors on soil EEAs and EES

The first two RDA axes explained 69.86 % of the variation in soil EEAs and 75.47 % of the variation in soil EES (Fig. 7). The analysis results of VPA indicated that TN had the highest explanatory power for the soil EEAs (48.74 %) and EES (15.4 %). Compared to climate and plant factors (MAP, MAT and NDVI) which explained <20 %, only some edaphic factors (e.g., TN ($F = 126.54$; $P = 0.001$), SOC ($F = 12.89$; $P = 0.001$), TC ($F = 12.48$; $P = 0.001$), CQI ($F = 11.06$; $P = 0.001$), EC ($F = 9.41$; $P = 0.001$), NH_4^+-N ($F = 7.37$; $P = 0.002$), DON ($F = 5.22$; $P = 0.008$), NO_3^--N ($F = 4.61$; $P = 0.01$)) explained 70.24 % of the spatial variance in soil EEAs.

Similarly, some main edaphic factors explained 55.67 % of soil EES variation, among them, TN ($F = 22.30$; $P = 0.001$), DON ($F = 13.49$; $P = 0.001$), SOC ($F = 11.24$; $P = 0.001$), TC ($F = 10.94$; $P = 0.002$), EC ($F = 10.92$; $P = 0.002$), C:N ($F = 5.78$; $P = 0.02$), and CQI ($F = 4.90$; $P = 0.02$).

4. Discussion

4.1. Variation of soil EEAs and EES along altitude gradients in Qinghai-Tibet Plateau

Although most soil EEAs associated with C, N, and P cycles had significant spatial variation characteristics in experimental area, inconsistent with our first hypothesis, BG and NAG activity significantly increased with increasing altitude ($p < 0.05$) (Fig. 2). However, the result was consistent with our second hypothesis that plant microsites increased the soil EEAs ($p < 0.05$) (Fig. 5). The resource allocation theory pointed out that when a single element restricts microorganisms, there is a corresponding increase of soil extracellular enzymes to meet their nutrient requirements (Sinsabaugh et al., 2009). Therefore, the increase of C-cycle enzymes and N-cycle enzymes activity may indicate certain C and N restrictions with an increase of altitude and the formation of vegetation patches in this high altitude area. However, the soil C and N contents increased at higher altitude areas and under plant patches (Table 1S), so these results were inconsistent with the theory of resource allocation (Fujita et al., 2019). Jian et al. (2021) also indicated

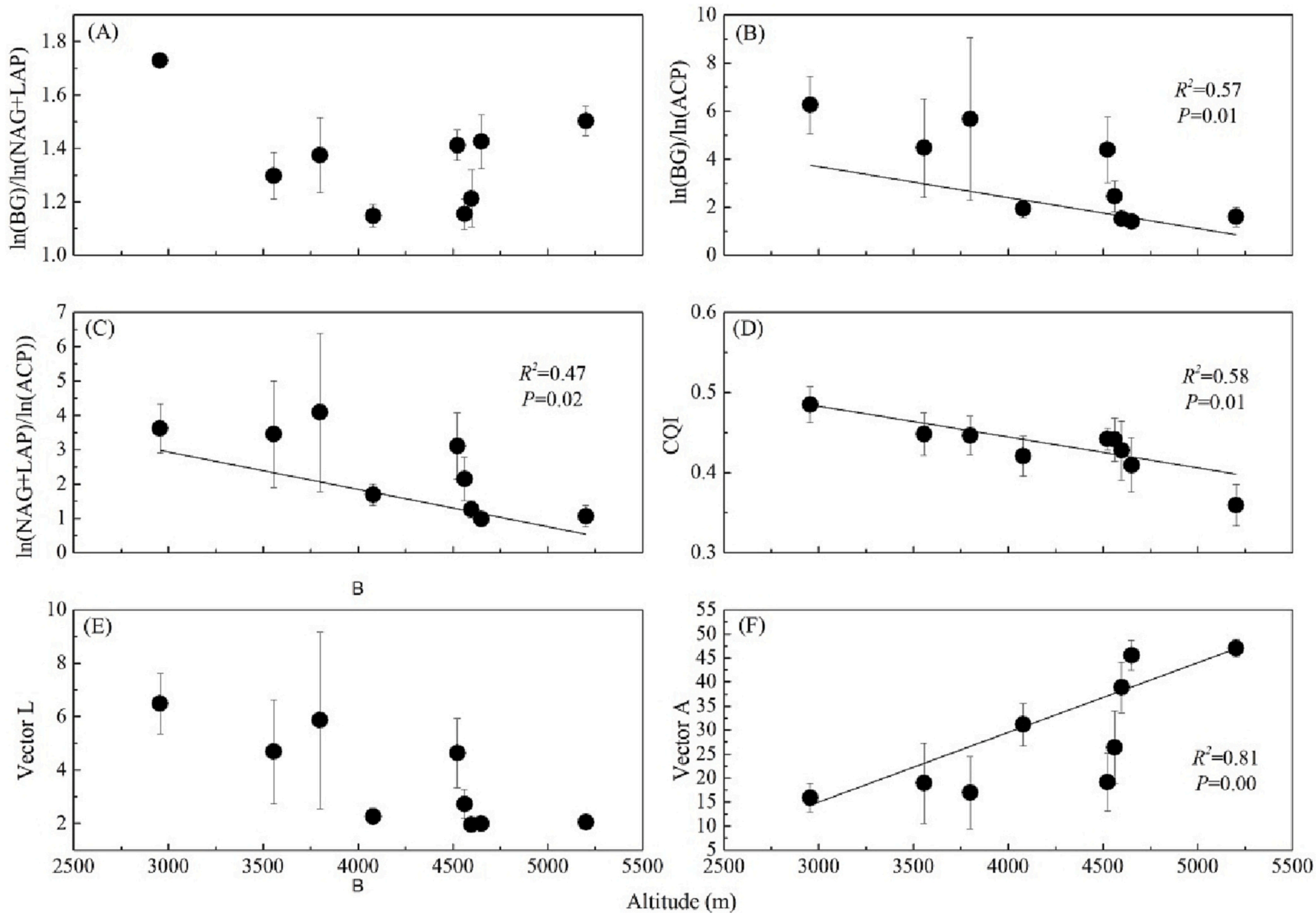

Fig. 4. Altitude patterns of soil ecoenzymatic stoichiometry (EES). (A) soil enzyme C:N ratio, E_{CN}; (B) soil enzyme C:P ratio, E_{CP}; (C) soil enzyme N:P ratio, E_{NP}; (D) Soil carbon quality index, CQI; (E) soil EES vector length, Vector L; (F) soil EES vector angle, Vector A. All ratios are expressed as the mean ± stdev (n = 6).

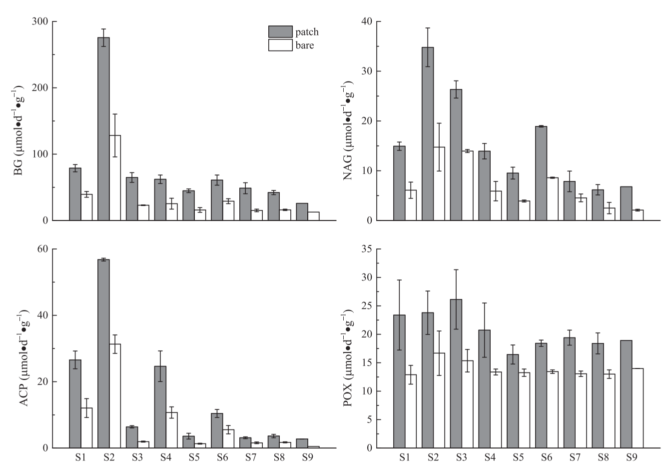

Fig. 5. Main soil extracelluar enzyme activities (EEAs) in vegetation patches (patch) and bare area at each study site (mean ± stdev, n = 3 for patch and bare, $P < 0.05$). BG, *β*-1,4-glucosidase; NAG, *β*-1,4-*N*-acetylglucosaminidase; ACP, acid phosphatase; POX, polyphenol oxidase.

that soil available P content and P-cycle enzymes activity increased along the altitude gradient in *Pinus massoniana* plantations in subtropical China which against the theory of resource allocation. The mechanism of these results need further analyzed.

As the carbon source for most microorganisms in soil organic matter, SOC provides an essential driving force for microbial activities (Wallenius et al., 2011). Specific soil EEAs (soil EEAs per g SOC) more appropriately represents the nutrient status. Although SOC and most soil EEAs all increased with increasing altitude (Table 1, Figs. 2 and 3), the increase rate of SOC was quicker than soil EEAs due to the variation of plant type with altitude, so some specific soil EEAs relating to C and N cycles showed different variation characteristics with soil EEAs in the study area. The specific soil CBH, NAG, and LAP decreased significantly with increasing altitude ($p < 0.05$, Fig. 3). Additionally, microorganisms mediate the effects of environmental factors on soil EEAs by altering the enzyme production efficiency (Bell et al., 2010). The significant variation of specific soil C and N cycle enzyme activities among altitudes suggested that the production efficiency of soil C and N cycle enzymes had distinct changes among soil microbes at different altitudes.

The E_{CP} and E_{NP} decreased significantly with altitude ($p < 0.05$) demonstrated that microbial metabolisms tended to involve higher investments in P-acquiring enzymes with altitude (Fig. 4). Therefore, soil EEAs and EES make a trade-off in nutrient acquisition among C, N, and P in response to variations in substrate quality and nutrient supply along an altitude gradient. Compared to other studies in Qinghai-Tibet Plateau (Wang et al., 2014; Wu et al., 2020), the soil EEAs values were different, which contributed to the differences in sampling time, plant type, and composition in study areas. Moreover, soil EEAs values and variation changed with soil depth and sampling time (Wu et al., 2020). The use of soil samples collected from only the 0–10 cm layer of soil in the summer limited the understanding of the temporal and spatial variation of soil EEAs in deeper soil layers. Sinsabaugh et al. (2008) presented results from 40 ecosystems on a globle scale, documenting that the most

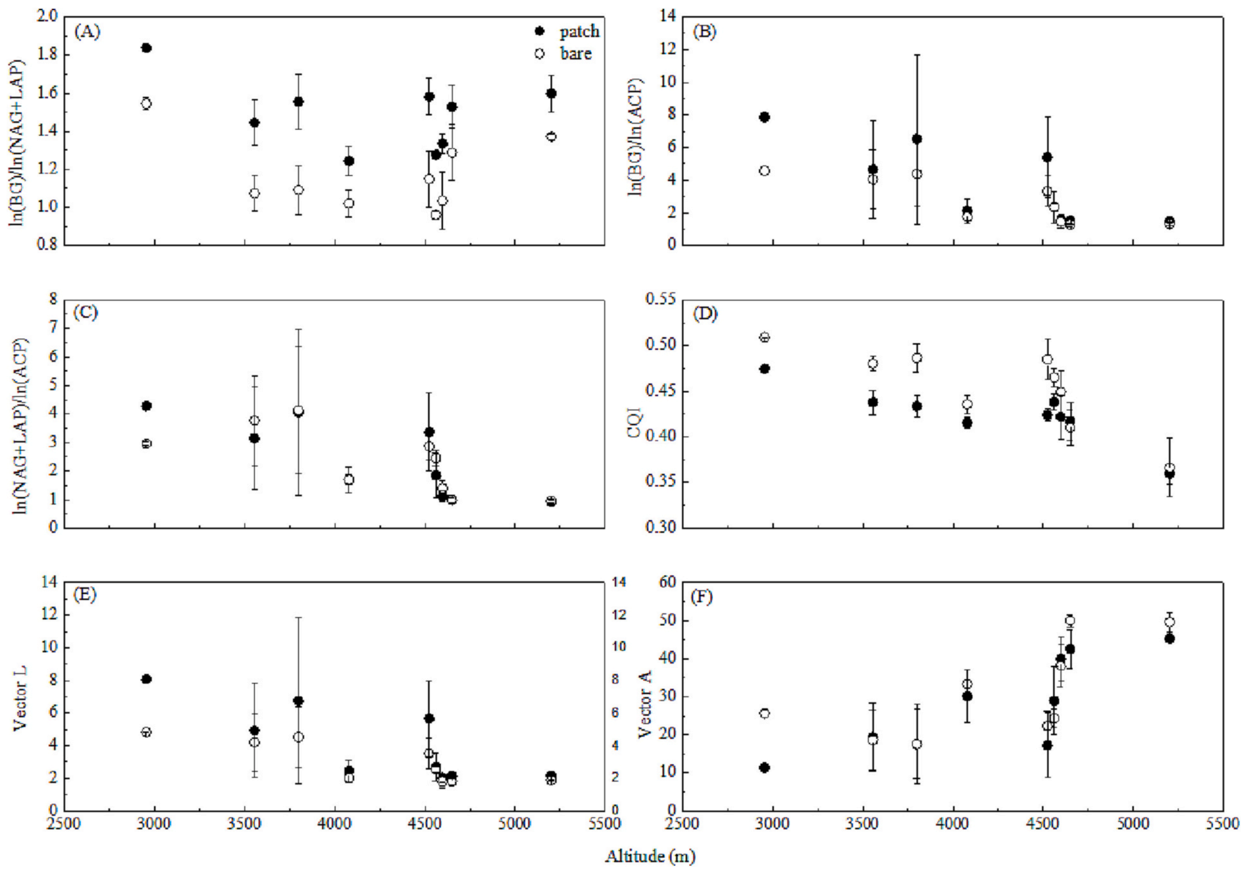

Fig. 6. Soil ecoenzymatic stoichiometry (EES) in vegetation patches (patch) and bare area (bare) at each study site (mean ± stdev, n = 3 for patch and bare). (A) soil enzyme C:N ratio, E_{CN}; (B) soil enzyme C:P ratio, E_{CP}; (C) soil enzyme N:P ratio, E_{NP}; (D) Soil carbon quality index, CQI; (E) soil EES vector length, Vector L; (F) soil EES vector angle, Vector A.

commonly measured soil EEAs showed different variation ranges and distributions characteristics as the change of ecosystem types. However, these different variation ranges and distributions characteristics converge on a common pattern linked to the stoichiometry of microbial growth. So the distribution and variation characteristics of microorganism are the focus of future research in study area, which helps us better understand the mechanisms of nutrient change with altitude in Qinghai-Tibet Plateau.

4.2. Drivers of spatial variation in soil EEAs and EES

Soil EEAs are affected by many physical, chemical, and biological factors (Jian et al., 2016). Within this study, soil EEAs and EES were significantly influenced by edaphic factors, especially TN (Fig. 7). This result supported previous findings (), showing that edaphic variables had a greater influence on soil EEAs and EES, such as SOC, total N, C:P, C:N, bulk density and so on (Jeon et al., 2012; Li et al., 2020a). In Swiss Alpine grasslands, soil EEAs were also positively related to most of the measured parameters indicating of organic matter quantity and quality (Park et al., 2014). Because soil nutrients affect the reproduction, growth, and development of plants and microorganisms and cause the secretion of various enzymes in the plant rhizosphere (Sun et al., 2021), these varying nutrient contents alter the microbial community structure and enzymatic systems (Loeppmann et al., 2016) and eventually lead to spatial heterogeneity in soil EEAs (Yang et al., 2018). Furthermore, these soil EEAs also participate in soil C, N, and P cycling, so there is a complementary relationship between soil enzyme and nutrient content. However, there was no clear relationship between soil P-cycle enzyme activity and soil P content in our study, similar to the result of Waring et al. (2014) based on regional data from tropical forests. This result was different from that found in phosphorus-limited ecosystems where phosphatase production and activity increase with soil microbial phosphorus demand. Furthermore, it is worth noting that the spatial distribution patterns of soil EEAs were not significantly influenced by soil pH in this study. Firstly, at all study sites, the soil pH value was >7 (alkaline soil), and the variation was not significant ($p > 0.05$). Secondly, the microbial activity may mediate the effects of soil pH on soil EEAs (Stark et al., 2014), which induced the influence of pH on soil EEAs was indirect and not significant in this area. On a global scale, most key soil EEAs (CBH, NAG, LAP, ACP and POX) had significant but weaker, univariate relationships with bulk soil pH (Sinsabaugh et al., 2008).

Soil EEAs have also been documented as sensitive to temperature and moisture (Steinweg et al., 2012). Previous research has indicated that the natural seasonal cycle of soil EEAs were amplified in a greenhouse setting for an arctic tundra soil (Sistla and Schimel, 2013). Although MAT and MAP were also important factors influencing soil EEAs in our study area, their contributions were small compared to edaphic factors (Fig. 7). Following the analysis of RDA, soil TN, SOC, TC, CQI, EC, NH_4^+-N, DON, and NO_3^--N explained 70.24 % of the spatial variance in soil EEAs. 55.67 % of soil EES variation was explained by soil TN, DON, SOC, TC, EC, C:N, and CQI (Fig. 7). Soil properties are not always altitudinal specific due to the influence of specific topography and vegetation conditions (Fierer et al., 2011; Tan et al., 2021). The altitudinal pattern of soil EEAs and EES could result from the long-term adaptation of soil microbes to altitude-specific and site-specific soil conditions (Jing et al., 2020). Therefore, edaphic factors, not climate factors, strongly determine the spatial changes in soil EEAs and EES along the altitudinal gradient in our study region (Cao et al., 2021).

Many researchers have found that plants greatly influenced the concentration of soil enzymes because of their intrinsic properties

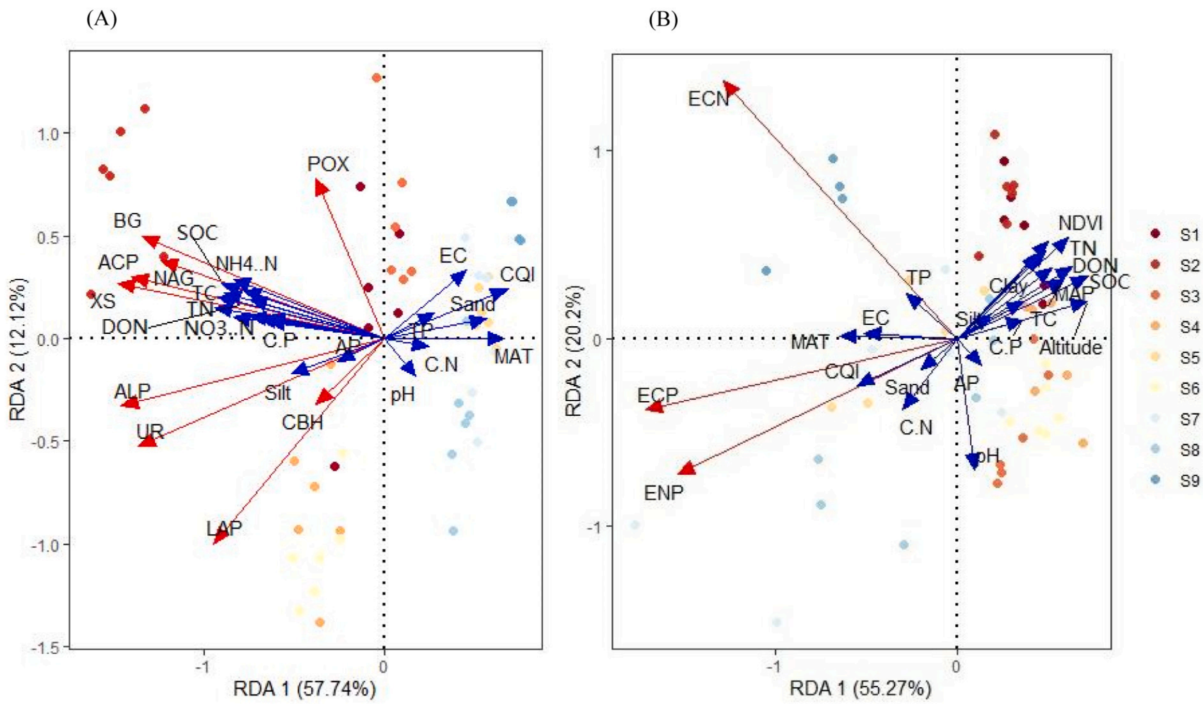

Fig. 7. Redundancy analysis for the relationship of soil extracelluar enzyme activities (EEAs) (A) and ecoenzymatic stoichiometry (EES) (B) with edaphic and climate parameters. BG, β-1,4-glucosidase; CBH, Cellobiohyrolase; XS, β-xylosidase; NAG, β-1,4-*N*-acetylglucosaminidase; LAP, Leucine aminopeptidase; UR, Urease; ACP, Acid phosphatase; ALP, Alkaline phosphatase; POX, Phenol oxidase. TN, Soil total nitrogen; TC, Soil total carbon; TP, Soil total phosphorus; NH_4^+-N, Soil ammonium; NO_3^--N, Soil nitrate nitrogen; DON, Soil dissolved organic nitrogen; SOC, Soil organic carbon; AP, Soil available phosphorus; C:N, C:P, N:P, The molar ratios of soil carbon to nitrogen, carbon to phosphorus and nitrogen to phosphorus, respectively; CQI, Soil carbon quality index; EC, Soil electric conductivity; Sand, Soil sand content; Silt, Soil silt content; Clay, Soil clay content; MAP, Mean annual precipitation; MAT, Mean annual temperature; NDVI, Normalized difference vegetation index; ECN, ECP and ENP are the ratios of soil extracelluar enzyme C:N, C:P and N:P, respectively.

(Zhang et al., 2013), like the effects of plant hormones and plant biomass on soil EEAs (Solangi et al., 2019; Li et al., 2020b). The effects of plants on the soil environment were greater than soil type in some study areas (such as the Loess Plateau in China) (Cui et al., 2018). Compared to other factors, the effect of NDVI on most soil EEAs was not prominent, but its effect was significant on EES (Fig. 7). The effect of NDVI may be indirect, taking effect by influencing soil nutrient content in different ecotones. CQI decreased significantly with the increasing altitude and the values were higher for bare than vegetation patches except at higher altitude sites (>4500 m) where the CQI value was similar (Figs. 4D and 6D), indicating that the abundance of readily decomposed carbon was higher at high altitudes and under plant patches.

Generally, the selected a set of abiotic and biotic factors explained most of the variations in all soil EEAs and EES in our study (Fig. 7). Other drivers, such as soil physical properties, chemical properties and biotic factors putatively contribute to the spatial variation of soil EEAs and EES (Cui et al., 2018; Zhou et al., 2020). Further exploration is needed to clarify the effects of abiotic and biotic factors on soil EEAs and EES across multiple scales in high-altitude areas of the Qinghai-Tibet Plateau.

4.3. Soil C, N limitation in Qinghai-Tibet Plateau

The mean value of soil enzyme stoichiometry ratio (E_{CNP}) was about 1:0.46:0.29, which deviated from the global ecosystem value of 1:1:1 (Sinsabaugh et al., 2008). The mean value of soil E_{CN}, E_{CP}, and E_{NP} at different altitudes was 1.36, 3.30, and 2.38, compared to the average E_{CN}, E_{CP}, and E_{NP} values of global terrestrial ecosystems (1.41, 0.62, and 0.44) (Sinsabaugh et al., 2008). These results indicated that soil microorganisms in this region have a high demand for carbon and nitrogen sources.

Generally, the soil carbon and nitrogen nutrient contents were higher with increasing altitude in study area (Table S1), that is to say, soil carbon and nitrogen restrictions were alleviated with increasing altitude, and the demand for phosphorus increased. Cao et al. (2021) found that the soil invertase, urease, and acid phosphatase activities in the mineral soil layer increased over altitude, while this variation trend was not significant in the soil organic layer on the eastern Qinghai-Tibet Plateau. Our results demonstrated that soil EES was resource-dependent in the study area. In response to the variation of substrate quality and nutrient supply at different altitudes, microbial metabolisms tended to involve higher investments of carbon and nitrogen acquiring enzymes than phosphorus acquiring enzymes. This outcome was consistent with research conducted in forest environments (Jian et al., 2021) and grassland environments (Peng and Wang, 2016).

In this study, vector A increased significantly with altitude, and most values were less than 45° except for the highest altitude area (site 1 and 2), where the values of Vector A were close to 45°. These calculations indicated that soil microorganisms were severely restricted by nitrogen, with this limitation being alleviated at increasing altitudes. Moreover, the soil C:N:P ratios in the 0–10 cm soil layer along the altitude gradient were 80:1:1 (Table S1). The corresponding values were 186:13:1 and 134:9:1 based on samples from surface soil (0-10 cm) on the global and national scale, respectively (Cleveland and Liptzin, 2007; Tian et al., 2010). These results also indicated that the carbon and nitrogen limitations were significant at Qinghai-Tibet Plateau of China.

Some soil characteristics were measured in this study, while others were not included, limiting the in-depth analysis and explanation of the variation pattern of soil EEAs and the influencing factors. The influence of the soil environment on enzymatic activity is complex in the alpine meadow ecotone. Therefore, to avoid extrapolation bias, it is necessary to consider various influencing factors to explore the trend and mechanisms behind enzyme activity changes, indicating a need for further experimentation and study. Furthermore, the soil microorganism work with soil enzymes to drive soil biogeochemical processes, and as living organisms in soil, soil microorganisms are sensitive to environmental changes and can indicate changes in ecosystem functions earlier, so the altitudinal variation characteristics of soil microorganism and their relationship with biological and abiotic factors will be the focus of future research.

5. Conclusions

Soil carbon, nitrogen and phosphorus cycle enzymes activity showed significantly varying characteristics at different altitude gradients in the eastern Qinghai-Tibet Plateau. These differences were mainly controlled by edaphic factors, especially TN. The C-cycle enzymes and N-cycle enzymes activity were higher for plant patches and increased with increases in altitude, this is consistent with the variation trend of soil carbon and nitrogen contents and inconsistent with the theory of resource allocation, which indicated that the productivity of soil C and N cycle enzymes by soil microorganisms varied significantly at different elevations. The decreased ECP and ENP in high altitude areas demonstrated that microbial metabolism tended to increase the input of P-acquiring enzymes with increasing altitudes, which made a trade-off in nutrient acquisition. The soil E_{CNP} value deviated from the global ecosystem value, with E_{CP} and E_{NP} being much higher, implying that the study area may suffer from carbon and nitrogen limitations affecting microbial and plant growth, and carbon and nitrogen limitations were gradually alleviated with increasing altitudes.

Our study provides valuable information on the spatial dynamics of soil EEAs and EES, determining the main influencing factors of their variations in high altitude areas and elucidating how soil EEAs may be affected by vegetation microhabitats. Our findings confirm the nutrient limitation characteristics of the Qinghai-Tibet Plateau. These results may help to develop the conservation and management techniques for fragile alpine ecosystems in the context of global climate change.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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References

Adamczyk, B., Kilpeläinen, P., Kitunen, V., Smolander, A., 2014. Potential activities of enzymes involved in N, C, P and S cycling in boreal forest soil under different tree species. Pedobiologia 57 (2), 97–102.

Bell, T.H., Klironomos, J.N., Henry, H.A.L., 2010. Seasonal responses of extracellular enzyme activity and microbial biomass to warming and nitrogen addition. Soil Sci. Soc. Am. J. 74, 820–828.

Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: current knowledge and future directions. Soil Biol. Biochem. 58, 216–234.

Cao, R., Yang, W.Q., Chang, C.H., Wang, Z., Wang, Q., Li, H., Tan, B., 2021. Differential seasonal changes in soil enzyme activity along an altitudinal gradient in an alpine-gorge region. Appl. Soil Ecol. 166, 104078.

Chen, H., Zheng, M.H., Mao, Q.G., Xiao, K.C., Wang, K.L., Li, D.J., 2019. Cropland conversion changes the status of microbial resource limitation in degraded karst soil. Geoderma 352, 197–203.

Cleveland, C.C., Liptzin, D., 2007. C:N:P stoichiometry in soil: is there a "Redfieldratio" for the microbial biomass? Biogeochemistry 85, 235–252.

Cui, Y.X., Fang, L.C., Guo, X.B., Wang, X., Zhang, Y.J., Li, P.F., Zhang, X.C., 2018. Ecoenzymatic stoichiometry and microbial nutrient limitation in rhizosphere soil in the arid area of the Northern Loess Plateau,China. Soil Biol. Biochem. 116, 11–21.

Fatemi, F.R., Fernandez, I.J., Simon, K.S., Dail, D.B., 2016. Nitrogen and phosphorus regulation of soil enzyme activities in acid forest soils. Soil Biol. Biochem. 98, 171–179.

Fierer, N., McCain, C.M., Meir, P., Zimmermann, M., Rapp, J.M., Silman, M.R., Knight, R., 2011. Microbes do not follow the elevational diversity patterns of plants and animals. Ecology 92, 797–804.

Fujita, K., Miyabara, Y., Kunito, T., 2019. Microbial biomass and ecoenzymatic stoichiometries vary in response to nutrient availability in an arable soil. Eur. J. Soil Biol. 91, 1–8.

German, D.P., Weintraub, M.N., Grandy, A.S., Lauber, C.L., Rinkes, Z.L., Allison, S.D., 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biol. Biochem. 43, 1387–1397.

He, Q.Q., Wu, Y.H., Bing, H.J., Zhou, J., Wang, J.P., 2020. Vegetation type rather than climate modulates the variation in soil enzyme activities and stoichiometry in subalpine forests in the eastern Tibetan Plateau. Geoderma 374, 114424.

Hill, B.H., Elonen, C.M., Jicha, T.M., Kolka, R.K., Lehto, L.L.P., Sebestyen, S.D., SeifertMonson, L.R., 2014. Ecoenzymatic stoichiometry and microbial processing of organic matter in northern bogs and fens reveals a common P-limitation between peatland types. Biogeochemistry 120, 203–224.

Jeon, S.M., Chandel, N.S., Hay, N., 2012. AMPK regulates NADPH homeostasis to promote tumourcell survival during energy stress. Nature 485 (7400), 661.

Jian, S.Y., Li, J.W., Chen, J., Wang, G.S., Mayes, M.A., Dzantor, K.E., Hui, D.F., Luo, Y.Q., 2016. Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: a meta-analysis. Soil Biol. Biochem. 101, 32–43.

Jian, Z.J., Ni, Y.Y., Zeng, L.X., Lei, L., Xu, J., Xiao, W.F., Li, M.H., 2021. Latitudinal patterns of soil extracellular enzyme activities and their controlling factors in Pinus massoniana plantations in subtropical China. For. Ecol. Manag. 495, 119358.

Jing, X., Chen, X., Tang, M., Ding, Z.J., Jiang, L., Li, P., Ma, S.H., Tian, D., Xu, L.C., Zhu, J.X., Ji, C.J., Shen, H.H., Zheng, C.Y., Fang, J.Y., Zhu, B., 2017. Nitrogen deposition has minor effect on soil extracellular enzyme activities in six Chinese forests. Sci. Total Environ. 607–608, 806–815.

Jing, X., Chen, X., Fang, J.Y., Ji, C.J., Shen, H.H., Zheng, C.Y., Zhu, B., 2020. Soil microbial carbon and nutrient constraints are driven more by climate and soil physicochemical properties than by nutrient addition in forest ecosystems. Soil Biol. Biochem. 141, 107657.

Li, B., Dong, S.C., Jiang, X.B., Li, Z.H., 2008. Analysis on the driving factors of grassland desertifcation in Zoige wetland. J. Soil Water Conserv. 15, 112–115.

Li, X.F., Zhang, Y., Ding, C.X., Liu, Y., Wu, K.X., Jiang, F.Z., Su, D.R., 2020a. Water addition promotes vegetation recovery of degraded alpine meadows by regulating soil enzyme activity and nutrients in the Qinghai-Tibetan Plateau. Ecol. Eng. 158, 106047.

Li, Y.Q., Wang, Z.X., Li, T., Jiang, S.H., Sun, Z.Y., Jiang, H., Qian, M.Y., Zhou, H.J., Xu, Q., 2020b. Changes in microbial community and methanogenesis during high-solid anaerobic digestion of ensiled corn stover. J. Clean. Prod. 242, 118479.

Liu, L., Li, D.Z., Sun, Y.M., Zhu, Y.Y., Li, L.L., Ren, Z.X., Kong, R.P., Jia, P., Peng, N.N., Jia, J., Ghulam, R.S., Rafique, T., He, J., Ji, Y.Q., Wang, X., 2021. Pattern of soil extracellular enzyme activities along a tidal wetland with mosaic vegetation distributions in Chongming Island,China. J. Clean. Prod. 315 (1), 127991.

Loeppmann, S., Blagodatskaya, E., Pausch, J., Kuzyakov, Y., 2016. Substrate quality affects kinetics and catalytic efficiency of exo-enzymes in rhizosphere and detritusphere. Soil Biol. Biochem. 92, 111–118.

Moorhead, D.L., Sinsabaugh, R.L., Hill, B.H., Weintraub, M.N., 2016. Vector analysis of ecoenzyme activities reveal constraints on coupled C, N and P dynamics. Soil Biol. Biochem. 93, 1–7.

Mori, T., 2020. Does ecoenzymatic stoichiometry really determine microbial nutrient limitations? Soil Biol. Biochem. 146, 107816.

Park, J.H., Meusburger, K., Jang, I., Kang, H., Alewell, C., 2014. Erosion-induced changes in soil biogeochemical and microbiological properties in Swiss alpine grasslands. Soil Biol. Biochem. 69, 382–392.

Peng, X.Q., Wang, W., 2016. Stoichiometry of soil extracellular enzyme activity along a climatic transect in temperate grasslands of northern China. Soil Biol. Biochem. 98, 74–84.

Sinsabaugh, R.L., Hill, B.H., Follstad Shah, J.J., 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462, 795–798.

Qiu, X.C., Peng, D.L., Tian, H.X., Wang, H.B., Liu, X., Cao, L., Li, Z., Cheng, S., 2021. Soil ecoenzymatic stoichiometry and microbial resource limitation driven by thinning practices and season types in Larix principis-rupprechtii plantations in North China. For.Ecol Manag. 482, 118880.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecol. Lett. 11, 1252–1264.

R Development Core Team, 2019. R version 3.6.1 R foundation for statistical computing. Vienna, Austria.
Sinsabaugh, R.L., Follstad Shah, J.J., 2012. Ecoenzymatic stoichiometry and ecological theory. Annu. Rev. Ecol. Evol. Syst. 43, 313–343.
Sistla, S.A., Schimel, J.P., 2013. Seasonal patterns of microbial extracellular enzyme activities in an arctic tundra soil: identifying direct and indirect effects of long-term summer warming. Soil Biol. Biochem. 66, 119–129.
Solangi, G.S., Siyal, A.A., Siyal, P., 2019. Spatiotemporal dynamics of land surface temperature and its impact on the vegetation. Civ.Eng.J. 5, 1753–1763.
Stark, S., Männistö, M.K., Eskelinen, A., 2014. Nutrient availability and pH jointly constrain microbial extracellular enzyme activities in nutrient-poor tundra soils. Plant Soil 383, 373–385.
Steinweg, J.M., Dukes, J.S., Wallenstein, M.D., 2012. Modeling the effects of temperature and moisture on soil enzyme activity: linking laboratory assays to continuous field data. Soil Biol. Biochem. 55, 85–92.
Sun, H.L., Zheng, D., Yao, T.D., Zhang, Y.L., 2012. Protection and construction of the national ecological security shelter zone on Tibetan Plateau. Acta Geograph. Sin. 67 (1), 3–12.
Sun, X.D., Ye, Y.Q., Ma, Q.X., Guan, Q.W., Jones, D.L., 2021. Variation in enzyme activities involved in carbon and nitrogen cycling in rhizosphere and bulk soil after organic mulching. Rhizosphere 19, 100376.
Tan, B., Yin, R., Zhang, J., Xu, Z.F., Liu, Y., He, S.Q., Zhang, L., Li, H., Wang, L.X., Liu, S. N., You, C.M., Peng, C.H., 2021. Temperature and moisture modulate the contribution of soil fauna to litter decomposition via different pathways. Ecosystems 24, 1142–1156.
Tian, H.Q., Chen, G.S., Zhang, C., Melillo, J.M., Hall, C.A.S., 2010. Pattern and variation of C:N:P ratios in China's soil: a synthesis of observational data. Biogeochemistry 98, 130–151.
Truong, C., Gabbarini, L.A., Corrales, A., Mujic, A.B., Escobar, J.M., Moretto, A., Smith, M.E., 2019. Ectomycorrhizal fungi and soil enzymes exhibit contrasting patterns along elevation gradients in southern Patagonia. New Phytol. 222, 1936–1950.
Wallenius, K., Rita, H., Mikkonen, A., Lappi, K., Lindström, K., Hartikainen, H., Raateland, A., Niemi, R.M., 2011. Effects of land use on the level, variation and spatial structure of soil enzyme activities and bacterial communities. Soil Biol. Biochem. 43 (7), 1464–1473.
Wang, X.X., Dong, S.K., Gao, Q.Z., Zhou, H.K., Liu, S.L., Su, X.K., Li, Y.Y., 2014. Effects of short-term and long-term warming on soil nutrients, microbial biomass and enzyme activities in an alpine meadow on the Qinghai-Tibet Plateau of China. Soil Biol. Biochem. 76, 140–142.
Waring, B.G., Weintraub, S.R., Sinsabaugh, R.L., 2014. Ecoenzymatic stoichiometry of microbial nutrient acquisition in tropical soils. Biogeochemistry 117, 101–113.
Wu, J.Q., Wang, H.Y., Li, G., Ma, W.W., Wu, J.H., Gong, Y., Xu, G.R., 2020. Vegetation degradation impacts soil nutrients and enzyme activities in wet meadow on the Qinghai-Tibet Plateau. Sci. Rep. 10, 21271.
Wu, P.F., Zhang, H.Z., Cui, L.W., Wickings, K., Fu, S.L., Wang, C.T., 2017. Impacts of alpine wetland degradation on the composition, diversity and trophic structure of soil nematodes on the Qinghai-Tibetan Plateau. Sci. Rep. 7 (1), 837.
Xiao, L., Liu, G.B., Li, P., Li, Q., Xue, S., 2020. Ecoenzymatic stoichiometry and microbial nutrient limitation during secondary succession of natural grassland on the Loess Plateau,China. Soil Tillage Res. 200, 104605.
Xu, Z.W., Yu, G.R., Zhang, X.Y., He, N.P., Wang, Q.F., Wang, S.Z., Wang, R.L., Zhao, N., Jia, Y.L., Wang, C.Y., 2017. Soil enzyme activity and stoichiometry in forest ecosystems along the North-South Transect in eastern China (NSTEC). Soil Biol. Biochem. 104, 152–163.
Xu, Z.W., Zhang, T.Y., Wang, S.Z., Wang, Z.C., 2020. Soil pH and C/N ratio determines spatial variations in soil microbial communities and enzymatic activities of the agricultural ecosystems in Northeast China: Jilin Province case. Appl. Soil Ecol. 155, 103629.
Yang, F., Tian, J., Fang, H.J., Gao, Y., Zhang, X.Y., Yu, G.R., Kuzyakov, Y., 2018. Spatial heterogeneity of microbial community and enzyme activities in a broad-leaved Korean pine mixed forest. Eur. J. Soil Biol. 88, 65–72.
Yang, Y., Liang, C., Wang, Y.Q., Cheng, H., An, S.S., Chang, S.X., 2020. Soil extracellular enzyme stoichiometry reflects the shift from P- to N-limitation of microorganisms with grassland restoration. Soil Biol. Biochem. 149, 107928.
Zhang, L.H., Song, L.P., Xu, G., Chen, P., Sun, J.N., Shao, H.B., 2013. Seasonal dynamics of rhizosphere soil microbial abundances and enzyme activities under different vegetation types in the coastal zone, Shandong, China. CLEAN-Soil, Air, Water 42 (8), 1115–1120.
Zheng, H.F., Liu, Y., Zhang, J., Chen, Y.M., Yang, L., Li, H.J., Wang, L.F., 2018. Factors influencing soil enzyme activity in China's forest ecosystems. Plant Ecol. 219, 31–44.
Zheng, H.F., Liu, Y., Chen, Y.M., Zhang, J., Li, H.J., Wang, L.F., Chen, Q.M., 2020. Short-term warming shifts microbial nutrient limitation without changing the bacterial community structure in an alpine timberline of the eastern Tibetan Plateau. Geoderma 360, 113985.
Zhou, L.L., Liu, S.S., Shen, H.H., Zhao, M.Y., Xu, L.C., Xing, A.J., Fang, J.Y., 2020. Soil extracellular enzyme activity and stoichiometry in China's forests. Funct. Ecol. 34, 1461–1471.
Zhu, X.M., Liu, M., Kou, Y.P., Liu, D.Y., Liu, Q., Zhang, Z.L., Jiang, Z., Yin, H.J., 2020. Differential effects of N addition on the stoichiometry of microbes and extracellular enzymes in the rhizosphere and bulk soils of an alpine shrubland. Plant Soil 449, 285–301.
Zuo, Y.P., Li, J.P., Zeng, H., Wang, W., 2018. Vertical pattern and its driving factors in soil extracellular enzyme activity and stoichiometry along mountain grassland belts. Biogeochemistry 141, 23–39.