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# Nitrogen mineralization in grazed BSC subsoil is mediated by itself and vegetation in the Loess Plateau, China



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### ABSTRACT

Biological soil crust (BSC) exists widely in many kinds of grassland, its effect on soil mineralization in grazing systems has well been studied, but the impacts and threshold of grazing intensity on BSC have rarely been reported. This study focused on the dynamics of nitrogen mineralization rate in biocrust subsoils affected by grazing intensity. We studied the changes in BSC subsoil physicochemical properties and nitrogen mineralization rates under four sheep grazing intensities (i.e., 0, 2.67, 5.33, and 8.67 sheep  $ha^{-1}$ ) in seasons of spring (May--early July), summer (July-early September), and autumn (September-November). Although this moderate grazing intensity contributes to the growth and recovery of BSCs, we found that moss was more vulnerable to trampling than lichen, which means the physicochemical properties of the moss subsoil are more intense. Changes in soil physicochemical properties and nitrogen mineralization rates were significantly higher under 2.67-5.33 sheep ha<sup>-1</sup> than other grazing intensities (Saturation phase). In addition, the structural equation model (SEM) showed that the main response path was grazing, which affected subsoil physicochemical properties through the joint mediation of BSC (25%) and vegetation (14%). Then, the further positive effect on nitrogen mineralization rate and the influence of seasonal fluctuations on the system was fully considered. We found that solar radiation and precipitation all had significant promoting effects on soil nitrogen mineralization rates, the overall seasonal fluctuation has a direct effect of 18% on the rate of nitrogen mineralization. This study revealed the effects of grazing on BSC and the results may enable a better statistical quantification of BSC functions and provide a theoretical basis to formulate grazing strategies in the grazing system of sheep in Loess Plateau even worldwide (BSC symbiosis).

### 1. Introduction

Biological soil crusts (BSCs) occur worldwide in grasslands, which cover approximately 50 million km<sup>2</sup> (~40% of the terrestrial area on Earth) (Liang et al., 2021), and occupy approximately 40% of arid and semi-arid areas (Stephens et al., 2019). BSCs play important roles in controlling surface runoff, preventing soil erosion, improving soil moisture, and mediating soil temperature and element transformations (Bi et al., 2021). Grazing is one of the main uses of grasslands, livestock dependent on grasslands provide livelihoods for more than one billion people worldwide and account for one-third of the global protein consumption requirements (Taugourdeau et al., 2016), it has a direct crushing effect on the BSC and indirectly affects the fertility of grasslands through fecal and urine matter by influencing soil mineralization (Bethany et al., 2019). Crushing and fertility both affect the structure and function of grassland ecosystems. Previous studies on the effect of grazing on nitrogen mineralization rate under BSCs have focused on different pathways, such as microbial and subsurface pathways (Williams et al., 2021). However, the response of varying grazing intensities on soil nitrogen mineralization rate at seasonal scales remains unknown.

In undisturbed grassland systems (primary succession systems), BSCs can represent grassland succession process to some extent, while in artificially disturbed grassland systems, the types and coverage of BSCs can represent grassland health status. The succession of BSC under undisturbed circumstances begins with the colonization of algae, gradually followed by moss and lichen after the ground surface is fixed, moss are more sensitive than lichen to grazing (Maestre et al., 2011; Chamizo et al., 2012). Some cyanobacteria in the BSC are capable of fixing

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atmospheric nitrogen and making it available to plants (Robinson, 2021), and can also fix carbon dioxide by photosynthesis, eventually converting it into soil organic carbon (SOC) (Chamizo et al., 2021). In grazing grasslands, the BSC is fragile during dry periods and its destruction by overgrazing cannot be naturally restored in a short period of time (Bo et al., 2019). Grazing management is therefore critically important to regulate the BSC (Robinson, 2021). Moderate grazing is beneficial to BSC development, whilst a high grazing intensity leads to their decline, or even complete disappearance (Ayuso et al., 2019). The nitrogen fixation effect of BSCs primarily results from the type and composition of coverage. At the same time, grazing reduced the abundance of cyanobacteria and the proportion of lichen, thereby significantly reducing the nitrogen fixation rate of the BSC (Kuske et al., 2012). Mineralization has important agricultural significance, and mineralized nitrogen is an important source of nitrogen for absorption by plants.

Although some studies have focused on the effect of grazing on the structural composition and soil mineralization under BSCs, further research is needed in order to predict the threshold levels of factors that influence trends in the BSC (Root et al., 2019; O'Connor and Germino, 2020; Robinson, 2021). The effects of grazing on soil moisture and BSC type tend to be synchronized because the BSCs have a certain protective effect on soil moisture (Li et al., 2021). So, we hypothesize (1) the mineralization rate of the soil under the BSC correspondingly changes as 'Hump curve' with the increase in grazing intensity; (2) The length of time the two curves reach their peak shows the difference between the two BSCs and because the sensitivity of moss is higher than that of lichen (concluding coverage and their subsoil physicochemical property); (3) Before the peak value of the curve rises rapidly, the grazing intensity promote the soil mineralization rate, crossing the threshold shows the opposite trend, in addition, warm season and cold season have synergistic or antagonistic effects on mineralization rate with grazing intensity (Fig. 1).

The typical steppe of China Loess Plateau is one of the most important parts in the Eurasian steppe, with a long grazing history of over 7000 years (Chen et al., 2017), and the high sensitivity to rainfall because the inter-annual precipitation fluctuates greatly (Huang et al., 2022). To test the above hypotheses (Fig. 1), a long rotational grazing experiment of Tan sheep in the loess Plateau was conducted to identify how the grazing intensity and growing season affect (1) the coverage of BSCs and vegetation and both relationships, (2) the nitrogen mineralization rate and physicochemical properties of soil under BSCs and the mechanism of climate, vegetation and soil properties on soil nitrogen mineralization. Our research results were expected to provide the key



Grazing intensity

Fig. 1. Diagram of the grazing effects on BSCs and physicochemical characteristics

**Note:** The blue and red curves show the response of soil nitrogen mineralization under moss and lichen to grazing intensity, respectively. The solid line and dotted line of the arrow respectively represent the response of soil mineralization rates in the warm season and cold season under different grazing intensities. threshold value of grazing intensity and the seasonal grazing period for the sustainability of BSC in global grazing lands, especially in arid and semiarid ecoregions.

### 2. Materials and methods

#### 2.1. Study site

The field experiments were conducted at Huanxian Grassland Agriculture Research Station of Lanzhou University, Gansu Province, China (37°07'N, 106°48'E; altitude 1700 m). The climate is characterized as temperate continental monsoon and the soil is classified as sandy, freedraining loess (Gong, 2007; Ren et al., 2008). The average annual temperature was 7.5  $^\circ \text{C}$  and the average annual rainfall was 269 mm yr<sup>-1</sup>, over 70% of which took place between late June and September (Fig. 2). The frost-free period was 125 days  $yr^{-1}$ , in accordance with the Comprehensive and Sequential Classification, the grassland is a typical temperate steppe (Ren et al., 2008). Spring and autumn are typically short, summer hot and humid, and winter long and cold. The soil is classified as sandy, free-draining loess. The plant growth period was from late March to early September. The main vegetation species were Lespedeza bicolor Turcz. (Lespedeza davurica (Laxm.) Schindl.). Stipa bungeana Trin., Artemisia capillaries Thunb. (Artemisia capillaris f. glabra), Heteropappus altaicus (Willd.) Novopokr., and Potentilla bifurca Linn. (Hu et al., 2019). The dominant agricultural system in the region is the integrated crop/grassland livestock production system (Hou et al., 2021).

### 2.2. Study design

A group of paddocks were set up for rotational Tan sheep grazing and every paddock was 50  $\times$  100 m (Chen et al., 2015). There were four grazing intensities, including 0 (control), 2.67, 5.33, and 8.67 sheep ha<sup>-1</sup>, with 3 replicates. The rotational grazing of Tan sheep started from the beginning of June and ended in late September each year, with a rotation period of 30 days and a grazing period of 10 days in each paddock for each rotation. The physicochemical properties under different BSCs were measured four times, before grazing in May, during grazing in July, after grazing in September, and two months after grazing in November. These months represented the spring (May to early July), summer (July to early September), and autumn (September to November) seasons of the study area.

### 2.3. Soil sample collection

In September 2015 and 2016, five  $(0.5 \times 0.5 \text{ m})$  sampling areas were designed according to the "W" form in each pasture. The quadrat was then further divided into 36 small squares and each one was tested using acupuncture methods. Specifically, the same sized small sample cross intersects between the vertical needle were taken through the whole community, needle samples of moss, lichen, and bare land were taken, and the number of needles that hit a species in the samples (N) and the ratio of the total number is the coverage of the species (Eq. (1)) (Zhang et al., 2001).

$$\operatorname{Cn}(\%) = \frac{N_n}{N_1 + N_2 + N_3 + N_4 + N_5} \times 100\%$$
(1)

In this experiment, the resin core in-situ culture method was used to test the influence of different types of BSC on the physicochemical properties of the soil. This began with an in-situ soil culture in May, when three quadrats measuring  $3 \times 3$  m were set diagonally in each grazing plot. Moss and lichen in a good growth state were selected for culture. The soil in-situ culture was carried out in May, July, September, and November to measure nitrate and ammonia nitrogen. All soil samples (0–10 cm) were divided into two parts; one part was used to measure the soil moisture (SM) content using the weighing method, the second parts were air-dried and then taken to the laboratory to measure



Fig. 2. The air temperature and precipitation of the study site in 2015 and 2016 (Data sources: the experiment station weather experimental station).

the soil pH, nitrate-nitrogen ( $NO_3^-N$ ), ammonium nitrogen ( $NH_4^+-N$ ), soil organic carbon (SOC) and total nitrogen (TN) contents. We also did a vegetation survey during the growing season.

#### 2.4. Measurement method and index calculation

The samples used to determine the physicochemical properties of the subsoil of BSCs were air-dried, the plant roots and insects growing in the soil were removed with an 80-mesh sieve. SM was measured using a soil multi-parameter rapid measuring instrument (TZS-ECW-G, China). The pH value of the soil was determined by a pH meter, and SOC was determined using the Walkley–Black dichromate oxidation method (Hu et al., 2020). TN was measured using the Kjeldahl method (Pruden et al., 1985). NH<sup>4</sup><sub>4</sub>-N and NO<sup>3</sup><sub>3</sub>-N were extracted with 2-M KCl solution and analyzed with a flow injection auto-analyzer (Sah, 1994). The nitrogen mineralization rate of soil was calculated by the formula used in Magill and Aber (2000).

Potential evapotranspiration ( $ET_0$ ) was measured using the Mc Cloud method (Mc method for short), and  $ET_0$  was an exponential function of temperature (Eq. (2)) (Mccloud, 1955).

$$ET_{0MC} = K \times W^{1.8T} \tag{2}$$

 $ET_{0MC}$  is the  $ET_0$  value calculated by the Mc method, mm/d; K = 0.254; W = 1.07. T<sub>rage</sub> represents the average temperature range in each month (Eq. (3)) (Sharafkhani et al., 2017).

$$T_{rage} = Average \left( T_{max} - T_{min} \right) \tag{3}$$

### 2.5. Statistics analysis

We used Two-way ANOVA with Duncan's significant difference (P <0.05) test to assess the effects of grazing intensity on the mean SM, pH, SOC, TN, NO<sub>3</sub><sup>-</sup>-N, NH<sub>4</sub><sup>+</sup>-N, and nitrogen mineralization rate in our study. We use the Mixed model to consider the grazing intensity and growth seasons interactions, and the contribution rate and effect size of environmental factors, grazing intensity and growth season are also considered, interannual variations were treated as duplications. For vegetation conditions, we considered the interaction of years and grazing intensity. All statistical analyses were conducted using SPSS 25.0 (SPSS Inc., Chicago, IL, USA), and scatter plots and bar graphs were drawn using Origin 2021. The structural equation model (SEM) was used to evaluate the direct and indirect effects of grazing intensity on BSC, vegetation status, and soil nitrogen mineralization rates under BSCs, and to consider their seasonal changes. We considered all possible paths, arranged them in a logical order from top to bottom and obtained the final structural equation model after model verification. Before

building the SEM model, the least square correlation matrix was derived for all variables, and the analysis was performed using AMOS 23.0 (SPSS Inc., Chicago, IL, USA).

### 3. Results

### 3.1. The coverage of BSC and vegetation under four grazing intensities

With the increase in stocking rate, the coverage of moss, lichen, vegetation, bare land and litter generally showed the same trend, while the regression trend of lichen coverage in 2015, lichen and vegetation in 2016 was not significant (P > 0.05). The coverage of moss and vegetation showed a trend of first increased then decreased. When the stocking rate was 2.67 sheep ha<sup>-1</sup>, the moss coverage reached the highest value (17.22% and 22.22%, respectively), whereas the vegetation reached the highest value of 33.14% and 32.81% respectively. The coverage of lichen was lowest (27.51%) in 2015 and rose to 37.82% in 2016 under 2.67 sheep ha<sup>-1</sup>. The litter coverage and bare land increased gradually as the stocking rates increased (Fig. 3).

### 3.2. Monthly fluctuations of soil physicochemical properties under different grazing intensities

Grazing intensity (GI) had significant effects on SOC and SM under BSCs and bare land soil, with the contribution rate of about 40% (P < 0.05). In addition, solar radiation (SR), precipitation (PR), temperature range ( $T_{rage}$ ), ET<sub>0</sub> and excreta (EX) had significant effects on SOC, TN, SM and pH to varying degrees (P < 0.05).

SOC and TN showed the same trends with no significant difference under 0, 2.67, and 5.33 sheep  $ha^{-1}$  grazing intensities, however, the SOC and TN in the control group (0 sheep  $ha^{-1}$ ) were significantly higher than that under 8.67 sheep ha<sup>-1</sup> (P < 0.05) (Fig. 4 A and B). Furthermore, the SOC content under moss and lichen was higher than that under bare land, and the SOC under moss was higher than that under lichen (P < 0.05). Under each grazing treatment, the SM under moss, lichen, and bare land decreased in July, then increased in September, followed by a small decrease in November (Fig. 4 C). As for the SM in the same season, the value of all BSC types showed a similar trend. The lower grazing intensities (0 and 2.67 sheep  $ha^{-1}$ ) had significantly higher SM than those under 5.33 and 8.67 sheep ha  $^{-1}$  (P <0.05). The soil pH under moss, lichen, and bare land decreased from May to July, and there were no significant differences between July, September, and November under each grazing treatment. Overall, there was a small fluctuation in soil pH in the growing season (Fig. 4 D).



Fig. 3. The coverage change of BSC and vegetation on the aboveground with the increasing stocking intensity. Note: Y, GI represent the year and grazing intensity, respectively. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, the same below.

## 3.3. Changes of BSCs subsoil nitrate, ammonium and mineralization rate under different grazing intensities

mineralization rate, while soil physicochemical properties showed direct positive effect (Fig. 6B).

SR, PR, T<sub>rage</sub>, ET<sub>0</sub>, EX all had significant effect on the content of nitrate and ammonium, but almost all of them have no significant effect on the rate of nitrogen mineralization (P < 0.05). For each grazing treatment, the soil NH<sup>4</sup><sub>4</sub>-N, and NO<sup>3</sup><sub>3</sub>-N content under moss, lichen, and bare land increased from May to September, then showed a slight decline in November (Fig. 5A and B). The soil NH<sup>4</sup><sub>4</sub>-N content under moss at 2.67 sheep ha<sup>-1</sup> in 2015 increased to 13.18 mg/kg in July, continued to rise to 18.81 mg/kg in September, and then sharply declined to 14.59 mg/kg in November. The soil NH<sup>4</sup><sub>4</sub>-N under the grazing intensity of 2.67 sheep ha<sup>-1</sup> was higher than that of the other grazing intensities in the same season (P < 0.05).

We also found that the soil nitrogen mineralization rates under moss and lichen increased, then decreased as the grazing intensities increased (Fig. 5 C). Additionally, the mineralization rate under 2.67 sheep ha<sup>-1</sup> was the highest when compared to the other grazing intensities in the same season (P < 0.05). The mineralization rates under moss were 0.183, 0.213, 0.194, and 0.138 mg/kg·d in spring as the grazing intensity increased, respectively. As for the different seasons, the mineralization rates in spring and summer were significantly higher than that in autumn (P < 0.05), besides, the negative value appeared in autumn (P < 0.05). In addition, the soil nitrogen mineralization rates under moss, lichen, and bare land showed a gradually decreasing trend.

### 3.4. Response mechanism of grazing intensity to BSCs subsoil mineralization in different grazing seasons

When the data from both years of the experiment are combined, the SEM revealed that grazing intensity had significant inhibitory effect on vegetation and biocrust coverage (P < 0.001, P < 0.01), which directly explain the 28% and 24% change in vegetation status and BSCs. But environmental change (rainfall, air temperature etc.) did not significantly affect them directly. Both the BSCs and vegetation layer had a significant effect on the physicochemical properties of the BSCs subsoil. Moreover, the grazing intensity and seasonal dynamic all had a significant promoting effect on the physicochemical properties of the soil, which directly explained the changes of 25% and 14%, respectively (P <0.001). The changes in the physicochemical properties of the soil significantly promoted the change in its mineralization rate, and the mineralization rate was significantly affected by seasonal dynamics (P < 0.001) (Fig. 6 A). Grazing intensity and seasonal dynamics showed indirect negative effect on the soil mineralization rate. The BSCs and vegetation status had the indirect positive effect on the soil

### 4. Discussion

### 4.1. BSCs differ in their tolerance to grazing intensity

Trampling by grazing animals is one of the most common and direct disturbances to BSC (Maestre et al., 2016). This was confirmed by our study that found that the BSC coverage was destroyed by an increasing grazing intensity (Yang et al., 2020). Notably, it was previously thought that lichen coverage would decrease with the increasing grazing intensity (Bao et al., 2019), but the current study showed the opposite effect, the lichen gradually increased with the increasing grazing intensities (Fig. 3). However, it is still reasonable that lichen in this study has not shown a downward trend, it probably because the grazing intensity in this grazing system has not reached the critical point where lichen coverage begins to decline. Some studies about red deer and BSCs are justify our case of our experiment (Joly et al., 2010: Moore and Crawley, 2014). The results of this experiment could prove that lichen has stronger resistance to trampling than moss, and that the grazing intensity in the current study may not have reached the tolerance threshold of lichen. This might be due to the high tolerance of lichens in comparison to mosses in various situations (Mcclelland, 2011; Pentecost and Whitton, 2012), which was consistent with our first hypothesis. Grazing not only directly affected the coverage of BSCs but also affected their function in other ways. For example, litter first decreased as the grazing intensity increased and this was attributed to foraging and trampling by animals (Larreguy et al., 2017), then it increased, possibly because the trampling was higher than foraging. However, litter and vegetation can somewhat protect BSCs under trampling conditions. BSCs need to fix solar energy for photosynthesis (Munzi et al., 2019) and a high litter coverage may have affected the existence of moss and lichen by limiting photosynthesis. Alternatively, grazing may also have increased the gaps between plants through foraging, leading to an increase in bare land and the amount of light reaching the soil, thereby affecting the BSCs, which can offset some of the damage caused by trampling. For the interannual differences, we found that the variation in trends of vegetation coverage and BSC were generally in agreement between 2015 and 2016 (Fig. 3). Interannual differences were most likely caused by variations in precipitation because there is sufficient evidence of the impact of precipitation on BSC (Scholer et al., 2017).



**Fig. 4.** The BSC subsoil organic carbon (A), total nitrogen content (B), moisture (C) and pH (D) in different grazing intensities **Note:** SR, PR, Trage, ET<sub>0</sub>, and EX represent Solar radiation, Precipitation, Temperature range, and Excreta, respectively. The same below.

### 4.2. Threshold of grazing intensity on soil properties and soil nitrogen characteristics under BSCs

SM under BSC increased first and then decreased with the increase in grazing intensities (Fig. 4 C), indicating that moderate grazing was beneficial to maintaining SM (Bowker et al., 2013). Trampling by livestock forms localized hoof marks on the soil surface, which could accumulate more water and reduce the evaporation area (Yang et al., 2019), thus providing a more suitable growth environment for fungi in BSCs. Moreover, previous studies have suggested that the BSC separates the soil from the atmosphere and reduces SM loss through evaporation (Xiao and Hu, 2017). This supports the findings in our study, which showed changes of the above crust coverage and SM under moderate grazing intensities. As for the seasonal dynamics of SM, the trend was associated with the change of rainfall in the Loess Plateau, whereby rainfall is mainly concentrated from July to September; the SM value reached its maximum in September and then declined from November to the following July and stayed low until the next rainy season (Zhi et al., 2010). Our results also demonstrated the water retention performance of the BSC and its high correlation with precipitation (Li et al., 2021). Moreover, the fluctuation of SM also explained the change in soil pH, and our findings are supported by previous research that showed how soil pH was affected by the excrement of poultry (Olden et al., 2016), while the increase in precipitation in the rainy season diluted the effect of feces and urine on soil pH, keeping it within a reasonable range to ensure plant growth (Fig. 4D).

Taking full account of its separation and blocking function, the BSC also plays an energy supply role by fixed energy (Mónica et al., 2014). BSCs are an important carbon and nitrogen source in the ecosystems of arid and semi-arid regions (Chamizo et al., 2015; Aanderud et al., 2018)





to improve soil fertility, which explains why the change in SOC and TN under the BSC was higher than that under bare land (Fig. 4 A, B). BSCs had a certain protective effect on the soil at lower grazing intensities. However, the protective effect was lost when the tolerance threshold of BSCs was broken with the increase in grazing intensity, but its energy could be directly returned to the soil. However, this energy return was not enough to compensate for the trampling and damage caused by a high grazing intensity. Compared with the SM fluctuations, the stability of SOC, TN and pH were relatively high, which also demonstrated the strong stability and buffer capacity of the soil.

In addition, it was observed that soil inorganic nitrogen ( $NH_4^+$ -N and  $NO_3^-$ -N) in different growing seasons changed significantly under the four grazing intensities (Fig. 5 A, B), and this phenomenon has been widely recognized (Britto and Kronzucker, 2013; Jiang et al., 2016). Nitrogen mineralization rate is a multi-factor process (Maes et al., 2014); the two most important factors are the associated microorganisms and oxygen (Uteau et al., 2015). This could explain the effects of

grazing intensity and season on nitrogen mineralization rate from three aspects (Fig. 5 C). First, the excreta of grazing animals increased soil nutrients, and the breaking of the BSC increased the available raw material for mineralized nitrogen (Wang et al., 2012), which was conducive to the mineralization rate process. Second, grazing affects nitrogen mineralization rate by changing the microbial community, moderate grazing promoted the activity of rhizosphere microorganisms by improving the soil temperature and moisture (Zhang et al., 2017), whereas high-intensity grazing could not, which explains the advantages of maintaining grazing intensities of 2.67–5.33 sheep  $ha^{-1}$  (Saturation phase) for promoting the mineralization rate in this experiment. The third factor is oxygen, which affects the conditions of microbial activities in the process of mineralization, and directly affects the process of soil ammonification (Haney et al., 2008), especially nitrification. Mild grazing may promote the increase in soil porosity, but high grazing intensity changes the spatial pattern and size of soil pores on bare land (Rauber et al., 2021), which led to changes in soil oxygen content and



Fig. 5. Seasonal dynamics of soil nitrate-nitrogen content(A), ammonium nitrogen content (B) and nitrogen mineralization rates (C) under moss, lichen, and bare land in different years

**Note:** Use the nitrate nitrogen and ammonium nitrogen content in May, July, September, and November in 2015 and 2016 to calculate the inorganic nitrogen content and their respective changes, then calculate the mineralization rates of May-early July (spring), July-early September (summer), and September-early November (autumn) is used to analyze the nitrogen utilization characteristics under BSCs and bare land. GI, GS, GI  $\times$  GS represent the grazing intensity, grazing season and the interaction between them, respectively. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001.



Fig. 6. The structural equation model of grazing intensity and seasonal response on the soil mineralization (A) and the total, direct, and indirect effect of factors on the soil nitrogen mineralization (B)

**Note:** (A) The structural equation model considered all possible ways and was screened by variance expansion coefficient (VIF <10). The numbers indicate the standard path coefficients. The arrow width is proportional to the strength of the relationship, blue indicates a positive effect, and orange indicates a negative effect.  $R^2$  represents the proportion of variance explained for each dependent variable in the model. CHI/DF = 1.074, P = 0.377, RMSEA = 0.023. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001. The double-layer rectangle represents the first principal component of PCA, where the symbols ' $\uparrow$ 'and ' $\downarrow$ 'represent the positive or negative relationship between variables and the first principal component in PCA, respectively. (B) SD, VS, SC and NM represent seasonal dynamics, vegetation status, soil characteristics, and nitrogen mineralization, respectively.

directly affected the rate of nitrogen mineralization. For the seasonal dynamics of nitrogen mineralization, the microbial activity and inorganic nitrogen content increased correspondingly under high temperatures and suitable water conditions in the growing season (Yan et al., 2011). When combined with the seasonal response by nitrogen mineralization, the obtained results are consistent with our third hypothesis. The changes in SM and pH might also have a certain influence on nitrogen mineralization. Regarding their specific roles, it has been reported that they mainly affect the living environment of microorganisms in the process of mineralization (Zhang et al., 2016; Li et al., 2020). In general, the intensity and trend of moss variation compared with lichen also verified the correctness of the second hypothesis, and the advantages of moss and lichen compared with bare soil were also obvious.

### 4.3. Path and mechanism of grazing management strategy for soils under BSCs

In this study, the diversity and complexity of the influencing factors of soil nitrogen mineralization rates were recognized, therefore, the effects of different grazing intensities and seasonal fluctuations on soil nitrogen mineralization rates were quantified using an SEM structural equation, which included vegetation coverage changes to ensure the study design was thorough (Fig. 6 A). The fitted SEM results were strongly consistent with the change of physicochemical properties and nitrogen mineralization rates mentioned above, clearly demonstrating the weakening effect of grazing intensity on BSC and vegetation coverage. Furthermore, the results clearly showed the protective effect of BSC coverings compared with bare land, which not only supported the protective effect of BSCs (Jian et al., 2015) but also showed the vulnerability of bare land in the grazing system (Lazaro et al., 2008). However, the physicochemical properties of soil in subcutaneous layer are not mediated by the crust alone, but also by vegetation status. The fluctuation of physicochemical properties will further promote the change of mineralization rate. In addition, seasonal fluctuations also indirectly affect the change of mineralization rate through the physicochemical properties of soil.

The main effects of grazing on the soil were primarily caused by livestock excretion and trampling (Butler-Lapointe, 2014). Their impact on the BSCs and vegetation were mainly caused by the feeding and trampling. BSCs are also sheltered and protected by the plants to a certain extent due to their growth environment. The grazing intensity indirectly affects the mineralization rates of the underlying soil through the physicochemical properties of the BSC and vegetation. The change in the mineralization rate will affect the cycle of the entire grazing system. When the grazing intensity is the main influencing factor, we also considered the impact of different environmental factors (SR, PR, T,  $T_{rage}$  and  $ET_0$ ) on the entire grazing system.

As for the response to different growth seasons, it has previously been confirmed that the BSC is highly sensitive to precipitation and temperature (Coe et al., 2012), and their effects will be more prominent in the season with a rainfall deficit, such as that experienced on the Loess Plateau (Mha et al., 2021). It was also observed that the physicochemical properties under BSCs influence the mineralization rate, which is recognized widely (Ugawa et al., 2020).

Although such experimental results were consistent with our hypotheses, there were still some differences. However, these differences can be logically explained. The experimental results confirmed the protective effect of BSC on soil in the grazing system, at the same time, the segmentation function and energy source function of BSCs have been digitally presented, which demonstrated that the increase of grazing intensity will remove the barrier effect, but the change was not large enough to detect in this study. In other words, compared with soil and plant fertility, the effect of the BSC in the microenvironment was still small. As for the comparison between the two types of BSC, the combined effect of moss was better than that of lichen, which may be due to their structural differences (Capozzi et al., 2020). Although the results of this study demonstrated the importance of the BSC, they also identified its limitations, which strongly highlighted the importance of grazing management measures in the Loess Plateau. However, it is worth noting the diversity and extensive existence of BSCs (Belnap and Lange, 2017), which deserve study.

### 4.4. Future perspectives

It is also important to note the limitations of this study, which was conducted as part of a BSC study in a long-term grazing trial. The reliability of the results was supported by long-term and stable observations, but we believe that multi-site observations may improve the results, although environmental factors are also taken into account. In addition, although many studies have been conducted on microbial and soil mineralization pathways, we believe that the role of livestock-grass-BSC-soil surface adhesion microorganisms in the matter and energy cycle of the whole grazing system needs further attention.

### 4.5. Concluding remarks

Biocrust acts as the connection part between vegetation and soil and it covers 40% of the terrestrial areas in the world (Liang et al., 2021). It has been estimated that the annual import of N from BSCs into the soil can reach 0.7–100 kg hm<sup>-2</sup> (Barger et al., 2016), which improvement effect on soil cannot be ignored. Therefore, this study revealed the threshold range of soil nitrogen mineralization of BSC under different grazing intensities, and further revealed the importance of BSC on soil nitrogen mineralization rate. The results of this study can provide some new ideas for the formulation of grazing strategy in sheep grazing system, and the combination of BSC type and coverage may make the decision more scientific.

### 5. Conclusion

The results showed that the grazing intensity promoted the BSC status significantly in the grazing saturation phase (2.67–5.33 sheep  $ha^{-1}$ ), and moss was more effective than lichen at promoting soil mineralization, but it was more vulnerable to trampling than lichen. Moreover, grazing had a 25% and 14% effect on soil physicochemical properties by regulating vegetation and BSCs. In addition, the soil nitrogen mineralization is also indirect affected by seasonal fluctuations in the environment (solar radiation etc.). The three pathways together account for 25% of the variation in the mineralization rate through perturbation of physical and chemical properties. Therefore, the results of the current study have revealed the effects of grazing intensity on soil physicochemical properties and nitrogen mineralization rates under BSCs. The crusting effect under grazing intensity was tracked and quantified, which provided a baseline for the establishment of sustainable sheep grazing systems worldwide.

#### Author contributions

Fujiang Hou designed and directed the study, Jing Zhang carried out the data analysis and wrote the manuscript. Qianwen Duan and Jie Ma collected samples, analyzed the data, and contributed to the final writing of the manuscript.

### 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

Data will be made available on request.

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