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Article

Grazing Intensity Affects the Composition and Stability of Soil Aggregates in Alpine Meadow of the Qilian Mountains

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Abstract

To explore the responses of soil aggregate composition and stability to different grazing intensities in alpine meadow of the Qilian Mountains, no grazing (CK) was set as the control, with four treatments including light grazing (LG), moderate grazing (MG), heavy grazing (HG) and extreme grazing (EG) established. The characteristics of soil aggregates in the 0–10 cm and 10–20 cm soil layers were determined by the dry sieving method and wet sieving method, and three stability parameters including the mean weight diameter (MWD), geometric mean diameter (GMD) and fractal dimension (D) were analyzed. Combined with environmental and biological factors, the mechanisms underlying the effects of grazing on soil aggregates structure and stability were elucidated. The results showed that: (1) Soil aggregates with the particle size of 5–10 mm were the dominant fraction in the soil structure of the alpine meadow, and this fraction changed drastically with grazing intensity. CK maintained relatively high aggregate mechanical stability but exhibited weaker resistance to water erosion compared to grazed plots. Under the CK condition, the content of water-stable aggregates with the 5–10 mm particle size decreased significantly compared with mechanical-stable aggregates (by 60.07% in the topsoil and 70.66% in the subsoil). Light and moderate grazing maintained a dynamic balance and high stability of soil structure. Heavy and extreme grazing intensified soil structure fragmentation and overall stability declined. (2) Soil aggregate stability was correlated with environmental factors. Altitude and soil bulk density were significantly positively correlated with aggregate stability ($P < 0.001$). Root biomass exerted a significant effect on the stability indices of mechanically stable aggregates in the topsoil ($P < 0.05$); high root biomass destroyed soil macroaggregates but enhanced the resistance to water erosion. Soil microbial biomass carbon (SMBC), nitrogen (SMBN), phosphorus (SMBP) were significantly positively correlated with GMD of water-stable aggregates, but negatively correlated with GMD, MWD and D of mechanical-stable aggregates, also MWD and D of water-stable aggregates. Nitrate nitrogen had a positive effect on aggregate stability, while ammonium nitrogen had a negative effect. (3) The stability of aggregate in different soil layer varied under different grazing intensity. Under LG and MG conditions, the subsoil exhibited higher aggregate stability than the topsoil, whereas the opposite pattern was observed under HG, EG and CK conditions. Therefore, from the perspective of soil structural stability and sustainable utilization, light and moderate grazing are the optimal utilization patterns for alpine meadow in the Qilian Mountains. It not only maintains the structural stability of subsoil aggregates but also balances biological cementation and physical disturbance, avoiding aggregate water stability insufficiency under no grazing and the risk of structural fragmentation under heavy or extreme grazing. The findings provide a scientific basis for rational grazing management and soil conservation in alpine meadow of the Qilian Mountains.

Keywords: alpine meadow; grazing intensity; soil aggregate; mechanical and water stability

1. Introduction

The Qilian Mountains have a special geographical location and serve as an important ecological security barrier and a national key ecological function zone in China, with the alpine meadow as the dominant grassland type. This type of grassland is one of the grassland ecosystems with the largest area and the highest altitude in the world [1]. The alpine meadow ecosystem is sensitive and fragile, being subject to the dual impacts of climate change and human activities. Grazing directly affects the vegetation community, soil structure and ecosystem multifunctionality of the alpine meadow [2]. Soil structure is a core regulator of the ecological functions of the alpine meadow ecosystem, exerting important effects on nutrient cycling, microbial communities, hydrological processes, productivity and ecosystem stability.

As the basic unit of soil structure, the formation of soil aggregates is associated with oxide cementation [3], and the formation process is dynamic and complex [4,5]. The composition and stability of soil aggregates influence the physical and chemical properties as well as biological characteristics of soils. Soil aggregates act as carriers of nutrients [6], regulating nutrient storage and soil and water conservation, and also serve as habitats for microorganisms [7]; microbial biomass affects nutrient storage and aggregate stability [8]. Aggregates with different particle sizes perform distinct functions, and the correlation between nutrient content and particle size remains controversial [9–13]. The force stability and water stability of soil aggregates are the key criteria for evaluating soil structure quality [15], which can reflect the resistance of soil aggregates to external damage and water erosion, and are of great significance for maintaining productivity and mitigating ecosystem degradation [16]. Their composition and stability are affected by soil organic matter, land use patterns and other factors [17]. Studies have shown that grassland degradation leads to a decrease in the proportion of macroaggregates and an increase in microaggregates [14], which damages soil structure and stability and seriously affects ecosystem stability. Therefore, clarifying the effects of grazing intensity on the composition and stability of soil aggregates is crucial for the conservation and restoration of alpine meadows.

In this study, the composition and structure of mechanically stable and water-stable soil aggregates with different particle sizes in alpine meadows under different grazing intensities were determined. Combined with the changes in environmental factors such as biomass, soil bulk density and soil microbial biomass C, N, P in alpine meadows, the variation characteristics of soil aggregate stability indices were analyzed, and the effects of grazing utilization on soil structure in alpine meadows were elucidated.

2. Materials and Methods

2.1. Study Area Overview

The study area is located in Tianzhu Tibetan Autonomous County, Wuwei City, Gansu Province, in the eastern section of the Qilian Mountains (102°07'–103°46' E, 36°31'–37°55' N), with an altitude ranging from 2040 m to 4874 m. The annual average sunshine duration in the study area is 2500–2700 h, the annual average temperature is -8–4 °C, the relative frost-free period is 90–145 d, and the annual average precipitation is 265–632 mm. During the index monitoring and sampling period in August 2024, the monthly average temperature was 17.3 °C, the average air relative humidity was 65%, and the monthly precipitation was 118.0 mm.

The stocking rate was calculated based on field surveys, and combined with survey data, five different grazing gradients were selected (Figure 1). The seasonal pasture in Honggeda Village was set as the light grazing (LG) site, with *Elymus nutans*, *Polygonum viviparum* and *Kobresia humilis* as the dominant species. The rotational grazing experimental field in the Tianzhu Alpine Grassland Ecosystem Experimental Station of Gansu Agricultural University was the moderate grazing (MG) site, where *Kobresia humilis*, *Melilotoides ruthenica* and *Elymus nutans* dominated. The public pasture in Shizhuanggou Village was the heavy grazing (HG) site, with *Potentilla candicans*, *Kobresia humilis* and *Polygonum viviparum* as the dominant species. The livestock track in Daiqian Village was the

extreme grazing (EG) site, where *Kobresia humilis*, *Potentilla candidans* and *Leymus secalinus* were the dominant species. The long-term no-grazing alpine meadow in the core area of the Qilian Mountains National Park was set as the control (CK), with *Elymus nutans*, *Polygonum viviparum* and *Kobresia humilis* as the dominant species. The sampling sites in this study had consistent site conditions and long-term fixed grazing patterns and intensities, which avoided the impacts of short-term artificial control and microclimate differences.

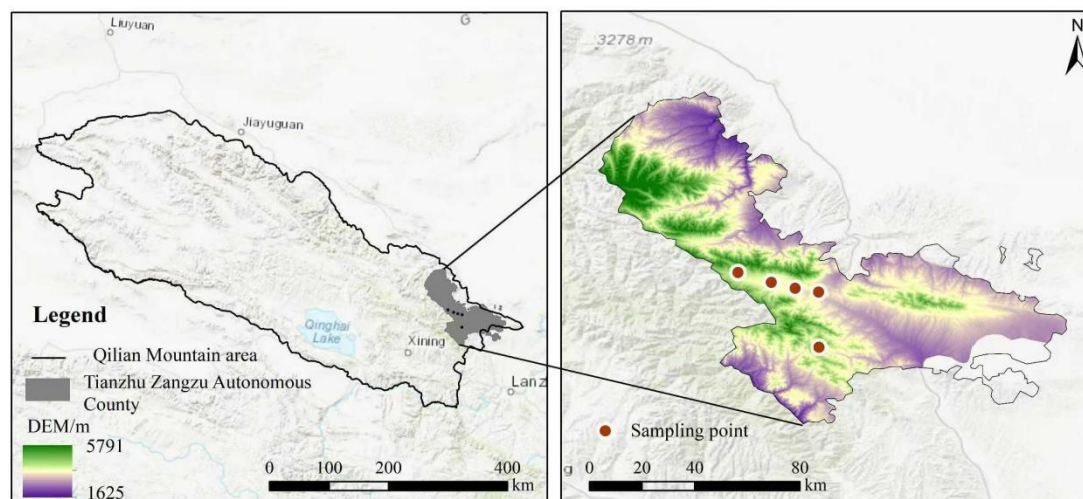


Figure 1. Overview of the Sampling Sites.

2.2. Experimental Methods

Six replicates were set for each of the five alpine meadow plots with different grazing gradients, and five sampling points were randomly distributed in each replicate plot. The 0–20 cm soil layer is the dense distribution layer of plant roots in the alpine meadow of the study area, so soil samples were collected separately from the topsoil (0–10 cm) and subsoil (10–20 cm). Soil aggregates were divided into six particle size fractions for determination: 5–10 mm, 2–5 mm, 1–2 mm, 0.5–1 mm, 0.25–0.5 mm and 0–0.25 mm. Referring to the method of Tisdall and Oades (1982) [6], a set of sieves with apertures of 5 mm, 2 mm, 1 mm, 0.5 mm and 0.25 mm was used to determine the composition and structure of mechanically stable soil aggregates by the dry sieving method and water-stable soil aggregates by the wet sieving method.

Meanwhile, the vegetation and soil environmental factors of the alpine meadow in the study area were determined. Soil bulk density was measured by the cutting ring method, and soil mineralization rate (SMR) was determined by the alkali absorption method. The contents of ammonium nitrogen ($\text{NH}_4^+\text{-N}$) and nitrate nitrogen ($\text{NO}_3^-\text{-N}$) were measured using a flow injection analyzer (San++Compact). Aboveground biomass was determined by clipping plants at ground level in 50×50 cm quadrats, and belowground root biomass was measured by excavating all soil to a depth of 50 cm in the clipped quadrats. The altitude (ALT) of each sampling point was measured with a GPS.

2.3. Data Analysis

Soil aggregate stability was quantified by the mean weight diameter (MWD), geometric mean diameter (GMD) and fractal dimension (D), which reflect the particle size distribution characteristics and structural stability of soil aggregates from different dimensions.

The calculation formulas for MWD, GMD and D are as follows:

$$\text{MWD} = \sum_{i=1}^n (\bar{R}_i W_i)$$

$$\text{GMD} = \exp\left[\frac{\sum_{i=1}^n (W_i \ln \bar{R}_i)}{\sum_{i=1}^n W_i}\right]$$

where R_i is the mean diameter of soil aggregates in the i -th particle size fraction (mm); W_i is the mass percentage of soil aggregates in the i -th particle size fraction (%).

$$D = 3 - \frac{\lg \frac{M_i}{M_0}}{\lg \frac{d_i}{d_{max}}}$$

where D is the fractal dimension; M_i ($i=1,2,3,\dots$) is the total mass of aggregates with particle size smaller than d_i (g); M_0 is the total mass of all soil aggregates (g); d_i is the mean diameter between adjacent particle size fractions of aggregates (mm); d_{max} is the diameter of the largest particle size fraction of aggregates (mm). The linear relationship between the logarithm of soil aggregate particle size and the logarithm of cumulative mass was fitted using Origin 2021 software, and the fractal dimension D was solved based on the fitting equation.

The Aggregate Water Stability Relative Change Index (AWSRCI) was used to quantify the stability change of soil aggregates with specific particle sizes under the action of water relative to that under physical mechanical force. The calculation formula is as follows:

$$\text{AWSRCI} = \frac{DS_d - WS_d}{DS_d} \times 100\%$$

where DS_d is the mass percentage of aggregates with particle size d determined by dry sieving, and WS_d is the mass percentage of aggregates with the corresponding particle size determined by wet sieving.

IBM SPSS Statistics 27.0 software was used to conduct one-way analysis of variance (One-way ANOVA) on the mass proportion of soil aggregate fractions to test the significance of differences in indices (gas flux data and influencing factors) among different treatment groups. When there were significant differences among groups ($P < 0.05$), multiple comparisons were further conducted to identify the sources of differences, with Duncan's new multiple range test used for post-hoc multiple comparisons. Two-way analysis of variance (Two-way ANOVA) was applied to explore the effects of soil depth and grazing intensity on the mass proportion of soil aggregate fractions and soil aggregate stability indices, as well as their interaction. Pearson correlation analysis was used to analyze the correlations between soil aggregate particle size distribution, stability and environmental factors.

3. Results

3.1. Composition and Distribution of Soil Aggregate Fractions in Alpine Meadows Under Different Grazing Intensities

As shown in Figure 2a,b, the mass proportion of mechanically stable soil aggregates with the 5-10 mm particle size was the highest under all grazing intensities and varied obviously. In the topsoil, the mass proportion of this fraction followed the order of $HG > CK > EG > LG > MG$; there was no significant difference between HG and CK ($P > 0.05$), but both were significantly higher than that under other grazing intensities ($P < 0.01$), being approximately twice that under LG and MG . Except for the 5-10 mm fraction, the mass proportion of all other aggregate fractions fluctuated between 10% and 20%, and there were no significant differences in these fractions under LG and MG ($P > 0.05$). The mass proportion of mechanically stable soil aggregates with the 0-0.25 mm particle size under LG and MG was significantly higher in the topsoil than that under HG , EG and CK , and significantly higher in the subsoil than that under HG and CK . The variation in soil structure with grazing intensity was greater in the topsoil than in the subsoil.

As shown in Figure 2c,d, the mass proportion of water-stable soil aggregates with the 5-10 mm particle size was the lowest under the no-grazing condition, while that of the 0.5-1 mm fraction was the highest. The mass proportion of water-stable soil aggregates with the 5-10 mm particle size under

LG and MG was approximately twice that under the no-grazing condition (14.85% in the topsoil and 21.70% in the subsoil). The mass proportion of water-stable aggregates with particle size >1 mm in the subsoil was higher than that in the topsoil under light and moderate grazing, while the opposite was true under heavy, extreme grazing and no grazing. The soil layer difference of aggregates under LG was the largest (coefficient of variation 24.8%), while that under CK was the smallest (coefficient of variation 5%).

Two-way ANOVA on the structural characteristics of soil aggregates under different grazing intensities and soil depths showed that: grazing intensity had an extremely significant effect on the content of mechanically stable soil aggregates in all particle size fractions ($P < 0.001$), and a significant effect only on the content of water-stable aggregates with the 5–10 mm and 0–0.25 mm particle sizes ($P < 0.05$). Soil depth had no significant effect on the content of soil aggregates ($P > 0.05$). The interaction between grazing intensity and soil depth only had a significant effect on the content of mechanically stable aggregates with the 5–10 mm particle size ($P < 0.05$).

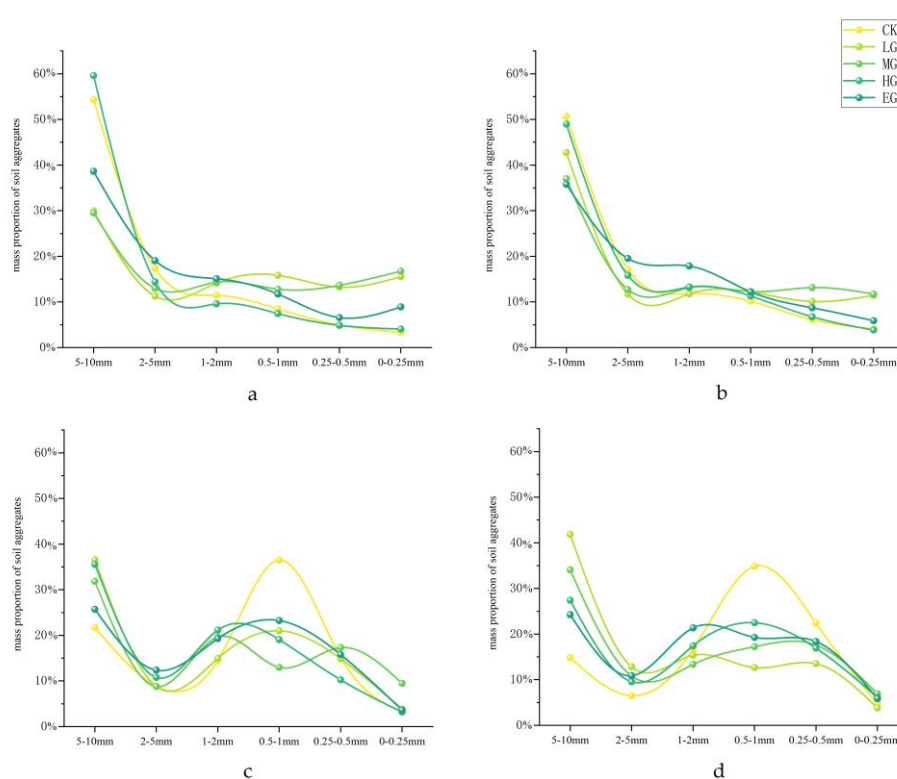


Figure 2. Effects of Grazing Intensity on the Mass Proportion of mechanically stable and Water-Stable Soil Aggregates in Different Soil Layers. (a) Topsoil (0–10 cm), mechanically stable soil aggregates; (b) Subsoil (10–20 cm), mechanically stable soil aggregates; (c) Topsoil (0–10 cm), water-stable soil aggregates; (d) Subsoil (10–20 cm), water-stable soil aggregates. Note: Different colors represent different grazing intensities: CK = no grazing (control), LG = light grazing, MG = moderate grazing, HG = heavy grazing, EG = extreme grazing. The x-axis denotes soil aggregate particle size fractions (unit: mm), and the y-axis represents the mass proportion of soil aggregates (%).

As shown in Figure 3, the AWSRCI of soil aggregates with the 5-10 mm and 2-5 mm particle sizes were all positive, and the values approached 0 under LG and MG. The AWSRCI of 1-2 mm soil aggregates in the subsoil under HG was much lower than that under other treatments. The AWSRCI of soil aggregates with the 0.5-1 mm and 0.25-0.5 mm particle sizes varied most significantly under different grazing intensities: the values approached 0 under LG and MG, while negative values were observed under other treatments, with the values under CK (330.54% in the topsoil and 205.43% in

the subsoil) being extremely significantly different from those under other treatments ($P < 0.001$). The AWSRCI of 0-0.25 mm soil aggregates was positive under all treatments except for the topsoil under HG.

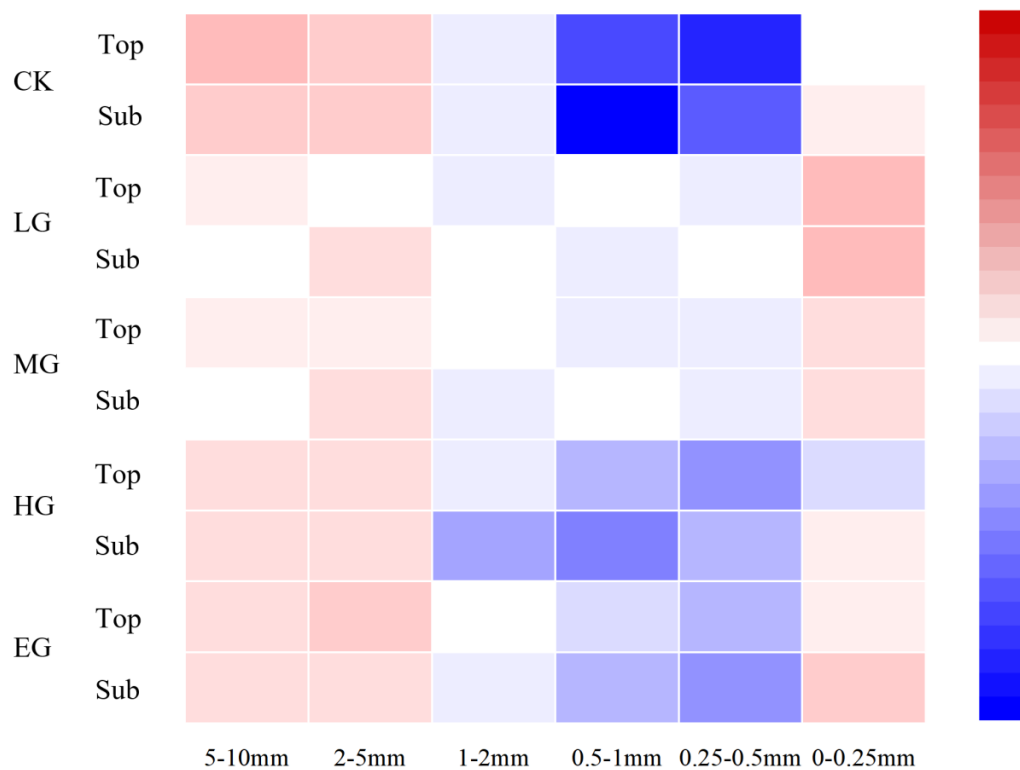


Figure 3. Heatmap of Soil Aggregate Water Stability Relative Change Index (AWSRCI) under Different Grazing Intensities and Soil Depths. Note: The color scale indicates the Aggregate Water Stability Relative Change Index (AWSRCI). Blue represents negative values, red represents positive values, and colors closer to white indicate values approaching 0. Abbreviations: Top = Topsoil; Sub = Subsoil; DS = Dry Sieving method; WS = Wet Sieving method; CK = Control (no grazing); LG = Light Grazing; MG = Moderate Grazing; HG = Heavy Grazing; EG = Extreme Grazing.

3.2. Soil Aggregate Stability Parameters in Alpine Meadows Under Different Grazing Intensities

As shown in Figure 4, the variation trends of MWD and GMD were highly consistent (correlation coefficient > 0.9), while the trend of D was basically opposite to that of the two indices. Under light and moderate grazing, the MWD and GMD of mechanically stable soil aggregates were smaller and the D value was larger, while the opposite was true under heavy and extreme grazing. There was a significant difference in indices between extreme grazing and no grazing ($P < 0.05$), while no significant difference was found between heavy grazing and the no-grazing control ($P > 0.05$).

The variation in stability indices of water-stable aggregates under different grazing conditions was smaller than that of mechanically stable aggregates. No grazing was the treatment with the smallest MWD (2.09 mm in the topsoil and 1.96 mm in the subsoil) and GMD (1.29 mm in the topsoil and 1.08 mm in the subsoil) of water-stable aggregates. The MWD and GMD of water-stable aggregates in the subsoil decreased with the increase in grazing intensity, with the highest values observed under light grazing (MWD 4.00 mm, GMD 2.37 mm), which were approximately twice those under no grazing. In the topsoil, the MWD and GMD slightly increased under heavy grazing, and the remaining variation trends were consistent with those in the subsoil. The inter-treatment difference in MWD (2.04 mm) was 1.58 times that in GMD (1.29 mm). The stability indices of soil aggregates in the subsoil were higher than those in the topsoil under light and moderate grazing, while the opposite was true under other treatments.

Two-way ANOVA results showed that: grazing intensity had an extremely significant effect on all mechanically stable parameters of soil aggregates ($P < 0.001$), and a significant effect on the MWD and GMD among the water-stable parameters of soil aggregates ($P < 0.05$). The interaction between grazing intensity and soil depth only had a significant effect on the MWD of mechanically stable aggregates ($P < 0.05$).

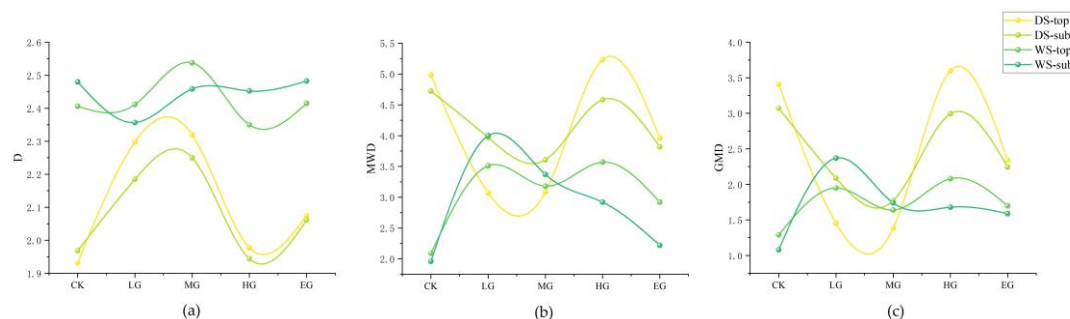


Figure 4. Stability Indices of Soil Aggregates Separated by Different Sieving Methods at Various Depths in the Alpine Meadow of Qilian Mountains Under Different Grazing Intensities. (a) Changes in Fractal Dimension (D) under different grazing intensities; (b) Changes in Mean Weight Diameter (MWD) under different grazing intensities; (c) Changes in Geometric Mean Diameter (GMD) under different grazing intensities. Note: DS = Dry Sieving, WS = Wet Sieving; Top = Surface soil layer (0–10 cm), Sub = Subsurface soil layer (10–20 cm); MWD = Mean Weight Diameter, GMD = Geometric Mean Diameter, D = Fractal Dimension; CK = no grazing (control), LG = light grazing, MG = moderate grazing, HG = heavy grazing, EG = extreme grazing.

3.3. Correlation Analysis Between Soil Aggregate Stability Indices and Environmental Factors

Correlation analysis (Figure 5) showed that the D value was significantly negatively correlated with altitude and soil bulk density ($P < 0.05$) except for that of water-stable aggregates in the subsoil. Altitude was significantly positively correlated with the MWD value ($P < 0.01$), and extremely significantly positively correlated with the GMD value of mechanically stable soil aggregates ($P < 0.001$), while the correlation with the GMD of water-stable soil aggregates was not significant ($P > 0.05$). Soil bulk density was extremely significantly positively correlated with the MWD value of aggregates in the topsoil ($P < 0.01$), while the correlation with the MWD of mechanically stable aggregates in the subsoil was not significant ($P > 0.05$). Except for water-stable aggregates in the topsoil, soil bulk density was significantly positively correlated with the GMD value ($P < 0.05$). The correlations between soil microbial biomass carbon, nitrogen, phosphorus and soil aggregate stability indices were not uniform: they were significantly positively correlated with the GMD of water-stable aggregates, but negatively correlated with mechanically stable indices and other water-stable indices. Belowground biomass, soil mineralization rate and nitrate nitrogen showed the same correlation trend as microbial biomass carbon, nitrogen and phosphorus. Belowground biomass was significantly correlated with all stability indices in the topsoil except for the GMD of water-stable aggregates ($P < 0.05$), and extremely significantly correlated with the GMD of water-stable aggregates in the subsoil ($P < 0.01$), while the correlations with other indices were not significant ($P > 0.05$). Soil mineralization rate was only significantly positively correlated with the D and GMD of water-stable aggregates in the topsoil ($P < 0.05$). Nitrate nitrogen was only extremely significantly positively correlated with the GMD of water-stable aggregates in the subsoil ($P < 0.001$), while the correlations with other stability indices were not significant ($P > 0.05$). Aboveground biomass and ammonium nitrogen showed the opposite correlation trend to microbial biomass carbon, nitrogen and phosphorus, and the correlations were not significant ($P > 0.05$). The correlations between soil aggregate stability indices and environmental factors were generally higher in the topsoil than in the subsoil, and the correlations of mechanically stable aggregate stability indices with environmental factors were overall higher than those of water-stable aggregate stability indices.

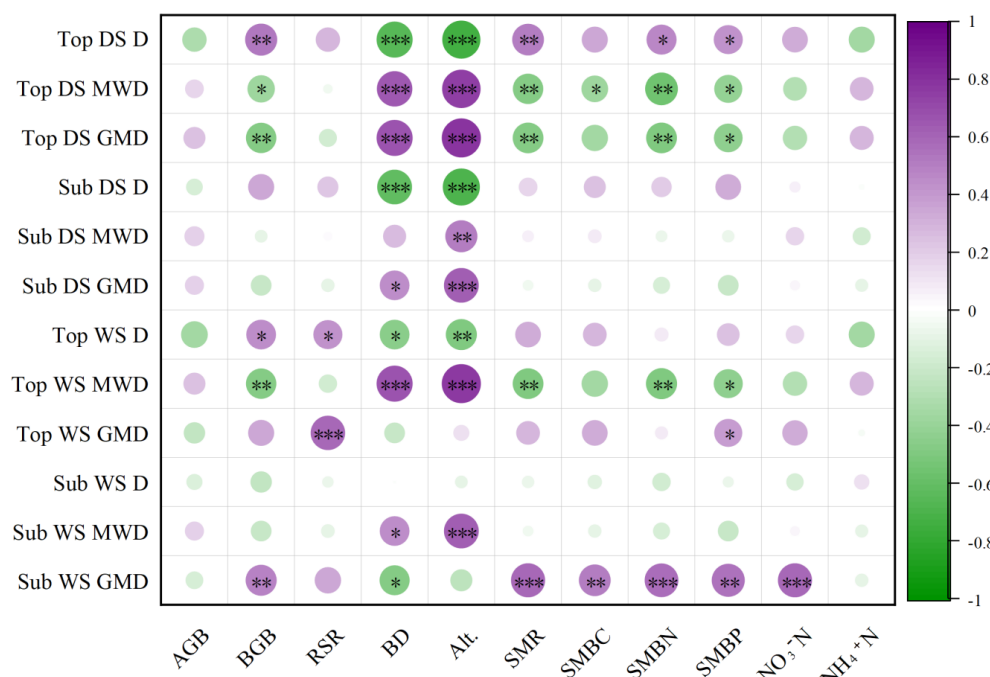


Figure 5. Correlation heatmap between soil aggregate stability indices under different sieving methods and environmental factors. Notes: Note: The abbreviations used in this correlation heatmap are defined as follows: Top = Topsoil; Sub = Subsoil; DS = Dry Sieving method; WS = Wet Sieving method; CK = Control (no grazing); LG = Light Grazing; MG = Moderate Grazing; HG = Heavy Grazing; EG = Extreme Grazing; MWD = Mean Weight Diameter; GMD = Geometric Mean Diameter; D = Fractal Dimension; RSR = Root-Shoot Ratio; AGB = Aboveground Biomass; BGB = Belowground Biomass; Alt. = Elevation; SMR = Soil Mineralization Rate; SMBC = Soil Microbial Biomass Carbon; SMBN = Soil Microbial Biomass Nitrogen; SMBP = Soil Microbial Biomass Phosphorus. Asterisks indicate significant correlation: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

4. Discussion

Soil structure is a core element maintaining ecosystem functions and soil quality, and directly regulates the processes of water transfer, gas exchange and nutrient cycling [18]. Essentially, soil structure is the spatial arrangement pattern of soil aggregates and particles with different particle sizes [6], and its formation and evolution are comprehensively regulated by environmental conditions, biological processes and human disturbances (e.g., grazing) [19]. Soil aggregates are the core indices characterizing soil structure quality [20], and their compositional characteristics are of great significance for evaluating changes in soil quality under grazing disturbance. This study found that grazing intensity had a significant effect on the composition of soil aggregates in the alpine meadow, which was consistent with the conclusions of previous studies on alpine steppes [21]. However, the effect of soil depth was not significant, and the effect of their interaction was also weak.

In this study, soil aggregates with the 5–10 mm particle size were the dominant fraction in the soil aggregate structure, and the variation in their mass proportion could directly reflect the interference effect of grazing on soil structure. The mass proportion of 5–10 mm aggregates in the topsoil was the highest under heavy grazing, which may be due to the accumulation of a large amount of animal and plant residues as well as livestock manure on the soil surface caused by long-term high-intensity grazing. This leads to the massive reproduction of microorganisms that secrete extracellular polymers, and microaggregates are bonded to form macroaggregates through chemical cementation combined with the physical entanglement of plant roots [25]. The higher proportion of macroaggregates under heavy and extreme grazing may be attributed to the increased soil bulk density caused by physical compaction from overgrazing [22]. The mass proportion of macroaggregates under extreme grazing was still lower than that under heavy grazing due to the

significant reduction in plant biomass caused by severe grassland degradation. The variation trends of soil aggregate composition with grazing intensity overlapped under light and moderate grazing, indicating that mechanically stable aggregates of different particle sizes were in a dynamic balance of fragmentation and cementation. Soil macroaggregates were moderately crushed into small and medium-sized aggregates by trampling, and meanwhile, small and medium-sized aggregates were re-polymerized through stable cementing substances such as humus and polyvalent cations. This is a key stage for maintaining soil structural stability. The high mass proportion of 5–10 mm mechanically stable soil aggregates under no grazing indicated that no grazing is beneficial to improving soil force stability, which is consistent with the research results of Wen et al. in the Inner Mongolia grassland [26]. The proportion of 5–10 mm water-stable aggregates under no grazing was lower than that under all other treatments and much lower than that of 5–10 mm mechanically stable aggregates under the same condition. The reduced resistance of grasslands to water erosion under no grazing may be due to the cut-off of manure input from livestock [25], which reduces the substrates for microbial survival. In addition, the underground root system of grasslands under no grazing does not grow as well as that under moderate grazing [27], resulting in a weak maintenance effect of roots on water-stable aggregates and poor resistance to water erosion. LG and MG were the least affected by the difference in sieving methods, and the subsoil was more stable than the topsoil, which proved that suitable grazing is beneficial to improving the water erosion resistance of grasslands [30].

Soil aggregate stability can directly reflect the anti-interference ability and water and nutrient retention potential of soil structure [20]. In this study, the characteristics of soil aggregates were determined by the dry and wet sieving methods, and three stability parameters including MWD, GMD and D were calculated to analyze the effects of grazing intensity and soil depth on aggregate stability. Combined with environmental factors, the internal driving mechanism was revealed. Among the stability indices of mechanically stable aggregates, the MWD and GMD under light and moderate grazing were lower than those under other treatments, and the D value was higher. This is because no grazing reduces biological damage, while animal trampling and manure input under overgrazing lead to an increase in soil macroaggregates [22,25]. Among the stability indices of water-stable aggregates, the no-grazing control was significantly lower than all other treatments and also lower than the mechanically stable indices of the no-grazing control, which was consistent with the changes in soil structure. The stability indices of water-stable soil aggregates in the subsoil decreased with the increase in grazing intensity, while those in the topsoil increased under heavy grazing with the remaining trends unchanged. Field observations found that heavy and extreme grazing led to the concentrated foraging of livestock or their aggregation during transhumance, and a large amount of manure covering the soil surface improved the water stability of the topsoil [25], which can explain this phenomenon.

Two-way ANOVA in this study found that soil aggregate structure and stability were extremely significantly affected by grazing intensity ($P < 0.001$), while the effect of soil depth was small, which was consistent with the findings of multiple studies [28–31]. Mechanically stable soil aggregates were more sensitive to grazing than water-stable soil aggregates [32], because the damage of grazing to aggregates is mainly mechanical fragmentation [22], and re-aggregation occurs under wet sieving, leading to a reduction in differences.

Correlation analysis of environmental factors showed that altitude was significantly positively correlated with soil aggregate stability ($P < 0.05$), which was consistent with the research results of Zhu et al. [33] in Xuebaoding Mountain, Sichuan Province. Relevant studies in the Helan Mountains have also confirmed this phenomenon [34]. Li et al. [35] further pointed out that the high-altitude environment optimizes the arrangement of soil particles by regulating soil bulk density and thus improves structural stability, and soil bulk density was significantly positively correlated with aggregate stability in this study ($P < 0.05$).

Soil mineralization rate was significantly positively correlated with aggregate stability ($P < 0.05$). A higher mineralization rate reflects vigorous microbial activity [36], and grazing has an extremely

significant effect on the mineralization process [37]. The internal mechanism is that humus and polysaccharides produced by microorganisms during organic matter decomposition bond soil particles through chemical bridging and physical entanglement. Nutrients released by mineralization promote the growth of plant roots and mycorrhizal fungi, and the secretions of mycorrhizal fungi can significantly improve aggregate stability [38]. The significant positive correlation between grassland belowground biomass and aggregate stability ($P < 0.05$) also confirms this mechanism, as the physical entanglement of roots is an important driving force for aggregate formation [19]. In this study, the stability of water-stable aggregates decreased with the increase in grazing intensity, which was consistent with the research conclusion of Zhang et al. [39] in the *Stipa krylovii* steppe of Inner Mongolia. The core reason is that overgrazing inhibits root growth and microbial activity, thus weakening biological cementation.

Soil microbial biomass carbon, nitrogen and phosphorus had negative effects on most aggregate stability indices, which is related to the dual effects of microorganisms. Although microorganisms can stabilize soil structure by regulating the arrangement of soil particles, the decomposition of aggregate cementing agents by excessive microorganisms plays a dominant role [30]. Among the water-stable indices, GMD was significantly positively correlated with microbial biomass because extracellular polymers secreted by microorganisms can directly promote the increase in geometric mean diameter [42,43].

In this study, nitrate nitrogen was positively correlated with aggregate stability, while ammonium nitrogen was negatively correlated, and the mechanism is related to the regulation of soil environment and microbial community by different nitrogen forms. Nitrate nitrogen can promote the growth of fungi, and their hyphal networks and secreted extracellular polymers are the key to the formation of macroaggregates. Meanwhile, OH^- released by plants during nitrate nitrogen absorption can maintain soil pH stability and indirectly enhance aggregate structure [41]. Ammonium nitrogen has the opposite effect to nitrate nitrogen, as H^+ produced during its nitrification leads to soil acidification and changes in microbial community structure [44]. Nitrate nitrogen can inhibit the degradation of unstable organic carbon more effectively than ammonium nitrogen [38], further enhancing aggregate stability.

5. Conclusions

(1) Soil aggregates with the 5–10 mm particle size are the dominant fraction in the soil structure of the alpine meadow, and this fraction changes drastically with grazing intensity. No grazing (CK) can maintain high force stability of soil aggregates, but its resistance to water erosion is weaker than that of grazed plots. Under the no-grazing condition, the content of water-stable soil aggregates with the 5–10 mm particle size decreases significantly compared with mechanically stable aggregates (by 60.07% in the topsoil and 70.66% in the subsoil). Light and moderate grazing result in a stable soil structure with dynamic balance and the ability to resist adverse external factors. In contrast, heavy and extreme grazing aggravate the fragmentation of soil structure and lead to an overall decline in stability.

(2) Altitude and soil bulk density are extremely significantly positively correlated with soil aggregate stability ($P < 0.001$); belowground biomass exerted a significant effect on the stability indices of mechanically stable aggregates in the topsoil ($P < 0.05$); high root biomass destroyed soil macroaggregates but enhanced the resistance to water erosion. Nitrate nitrogen has a positive effect on aggregate stability, while ammonium nitrogen has a negative effect; soil microbial biomass carbon, nitrogen and phosphorus exhibit dual effects, being significantly positively correlated with the GMD of water-stable soil aggregates but negatively correlated with mechanically stable indices and other water-stable indices.

(3) Mechanically stable soil aggregates in the alpine meadow are more sensitive to grazing intensity than water-stable soil aggregates, and the dry sieving method is more effective in reflecting the effects of grazing on grasslands. The topsoil of the alpine meadow is more sensitive to grazing intensity than the subsoil; the stability of soil aggregates in the subsoil is superior to that in the topsoil

under light and moderate grazing, while the opposite is true under no grazing, heavy and extreme grazing, as the subsoil is less disturbed by grazing trampling.

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Abbreviations

The following abbreviations are used in this manuscript:

CK	No Grazing (Control)
LG	Light Grazing
MG	Moderate Grazing
HG	Heavy Grazing
EG	Extreme Grazing
MWD	Mean Weight Diameter
GMD	Geometric Mean Diameter
D	Fractal Dimension
AWSRCI	Aggregate Water Stability Relative Change Index
SMR	Soil Mineralization Rate
NH ₄ ⁺ -N	Ammonium Nitrogen
NO ₃ ⁻ -N	Nitrate Nitrogen
ALT	Altitude
SMBC	Soil Microbial Biomass Carbon
SMBN	Soil Microbial Biomass Nitrogen
SMBP	Soil Microbial Biomass Phosphorus
AGB	Aboveground Biomass
BGB	Belowground Biomass
RSR	Root-Shoot Ratio
BD	Bulk Density
DS	Dry Sieving method
WS	Wet Sieving method
Top	Topsoil (0–10 cm)
Sub	Subsoil (10–20 cm)

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