Variation Characteristics of Soil Extracellular Enzyme Activity and Stoichiometry During Biological Soil Crust Formation on the Loess Plateau (Postprint)

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Abstract

Biological soil crusts play crucial roles in enhancing soil resistance to water and wind erosion and improving soil nutrient conditions. Soil extracellular enzyme activity can serve as a microbial indicator of soil biochemical reaction intensity and is of great significance for understanding nutrient cycling processes mediated by microorganisms in desert ecosystems. Soils from five biological crust development stages (bare sand, full algae crust, algae-moss mixed crust, moss-algae mixed crust, and full moss crust) in the Liudaogou watershed of Shenmu County on the Loess Plateau were selected as research objects to investigate the variation characteristics of soil extracellular enzyme activity (β-1,4-glucosidase, β-1,4-N-acetylglucosaminidase, leucine aminopeptidase, and alkaline phosphatase) and its stoichiometry during biological crust development. The results showed that: (1) The activities of all four soil extracellular enzymes increased significantly along the biological crust development sequence, with full moss crust soils being significantly greater than full algae crust soils ($P<0.05$). (2) Extracellular enzyme activities in the biological soil crust layer were significantly higher than in the underlying soil, and enzyme activities decreased continuously with increasing soil depth. (3) Correlation analysis indicated that soil C, N, P, soil C:P, and soil N:P all showed highly significant positive correlations with soil extracellular enzyme activity ($P<0.05$). (4) Nutrient contents and soil extracellular enzyme activities in crust-covered soils were significantly higher than in bare sand, and nutrient contents and soil extracellular enzyme activities in full moss crust soils were significantly higher than in full algae crust soils. (5) Standardized major axis estimation showed that soil extracellular enzyme activities were significantly enhanced with biological crust development and exhibited homeostatic characteristics. The slopes between N-acquisition enzymes and P-acquisition enzymes relative to C-acquisition enzymes showed an isometric relationship, suggesting

that soil microorganisms play an important role in nutrient cycling processes in arid and semi-arid ecosystems through homeostatic regulation of extracellular enzyme stoichiometric characteristics.

Full Text

Variation in Soil Extracellular Enzyme Activities and Stoichiometry During Biological Soil Crust Formation in the Loess Plateau

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Abstract

Biological soil crusts (BSCs) play an important role in enhancing soil resistance to water and wind erosion and improving soil nutrient conditions. Soil extracellular enzyme activity serves as a microbial indicator of soil biochemical reaction intensity, which is crucial for understanding nutrient cycling processes involving microorganisms in desert ecosystems. This study investigated soils from five biological crust development stages (bare sand, algal crust, algal-moss mixed crust, moss-algal mixed crust, and moss crust) in the Liudaogou watershed of Shenmu County on the Loess Plateau to characterize the variation in soil extracellular enzyme activities [β-1,4-glucosidase (BG), β-1,4-N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), and alkaline phosphatase (AP)] and their stoichiometry during biocrust formation. The results demonstrated that: (1) The activities of all four extracellular enzymes increased significantly along the biological crust developmental sequence, with moss crust soils exhibiting significantly greater activity than algal crust soils ($P<0.05$). (2) Extracellular enzyme activities were significantly higher in the BSC layer than in underlying soil layers, decreasing continuously with soil depth. (3) Nutrient contents and soil extracellular enzyme activities in crust-covered soils were significantly higher than in bare sand, with moss crust soils showing significantly higher values than algal crust soils. (4) Correlation analysis revealed that soil C, N, P, C:P, and N:P were all extremely significantly positively correlated with soil extracellular enzyme activities ($P<0.01$). (5) Standardized major axis estimation

indicated that soil extracellular enzyme activities were significantly enhanced and exhibited homeostatic characteristics with BSC development. The slopes between N- and P-acquiring enzymes relative to C-acquiring enzymes showed isometric relationships, suggesting that soil microorganisms play an important role in nutrient cycling in arid and semi-arid ecosystems through homeostatic regulation of extracellular enzyme stoichiometry.

Keywords: biological soil crusts; developmental sequence; extracellular enzymes; stoichiometry; Loess Plateau

Introduction

Biological soil crusts (BSCs) are organic complexes formed by cyanobacteria, fungi, algae, lichens, mosses, and soil particles, covering approximately 12% of the global terrestrial surface area. They enhance soil aggregation and stability, improve soil aeration and porosity, promote vascular plant establishment, and increase microbial community relative abundance, thereby playing crucial ecological roles in desert ecosystems. Through microbial photosynthetic carbon fixation, BSCs improve soil quality and contribute significantly to global soil carbon cycling. As BSCs develop, the complexity of soil substrates increases, promoting enzyme substrate supply and enhancing soil enzyme activities.

Soil extracellular enzymes, primarily derived from the decomposition and release of soil fauna, flora, and microorganisms, degrade high-molecular-weight organic compounds into assimilable molecules, playing vital roles in regulating soil organic matter degradation and nutrient cycling. Soil enzyme stoichiometry refers to the ratios of enzyme activities involved in nutrient element cycling within ecosystems, including "C-acquiring enzymes" (such as hemicellulase, cellulase, and glucosidase), "N-acquiring enzymes" (such as urease, chitinase, and peptidase), and "P-acquiring enzymes"(such as phosphatase). The most common extracellular enzymes involved in C, N, and P cycling include β-1,4-glucosidase, β-1,4-N-acetylglucosaminidase, leucine aminopeptidase, and phosphatase.

Soil enzyme ecological stoichiometry reflects the biogeochemical equilibrium between microbial metabolic processes and nutrient demands, providing a theoretical framework for understanding soil nutrient limitation and cycling dynamics. Previous research has demonstrated that soil extracellular enzyme activities gradually decrease during desert grassland desertification. However, the effects of BSC formation on soil extracellular enzyme activities and stoichiometric characteristics remain poorly understood. During BSC development, soil properties and microbial communities exhibit coupling and synergistic changes. Because BSCs exist at the atmosphere-soil interface, microorganisms are more susceptible to disturbance, necessitating consideration of microbial homeostasis when studying extracellular enzyme activities.

BSCs have become widespread surface covers following the Grain for Green Project on the Loess Plateau, comprising various types including algal, lichen, moss, and mixed crusts. While numerous studies have documented the effects of

BSC types and succession on soil nutrients, how BSCs regulate soil extracellular enzyme activities and physicochemical properties to influence enzyme stoichiometric characteristics requires further investigation. Therefore, this study examined changes in soil extracellular enzyme activities across BSC developmental sequences on the Loess Plateau to elucidate the effects of BSC formation on enzyme stoichiometric characteristics and to deepen understanding of BSCs' role in nutrient cycling in sandy ecosystems.

Materials and Methods

1.1 Study Area Overview The Liudaogou watershed is located in the northern Loess Plateau within Shenmu County, Shaanxi Province (110°21 ~110°23 E, 38°46 ~38°51 N). The watershed covers approximately 6 km^2 and features a semi-arid temperate climate characterized by dry, windy winters and springs. Elevations range from 1094.0 to 1273.9 m, with an average annual temperature of 8.4°C and average annual precipitation of 408.5 mm exhibiting high interannual variability. Heavy rainfall is concentrated in summer and autumn, accounting for 70%-80% of annual precipitation, with annual potential evaporation reaching 1000 mm. Following years of afforestation and grassland restoration, BSCs are widely distributed throughout the area, primarily including algal crusts, mixed crusts, and moss crusts with coverage typically ranging from 60% to 70%. These BSCs are mainly distributed on hill slopes and summits, occupying 60.7% of the watershed area.

1.2 Soil Sample Collection Five BSC development stages were selected in Liudaogou watershed: bare sand (CK), algal crust (dominated by cyanobacteria, A), algal-moss mixed crust (with greater algal crust coverage, AM), moss-algal mixed crust (with greater moss crust coverage, MA), and moss crust (dominated by *Didymodon vinealis*, M). Three representative plots were established at each stage with consistent slope aspect and position and similar gradients. Each plot measured 40 m × 40 m, with distances exceeding 100 m between plots. Within each plot, 12-15 sampling points were selected using an S-shaped random sampling pattern. Soil samples from these points were combined to obtain approximately 2 kg of representative material. Sampling was conducted in July 2019, yielding 75 representative samples (5 stages × 3 replicates × 5 soil layers). Samples included the BSC layer (0-2 cm) and underlying soil layers (2-10 cm, 10-20 cm). The BSC layer comprised cryptogams and associated soil microorganisms cemented with surface soil particles. BSC thickness averaged $11.1\$\pm1.9mmforalgalcrust, 10.9\pm1.8mmforalgal-mossmixedcrust, 7.2\pm2.2mmformoss-algalmixedcrust, and 9.6\pm\$1.9$ mm for moss crust. After carefully removing moss tissues and residues, samples were placed in foam boxes with ice packs and transported to the laboratory within four hours. Soil samples were divided into two portions: one for immediate extracellular enzyme activity analysis within one week, and the other air-dried and passed through a 0.15 mm sieve for physicochemical property analysis.

1.3 Soil Sample Analysis Soil organic carbon (SOC) content was determined by the potassium dichromate volumetric method. Soil total nitrogen (TN) content was measured using the Kjeldahl method with H_2SO_4 mixed accelerator digestion. Soil total phosphorus (TP) content was determined by the H_2SO_4-$HClO_4$ digestion-molybdenum blue colorimetric method.

Soil extracellular enzyme activities were measured using a multifunctional microplate reader (Tecan Infinite M200 PRO, Switzerland) with microplate fluorometric methods. The assay principle employs fluorescent substances (4-methylumbelliferone, MUB; 7-amino-4-methylcoumarin, AMC) conjugated to specific substrates. Four C-cycling enzymes (β-1,4-glucosidase, BG), two N-cycling enzymes (β-1,4-N-acetylglucosaminidase, NAG; leucine aminopeptidase, LAP), and one P-cycling enzyme (alkaline phosphatase, AP) were measured. Enzyme activities were expressed as nmol substrate per gram dry soil per hour ($nmol \cdot g^{-1} \cdot h^{-1}$).

1.4 Data Analysis Microsoft Excel 2010 was used for data organization and calculation. Soil and enzyme stoichiometry were calculated using mass ratios. Data were log-transformed prior to analyzing linear relationships between soil C, N, P contents and their acquiring enzymes. Statistical analyses were performed using SPSS 25.0. One-way ANOVA was employed to test the effects of BSC formation stage, soil layer depth, and their interactions on soil C, N, P contents, stoichiometry, and extracellular enzyme activities. Origin 2018 and R 3.5.1 were used for graphical presentation. Redundancy analysis (RDA) was conducted to examine relationships between soil C, N, P contents, stoichiometry, and enzyme activities. Standardized major axis (SMA) estimation using SMATR 2.0 software was applied to explore proportional relationships between enzyme activities involved in C, N, and P cycling. The model $\log(y) = a + b \cdot \log(x)$ was used, where x and y represent enzyme activities, b is the slope, and a is the intercept. When the slope was not significantly different from 1.0, the relationship was described as isometric.

Results

2.1 Variation Characteristics of Soil C, N, and P Contents Across different BSC development stages, SOC, TN, and TP contents all decreased gradually with soil depth (Table 3). Significant differences existed among development stages ($P<0.05$). In the 0-2 cm layer, SOC and TN contents followed the order: moss crust > moss-algal mixed crust > algal-moss mixed crust > algal crust > bare sand, with significant differences between moss crust and bare sand ($P<0.05$). TP content showed no significant variation among development sequences. Throughout the 0-20 cm profile, SOC and TN contents were significantly higher in crust-covered soils than in bare sand, and significantly higher in moss crust than in algal crust ($P<0.05$).

Table 3 Variation characteristics of soil C, N, and P contents at different formation stages (mean ± SD)

Stage	Depth (cm)	SOC ($g \cdot kg^{-1}$)	TN ($g \cdot kg^{-1}$)	TP ($g \cdot kg^{-1}$)
CK	0-2	1.29$±0.28*Ab*	0.10±0.01*Ba*	0.19±0.02*ABd*|||2−
	10	1.28±0.43*Ac*	0.09±0.02*Bb*	0.19±0.06*ABc*|||10−
	20	1.10±0.27*Ba*	0.08±0.01*Bc*	0.16±0.01*Bb*||*A*|0−
	2	15.2±3.08*Ba*	0.46±0.14*Ab*	0.40±0.03*Ba*|||2−
	10	1.72±0.44*Ac*	0.18±0.05*Ac*	0.32±0.03*Ab*|||10−
	20	1.61±0.37*Ac*	0.17±0.05*Ac*	0.32±0.04*Ab*||*AM*|0−
	2	17.8±3.20*ABa*	0.40±0.04*ABa*	0.34±0.05*Ab*|||2−
	10	1.47±0.47*Ac*	0.16±0.03*Ac*	0.31±0.03*Ab*|||10−
	20	1.38±0.16*Aa*	0.15±0.03*Ac*	0.27±0.12*Ab*||*MA*|0−
	2	20.0±2.20*Aa*	0.45±0.06*Aa*	0.39±0.03*Ba*|||2−
	10	1.30±0.26*ABa*	0.18±0.05*Ac*	0.32±0.04*Ab*|||10−
	20	1.15±0.23*Ba*	0.16±0.05*Ac*	0.23±0.03*ABc*||*M*|0−
	2	19.8±3.97*Aa*	0.49±0.14*Ab*	0.40±0.03*Ba*|||2−
	10	1.72±0.44*Ac*	0.18±0.05*Ac*	0.32±0.04*Ab*|||10−
	20	1.61±0.37*Ac*	0.17±0.05*Ac*	0.32±$0.04Ab

Note: Different uppercase letters indicate significant differences among development stages within the same soil layer, while different lowercase letters indicate significant differences among soil layers within the same development stage (P<0.05). The same below.

2.2 Variation in Soil Extracellular Enzyme Activities and Relationships with Soil C, N, and P Extracellular enzyme activities differed significantly among development stages (P<0.05) (Table 4). In the 0-2 cm layer, enzyme activities followed the order: moss crust > moss-algal mixed crust > algal-moss mixed crust > algal crust > bare sand, with all crust stages showing significant increases compared to bare sand (P<0.05). The algal-moss mixed crust and moss crust stages differed significantly from bare sand (P<0.05), while enzyme activities did not vary significantly with development sequence in the 2-20 cm layer.

Linear regression analysis revealed that C-, N-, and P-cycling enzyme activities were extremely significantly positively correlated with soil C, N, and P contents (P<0.001) (Fig. 2). Standardized major axis analysis indicated that the slope between NAG+LAP and BG activities was greater than 1.0 and statistically significant in the algal-moss mixed crust stage (P<0.001), while slopes in other BSC development stages were not significantly different from 1.0, demonstrating constrained relationships (Table 5). Additionally, slopes between SOC, TP contents and BG, NAG+LAP activities were significantly greater than 1.0.

Table 4 Variation characteristics of soil extracellular enzyme activities at different formation stages (mean ± SD)

Stage	Depth (cm)	BG (nmol · h^{-1} · g^{-1})	NAG (nmol · h^{-1} · g^{-1})	LAP (nmol · h^{-1} · g^{-1})	AP (nmol · h^{-1} · g^{-1})
CK	0-2	80.1$±10.5*ABa*|19.5±1.65*Ba*|167±13.5*Aa*|145±12.3*Aa*|||2−10|71.6±9.81*Ba*|41.5±6.83*ABb*|67.7±5.23*Ab*|60.8±8.08*Bb*|||10−20|117±6.89*Aa*|26.9±5.11*ABb*|42.6±5.68*Ab*|42.4±7.69*Ab*||*A*|0−2|111±19.3*Aa*|36.5±12.5*ABb*|61.0±8.45*Aa*|86.2±10.31*Ab*|||2−10|13.7±1.95*Ab*|15.9±3.21*Ac*|12.3±1.60*Ab*|12.5±3.90*Ab*|||10−20|12.2±2.51*Ab*|13.6±3.14*Ab*|12.5±3.90*Ab*|9.68±1.74*Ab*||*AM*|0−2|6.73±1.97*Ab*|5.45±1.24*Ab*|6.14±2.76*Ab*|5.80±1.97*Ab*|||2−10|2.44±0.29*Ba*|0.46±0.14*Ab*|1.53±0.37*Bb*|2.44±0.65*Ac*|||10−20|2.27±0.46*ABc*|0.40±0.04*ABa*|2.09±0.69*ABc*|2.27±0.46*ABc*||*MA*|0−2|5.80±1.97*Ab*|6.14±2.76*Ab*|1.53±0.37*Bb*|2.41±0.69*Ac*|||2−10|2.44±0.65*Ac*|0.40±0.04*ABa*|2.09±0.69*ABc*|2.27±0.46*ABc*|||10−20|2.27±0.46*ABc*|0.34±0.05*Ab*|2.41±0.69*Ac*|2.27±0.46*ABc*||*M*|0−2|20.0±2.20*Aa*|0.45±0.06*Aa*|0.49±0.14*Ab*|0.40±0.03*Ba*|||2−10|1.30±0.26*ABa*|0.18±0.05*Ac*|1.15±0.23*Ba*|0.32±0.04*Ab*|||10−20|1.15±0.23*Ba*|0.16±0.05*Ac*|1.38±0.16*Aa*|0.32±$0.04Ab

2.3 Variation in Soil Stoichiometry and Enzyme Stoichiometry In the BSC layer, C:N, C:P, and N:P ratios increased gradually with development sequence: A<AM<MA<M (Fig. 3). Soil C:N ratio showed no significant variation among development sequences. Compared with bare sand, C:P and N:P ratios in the 0-2 cm layer decreased by 55.2% and 67.9% in algal crust, and by 56.7% and 76.9% in moss-algal mixed crust. The BG:AP and (NAG+LAP):AP ratios reached maximum values in algal crust and minimum values in moss-algal mixed crust.

Table 5 Standardized major axis analysis between extracellular enzyme activities involved in C, N, and P cycles and soil C, N, and P contents

Stage	Relationship	Slope	Intercept	R^2	P (test)
A	NAG+LAP vs BG	1.23	0.15	0.89	<0.001
AM	NAG+LAP vs BG	1.45	0.22	0.92	<0.001
MA	NAG+LAP vs BG	1.12	0.08	0.85	<0.01
M	NAG+LAP vs BG	1.18	0.11	0.88	<0.001
All stages	SOC vs BG	1.35	0.31	0.76	<0.001
All stages	TP vs AP	1.28	0.19	0.71	<0.001

Note: P(test) indicates significance testing of the slope (compared to 1.0). Slopes significantly greater than 1.0 are shown in bold (P<0.05).

2.4 Relationships Between Soil C, N, P Contents, Stoichiometry, and Enzyme Activities Correlation analysis demonstrated that soil C, N, P contents and stoichiometric ratios were extremely significantly positively correlated

with soil extracellular enzyme activities and stoichiometry (P<0.01) (Table 7). Redundancy analysis (RDA) showed that soil C, N, P contents and enzyme activities explained 88.17% of the variation in enzyme stoichiometry, with the first and second axes accounting for 77.00% and 11.17% of the variation, respectively (Fig. 4). In the BSC layer, soil C:N, C:P, and N:P ratios showed strong positive correlations with enzyme stoichiometric ratios (P<0.01).

Table 6 Effects of stage, soil layer, and their interaction on soil ecological stoichiometry and soil extracellular enzymes and their stoichiometric characteristics

Factor	SOC	TN	TP	C:N	C:P	N:P	BG	NAG+LAP	AP	BG:(NAG+LAP)	BG:AP	(NAG+LAP):AP
Stage	***	***	***	ns	***	***	***	***	***	***	***	***
Layer	***	***	***	ns	***	***	***	***	***	***	***	***
Stage×Layer	***	***	ns	ns	***	***	***	***	***	***	***	***

Note: ** indicates significant difference at P<0.001 level, ** at P<0.01 level, * at P<0.05 level; ns indicates no significant difference.*

Table 7 Correlation between C, N, P and their stoichiometry and soil extracellular enzyme activity and stoichiometry

Parameter	BG	NAG+LAP	AP	BG:(NAG+LAP)	BG:AP	(NAG+LAP):AP
SOC	0.89***	0.85***	0.76***	0.45**	0.38**	-0.32**
TN	0.87***	0.83***	0.74***	0.41**	0.35**	-0.28*
TP	0.76***	0.71***	0.68***	0.38**	0.42**	0.15ns
C:N	0.32**	0.28*	0.24*	0.18ns	0.15ns	-0.12ns
C:P	0.85***	0.81***	0.73***	0.52***	0.45**	-0.35**
N:P	0.83***	0.79***	0.71***	0.48**	0.41**	-0.31**

Note: ** indicates correlation significant at the 0.001 level (two-tailed), ** at the 0.01 level, * at the 0.05 level.*

Discussion

3.1 Effects of BSC Formation on Soil C, N, P Contents and Extracellular Enzyme Activities The formation of BSCs reflects the transformation of soil properties and regional ecological health in arid and semi-arid ecosystems. In these regions, BSCs covering sand surfaces promote fine particle accumulation and play a crucial role in improving sandy soil nutrients. Our results demonstrate that BSC formation significantly affects soil C, N, and P contents, consistent with previous studies. These effects likely relate to differences in BSC types and their biological composition and carbon fixation capacity. Moss crusts possess rougher surface structures that better capture organic matter, leading to nutrient accumulation beneath the crust layer. The biological composition

and carbon fixation capacity of moss crusts are significantly higher than those of algal crusts.

SOC and TN contents were higher in surface layers and decreased with depth, consistent with findings from other studies. The BSC layer exhibited significantly higher enzyme activities than underlying soil layers, with significant differences among development sequences. This pattern primarily results from: (1) relatively abundant nutrient content in BSC layers providing more substrates for enzymatic reactions; (2) higher enzyme activities in more developed BSC stages; (3) moss crusts' greater capacity to improve C and N input and exchange compared to algal crusts; and (4) BSCs moderating soil climate fluctuations through dark pigment absorption of solar radiation, thereby increasing enzyme activities. Enzyme activities decreased with soil depth, consistent with Maxwell et al. (2020). In the 2-20 cm layer, no significant differences were observed among development stages, indicating that BSC influence on underlying soil is limited and primarily related to soil parent material.

3.2 Effects of BSC Formation on Soil Stoichiometry and Enzyme Stoichiometry Soil ecological stoichiometry effectively indicates nutrient limitation types and soil quality status. BSC development significantly affected soil ecological stoichiometry (Table 6). During BSC development, vegetation nutrient return is dynamic, and soil physicochemical properties and microbial activity change significantly. Lower C:N and C:P ratios favor microbial decomposition of organic matter. In this study, C:N and C:P ratios in the BSC layer were higher than in underlying soils, indicating weaker organic matter decomposition rates in the BSC layer.

The average C:N, C:P, and N:P ratios in BSC layers were 14.6, 42.8, and 3.0 respectively, exceeding national averages (10.6, 24.0, 2.4), suggesting lower soil carbon source availability and organic matter decomposition rates compared to national levels. With BSC development, organic matter decomposition rates gradually weakened, increasing organic matter accumulation. Soil enzyme stoichiometry reveals the balance between microbial nutrient demand and environmental nutrient availability. When an element becomes limited in soil, microorganisms may release more enzymes to acquire that element in response to changes in soil substrate and nutrient supply. In this study, the BG:(NAG+LAP) ratio decreased with BSC development, similar to patterns observed in other studies, indicating greater microbial demand for N. The NAG+LAP:AP ratio remained relatively stable. Except in the BSC layer, the N:P ratio was significantly higher in underlying soils, suggesting relatively weaker N supply in subsoil that may become a limiting element for vegetation growth. The decrease in BG:(NAG+LAP) ratio with depth indicates that microorganisms release fewer N-cycling enzymes with increasing soil depth. Although enzyme stoichiometric ratios were significantly affected by BSC formation, microorganisms regulated enzyme release to maintain homeostasis.

3.3 Correlation Analysis of Soil C, N, P-Enzyme Activity-Enzyme Stoichiometry During vegetation-soil co-evolution, enzymes regulating C, N, and P cycling are influenced by soil nutrient status. Soil nutrient content indirectly regulates enzyme secretion through microorganisms, creating correlations between nutrients and enzyme activities. This study showed significant positive correlations between soil C, N, P contents and their acquiring enzymes, indicating that soil microbial activities are regulated by nutrient content. Higher nutrient content leads to higher enzyme activity, reflecting the energy source for enzyme production and secretion.

Soil C:N and C:P ratios showed significant correlations with enzyme stoichiometry, indicating different response mechanisms of enzyme activities to soil nutrient changes during BSC development. Microorganisms adjust their ecological stoichiometry to adapt to these changes. BSC development and soil depth were significantly correlated with enzyme activities and stoichiometry (Table 6), with interactive effects between these factors, demonstrating that enzyme activities and stoichiometry are mediated by nutrient availability. Different nutritional supplies at various BSC development stages affect microbial reproduction and growth, thereby determining enzyme secretion.

Conclusion

1. With BSC development, C-, N-, and P-cycling enzyme activities were significantly enhanced, with moss crust showing significantly higher activities than algal crust. This reflects that BSC formation enriches soil microbial diversity and quantity, thereby increasing soil extracellular enzyme activities. Within the same BSC development stage, enzyme activities decreased with soil depth.

2. Standardized major axis analysis revealed that slopes between soil organic carbon, total phosphorus and C-, N-acquiring enzyme activities were significantly greater than 1.0, showing non-isometric models and indicating dependence of soil enzymes on soil nutrients. This reflects the close relationship between soil extracellular enzymes, their stoichiometry, and soil nutrient cycling.

3. Across BSC development stages, slopes between N- and P-acquiring enzymes relative to C-acquiring enzymes showed isometric relationships, indicating that extracellular enzyme stoichiometry maintains homeostasis under changing environmental conditions. Microbial homeostasis plays an important role in regulating nutrient resource acquisition, enabling better adaptation to nutrient deficiency and maintenance of soil nutrient dynamic balance in relatively constrained environments.

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