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Associated soil aggregate nutrients and controlling factors on aggregate stability in semiarid grassland under different grazing prohibition timeframes

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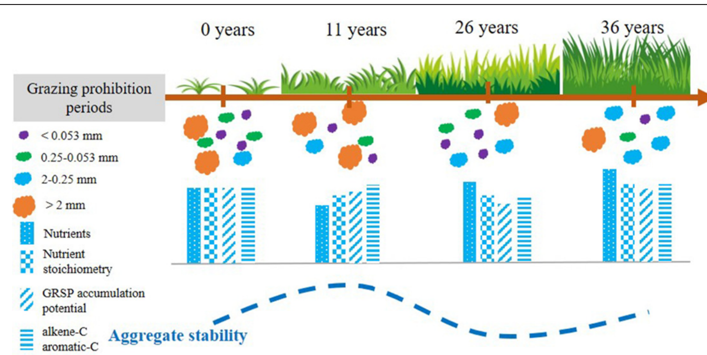
HIGHLIGHTS

- Soil aggregates have more nutrients after 26 years grazing prohibition.
- Soil aggregates' stability index MWD showed an order of 11 > 36 > 26 years.
- GRSP potential and nutrient stoichiometry are the main factors controlling MWD.
- High aggregate stability of semi-arid grasslands has more alkene-C and aromatic-C.
- Grazing prohibition after 26 years favors the ecological quality of the grassland.

GRAPHICAL ABSTRACT



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ABSTRACT

Grazing prohibition is an effective measure in improving soil stability and ecological quality. However, only a limited number of studies have been published on the dominant factors that impact soil aggregate stability and their associated effects on nutrient distribution for different size soil aggregates under long-term grazing prohibition management. In this study, we investigated variation in soil aggregate stability and nutrient distribution characteristics in semiarid grassland sites under different grazing prohibition timeframes (0 years [GP0], 11 years [GP11], 26 years [GP26], and 36 years [GP36]). Results showed that organic carbon (C) and total nitrogen (TN) concentrations in soil aggregates decreased at GP11 before progressively increasing and reaching its highest value at GP36, and the total phosphorus (TP) concentration did not change significantly. Most nutrients accumulated in macroaggregates (> 0.25 mm) under grazing prohibition, and the nutrient stoichiometry in soil aggregates increased after 26 years. Compared to the control (GP0), the mean weight diameter (MWD) value of the soil stability index increased at GP11 (21.7%) and decreased at GP26 (18.9%). Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) also showed that the proportion of stable organic C-related functional groups (i.e., alkene-C and aromatic-C) in macroaggregates were higher at GP11 and GP36 than at GP26. Furthermore, principal component analysis (PCA), partial least squares path modeling (PLS-PM), and the relative importance of regressors all showed that glomalin-related soil proteins (GRSP) and nutrients indirectly improved

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aggregate stability in semiarid grassland through their influence on the GRSP accumulation potential and nutrient stoichiometry. Generally, after 26 years grazing prohibition had a positive effect on soil aggregate stability and nutrient accumulation in the semiarid grassland sites investigated for this study. Results from this study provide a theoretical basis to select appropriate grazing prohibition timeframes under grassland management initiatives to optimize ecological quality measures in semiarid regions.

1. Introduction

Grassland is an indispensable terrestrial ecosystem that maintains the ecological balance of arid and semiarid regions under global climate change (Cui et al., 2019; Dixon et al., 2014). Global climate change, anthropogenic disturbances, and desertification have all led to the continuous degradation of grassland ecosystems, for which ecosystem services and functions have effectually decreased (Pereira, 2020; Yu et al., 2020). Misguided grazing practices are an artificial driving force of grassland degradation, affecting both the ecological sustainability and the development of animal husbandry (Li et al., 2020; Pan et al., 2018). Some ecological engineering measures have been recently introduced to protect and restore grassland ecosystems (Pereira, 2020; Mganga et al., 2015). Globally, grazing prohibition has become both a leading and an effective method by which to restore natural grassland vegetation. Grazing prohibition can increase plant diversity and aboveground productivity, improve soil erosion resistance and aggregate stability, and enrich soil nutrient concentrations (Hancock and Vallely, 2020; Listopad et al., 2018). However, inappropriate grazing prohibition practices will result in an imbalance between soil inputs and outputs that will eventually lead to grassland degradation, especially in ecologically fragile arid and semiarid regions (Bi et al., 2018; Deng et al., 2014).

To a certain extent, the stability of soil aggregates reflects the physical properties of soil and plays an essential role in maintaining soil porosity, water holding capacity, permeability, soil erosion reduction, etc., which can be used as a basis to evaluate soil quality in semiarid regions (Liu et al., 2020; Ye et al., 2019; Zhu et al., 2019). Moreover, the mean weight diameter (MWD) is a key indicator used to evaluate soil aggregate stability. Cheng et al. (2015) reported that over progressive years, the trend in MWD values and the soil organic carbon (SOC) content of aggregates in abandoned overgrazed grassland in semiarid regions on the Chinese Loess Plateau was to first increase before decreasing. Relative to the prohibition of grazing, a lower degree of grazing has resulted in higher soil MWD, geometric mean diameter (GMD), and nutrient accumulation in arid grassland of Iran (Molaeinasab et al., 2018). However, a longer grazing prohibition timeframe will not necessarily be conducive to soil stability and total carbon (TC) and total nitrogen (TN) accumulation as reported in a Canadian grassland study (Evans et al., 2012). For example, aggregate stability increased significantly after short-term restoration of natural vegetation on the Loess Plateau (An et al., 2013). However, a previous study also reported that as the grazing prohibition timeframe increased (from 4 to 31 years), soil MWD values, macroaggregate proportions, and macroaggregate-associated C content all increased (Wen et al., 2016). Therefore, it is important to determine the optimal artificial grazing prohibition timeframe to achieve the ideal ecological mode for grassland management. Moreover, it is necessary to clarify the stability of soil aggregates and associated influencing factors during long-term grazing prohibition measures in semiarid grassland regions.

Aggregates, as the basic unit of soil structure, dominate changes in soil nutrient accumulation. Moreover, dynamic SOC changes affect soil aggregate stability (Wang et al., 2018; Yao et al., 2019). Following 15 years of vegetation restoration, both SOC and TN content significantly increased in one related study, for which the content in macroaggregates exhibited the greatest increase (Wang et al., 2018). The protection of large aggregates is considered the basis for maintaining high SOC reserves, which many ecological models have incorporated into their design (Kurmi et al., 2020; Wen et al., 2016). Soil organic matter (SOM) can combine with mineral particles to form complex adhesives, improving soil aggregate stability through a reduction in wettability (Cheng et al., 2015). Moreover, soil nutrients in aggregates play an essential role in improving soil fertility while also changing the particle size distribution of soil aggregates, thus influencing aggregate stability (Spohn and Giani, 2010; Zhong et al., 2021). Yao et al. (2019) reported that both SOC and TN content in surface soil aggregates mostly accumulated in macroaggregates under three different vegetation recovery measures wherein farmland was converted back to grassland, forestland, and artificial grassland, thus improving the macroaggregate and soil structure mass ratios. Characteristics and interactions associated with soil aggregate C, N, and phosphorus (P) cycling all play an essential role in clarifying the limiting trends and balancing mechanisms of soil nutrients (Liu et al., 2017). Additionally, soil stoichiometry has a significant impact on ecosystem multifunctionality during ecosystem succession (Lucas-Borja and Delgado-Baquerizo, 2019). Our previous studies found that climate, soil characteristics, and management practices can all affect soil nutrient accumulation and its stoichiometry (Cui et al., 2019; Zhao et al., 2019). For different vegetation communities, small microaggregate C:N value ranges indicate that microaggregate SOC content is more stable (An et al., 2010). Since nutrients play an important role in soil structure stability, it is necessary to determine the nutrient distribution of different soil aggregate sizes.

Many studies have shown that glomalin-related soil proteins (GRSP) and soil components/functional groups both play a critical role in stabilizing aggregates (Galazka et al., 2017; Xue et al., 2019). Soil GRSP is a thermostable glycoprotein released by arbuscular mycorrhizal fungi (AMF), which is widely distributed in soil (Zhu et al., 2019). It has been shown that GRSP can bind together through hydrophobic interactions and adhere to the soil to improve soil structure stability (Qiao et al., 2019). As a biological "glue", GRSP participates in internally combining aggregates, which helps microaggregates to form into macroaggregates (Zhu et al., 2019). However, it is difficult for easily extracted GRSP components to accumulate. Conservation tillage and organic amendment practices redistribute different size GRSP aggregates to promote soil aggregate formation and stabilization. Soil aggregate stability is associated with the content of the primary cementing agent, such as clay minerals, SOM, and carbonates (Peltre et al., 2014; Xue et al., 2019). At present, attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) is widely used to determine the C functional group chemistry of SOM (Dhillon et al., 2017). The ATR-FTIR technique is favored by researchers due to its advantages, such as the simplicity of sample preparation, no special sample state requirements, and high detection sensitivity (Xu et al., 2020b). For example, James et al. (2019) used ATR-FTIR to determine the composition of water-soluble organic matter under different land-use patterns in the Brazilian Cerrado. Moreover, land management practices have been shown to affect organic C functional group content, namely, alkene-C, aromatic-C, and carboxylic-C, as measured by FTIR (Xue et al., 2019). However, few studies have focused on the role that GRSP and SOC functional groups play on aggregate stability in semiarid grassland. Accordingly, a comprehensive investigation on the effects that nutrients, GRSP, and SOC have on soil aggregate stability is necessary, which will help us better understand the underlying mechanisms.

Four grassland areas were selected for this study that represent four different grazing prohibition timeframes (i.e., 0, 11, 26, and 36 years) in a typical semiarid region on the Loess Plateau to investigate long-term grazing prohibition effects on soil aggregate-associated nutrients and stability. The objectives of this study were: (1) to reveal long-term grazing prohibition effects on soil aggregate-associated nutrients and stability in a semiarid grassland region, and (2) to ascertain the main factors that affect soil aggregate stability following grazing prohibition measures. For this study, we hypothesized that: (1) there is an optimal grazing prohibition timeframe that will increase soil aggregate stability and aggregate-associated nutrients following long-term grazing prohibition; (2) soil stability improvements can potentially be attributed to changes in nutrients and GRSP, which will increase stable C within organic C.

2. Materials and methods

2.1. Study area

The study area is in the largest typical semiarid steppe region on the Loess Plateau, within the Yunwu Shan Nature Reserve, the Ningxia Hui Autonomous Region (NHAR), China (Fig. 1). The region is characterized by a moderate temperate semiarid climate and a variety of topographic features, sparse vegetation, severe soil erosion, and water loss conditions, for which the erosion modulus is as high as 5000–10,000 t/a·km^{-2}, which greatly affects agricultural development. Mean annual total sunshine hours, mean annual evaporation, and average frost-free days per year are 2518 h, 1600 mm, and 137 days, respectively. The mean annual temperature range is 4–6 °C. The highest mean monthly temperature is in July (24 °C) and the lowest is in January (−14 °C). The soil is a montane-type gray-cinnamon colored soil. The dominant plant species are *S. bungeana*, *T. mongolicus, Potentilla acaulis*, and *A. sacrorum* (Jing et al., 2014).

2.2. Sampling design

In September 2018, four grassland sites were selected for investigation along a grazing prohibition chronosequence. All sites surveyed were intensively grazed (> 50 sheep/ha) before the enactment of grazing prohibition (Jing et al., 2014). According to the grazing prohibition timeframe of each site, three grassland sites were subdivided into representative grazing prohibition treatments (i.e., short-term [GP11], medium-term [GP26], and long-term [GP36]), while the fourth under consecutive moderate grazing (4 sheep/ha/year) was used as the control (GP0). Table S1 provides the coordinates of each study site. The soils of all sites have similar bulk density, clay, silt, and sand content, which has been reported in our previous study (Zhang et al., 2019). Similar soil types, soil texture, slope gradients, slope aspects, and elevations were selected at all four sites. Additionally, no fertilization or crop planting activities have taken place in any of these sites since 1982. Each site consisted of three randomly-established replicate plots (100 × 50 m), where each replicate plot was separated by 100 m. For all plots, litter was entirely removed prior to soil sampling. We used an aluminum box (15 cm diameter, 10 cm height) to collect soil samples at a 0–10 cm soil depth. The "S" sampling pattern approach was applied to each plot, wherein five sampling points were randomly selected. Soil samples were then transferred to the laboratory and dried naturally for soil aggregate determination.

2.3. Soil aggregate size distribution and analysis

Aggregate stability was determined using the wet sieving method (Elliott, 1986). Air-dried soil samples (100 g) were immersed in distilled water for 30 min and then transferred into three different size sieves (2, 0.25, and 0.053 mm). The wet sieving procedure consisted of an up and down movement (5 cm amplitude, 40 times min^{-1} frequency). The aggregates retained in the sieve were collected in the aluminum box before being dried and weighed at 60 °C. The mass

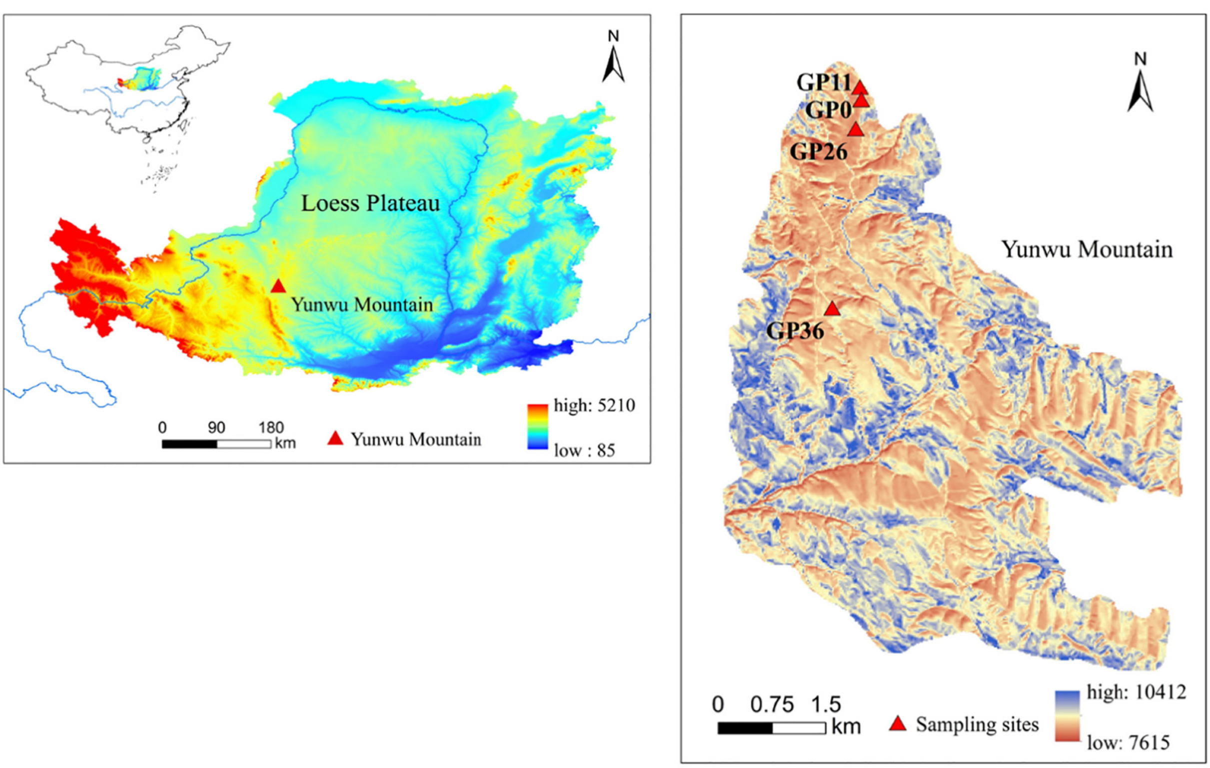

Fig. 1. Map of the sampling site.

percentage of each aggregate fraction was then calculated. Finally, a total of four aggregate classes of differing diameters were obtained, including: >2, 2–0.25, 0.25–0.053, and <0.053 mm. The MWD was then used to evaluate the water stability of soil aggregates, using Eq. (1):

$$\mathrm{MWD} = \sum_{i=1}^{n} \mathrm{X}i * \mathrm{W}i \quad (1)$$

where X*i* is the mean diameter of the aggregate fraction *i*, and W*i* is the mass proportion of the aggregate fraction *i*.

2.4. Measurement of nutrients and GRSP content in soil aggregates

The potassium dichromate oxidation method was used to determine aggregate SOC content, while the Kjeldahl method was used to determine aggregate TN content. Additionally, the ammonium molybdate colorimetric method was used to determine total phosphorus (TP) content. GRSP was extracted using the method described by Wright and Upadhyaya (1998). In brief, this method obtains *E*-GRSP (easily extractable GRSP) by autoclaving 1 g of air-dried soil samples in 8 ml of a 20 mM citrate solution (pH = 7.0) at 121 °C for 30 min and then centrifuging the solution at 10000 rpm for 5 min to obtain the supernatant. To determine T-GRSP (total extractable GRSP) content, 1 g of the air-dried soil samples was poured into a 10 ml centrifuge tube wherein 8 ml of a 50 mM sodium citrate buffer (pH = 8.0) was added. Following this, the solution was extracted at 121 °C for 60 min and then centrifuged at 10000 rpm for 5 min. The above process was replicated five times, after which the supernatant was mixed evenly. Following this, 1 ml of the extract was added to 5 ml of Coomassie Brilliant Blue, after which a spectrophotometer (UV3200, Shimadu Corporation, Japan) was used to conduct colorimetry at 595 nm after 2 min.

2.5. ATR-FTIR spectroscopy

Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) was used to analyze both the soil component types and the soil aggregate functional bonds (Xu et al., 2020b). The ATR (Nicolet iS10 FTIR Spectrometer, the United States of America) crystal with a DTGS detector was cleaned with anhydrous ethanol, while the spectrum was calibrated against the ambient air background. Dried soil samples were then pressed onto the crystals for scanning, using a scanning range of 4000–400 cm^{-1} and a wavelength resolution of 4 cm^{-1}. Based on Solomon et al. (2007), we conducted semi-quantitative analysis of organic C functional groups. OriginPro 2020 (OriginLab Corporation, Northampton, MA, USA) was used to perform multi-peak fitting analysis. The absorption peak area was analyzed by subtracting the baseline, searching for the peak and multi-peak fitting.

2.6. Statistical analysis

One-way analysis of variation (ANOVA) was used to evaluate the effects of grazing prohibition on the stability, nutrient content, and GRSP of soil aggregates. Tukey's HSD test was used to determine the significance level ($P < 0.05$). Values were the means ($n = 3$) ± the standard error (*SE*). Additionally, IBM SPSS 25.0 software (IBM, Armonk, the United States of America) was used to perform one-way ANOVA and multiple comparisons. Pearson correlation analysis was used to examine relationships between soil aggregate stability (MWD) and nutrients and GRSP. The "relaimpo" package was used in R software (version 3.6.3) to determine the relative importance of predictive variables to explanatory variables. The "plspm" package was used to construct partial least squares path modeling (PLS-PM), which determines the major pathways of predictive variables to explanatory variables. Principal component analysis (PCA) was used to analyze relationships between environmental factors and MWD. Principal coordinates analysis (PCoA) based on Bray–Curtis dissimilarity was used to analyze MWD differences among the different grazing prohibition treatments. Lastly, Origin Pro 2020 was used to generate the figures.

3. Results

3.1. Aggregate particle distribution and stability

Aggregates >0.25 mm constituted the largest proportion of all soil aggregates (69.6–85.9%), followed by aggregates <0.053 mm (11.9-23.9%) (Table 1). Proportions <0.053 mm significantly decreased from 23.9% to 11.9%, whereas proportions >0.25 mm significantly increased from 69.6% to 85.5% after 36 years of the grazing prohibition. The MWD is used as an indicator to assess soil aggregate stability. Additionally, PCoA was used to further analyze differences in MWD among the different grazing prohibition treatments (Fig. S1). The first and second axes clearly illustrate a separation in the MWD index among grazing and prohibited grassland grazing sites, indicating that grazing prohibition can significantly affect soil aggregate stability. Compared to GP0 (the control), the MWD value of GP11 significantly increased (by 21.7%). The lowest MWD value was observed in GP26, followed by GP36. In general, short-term (GP11) and long-term (GP36) grazing prohibition treatments were both beneficial to soil water aggregate stability, while the medium-term (GP26) grazing prohibition treatment was potentially detrimental to soil water aggregate stability.

3.2. Soil aggregate nutrients

The timeframe of the different grazing prohibition treatments had a significant effect on soil aggregate nutrient concentrations (Table 2), for which its effect on SOC and TN concentrations was significant, but its effect on TP concentrations was generally weak. Results from PCA further showed that GP36 may have potentially had a greater effect on SOC and TN concentrations in soil aggregates and that both GP11 and GP26 affected TP (Fig. 2A). Moreover, SOC and TN concentrations during the grazing prohibition treatments were generally highest for aggregate sizes between 2 and 0.25 mm. Conversely, SOC concentrations for aggregate sizes between 0.25 and 0.053 mm were the lowest in all four grazing prohibition treatments, ranging from 22.8 to 32.8 g kg^{-1}. For soil aggregates under grazing prohibition, SOC and TN concentrations significantly decreased during the first 11 years before significantly increasing thereafter (i.e., from 11 to 36 years). For GP36, there was still

Table 1
Soil aggregate fraction (%) and MWD following grazing prohibition.

Study sites	Soil aggregate fraction				MWD (mm)	>0.25 mm (%)
	>2 mm (%)	2–0.25 mm (%)	0.25–0.053 mm (%)	<0.053 mm (%)		
GP0	39.96 ± 2.78 b	29.62 ± 2.65 c	6.43 ± 0.67 a	23.90 ± 0.49 a	1.75 ± 0.07 b	69.65 ± 0.17 c
GP11	51.64 ± 0.95 a	27.64 ± 1.07 c	3.17 ± 0.03 bc	17.54 ± 0.09 b	2.13 ± 0.02 a	79.29 ± 0.12 b
GP26	25.52 ± 1.86 c	45.09 ± 0.04 b	5.63 ± 1.26 ab	23.75 ± 0.64 a	1.42 ± 0.06 c	70.62 ± 1.90 c
GP36	26.49 ± 2.50 c	59.36 ± 2.03 a	2.29 ± 0.03 c	11.86 ± 0.43 c	1.60 ± 0.06 bc	85.85 ± 0.46 a

Note: MWD: mean weight diameter. Values are the means ± *SE* ($n = 3$). Different lowercase letters indicate significant differences among the study sites for the same aggregate size using Tukey's HSD test ($P < 0.05$).

Table 2
Distribution of nutrients in soil aggregates following grazing prohibition.

Study sites	>2 mm	2–0.25 mm	0.25–0.053 mm	<0.053 mm
SOC (g kg^{-1})				
GP0	28.42 ± 0.34 bC	34.63 ± 0.31 aA	28.05 ± 0.73 bC	32.40 ± 0.38 aB
GP11	24.82 ± 0.13 cB	28.08 ± 0.37 cA	22.75 ± 0.29 cC	26.56 ± 0.76 bAB
GP26	27.55 ± 0.08 bB	31.71 ± 0.47 bA	26.50 ± 0.64 bB	26.94 ± 0.41 bB
GP36	30.64 ± 0.50 aC	34.45 ± 0.23 aA	32.79 ± 0.22 aB	33.56 ± 0.42 aAB
TN (g kg^{-1})				
GP0	2.83 ± 0.03 bB	3.22 ± 0.03 bA	2.75 ± 0.02 cB	3.16 ± 0.08 aA
GP11	2.61 ± 0.01 cB	2.80 ± 0.03 cA	2.38 ± 0.03 dC	2.57 ± 0.04 dB
GP26	3.00 ± 0.02 aB	3.32 ± 0.02 bA	2.89 ± 0.03 bC	2.85 ± 0.03 bC
GP36	3.06 ± 0.01 aC	3.46 ± 0.03 aA	3.22 ± 0.03 aB	3.09 ± 0.06 abBC
TP (g kg^{-1})				
GP0	0.25 ± 0.03 bB	0.50 ± 0.00 aA	0.42 ± 0.07 aAB	0.48 ± 0.08 aAB
GP11	0.61 ± 0.02 aA	0.51 ± 0.03 aAB	0.44 ± 0.02 aB	0.49 ± 0.04 aB
GP26	0.63 ± 0.02 aA	0.55 ± 0.02 aAB	0.51 ± 0.04 aAB	0.47 ± 0.05 aB
GP36	0.51 ± 0.07 aA	0.47 ± 0.02 aA	0.50 ± 0.00 aA	0.48 ± 0.04 aA

Note: SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus. Values are the means ± *SE* ($n = 3$). Different uppercase letters indicate significant differences among different aggregate sizes in same study site; different lowercase letters indicate significant differences among study sites for the same aggregate size using the Tukey's HSD test ($P < 0.05$).

a mark increase in SOC and TN concentrations in aggregate sizes >2 and between 0.25 and 0.053 mm compared to the free grazing site (GP0, the control) but no significant difference in aggregate sizes <0.053 mm. Furthermore, we observed significant increases in SOC concentrations at GP36 for aggregate sizes >2 and between 0.25 and 0.053 mm (i.e., 7.8 and 16.6%, respectively), but TN concentrations for the corresponding aggregate sizes at the same site were higher (8.1 and 17.1%). Additionally, aggregate sizes between 2 and 0.25 mm affected both SOC and TN accumulation in grassland (Fig. 2B). Compared to GP0, soil TP concentrations for aggregate sizes >2 mm at GP11 significantly increased and then remained stable throughout the subsequent timeframes (i.e., 11–36 years). However, we observed no change in TP concentrations in aggregate sizes between 2 and 0.25, 0.25–0.053, and < 0.053 mm throughout entire study period (i.e., 0–36 years grazing prohibition).

Grazing prohibition also altered nutrient stoichiometry in soil aggregates (Table S2). Soil C/N ratios were lowest for all soil aggregate sizes at GP26, whereas C/P ratios and N/P ratios were lowest at GP11. Compared to the control (GP0), we observed an evident reduction in soil C/N ratios for aggregate sizes >2, 2–0.25, and 0.25–0.053 mm at GP26. Furthermore, we observed a significant decrease in C/N ratios for aggregate sizes between 2 and 0.25 mm at GP11, GP26, and GP36 (i.e., 6.76, 11.14, and 7.25%, respectively) compared to GP0. Soil C/P and C/N ratios for aggregate sizes >2 and between 2 and 0.25 mm significantly increased during the first 11 years of grazing prohibition. The soil N/P ratio for aggregate sizes >2 mm significantly increased at GP11.

3.3. GRSP concentrations in soil aggregates

Over a yearly progression in grazing prohibition, T-GRSP concentrations in aggregates >0.25 mm decreased at GP11, which was followed by a significant increase at GP26 before another sharp decrease at GP36, while the trend in T-GRSP concentrations for aggregates <0.25 was to first increase and then decrease (Fig. 3A). T-GRSP concentrations for aggregate sizes between 0.25 and 0.053 and > 2 mm increased significantly between 11 and 26 years, while all aggregate sizes significantly decreased between 26 and 36 years. Moreover, GP26 yielded the highest T-GRSP concentrations out of all aggregate sizes. After GP36, we observed a significant increase in T-GRSP concentrations for aggregate sizes between 0.25 and 0.053 mm compared to the control (GP0), while we observed no significant changes for the other aggregate sizes. As the grazing prohibition timeframe (treatments) progressed, *E*-GRSP concentrations for aggregate sizes >2 mm decreased, while E-GRSP concentrations for aggregate sizes <2 mm tended to first decrease before increasing once again (Fig. 3B). Moreover, *E*-GRSP concentrations for all soil aggregate sizes significantly decreased in the first 26 years. For GP26, *E*-GRSP concentrations in all soil aggregate sizes were generally lower than those in the other treatments and significantly lower than those in the control (GP0). Following an extended grazing prohibition period, E-GRSP concentrations in aggregate sizes >0.053 mm decreased significantly. E-GRSP/T-GRSP in aggregate sizes >2, 2–0.25, and

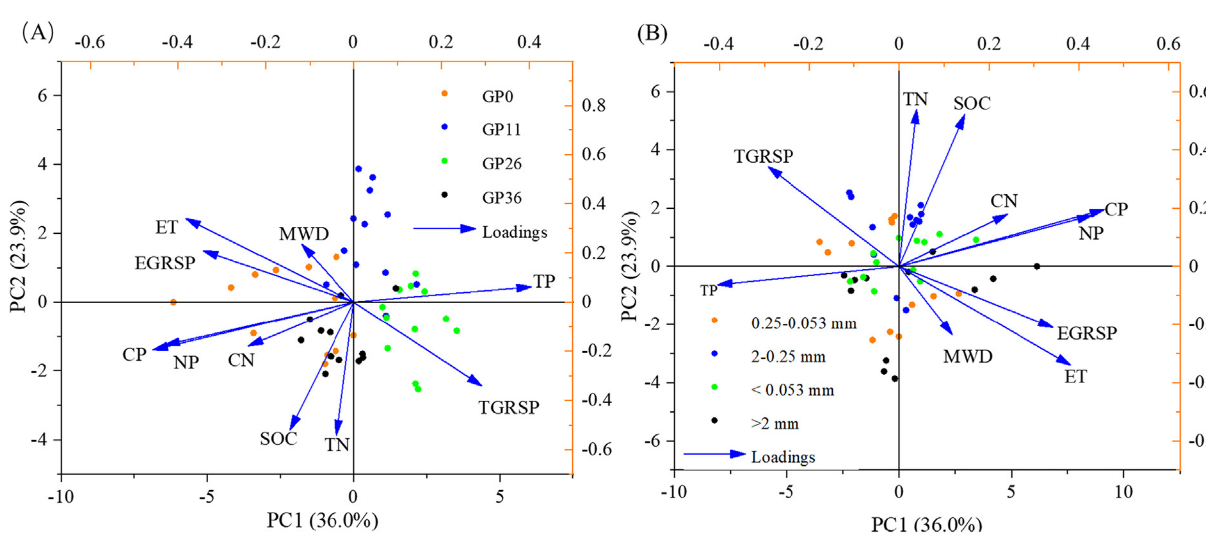

Fig. 2. Principal component analysis (PCA) revealing the relationship between MWD and environmental factors. (A), grazing prohibition treatments; (B), different aggregate components. MWD: mean weight diameter. Environmental factors include: TGRSP (total extractable GRSP), EGRSP (easily extractable), EGRSP/TGRSP, SOC (soil organic carbon), TN (total nitrogen), TP (total phosphorus), CN (SOC/TN); CP (SOC/TP), and NP (TN/TP). Arrows denote vectors of MWD and environmental factors, with longer arrows representing a greater influence of these parameters. The direction of an arrow indicates the steepest increase in the variable, and the length indicates the strength relative to the other variables.

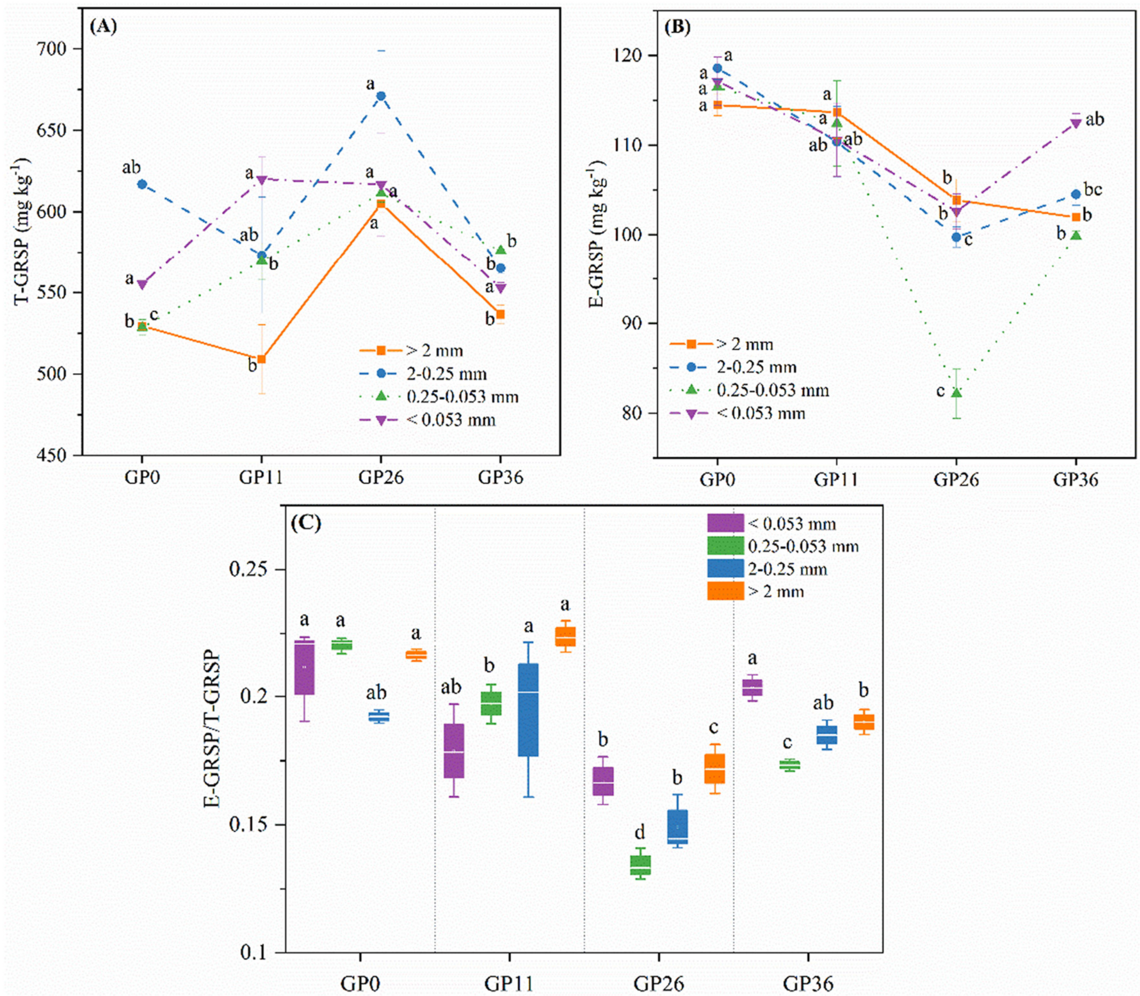

Fig. 3. Glomalin-related soil proteins (GRSP) concentration in soil aggregates following the enactment of grazing prohibition. T-GRSP: total extractable GRSP; *E*-GRSP: easily extractable GRSP. Values are the means ± *SE* ($n = 3$). Different lowercase letters indicate significant differences among study sites for the same aggregate size using Tukey's HSD test ($P < 0.05$).

0.25–0.053 mm significantly decreased between 11 and 26 years (Fig. 3C). E-GRSP/T-GRSP ratios for aggregate sizes >2 mm were generally higher than for aggregate sizes between 2 and 0.25 and 0.25–0.053 mm. We observed a significant reduction in E-GRSP/T-GRSP ratios for aggregate sizes >2 mm and between 0.25 and 0.053 in GP36 compared to the control (GP0), while corresponding ratios in aggregate sizes between 2 and 0.25 and < 0.053 mm remained for the most part unchanged.

3.4. Soil components or functional group

Generally, the ATR-FTIR spectra of soil aggregates over the different grazing prohibition years were similar (Fig. 4). Table S3 summarizes the functional group type or soil component that corresponds to absorption peak characteristics. The spectra featured a peak at 1634 cm^{-1}, which was attributed to the C═O vibration in carboxylic acids, while a peak at approximately 1539 cm^{-1} was attributed to the C═C vibration in aromatic compounds. Corresponding peaks at 1000 and 775 cm^{-1} were attributed to the C—H vibration in alkene and NH_2 in primary amine, respectively. Grazing prohibition increased both aromatic-C and carboxylic-C concentrations (Table S4). Moreover, the aromatic-C concentration increased the most at GP11 and GP36. Additionally, the alkenes-C concentration for all aggregate sizes decreased in GP11, GP26, and GP36 compared to the control (GP0).

3.5. Nutrient and GRSP effects on soil aggregate water stability

For aggregate sizes >2, 2–0.25, and <0.053 mm, a linear relationship was observed in soil nutrients and GRSP and aggregate water stability, while no significant linear relationship was observed for aggregate sizes between 0.25 and 0.053 mm (Table 3). For aggregate sizes >2 mm, the MWD was significantly negatively correlated to SOC ($P < 0.01$) and TN ($P < 0.01$) and significantly positively correlated to *E*-GRSP ($P < 0.05$). TN ($P < 0.01$) and TP ($P < 0.05$) concentrations for aggregate sizes between 2 and 0.25 mm were linearly correlated to MWD. For aggregate sizes <0.053 mm, MWD was negatively correlated to the C/N ratio ($P < 0.05$). PCA also found a negative correlation between MWD and nutrient content and a positive correlation with *E*-GRSP and E-GRSP/T-GRSP (Fig. 2).

For linear models, the relative importance of regressors showed that soil GRSP, nutrients, and their associated stoichiometry, used as environmental variables, explained 65.4% of MWD (Fig. 5). Moreover, C/P, TP, and N/P were identified as the most important variables (with a 50.8% relative influence). T-GRSP, C/N, and E-GRSP/T-GRSP were also key variables that affected soil aggregate stability (with a 35.0% relative influence). Moreover, PLS-PM showed that nutrient stoichiometry had a significant and direct effect ($P < 0.001$) on MWD (Fig. 6). The total effect of both E-GRSP/T-GRSP (with a 1.19 total effect) and nutrient stoichiometry (with a 1.54 total effect) was positive on MWD, whereas the total effect of GRSP (with a -0.22 total effect) and nutrients (with a -0.15 total effect) was negative.

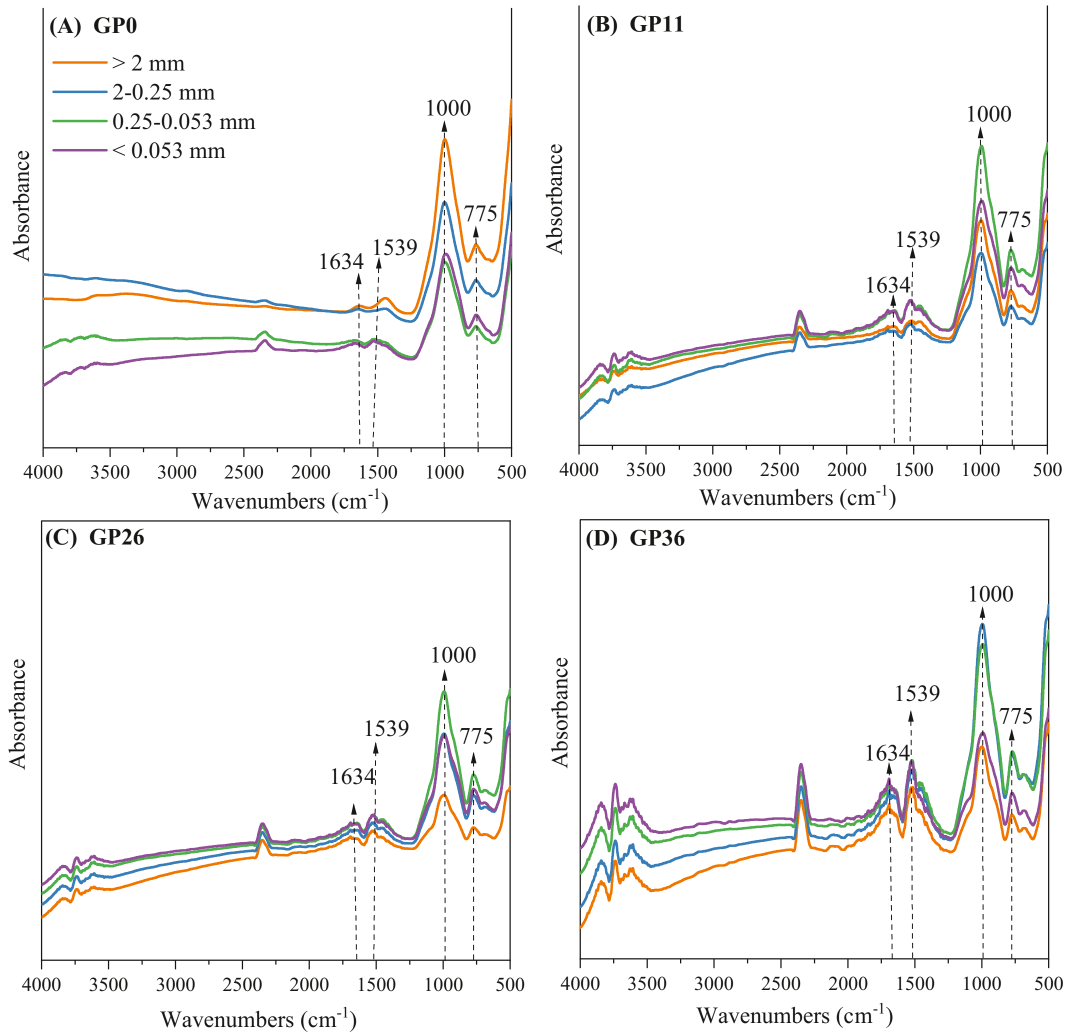

Fig. 4. Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) of soil aggregates following the enactment of grazing prohibition.

4. Discussion

4.1. Grazing prohibition effects on nutrient distribution in soil aggregates

Both soil aggregate SOC and TN concentrations decreased significantly in the first 11 years of grazing prohibition, which was due to a reduction in organic matter inputs from livestock manure. However, after 11 years, SOC and TN concentrations in the different size aggregates increased significantly, which could potentially be attributed to a release

Table 3
The relationship between MWD and environmental factors in soil aggregate fractions.

Aggregate	Equation	R^2	F	P
>2 mm	MWD = 15.674 E/T - 1.885	0.736	27.898	<0.01
	MWD = −0.131 SOC + 4.918	0.515	10.611	<0.01
	MWD = −2.145 TN + 7.424	0.939	154.06	<0.01
	MWD = 0.044 EGRSP - 3.465	0.491	9.629	<0.05
2–0.25 mm	MWD = 0.126 TP - 0.342	0.417	7.151	<0.05
	MWD = 0.467 TN - 1.040	0.599	14.926	<0.01
	MWD = −0.013 EGRSP +1.811	0.419	7.223	<0.05
<0.053 mm	MWD = −0.001C/N + 0.019	0.445	8.006	<0.05

Note: MWD: mean weight diameter. Environmental factors include: GRSP, EGRSP/TGRSP, soil nutrients, and nutrient stoichiometry. GRSP, glomalin-related soil protein; EGRSP, easily extractable GRSP; TGRSP, total GRSP; E/T, EGRSP/TGRSP; SOC, soil organic carbon; TN, total nitrogen; TP: total phosphorus.

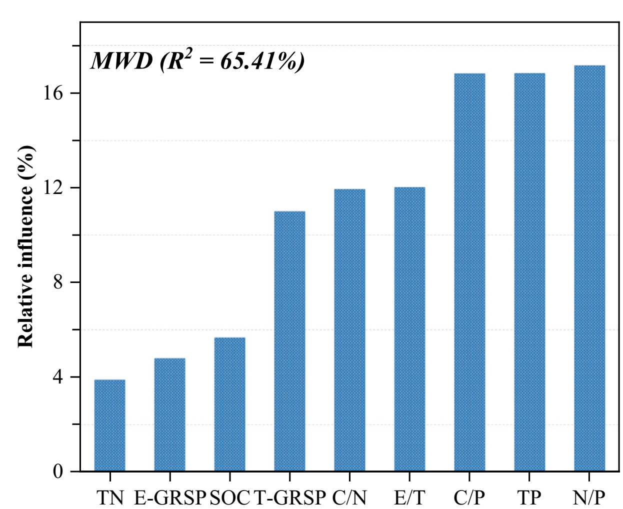

Fig. 5. Relative influence (%) of environmental drivers associated with MWD. MWD: mean weight diameter. Environmental drivers include: T-GRSP (total extractable GRSP), E-GRSP (easily extractable GRSP), E/T (E-GRSP/T-GRSP), SOC (soil organic carbon), TN (total nitrogen), TP (total phosphorus), C/N (SOC/TN); C/P (SOC/TP), and N/P (TN/TP). R^2 represents the proportion of variance explained by the linear model.

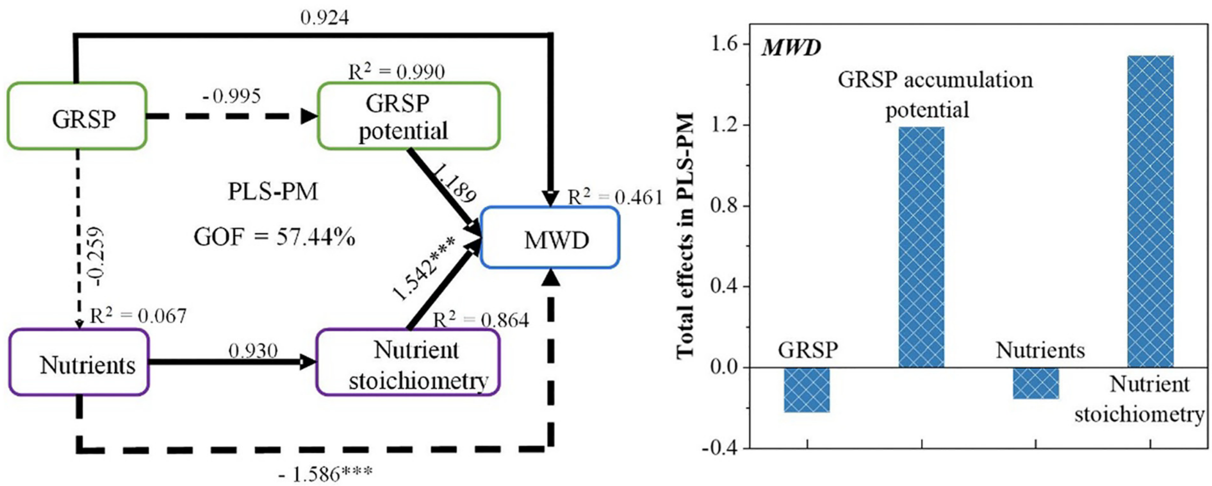

Fig. 6. Partial least squares path modeling (PLS-PM) results explaining major pathways of influence of environmental variables on the MWD of soil aggregates. MWD: mean weight diameter. GRSP include: T-GRSP (total extractable GRSP) and E-GRSP (easily extractable GRSP); GRSP accumulation potential include: E-GRSP/T-GRSP; nutrients include: SOC (soil organic carbon), TN (total nitrogen), TP (total phosphorus); nutrient stoichiometry include: C/N (SOC/TN), C/P (SOC/TP), and N/P (TN/TP). Standardized total effects (i.e., direct plus indirect effects) were calculated using PLS-PM. Solid and dashed lines denote positive and negative flows of causality, respectively. Numbers on arrow lines denote significant standardized path coefficients. * denotes significance at $P < 0.05$; ** denotes significance at $P < 0.01$; *** denotes significance at $P < 0.001$. R^2 values represent the variance of dependent variables explained by the inner model. GOF denotes the goodness of fit index.

in accumulated C and N during litter, root exudate, and dead root decomposition (Ayoubi et al., 2012). At the same time, the prohibition of grazing safeguarded aggregates from being trampled on and consequently destroyed by livestock, inhibited grassland soil respiration, and reduced the release of soil organic C to the atmosphere (Wen et al., 2016). Furthermore, as SOC concentrations increase, microorganisms will prioritize fixed N rather than mineralized N, thereby reducing net N mineralization rates. Although the prohibition of grazing initially decreased SOC and TN concentrations in all soil aggregates, increases in C and N accumulation occurred at a later stage (26–36 years). PCA also showed that GP36 had a significant effect on SOC and TN concentrations in soil aggregates. These results indicated that long-term grazing prohibition will improve SOC and TN accumulation in soil aggregates. Our previous research reported that the 35-years grazing prohibition is beneficial to increase the accumulation of nutrients in the bulk soil (Zhang et al., 2019). Moreover, the SOC content of water-stable aggregates was mainly distributed in macroaggregates (i.e., aggregates >0.25 mm), which is associated with the macroaggregate formation process (Ye et al., 2019). Moreover, SOC accumulation is associated with the physical protection of organic C in macroaggregates (Six and Paustian, 2014). The SOC content of aggregates <0.053 mm was relatively high, wherein Zheng et al. (2011) reported similar results. The smaller the aggregate size is, the larger its specific surface area will be. In other words, aggregate size can absorb more organic matter. The SOC content in aggregates <0.053 mm also play an essential role in forming soil aggregates. In general, SOC, TN, and TP content in grazing prohibited grassland primarily accumulated in aggregates >0.25 mm. Ge et al. (2019) reported that organic C, N, and P concentrations in soil macroaggregates were higher than those of microaggregates in cropland and woodland sites on the Loess Plateau. Results from our study showed that early-stage grazing prohibition was essential for soil TP recovery, while TP was primarily enriched in aggregates >2 mm. In grassland ecosystems where organic matter accumulates in soil, macroaggregate P content will increase (Fonte et al., 2014). Macroaggregate formations are essential for P accumulation, while P distribution in aggregates will affect both the availability and loss of soil P. Xu et al. (2020a) found that large macroaggregates (>2 mm) had a significant effect on soil nutrients in woodland based on their PCA results. PCA results from our study also showed that macroaggregates can affect the accumulation of soil nutrients in grassland.

Results from this study showed no significant difference in soil C/N for all soil aggregates during the initial 26 years, indicating that the SOC decomposition rate of different size soil aggregates was similar, which may result from the close connection between C and N and their synchronous response to environmental changes (Fonte et al., 2014; Ma et al., 2020b). Compared to the control (GP0), there was an evident reduction in soil C/N ratios in aggregates >0.053 mm at GP26, indicating that the 26 years medium-term grazing prohibition treatment was not conducive to organic matter accumulation. For soil aggregates, soil C/P ratios ranged from 40.52 to 119.29 (<200), indicating net nutrient mineralization (Liu et al., 2020). The N/P ratio of aggregates >2 mm decreased significantly during the early grazing prohibition stage, which primarily resulted from a decrease in the N content of aggregates >2 mm and an increase in P content. Also, C/P and N/P ratios in aggregate sizes between 2 and 0.25 mm clearly increased from 11 to 36 years. During the latter stage of grassland vegetation restoration, nutrient immobilization is promoted and N limitation is alleviated. In general, nutrient accumulation in grassland aggregates clearly increased after 26 years of grazing prohibition and was mainly concentrated in macroaggregates. These results support our first hypothesis, namely, the importance in selecting an optimal grazing prohibition timeframe and particle size composition for grassland management of soil nutrient accumulation.

4.2. Control factors and ecological implications for soil aggregate stability

The MWD index increased significantly during the early grazing prohibition stage (0–11 years), reaching its maximum at GP11 and decreasing thereafter. It has been reported that aggregate stability increases during the early vegetation succession stage and decreases during the middle and late stages (Cheng et al., 2015). Evans et al. (2012) reported that the MWD index of soil aggregates was lower in a semiarid area for which grazing was prohibited for 30 years compared to a corresponding area where grazing was prohibited for 20 years. Conversely, one study reported that soil aggregate stability in semiarid grassland in Inner Mongolia increased over a progressive grazing prohibition timeframe (i.e., 7, 11, and 31 years) (Wen et al., 2016). Generally, aggregates >0.25 mm are referred to as macroaggregates, which are regarded as those aggregates that possess the best structure and the strongest corrosion resistance, and their size is positively correlated to soil erosion resistance and stability (Deng et al., 2018). Our results suggest that long-term grazing prohibition (GP36) can increase the percentage of aggregates >0.25 mm. Long-term grazing prohibition allows grassland to recuperate and provides more plant residue that can act as organic binding agents that accumulate on the soil surface, promoting the formation of macroaggregates (Wiesmeier et al., 2012). Additionally, MWD and the percentage of aggregates >0.25 mm clearly decreased at GP26, indicating that timeframe is one of the key factors for grazing prohibition to improve the stability of soil aggregates.

Given that SOC is stored in soil aggregates, soil aggregates play a role in the physical protection of SOC, while SOC is also the primary factor associated with soil aggregate formation and stability (Dong et al., 2020). SOM can be used as a binding agent to stabilize aggregates and promote nutrient retention (Okolo et al., 2020). In this study, SOC content in soil aggregates decreased after 11-years grazing prohibition and then gradually increased after 26 years. We speculated that this phenomenon derived from the early grazing prohibition stage when grassland was undisturbed by livestock, allowing the plant to continuously use the accumulated soil nutrients for plant growth. Concurrently, no livestock-based fertilizer or exogenous input entered the soil of this closed system, which resulted in greater overall C and N outputs. Short-term grazing prohibition initiatives will not alter SOC and TN content in surface grassland soil (0–20 cm) in semiarid regions (Wen et al., 2013). For semiarid regions, one study found that an increase in the macroaggregate C storage capacity under a grassland grazing prohibition timeframe from 11 to 31 years was beneficial to soil aggregate stability (Wen et al., 2016), which was consistent with the results of this study. Soil N promotes mucilage accumulation, which can stimulate the growth of soil microorganisms while acting as an additional binder in the rhizosphere (Wang et al., 2019), thus increasing aggregate water stability. Moreover, both the secretion and biomass of the vegetation root system in grassland under grazing restrictions was higher than those not under such restrictions, enhancing soil aggregate stability (Ma et al., 2020a). A recent study reported that both soil organic C and TN content was strongly associated with aggregate stability in a natural grassland system (Zhong et al., 2021). Additionally, the relative importance of regressors in linear models indicated that soil nutrient stoichiometry contributed the most to MWD. PLS-PM analysis also confirmed that nutrient stoichiometry had a significant and direct effect and the largest total effect on MWD. These results confirmed our second hypothesis, namely, that nutrients mainly change their stoichiometry to affect the stability of soil aggregates. This finding is similar to that of Xu et al. (2020a) who showed that the C/N ratio of large-size macroaggregates had an important influence on the MWD of aggregates in a woodland system. Furthermore, considering the important influence that soil nutrient stoichiometry has on MWD, soil aggregate stability can reflect the decomposition rate of SOM as well as the degree of soil nutrient limitation (Zechmeister-Boltenstern et al., 2015).

Both correlation analysis and PCA showed that MWD was positively correlated to aggregate *E*-GRSP concentrations, particularly aggregates >2 mm, which indicated that E-GRSP played a key role in promoting soil aggregate stability. GRSP penetrates into organic matter, combining it with sediment, sand, and clay particles to stabilize soil structure and prevent soil C loss (Dai et al., 2015). *E*-GRSP decreased during the early grazing prohibition stage and gradually increased thereafter, but it remained lower than the control (GP0) treatment. This phenomenon is caused by the following facts: the dominant species of grassland becomes more obvious as the grazing prohibition timeframes increases, and the abundance of vegetation decreases, resulting in a decrease in underground biomass (Zhang et al., 2019). GRSP is a product of AMF, and a reduction in plant root biomass will lead to a reduction in AMF symbiosis, resulting in a reduction in *E*-GRSP content (Dai et al., 2015). Also, E-GRSP is understood to be a recently produced fungal protein, which is more active than T-GRSP and is easy to use as a C source by plants (Liu et al., 2020; Vasconcellos et al., 2016). Additionally, E-GRSP/T-GRSP can reflect the potential of GRSP accumulation (Jorge-Araujo et al., 2015). Results from our study showed that the accumulation potential of GRSP was lowest at GP26, which confirmed that the lowest aggregate water stability point occurred during the medium-term (26 years) grassland grazing prohibition treatment. Previous studies have shown that the accumulation potential of GRSP in macroaggregates decreases during farmland conversion to grassland in semiarid regions (Liu et al., 2020). Furthermore, our study found a close positive correlation between E-GRSP/T-GRSP and MWD, confirming that the accumulation potential of GRSP is a critical limiting factor affecting MWD.

Additionally, SOC is the most important binding agent, and the type and number of its functional group can reflect the chemical stability of organic matter and some soil components during soil humification processes, subsequently providing evidence for aggregate stability (Peltre et al., 2014; Verchot et al., 2011). Compared to free grazing, grazing prohibition increased aromatic-C concentrations in aggregates, and this increase was highest at GP11 and GP36. Aromatic-C is produced by lignin oxidation, which is relatively stable and not easily oxidized by microbial and abiotic soil factors (Schmidt and Noack, 2000). Grazing prohibition can increase plant lignin inputs, while a stable lignin can selectively accumulate in soil, thus effectively increasing the proportion of aromatic compounds in the soil. The proportion of alkene-C and aromatic-C in aggregates >0.25 mm was higher at GP11 and GP36 than at GP26, which confirmed the poor stability of soil aggregates for the 26-year grazing prohibition treatment. This is because alkene-C and aromatic-C are the main binders of macroaggregates (Xue et al., 2019). The prohibition of grazing promoted the formation of macro-aggregation by increasing the accumulation of carboxylic-C, which is attributed to the preferential participation of carboxyl groups in organic matter and mineral interactions. Carboxylic-C can also promote aggregate stability through its surface blocking effect (Verchot et al., 2011). Therefore, the proportion of alkene-C, aromatic-C, and carboxylic-C in aggregates, especially in macroaggregates, can be used to predict soil stability in semiarid grassland under grazing prohibition management. Furthermore, improving soil aggregate stability is beneficial in helping to increase the proportion of stable-C functional groups in aggregates.

5. Conclusions

In this study, we evaluated the nutrient content and aggregate stability in semiarid grassland sites under the ecological management of different grazing prohibition timeframes. The long-term grazing prohibition treatment (36 years) markedly promoted nutrient accumulation in soil aggregates, and most nutrients accumulated in macroaggregates. The short-term (11 years) grazing prohibition treatment helped to improve soil aggregate stability, an improvement that continued after the medium-term grazing prohibition (26 years). Soil nutrients and GRSP indirectly improved soil structure stability through their influence on nutrient stoichiometry and the GRSP accumulation potential. Although grazing prohibition helped improve soil stability, this improvement can also be attributed to an increase in the stable organic C components in aggregates. Findings from this study will help us better understand issues related to nutrients and the stability of aggregates over long-term enclosed grassland grazing prohibition management, while also providing a theoretical basis for soil quality management in degraded grassland.

CRediT authorship contribution statement

Dongdong Liu: Methodology, Investigation, Data curation, Writing – original draft. **Wenliang Ju:** Methodology, Investigation, Data curation, Writing – original draft. **Xiaolian Jin:** Investigation, Formal analysis. **Mengdi Li:** Investigation, Formal analysis. **Guoting Shen:** Investigation, Formal analysis. **Chengjiao Duan:** Investigation, Formal analysis. **Liang Guo:** Investigation. **Yanyan Liu:** Writing – review & editing. **Wei**

Zhao: Writing – review & editing. **Linchuan Fang:** Conceptualization, Project administration, Funding acquisition, Writing – review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.146104.

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