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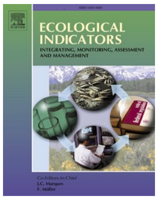

Distributions of medium mineral elements with long-term grazing exclusion in a semi-arid grassland of Inner Mongolia

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ABSTRACT

The variations of medium mineral elements in long-term grazing exclusion grassland have been few studied. Therefore, the contents, stocks, and proportions of calcium (Ca), magnesium (Mg), sulphur (S), and silicon (Si) in green plant-litter-root-soil continuum were evaluated by grazing exclusion for 18-, and 39-year (F18 and F39) and continue grazing (F0) in Mongolia grassland. Results showed that F18 and F39 decreased the stocks of Mg and Si in green plant and the stocks of Ca and Mg in 20–50 cm root ($p < 0.05$). F18 and F39 increased the stocks of S in 0–100 cm soil and the stocks of elements in litter ($p < 0.05$). Compared to F18, F39 increased the stocks of elements in 0–20 cm root, especially Si ($p < 0.05$), and a 30.3 % higher of Ca stock in green plant was observed ($p < 0.05$). The stock of Ca and Mg in 0–100 cm soil at F39 was 164.3 % and 72.6 % lower than that at F18, respectively ($p < 0.05$). F39 decreased the stocks of Mg, S, and Si in incomplete- and complete decomposed litter relative to F18. We concluded that four elements were shifted from root to other parts with long-term grazing exclusion. However, Ca, Mg, and Si were shifted from soil to plant with grazing exclusion for 39 years relative to 18 years, and Ca was shifted from root to litter, and Mg, S, and Si were shifted from litter to root or green plant.

1. Introduction

Grassland ecosystems play important roles in the balance of ecologic as well as human livelihood (Straton, 2006). However, about half of all global grasslands have been under threat from ongoing degradation, weakening their capacity to support biodiversity, ecosystem services and human well-being (Schönbach et al., 2011; Chen et al., 2014; Yang et al., 2019). Inner Mongolia grassland is one of the most important temperate grasslands, and the livestock production is the main livelihood. But the high stocking rate of livestock and the poor grazing management have caused various degrees of grassland degradation in this region (Zeng et al., 2015; Wang et al., 2017; Bai et al., 2015; Hafner et al., 2012; Liu et al., 2019; Chen et al., 2014).

Grazing exclusion becomes an effective management measure to improve grassland degradation in recent decades (Shrestha and Stahla, 2008; Hu et al., 2016; Xiong et al., 2016; Golodets et al., 2010; Tarhouni et al., 2017). Studies have found that grazing exclusion can boost the coverage, biodiversity, and biomass of vegetation, and improve the soil texture, soil microbial communities, and soil fertility, and thus promote the restoration of degraded grassland (Liu et al., 2020a; Liu et al., 2020b; Yan and Lu, 2015; Liu et al., 2017; Wang et al., 2018; Wang et al., 2014a; Wang et al., 2014b; Xiong et al., 2017; Zhao et al., 2014). The year of grazing exclusion has attracted a great deal of attention during the last few decades. Most studies have focused on the variations of carbon (C) and the key mineral elements such as nitrogen (N) and phosphorus (P) in grassland system in different years of grazing exclusion. Moreover, the results were inconsistent by reasons of variations in region, climate, pasture type, and grazing exclusion years (Wang et al., 2016; Wang et al., 2014a; Wang et al., 2014b; Zhou et al., 2011; Zeng et al., 2017). However, significant knowledge gaps still remain about the changes of other essential medium mineral elements, such as Calcium (Ca), magnesium (Mg), sulphur (S), and silicon (Si) in grazing exclusion plot, especially in long-term grazing exclusion plot.

Ca, Mg, S, and Si play the key roles in plant energy metabolism, photosynthesis, and membrane transport of plant. The contents of elements in vegetation are affected by soil, climate, botanical composition and grazing management (Perez Corona et al., 1995; Perez Corona et al., 1998; Jones and Tracy, 2013). Long-term grazing exclusion not only improves the diversity of species and the biomass of vegetation, but also results in excessive litter on the surface of soil, which certainly influences the cycles of mineral nutrients in grassland (Hu et al., 2020; Hu et al., 2021; Hou et al., 2019; Wang et al., 2017). Recent researches have

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reported that biodiversity can regulate the biomass production and the cycles and stocks of nutrients (Balvanera et al., 2006; Cardinale et al., 2006). The Ca is higher in the Fabales, while the Si is considerably higher in the Poales (Broadley et al., 2003; Bauer et al., 2011; Hodson et al., 2005; Schaller et al., 2016). The uptakes of Ca and Si by vegetation are large differences during grass and legume succession with long-term grazing exclusion. Moreover, long-term grazing exclusion accumulated lots of litter which was bound to influence the allocations of elements in plant and soil systems. Litter decomposition is a basic process that contribute to recycle nutrients held in dead plant biomass. Studies have reported that Ca and Mg are significantly correlated with litter mass loss, which play a crucial role during litter decomposition (Makkonen et al., 2012; García-Palacios et al., 2016; Berg and Laskowski, 2005; Lovett et al., 2016; Berg and McClaugherty, 2014). Si-accumulating plants can influence Si turnover rate in ecosystem by the uptake, storage, and release of Si during plant decomposition (Ehrlich et al., 2010; Lucas, 2001; Sommer et al., 2006; Schaller et al., 2012; Schaller and Struyf, 2013). The 38-year enclosing obviously decreased the stocks of Ca, Mg, and S (Hu et al., 2021), thereby changing the contents of these elements in grassland. The research on the cycle of medium mineral elements can help us maintain the community structure and ecosystem function of grassland, as well as better understand the deficiency of elements in long-term grazing exclusion grassland.

Ca, Mg, S, and Si are essential for maintaining livestock growth, reproduction and health (Jones and Tracy, 2013; Yoshihara et al., 2013). Ca deficiency results in a failure to mineralize new bone, contributing to rickets in younger animals and osteoporosis in older animals (NRC, 2000). Si can increase tooth and mandible wear and reduce the digestibility of plant material from grasses (Massey and Hartley, 2009; Seyfferth and Fendorf, 2012; Neu et al., 2017). Mg and S deficiency also results in various health problems in calves (NRC, 2000). Thus, deficiencies in certain minerals in vegetation can reduce animal productivity and result in economic losses (Spears and Weiss, 2014).

The functions of Ca, Mg, S, and Si in grassland highlighted the needs for knowledge regarding the variations of these elements in grazing exclusion grassland. However, this has been poorly assessed, especially in long-term grazing exclusion grassland. Therefore, the objectives of this study are to explore the contents, stocks, and distributions of Ca, Mg, S, and Si in green plant, litter, root, and soil in grassland plots with 18- and 39-year grazing exclusion. We hypothesis that the allocations of Ca, Mg, S, and Si in plots with different years of grazing exclusion would be altered. The research will provide scientific basis for the restoration of degraded grassland in semi-arid grassland of Inner Mongolia.

2. Material and methods

2.1. Study area

This study was conducted at the Inner Mongolia Grassland Ecosystem Research Station (IMGERS, 43°38′N, 116°42′E) of the Chinese Academy of Sciences, which is located in Xilin River Basin of Inner Mongolia, China (Fig. 1). The region has a semi-arid steppe climate with an annual average temperature of 2.3 °C, annual average precipitation of 330 mm, and annual evaporation of 4 ~ 5 times that of the precipitation. The soil is described as a dark chestnut, with a loamy sand texture. The original forage species were *Stipa grandis* and *Leymus chinensis*, and the mean percentage of *Stipa grandis* and *Leymus chinensis* was 57 % and 21 %, respectively (Hu et al., 2021). Strong winds occur in 3–5 month with an average monthly speed of up to 4.9 m·s^{-1}. Wind erosion and dust storms are the common phenomena in this region and contribute to matter balances (Hoffmann et al., 2008).

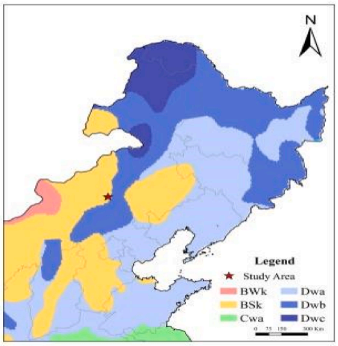

Fig. 1. The map of study area.

2.2. Experimental design

The experimental site is composed of three plots, including long term grazing plot continued 39 years outside of fence (F0), long term enclosing plot continued 18 years inside of fence (F18, livestock excluded from 2001) and long term enclosing plot continued 39 years inside of fence (F39, livestock excluded from 1980) (Fig. 2). The grazing intensity was about 5 sheep·hm^{-1}·year^{-1} in the long-term grazing plot. The information of density, species and biomass of plant in these three plots were showed in Table 1.

2.3. Sampling and analysis

A typical transect (100 m long) was randomly located in each plot in August 2019. Ten quadrats were established at 10 m intervals for vegetation and soil sampling within each transect. Plant, litter, and soil samples were collected within the same quadrat of 1 m × 1 m. Plant and litter were weighed after dried in 105℃ in laboratory. Litter was divided into undecomposed litter, incomplete decomposed litter, and complete decomposed litter according to the decomposition state of litter. From each soil sampling location, three soil cores were collected (0–10 cm, 10–20 cm, 20–30 cm, 30–50 cm, 50–70 cm, and 70–100 cm depth) to make a composite sample. Soil samples were placed into plastic bags and then transported to the laboratory where they were air-dried. Then the soils were sieved through a 2 mm sieve and used for determination. The Ca, Mg, S, and Si contents in plant and soil were determined using a flame atomic absorption machine (Varian Model AA240, Palo Alto, CA).

2.4. Statistical analysis

All data were statistically analyzed using a 10.0 SPSS software. One-way analysis of variance (ANOVA) was used to calculate the standard errors and compare the means of parameters among different treatments.

Stock of element was calculated as follows: Element stock in soil (g/m^2) = Element content (g/kg) × bulk density (g/cm^3) × soil deep (cm) × 10000. Element stock in plant (green plant, litter, and root) (g/m^2) = Element content (g/kg) × dry matter of plant (g/m^2) × 10^{-3} (Batjes and Dijkshoorn, 1999).

The element reuse efficiency was calculated as follows: Element reuse efficiency (%) = (element content in green plant - element content in litter) / element content in green plant × 100 % (Aerts, 1996; Killingbeck, 1996; Feller et al., 2002).

3. Results

3.1. Contents of elements in grassland

F18 obviously decreased the contents of Ca, Mg, S, and Si in green plant compared to F0. The content of Ca in green plant at F39 was 25.2 % higher than that at F18 (Table 2).

The contents of these four elements in litter showed L3 > L2 > L1. F18 obviously decreased the contents of Ca and Mg in L1 compared to F0. F39 obviously increased the contents of Ca and Si in L1 and L2

Fig. 2. Grazing plot (a), enclosing 18 years plot (b), and enclosing 39 years plot (c).

Table 1
The basic information of F0, F18, and F39 plots.

	F0	F18	F39
Species (number/m^{2})	9.40 ± 1.35^{b}	8.90 ± 2.13^{b}	14.50 ± 2.72^{a}
Density (plant/m^{2})	454.60 ± 33.41^{a}	148.70 ± 15.27^{b}	118.90 ± 6.23^{b}
Green plant biomass	88.62 ± 4.55^{b}	131.40 ± 8.88^{a}	128.77 ± 5.96^{a}
L1 biomass	25.42 ± 1.44^{b}	185.90 ± 31.79^{a}	229.09 ± 76.50^{a}
L2 biomass		182.80 ± 11.74^{a}	136.94 ± 9.94^{b}
L3 biomass		360.30 ± 105.15^{a}	246.23 ± 61.12^{b}
0–20 cm root biomass	15.89 ± 0.50^{a}	13.63 ± 0.15^{b}	14.42 ± 0.36^{b}
20–50 cm root biomass	9.31 ± 0.09^{a}	8.82 ± 0.11^{b}	9.15 ± 0.15^{a}
50–100 cm root biomass	8.90 ± 0.09^{a}	8.74 ± 0.08^{a}	8.98 ± 0.10^{a}

Note: L1, undecomposed litter; L2, incomplete decomposed litter; L3, complete decomposed litter. Lowercase letter in row indicates significant difference at 0.05 level among treatments (ANOVA).

compared to F18. The content of Ca in L3 at F39 was 24.7 % higher than that at F18, while a 19.4 % lower of Si was observed.

F18 obviously decreased these four elements contents in 20–50 cm root compared to F0. The Mg and Si content in 0–20 cm root at F39 was 30.2 % and 81.7 % higher than that at F18, respectively.

F18 decreased Ca contents in 0–50 cm soil and increased Ca contents in 50–70 cm soil compared to F0. F39 decreased the contents of Ca and Mg in deep soil layer compared to F18, while increased the S contents in 20–70 cm soil.

3.2. *Correlations of elements contents in grassland*

The relationships of Ca, Mg, S, and Si contents between green plant, litter, root, and soil were established (Table 3). Significantly correlation values of Mg and S were observed between the green plant and litter ($p < 0.01$). Significantly correlation value of Ca ($p < 0.01$), Mg ($p < 0.01$), and Si ($p < 0.05$) were obtained between root and litter. Ca content in root was significantly correlated with Ca content in soil ($p < 0.05$), and S content in litter was significantly related to S content in soil ($p < 0.05$).

Table 2
The contents of elements in grassland with long-term grazing exclusion (Mean ± standard error).

		Ca (g/kg)			Mg (g/kg)			S (g/kg)			Si (g/kg)		
		F0	F18	F39	F0	F18	F39	F0	F18	F39	F0	F18	F39
	Green plant	6.10 ±0.16^{a}	4.53 ±0.52^{b}	5.67 ±0.80^{b}	1.95 ±0.20^{a}	1.16 ±0.13^{b}	1.22 ±0.17^{b}	0.80 ±0.05^{a}	0.65 ±0.02^{b}	0.69 ±0.04^{b}	0.18 ±0.06^{a}	0.10 ±0.01^{b}	0.09 ±0.01^{b}
Litter	L1	11.36 ±1.74^{a}	4.59 ±0.79^{b}	6.07 ±1.09^{b}	2.14 ±0.60^{a}	1.71 ±0.38^{a}	1.88 ±0.91^{a}	0.46 ±0.08^{a}	0.57 ±0.01^{a}	0.51 ±0.04^{a}	0.30 ±0.05^{a}	0.32 ±0.06^{a}	0.43 ±0.15^{a}
	L2		6.33 ±0.10	7.67 ±0.96		2.49 ±0.42	2.69 ±1.82		0.67 ±0.45	0.63 ±1.86		0.55 ±0.09	0.61 ±0.17
	L3		8.15 ±0.25	10.16 ±0.59		4.39 ±0.58	4.03 ±0.51		0.72 ±0.09	0.70 ±0.04		1.13 ±0.23	0.91 ±0.14
Root (cm)	0-20	11.31 ±1.47^{a}	10.83 ±0.87^{a}	12.45 ±1.00^{a}	2.77 ±0.71^{a}	1.99 ±0.17^{a}	2.59 ±0.31^{a}	0.62 ±0.07^{a}	0.66 ±0.06^{a}	0.74 ±0.13^{a}	0.75 ±0.02^{a}	0.30 ±0.06^{b}	0.55 ±0.12^{ab}
	20-50	16.57 ±0.99^{a}	12.06 ±1.19^{b}	10.62 ±0.98^{b}	2.59 ±0.23^{a}	1.87 ±0.21^{b}	1.88 ±0.23^{b}	0.73 ±0.06^{a}	0.62 ±0.03^{a}	0.66 ±0.09^{a}	0.50± 0.01^{a}	0.33 ±0.09^{a}	0.38 ±0.09^{a}
	50-100	14.95 ±1.44^{a}	14.61 ±0.73^{a}	11.43 ±0.65^{b}	2.41 ±0.16^{a}	2.02 ±0.16^{b}	1.97 ±0.07^{b}	0.68 ±0.05^{a}	0.72 ±0.05^{a}	0.64 ±0.02^{a}	0.54± 0.01^{a}	0.29 ±0.05^{a}	0.45 ±0.13^{a}
Soil (cm)	0-10	6.14 ±0.17^{a}	5.53 ±0.54^{a}	5.34 ±0.21^{a}	4.85 ±0.05^{a}	4.91 ±0.27^{a}	4.96 ±0.011^{a}	0.45 ±0.10^{a}	0.50 ±0.20^{a}	0.51 ±0.08^{a}	1.72± 0.14^{a}	1.79 ±0.20^{a}	1.77± 0.25^{a}
	10-20	6.25 ±0.56^{a}	6.09 ±1.65^{a}	4.86 ±0.39^{a}	4.34 ±0.18^{a}	4.28 ±0.15^{a}	4.06 ±0.24^{a}	0.35 ±0.10^{a}	0.48 ±0.18^{a}	0.38 ±0.17^{a}	1.81 ±0.25^{a}	1.79 ±0.22^{a}	1.81 ±0.33^{a}
	20-30	5.94 ±0.16^{a}	4.90 ±0.22^{b}	4.63 ±0.03^{b}	3.98 ±0.08^{a}	4.39 ±0.80^{a}	3.73 ±0.12^{a}	0.30 ±0.17^{b}	0.60 ±0.19^{a}	0.73 ±0.18^{a}	1.79 ±0.26^{a}	1.77 ±0.28^{a}	1.79 ±0.18^{a}
	30-50	6.03 ±0.56^{a}	5.78 ±0.45^{a}	4.34 ±0.14^{b}	3.84 ±0.31^{a}	3.93 ±0.21^{a}	3.52 ±0.05^{a}	0.28 ±0.09^{a}	0.65 ±0.25^{a}	0.76 ±0.25^{a}	1.79 ±0.35^{a}	1.73 ±0.32^{a}	1.80 ±0.26^{a}
	50-70	6.23 ±0.43^{a}	7.41 ±0.86^{a}	4.45 ±0.28^{b}	3.92 ±0.13^{a}	4.02 ±0.09^{a}	3.51 ±0.12^{a}	0.31 ±0.18^{b}	0.63 ±0.19^{ab}	0.87 ±0.06^{a}	1.77 ±0.28^{a}	1.74 ±0.20^{a}	1.78 ±0.25^{a}
	70-100	7.17 ±0.96^{b}	10.71 ±0.62^{a}	4.64 ±0.30^{c}	4.27 ±0.12^{a}	4.15 ±0.37^{a}	3.59 ±0.03^{b}	0.24 ±0.04^{b}	0.83 ±0.02^{a}	0.79 ±0.11^{a}	1.74 ±0.10^{a}	1.72 ±0.15^{a}	1.78 ±0.22^{a}

Note: L1, undecomposed litter; L2, incomplete decomposed litter; L3, complete decomposed litter. Lowercase letter in row indicates significant difference at 0.05 level among treatments (ANOVA).

Table 3
Green plant-litter-root-soil relationship (correlation) in respect to Ca, Mg, S, and Si contents with long-term grazing exclusion.

	Correlation	Ca	Mg	S	Si
Green plant-Root	Pearson correlation value	−0.059	0.582	0.473	0.275
	Sig. (2-tailed)	0.880	0.100	0.199	0.474
Green plant-Litter	Pearson correlation value	−0.241	−0.830**	−0.798**	−0.657
	Sig. (2-tailed)	0.532	0.006	0.010	0.054
Green plant-Soil	Pearson correlation value	−0.428	−0.117	−0.622	−0.011
	Sig. (2-tailed)	0.250	0.764	0.074	0.978
Root-Litter	Pearson correlation value	−0.805**	−0.847**	−0.261	−0.750*
	Sig. (2-tailed)	0.009	0.004	0.498	0.020
Root-Soil	Pearson correlation value	0.724*	−0.100	−0.331	0.190
	Sig. (2-tailed)	0.027	0.798	0.385	0.625
Litter-Soil	Pearson correlation value	−0.569	0.026	0.721*	−0.073
	Sig. (2-tailed)	0.110	0.947	0.028	0.852

Note: * and ** indicates significant correlation at 0.05 and 0.01 level.

3.3. Stocks of elements in grassland

The stocks of elements in grassland showed Ca > Mg > Si > S (Fig. 3). F18 increased the stocks of Ca and S in grassland compared to F0, which were mainly decided by the increases of Ca and S stocks in litter and soil. The stock of Ca in grassland at F39 was decreased compared to F18, which was mainly caused by the decrease of Ca stock in soil.

3.4. Stocks of elements in root in different soil layers

The four elements mainly stocked in 0–20 cm root, and they accounted for 38.5 %-50.9 % of 0–100 cm root. F18 and F39 obviously decreased the stocks of Mg and Si in 0–20 cm root, and they were 14.9 %-38.3 % and 33.9 %-65.6 % lower than F0, respectively. The Si, Mg, Ca, and S stock in 0–20 cm root at F39 was 92.4 %, 37.8 %, 21.7 %, and 18.3 % higher than that at F18, respectively (Fig. 4).

3.5. Stocks of elements in litter in different layers

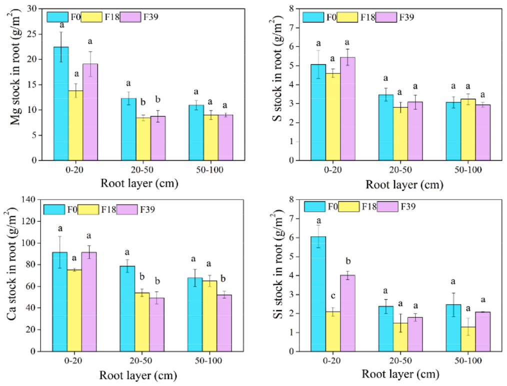

Fig. 4. The stocks of Ca, Mg, S, and Si in root with long-term grazing exclusion Note: lowercase letter indicated significant difference at 0.05 level (ANOVA). Error bars were one standard deviation.

The F18 and F39 greatly increased the stocks of these four elements in L1. The stock of Si, Ca, and Mg in L1 at F39 was 59.3 %, 54.4 %, and 26.5 % higher than that at F18 respectively. F39 obviously decreased the stocks of Mg, S, and Si in L2 and L3 relative to F18, and the stock of Si and Mg in L3 was 52.1 % and 33.6 % lower respectively. While the Ca stock in L3 at F39 was 76.7 % higher than that at F18 (Fig. 5).

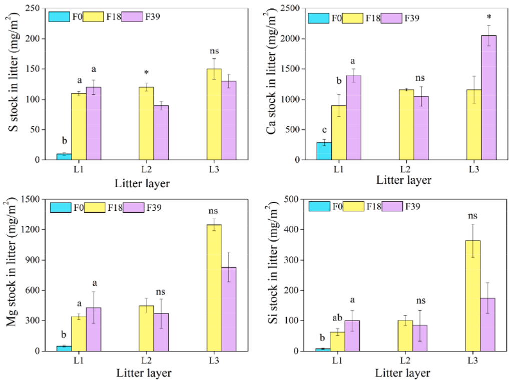

Fig. 5. The stocks of Ca, Mg, S, and Si in litter with long-term grazing exclusionNote: lowercase letter indicated significant difference at 0.05 level (ANOVA). * $p < 0.05$; ** $p < 0.01$; ns, no significance. Error bars were one standard deviation.

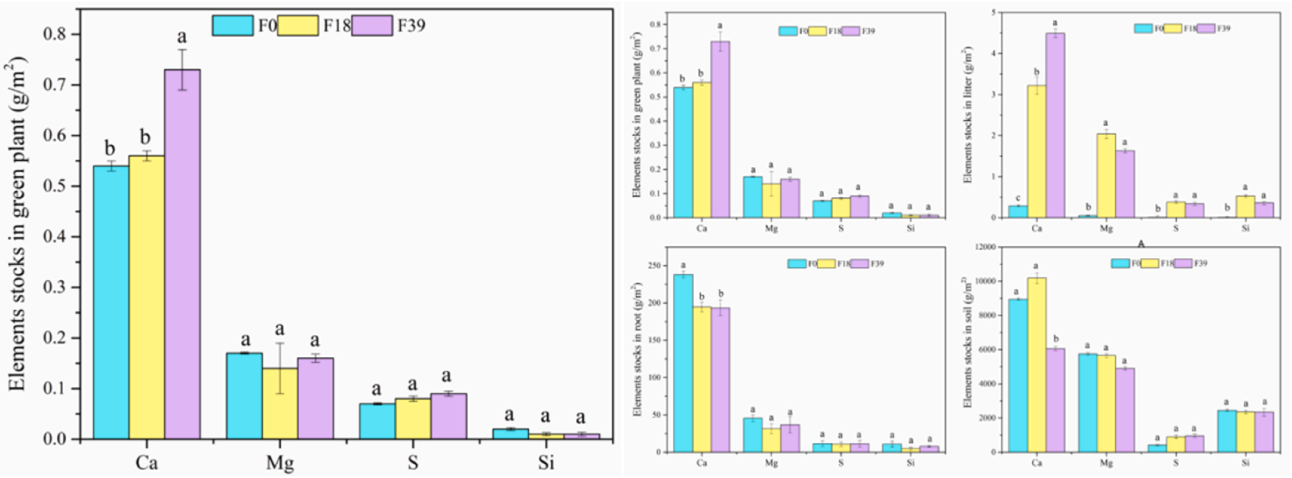

Fig. 3. The stocks of Ca, Mg, S, and Si in grassland with long-term grazing exclusionNote: The stock of each element in grassland was the sum of element stock in green plant, root, litter, and soil. Lowercase letter indicated significant difference at 0.05 level (ANOVA). Error bars were one standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.6. *Stocks of elements in soil in different layers*

F18 decreased Ca stock in 0–50 cm soil and increased Ca stock in 50–100 cm soil compared to F0. The S stock in each soil layer at F18 was increased compared to F0, especially deep soil. Compared to F18, F39 obviously decreased the stock of Ca in each soil layer, especially in 50–70 cm and 70–100 cm with 41.8 % and 57.8 % lower respectively. F39 also obviously decreased the stocks of Mg in 20–100 cm soil with 12.9 %-17.8 % lower than F18. The S stock in 10–20 cm soil at F39 was 23.6 % lower than that at F18. The S stock in 20–30 cm, 30–50 cm, and 50–70 cm soil at F39 was 18.3 %, 13.4 %, and 34.2 % higher than that at F18 (Fig. 6).

3.7. *Variations of elements stocks proportions in grassland*

The Ca, Mg, S, and Si mainly stocked in soil, and the stocks of them accounted for 96.83 %-99.77 % of grassland. In plant, the stocks of these four elements in root accounted for 90.02 %-99.78 %, and in litter accounted for 0.07 %-9.74 %, while only<1 % of them stocked in green plant. The proportions of Ca and S stocks in plant system were higher than that of Mg and Si.

F18 decreased the proportions of elements in plant and increased the proportions of elements in soil compared to F0 (Fig. 7). In plant, the elements were mainly accumulated in litters (especially in L3) at F18 compared to F0, which might be attributed to the decreases of root proportions. However, F39 increased the proportions of Ca, Mg, and Si in plant compared to F18. In plant, F39 obviously increased the proportions of Mg and Si in root and greatly decreased the proportions of Mg and Si in L3 compared to F18.

3.8. *Elements reuse efficiencies*

Long-term grazing exclusion increased the reuse efficiency of Ca ($p < 0.05$), while decreased the reuse efficiencies of Mg, S, and Si ($p < 0.05$) (Fig. 8). The reuse efficiencies of these four elements at F39 were no significant differences compared to F18.

4. Discussion

The elements of Ca, Mg, S, and Si mainly distributed in the soil, accounting for 96.83 %-99.77 % of grassland. In plant system, these elements mainly accumulated in the root, accounting for 90.02 %-99.78 %, while<1 % of them were stocked in green plant. These results were consistent with other reports (Wang et al., 2014a; Wang et al., 2014b; Lu et al., 2015). All of the contents and stocks of elements in green plant, litter, root, and soil showed Ca > Mg > S > Si. This because that a certain element is more abundant in soil, it will accumulate more in plants (Barceló and Poschenrieder, 2011). In addition, the Ca contents in green plant were 4.53–6.10 g/kg within the recommended range of 2.7–20.0 g/kg (NRC, 2000). The observed Mg contents in green plant of 1.16–1.95 g/kg were within the recommended range of 0.5–2.5 g/kg (NRC, 2000).

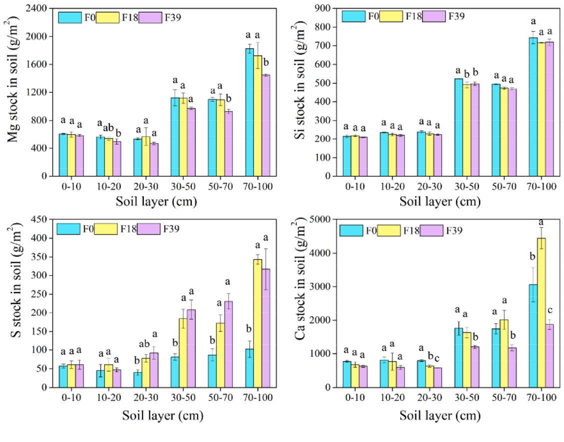

Fig. 6. The stocks of Ca, Mg, S, and Si in different soil layers with long-term grazing exclusion Note: lowercase letter indicated significant difference at 0.05 level (ANOVA). Error bars were one standard deviation.

This study found that different years of long-term grazing exclusion altered the density and species of vegetation, as well as the biomass of green plant, root, and litter (Table 1) (Hu et al., 2021; Hou et al., 2019; Wang et al., 2017), and thus certainly influenced the variations of Ca, Mg, S, and Si in grassland system (Cheng et al., 2016; Qasim et al., 2017; Zeng et al., 2017; Bai et al., 2021; Chen et al., 2021).

4.1. *Distributions of elements as affected by long-term grazing exclusion*

The contents of Ca, Mg, S, and Si in green plant were obviously decreased with 18- and 39-year grazing exclusion. These results were consistent with the reports of some studies, which showed that long-term grazing exclusion decreased the contents of mineral nutrients in vegetation, such as P, Fe, and Mn (Lu et al., 2015; Liu et al., 2020a; Liu et al., 2020b; Li et al., 2011; Marschner and Marschner, 2012). This might be attributed to the dilution effect of increased vegetation productivity (Table 1). Long-term grazing exclusion increased the biomass of vegetation directly related to the decreased livestock consumption by grazing animals (Alberti et al., 2017; Steffens et al., 2008), as well as the improvement of soil bulk density, water holding capacity of soil, and soil nutrients (Wu et al., 2010). The study found that excluding grazing for 18 years and 39 years reduced the Mg and Si stocks in green plant, which were mainly resulted from the reductions of Mg and Si contents in green plant. This might be attributed to the restriction of photosynthetic efficiency for the excess litter in long-term grazing exclusion plots (Hou et al., 2019; Liu et al., 2019; Zhang et al., 2019). As Mg and Si play important role in the formation of photosynthetic product, and can regulate the photosynthesis and improve the photosynthetic efficiency. Moreover, the Mg and Si had lower nutrient reuse efficiencies. This indicated that most Mg and Si were transferred from green plant to litter in long-term grazing exclusion plots. In addition, generally, legumes and forbs usually have higher mineral elements than grasses (Garcia-Ciudada et al., 1997; Pirhofer-Walzl et al., 2011). Some researches have reported that the lower grasses K, Ca and Mg contents under the fencing than continue grazing were related to the differences in botanic composition (Lyttelton, 1973; Norton, 1982). As a result, the increases of grasses compositions in long-term grazing exclusion might be responsible for the lower contents of mineral elements.

The stocks of Ca, Mg, and Si in 0–100 cm root with 18- and 39-year grazing exclusion were decreased because of the reductions of root biomass and elements contents. However, these results were inconsistent with other reports, which might owing to the types of grassland and the years of grazing exclusion (Wu et al., 2010; Deng et al., 2014; Lu et al., 2015; Wang et al., 2014a; Wang et al., 2014b). Firstly, long-term grazing exclusion inhibited the photosynthesis of plant and thus reduced the allocation of photosynthetic product into the root, leading to an increase in the root mortality (Wang et al., 2016; Dai et al., 2021; Zhu et al., 2021). Secondly, the decreases of Ca, Mg, and Si contents in the root might also be attributed to the increase of grasses species composition in long-term grazing exclusion plots (Perez Corona et al., 1995; Perez Corona et al., 1998). In further, among these four elements, the stocks of Ca and Mg in 20–50 cm root in 18- and 39-year grazing exclusion plots decreased most ($p < 0.05$). The main reason of this was the significant decreases of Ca and Mg contents in 20–50 cm root in long-term grazing exclusion plots. The large amounts of Ca and Mg in 20–50 cm root might be transferred to litter, as the significant relationships of Ca ($r = -0.805^{**}$) and Mg ($r = -0.847^{**}$) contents between root and litter were found.

Litter plays an important role in the balance of mineral nutrient in grassland with long-term grazing exclusion (Ren et al., 2016). Some

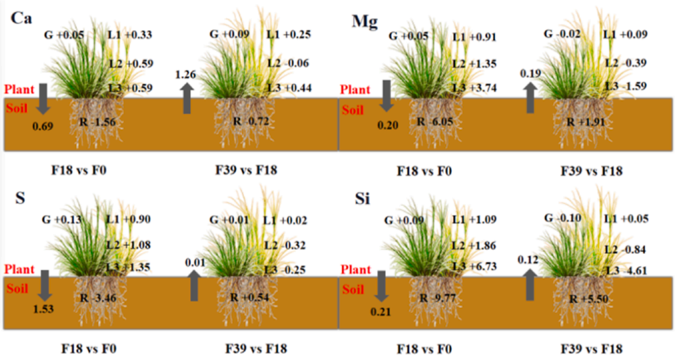

Fig. 7. Variations of the proportions of Ca, Mg, S, and Si stocks in grassland system with long-term grazing exclusionNote: G, proportion of element in green plant to plant; R, proportion of element in root to plant; L1, proportion of element in undecomposed litter to plant; L2, proportion of element in incomplete decomposed litter to plant; L3, proportion of element in complete decomposed litter to plant. The data in the figure was the difference value of element proportion among treatments. + indicated the different value was great than 0. - indicated the different value was<0. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

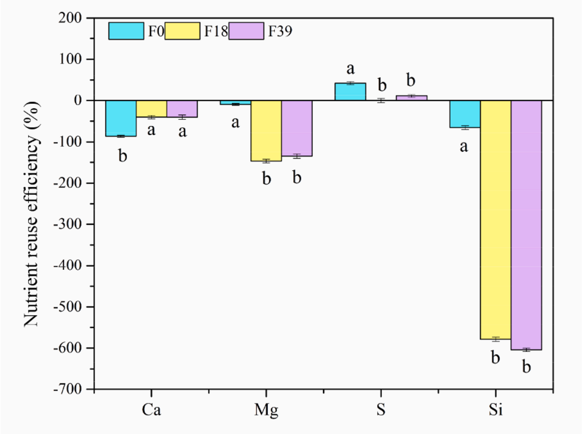

Fig. 8. The reuse efficiencies of Ca, Mg, S, and Si with long-term grazing exclusionNote: lowercase letter indicated significant difference at 0.05 level (ANOVA). Error bars were one standard deviation.

studies have reported that the contents of mineral elements in litter play a crucial role in litter decomposition (Lovett et al., 2016; Berg and McClaugherty, 2014). The nutrient will be transferred and retained in plant before the plant withering, which is called nutrient reuse (Millett et al., 2010; Aerts, 1996; Berg, 1986; Han et al., 2005). Therefore, the content of nutrient in plant may be depended on the nutrient reuse efficiency. The result found that long-term grazing exclusion greatly increased Si content in litter (undecomposed litter, L1). This indicated that great of Si might be retained in green plant before withering, and thus increased Si content in litter, owing to the lowest Si reuse efficiency.

The imbalance of mineral nutrients in soil could not only restrict plant growth, but also affect the structure and function of grassland ecosystems (Fan et al., 2015). Soil mineral nutrients in grassland are closely related to livestock consumption and decomposition rates of litter and dung (Gao et al., 2014; Wang et al., 2014a; Wang et al., 2014b). Oliveira et al., (2019) found that the content of Al^{3+} in soil in a semi-arid region in Brazil was decreased with grazing exclusion. This study also showed that grazing exclusion for 18 years decreased the contents and stocks of Ca in 0–50 cm soil. This might be attributed to the uptake of Ca by plant root. However, the contents and stocks of S in 0–50 cm soil were increased with 18- and 39-year grazing exclusion. This was consistent with Li et al., (2011) and He, (2019), who reported that the Fe, Mn, and B contents in soil were increased with long-term grazing exclusion in different types of grassland. Long-term grazing exclusion reduced the risks of soil nutrients loss caused by wind and water erosion for the plant coverage and litter accumulation (Hoffmann et al., 2008). Among these four elements, the S content in soil with long-term grazing exclusion was increased most. The large amounts of S in soil might be shifted from litter, and the significant relationship of S content between litter and soil ($r = 0.721^*$) verified this explanation. In addition, the Si content in 0–10 cm soil was increased with 18 year grazing exclusion, which might be caused by the highest content and stock of Si in litter.

4.2. Distributions of elements as affected by continue grazing exclusion for 39 years

Grazing exclusion for 39 years obviously increased the species diversity, root biomass, and undecomposed litter biomass, while decreased the biomass of incomplete-, and complete decomposed litter. Large amounts of undecomposed litter might inhibit the establishment of seedling and restrict the productivity of vegetation (Hovstad and Ohlson, 2008; Loydi et al., 2013; Ruprecht and Szabó, 2012). Therefore, more photosynthetic products might be distributed to the root in order to restore the growth of above-ground plant (Mcnaughton and Mcnaughton, 1998). For these reasons, grazing exclusion for 39 years altered the distributions of Ca, Mg, S, and Si in green plant-root-litter-soil relative to 18-year grazing exclusion.

The contents and stocks of Ca, Mg, and S in green plant with continue grazing exclusion for 39 years were increased, especially Ca, while these of Si were decreased. Firstly, this might be attributed to the concentration effect induced by the reduction of green plant biomass in 39-year grazing exclusion plot. Secondly, this might because of the increase of species diversity in 39-year grazing exclusion plot. Some studies have found that Ca content in plant vary considerably, but is generally lower in the grasses and higher in the legumes (Broadley et al., 2003, 2004; Bauer et al., 2011; Larcher, 2003). However, grasses are the highest Si accumulators, and Fabales, including legumes, are low accumulators (Epstein 1994; White and Broadley, 2003; Hodson et al., 2005; Brackhage et al., 2013). The obvious decreases of Ca content and stock in 0–100 cm soil in continue grazing exclusion for 39 years were observed in this study. This illustrated that lots of Ca in soil were absorbed by root of Ca-accumulated plant, and then transferred to green plant. The significant relationship of Ca content between root and soil ($r = 0.724^*$) proved this point. Continue grazing exclusion for 39 years increased the contents of Ca in undecomposed litter, incomplete decomposed litter, and complete decomposed litter. This because that Ca is transported passively with the transpiration stream and tends to accumulate at the end of the stream in the leaves, and increases in content with leaf aging. In addition, the increase of Ca stock in litter was mainly derived from deep root (20–100 cm), and the significant relationship of Ca content between root and litter ($r = -0.805^{**}$) was observed. In this study, we found that the dry matter of undecomposed litter with 39-year grazing exclusion was 16.94 % higher than that with 18-year grazing exclusion. However, the dry matter of incomplete decomposition litter and complete decomposition litter with 39-year grazing exclusion were 33.49 % and 38.00 % less than that with 18-year grazing exclusion, respectively. The decreases of Mg, Si, and S stocks in incomplete decomposition litter and complete decomposition litter at F39 were mainly resulted from the reductions of litter biomass.

The contents and stocks of Ca, Mg, S, and Si in 0–20 cm root with continue grazing exclusion for 39 years were obviously increased. This might be attributed to the optical compensation effect of restoring the above-ground plants. Among these four elements, the Si most accumulated in the root with grazing exclusion for 39 years relative to grazing exclusion for 18 years. Firstly, some grasses have active Si transporters, and the active uptake of Si into root xylem occurs via the transporters Lsi1 and Lsi2 (Ma et al., 2008; Ma and Yamaji, 2006, 2015). Secondly, Si stored in dead plant biomass has high dissolution rates in soils and sediments (Dixit and Van Cappellen, 2002; Haynes, 2014; Belanger et al., 2015), which provides abundant dissolved Si in soil during the decomposition of litter (Sommer et al., 2006; Fraysse et al., 2006, 2009; Lucas, 2001; Sommer et al., 2006). In addition, lots of Mg also accumulated in root owing to the increases of Mg content and root biomass. This might because that Mg can regulate photosynthesis and improve photosynthetic efficiency. The large amounts of Mg and Si in root were mainly derived from incomplete-, and complete- decomposed litter, as well as soil. The significant relationships of Mg ($r = 0.847^{**}$) and Si ($r = 0.750^{*}$) contents between root and litter verified this explanation.

5. Conclusion

The study first investigated the contents and stocks of Ca, Mg, S, and Si in grassland system with long-term grazing exclusion in Inner Mongolia. We confirmed our hypothesis that long-term grazing exclusion altered the distributions of medium mineral elements in green plant-root-litter-soil. The Ca, Mg, S, and Si with 18- and 39-year grazing exclusion were mainly shifted from root to green plant, litter, and soil. Grazing exclusion for 39 years, Ca, Mg, and Si were shifted from soil to plant relative to 18-year grazing exclusion. Moreover, in plant system, Ca was shifted from root to litter, and Mg and Si were shifted from litter to root, and S was shifted from litter to green plant.

CRediT authorship contribution statement

Juan Hu: Writing – original draft. **Qiang Li:** Investigation, Writing – review & editing. **Yingxin Huang:** Investigation, Writing – review & editing. **Qilin Zhang:** Software. **Daowei Zhou:** Conceptualization, Methodology.

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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