

# Change of Soil Microbial Biomass C, N between Longtime Free Grazing and Exclosure Pasture in Semiarid Grassland Ecosystem in Tongliao and Chifeng of Inner Mongolia

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## Abstract

Grassland degradation in the Inner Mongolia grassland became seriously since the end of 20<sup>th</sup> century, because of the rapid expansion of livestock numbers and the development of economy. Overgrazing in this region is one of the main causes of grassland degradation. In this paper, soil microbial biomass carbon ( $C_{mic}$ ) and nitrogen ( $N_{mic}$ ) under continuous free-grazing and exclusion of livestock were examined in Nuogusitai (NGST) of Tongliao and Haisilamu (HSLM) of Chifeng in Inner Mongolia. Our results showed that  $C_{mic}$  and  $N_{mic}$  content significantly decreased in grazing grassland compared to the exclosure grassland in both NGST and HSLM. In NGST, the average  $C_{mic}$  (79.2 to 881.1 mg C kg<sup>-1</sup>) and  $N_{mic}$  (6.1 to 64.8 mg C kg<sup>-1</sup>) were lower than  $C_{mic}$  (452.7 to 1218.1 mg C kg<sup>-1</sup>) and  $N_{mic}$  (48.4 to 152.6 mg C kg<sup>-1</sup>) in HSLM. Furthermore, after grazing  $C_{mic}$  and  $N_{mic}$  decreased 81.39, 69.51% and 75.48, 67.12% in 0-5 and 5-15 cm layers, respectively in NGST. These were all greater than in HSLM which were 42.12, 30.00% and 67.12, 31.40%, respectively. The lower  $C_{mic}$  and  $N_{mic}$  concentration in grazing grassland than in exclosure might due to reduction in organic matter into soil by livestock grazing and destruction of vegetation root by trampling and the lower  $C_{mic}$  in  $N_{mic}$  in NGST than HSLM was mainly the result of higher soil pH and soil substrate.  $C_{mic}/C_{org}$  ratio in HSLM (3.1 to 4.6) was higher than NGST (0.2 to 0.4). The higher  $C_{mic}$  concentration and  $C_{mic}/C_{org}$  in HSLM suggest a great potential for carbon sequestration in neutral soil than in alkaline soil. Therefore, to restore grassland and alleviate the grassland degradation, exclosure should be encouraged in semi-arid grassland.

**Keywords:** Grazing; Exclosure; Grazing; Microbial biomass C and N

## Introduction

Grassland covers an area is of around 4.8 billion ha on the earth, taking up one third of the global land surface [1]. It is an important component of the terrestrial ecosystem [2-4] and has a key role in ecology, food security [5] and carbon storage [5-7]. The grassland in China covers approximately 400 million ha, which is nearly 40% of its total land area [2]. However, because of climate change, population growth and a rapid expansion in livestock numbers [8], by the end of the twentieth century, most of these grasslands were degraded, especially for the semi-arid grasslands in Inner Mongolia [9-11]. Grassland degradation has become a serious environmental problem in China [12] and the degraded area increases by 15% each decade from the 1960s to the mid-2000s [13].

Grazing in the Inner Mongolia grassland is an important agricultural activity [14,15] and is considered as the most economical way of utilizing rangeland vegetation which can produce high quality food (meat and milk) for human consumption. Unfortunately, due to high-intension land use, grassland has been degraded worldwide in the past decades [16-18]. According to previous reports, overgrazing is thought to play a major role in increasing desertification [19-21]. The effects of overgrazing on the plant community and soils are considered destructive because of the reduction of canopy cover, the destruction of topsoil structure, and compaction of soil as a result of trampling [22,23]. In addition, these processes increase soil crusting, reduce soil infiltration, and enhance soil erosion susceptibility [23-25]. To control desertification and protect the regional environment, some measurements, e.g., planting local trees, shrubs and grasses, returning degraded farmland to grassland and fencing desertified sandy grassland. And livestock exclusion practices on desertified sandy grassland were shown to be good alternatives to recover vegetation and attenuate soil loss by wind erosion in these erodible grasslands [26,27].

Soil microbial biomass plays a crucial role in nutrient cycling [28,29]. Soil microbial biomass was found to be a sensitive indicator of the dynamics of soil C and N cycles [30,31]. The soil microbial biomass contributes 2-3% of the total organic carbon in soil and is a relatively labile fraction of soil organic matter [32]. It is a key component of soil, since it defines the functional component of the soil micro-biota primarily responsible for decomposition, soil organic C turnover, and nutrient transformations [33-36]. Soil microbial biomass does not respond uniformly to grazing by livestock or other large animals, and has been observed to increase or decrease in response to grazing of the plant community [37].

The study area, Tongliao and Chifeng located in the southeast part of Inner Mongolia, controlled by continental monsoon. Tongliao City is the main body of Horqin Sandy Land, and almost half of the area is covered by sand dunes [38]. The soils are sandy with light yellow color, coarse texture and loose structure [39] and alkaline soil (pH>8) accounting about 8.6% of total Tongliao land area [40]. We hypothesize that livestock grazing would have negative impacts on soil microbial biomass growth in Inner Mongolia, and may cause more severe degradation in the alkaline soil. The objectives of this study were:

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1. To evaluate soil microbial biomass C ( $C_{mic}$ ) and N ( $N_{mic}$ ) under the longtime free-grazing and exclusion of livestock in the neutral and alkaline grassland soil;
2. To establish relationships between  $C_{mic}$ ,  $N_{mic}$ , and the physicochemical characteristics of the soil, such as pH, EC, soil texture,  $C_{org}$  and TN.

## Materials and Methods

### Study site and soil sampling

Tongliao (42°14'37"-45°59'24"N, 119°14'28"-123°42'30"E) is located in the southeast part of Inner Mongolia, China which has an area of  $5.95 \times 10^4$  km<sup>2</sup>. The area of grassland covers  $3.35 \times 10^4$  km<sup>2</sup>, accounting for 56.21% of total area. And the area of degraded, desertification and salinization and alkaline grassland occupy 82% of total grassland area. The terrain is high in the southern and northern parts, low-lying and saddle-shaped in the central part. In the north is the south of Da Hinggan Mountains, the elevation is 400-800 m; in south is the edge of Liaoxi mountain, the elevation is 600-800 m; in the middle is the alluvial plain of Liaohe river, the elevation is 120-320 m. It belongs to a typical semi-arid continental monsoon climate. Annual mean temperature is between 0 and 6°C, and annual average precipitation is 350-400 mm. This region is arid with an annual water deficit of 350 mm, due to potential evaporation exceeding precipitation. The soil in region appeared to be the saline-alkaline, soil pH is between 8.5-10.6.

The Chifeng study area is located in southeast Inner Mongolia, between 41°17'10"-45°24'15"N, 116°21'07"-120°58'52"E. It has an area of  $9.03 \times 10^4$  km<sup>2</sup>. This area has a continental monsoon climate with long, cold, windy, but dry winters, and hot, humid summers. Seasonal dry and wet conditions alternate in response to intrusion of dry-cold air masses from high latitudes in winter and warm-humid air masses from low latitude oceans in summer. Droughts frequently occur in each season, especially in spring and summer. Sandstorms and dust storms frequently occur as well. The mean annual precipitation is around 300-460 mm, where summer rainfall accounts for 60%-70% of annual total precipitation. The annual mean maximum temperature in the area is 11.6°C, and minimum -1.8°C [41,42]. From north to south Chifeng City stretches 457.5 km, while from east to west it stretches 375 km. Elevations decrease from a high of 2,067 m in the west to less than 300 m in the east.

Field sampling was conducted in early-August of 2013 in Nuogusitai (NGST) of Tongliao and 2014 in Haisilamu (HSLM) of Chifeng. In each site, a long-term free grazing grassland plot (GG) and an exclosure grassland plot (EG) were selected. Three aboveground sampling quadrats (1 × 1 m) were random established in both grazing and exclosure. After identified the grass species, the grass was cut and collected from each quadrat. And then dried at 80°C for 24 h and weighted. In each plot, five quadrats (1 × 1 m) were selected for soil sampling. The soil samples were collected from five randomly selected points in the quadrat and mixed into one sample in both 0-5 and 5-15 cm layers. After carefully removing the surface organic materials and fine roots, each mixed sample

was divided into two parts. One part was air-dried for analysis of soil physicochemical properties and the other was sifted through a 2 mm sieve for microbial assays after transporting to laboratory at 4°C. The vegetation characteristics of sampling sites are summarized in Table 1.

### Laboratory analysis

**Soil physico-chemical properties:** Soil moisture was measured by the oven-dry method at 105°C for 24 h. Soil pH was measured by the glass electrode, at the soil solution ratio of 1:5 after shaking for 1 h. Electrical conductivity (EC) was measured after pH measurement, using an EC meter (CM-14P, TOA Electronics Ltd., Japan). Particle size distribution was determined by the pipette method [43]. Part of each sample was air-dried and finely ground to pass 0.1 mm sieve and analyzed for total nitrogen (TN) by the dry combustion method with NC-Analyzer (Sumigraph Nc-80, Sumika Chemical Analysis Service Co., Tokyo, Japan). Soil organic carbon ( $C_{org}$ ) was determined by Walkley and Black method [44]. The available phosphorus (Avail. P) content was determined by the Bray II method [45], where soil samples were extracted with an extraction solution (1 M  $NH_4F$  and 0.5 M HCl) then color developing reagent was added and determined available phosphorus by absorbance measurement with spectrophotometer at a wavelength of 710 nm (Shimadzu, UV-142-02, Kyoto, Japan).

**Microbial biomass C, N:** Microbial biomass C ( $C_{mic}$ ) and N ( $N_{mic}$ ) was estimated using the Fumigation extraction method. Two portions of moist soil (10 g oven-dry soil) were weighed after which the first portion (not fumigated) was immediately extracted with 40 ml of 0.5 M  $K_2SO_4$  for 30 min by oscillating, shaking at 200 rpm and then filtered (Advantec No.6); the second one was fumigated for 24 h at 25°C with ethanol-free  $CHCl_3$  and then extracted as described above. The contents of biomass C and N were calculated from the following equations, respectively:

$$\text{Biomass C} = 2.64 \times [(C \text{ from fumigated soil}) - (C \text{ from non-fumigated soil})]$$

$$\text{Biomass N} = 2.22 \times [(N \text{ from fumigated soil}) - (N \text{ from non-fumigated soil})]$$

### Statistical analyses

One-way ANOVA was used to analyze the means of the same soil layers between grazing and exclosure. Differences were evaluated at the 0.05 significance level. All statistical analyses were performed using the SPSS 19. Data were analyzed by correlation analysis to evaluate relationships between different soil parameters.

The diversity index was calculated by using the Shannon-Wiener diversity index (1949). Diversity index  $H' = -\sum Pi \ln Pi$ .

Where,

$Pi = S / N$ , S=number of individuals of one species

N=total number of all individuals in the sample.

		Elevation m.a.s.l.	Longitude	Latitude	Vegetation cover (%)	Aboveground biomass (g m <sup>-2</sup> )	Shannon diversity	Dominant species
NGST	EG	191	122°12'37.97"	43°20'27.75"	73.33 ± 11.54 a	86.94 ± 16.36 a	1.13 ± 0.33 a	<i>Artemisia capillaris</i>
	GG	186	122°13'56.31"	43°22'32.17"	30.00 ± 5.00 b	47.20 ± 12.57 b	1.60 ± 0.13 a	<i>Glaux maritima</i> ; <i>Carex duriuscula</i>
HSLM	EG	853	118°50'11.85"	43°54'24.18"	91.67 ± 2.89 a	965.00 ± 65.08 a	1.16 ± 0.47 a	<i>Leymus chinensis</i>
	GG	838	118°50'35.85"	43°54'13.30"	68.33 ± 7.63 b	156.67 ± 8.33 b	1.43 ± 0.27 a	<i>Carex duriuscula</i>

Different lowercase letters indicate significant differences between EG and GG (P<0.05)

**Table 1:** Vegetation characteristics for EG and GG in the NGST and HSLM.

## Results and Discussion

### Soil physico-chemical properties

Grazing significantly decreased soil moisture both in two study sites ( $P < 0.05$ ) (Table 2). Soil showed alkaline (8.83-9.62) in NGST, and it was highest in EG of 0-5 cm in NGST in combination with highest moisture and EC. In 0-5 cm, Soil pH in GG was significantly decreased in 0-5 cm in NGST ( $P < 0.05$ ), but increased in HSLM. Soil EC, organic C and TN in GG were all significantly lower than in EG both in the two sites ( $P < 0.05$ ). Soil silt and clay content decreased in GG compared with in EG, while soil sand increased both in the two study sites and it was significant in NGST. Available P in GG was higher than EG in NGST, but it was lower than EG in HSLM.

### Soil microbial biomass C and N

In NGST,  $C_{mic}$  varied from 79.2 to 881.1 mg C kg<sup>-1</sup> soil and  $N_{mic}$  varied from 6.1 to 64.8 mg C kg<sup>-1</sup> soil during the experimental period (Figures 1a and 1b). In GG,  $C_{mic}$  and  $N_{mic}$  decreased 81.39, 69.51% and

75.48, 67.12% in 0-5 and 5-15 cm layers, respectively, compared to EG. In HSLM,  $C_{mic}$  varied from 452.7 to 1218.1 mg C kg<sup>-1</sup> soil and  $N_{mic}$  varied from 48.4 to 152.6 mg C kg<sup>-1</sup> soil (Figures 2a and 2b). In GG,  $C_{mic}$  and  $N_{mic}$  decreased 42.12, 30.00% and 67.12, 31.40% in 0-5 and 5-15 cm layers, respectively, compared to EG.  $C_{mic}$  and  $N_{mic}$  in the EG were significantly higher than in GG ( $P < 0.05$ ) in both NGST and HSLM.  $C_{mic}$  and  $N_{mic}$  in surface layer were significantly higher than in the lower layer except for  $C_{mic}$  in NGST (Figures 1a and 1b, Figure 2a and 2b).

In NGST,  $C_{mic}$  positively correlated with moisture, EC,  $C_{org}$ , TN, silt and clay content and negatively correlated with sand content.  $N_{mic}$  also positively correlated with moisture, EC,  $C_{org}$ , TN and clay content and negatively correlated with sand content (Table 3). In addition, we observed a weak positive correlation between  $C_{mic}$  and pH, and a weak positive correlation between  $N_{mic}$  and silt content. On the other hand, in HSLM,  $C_{mic}$  positively correlated with moisture, EC,  $C_{org}$ , TN and Avail. P.  $N_{mic}$  also positively correlated with moisture,  $C_{org}$ , TN and Avail. P.  $C_{mic}$  and  $N_{mic}$  both negatively correlated with pH. Moreover, we observed a weak positively correlation between  $N_{mic}$  and EC (Table 4).

Soil characteristics	Unit	NGST		HSLM	
		EG	GG	EG	GG
<b>0-5 cm</b>					
Moisture	%	28.98 ± 7.11 a	4.22 ± 2.30 b	11.18 ± 1.13 a	6.94 ± 1.24 b
pH (H <sub>2</sub> O)		9.62 ± 0.20 a	8.83 ± 0.17 b	6.84 ± 0.07	6.97 ± 0.14
EC	mS/m	95.16 ± 30.86 a	11.12 ± 3.47 b	9.02 ± 1.05 a	4.76 ± 0.66 b
$C_{org}$	g/kg	32.80 ± 8.74 a	5.55 ± 1.29 b	26.39 ± 2.60 a	18.81 ± 1.84 b
TN	g/kg	3.16 ± 0.76 a	0.81 ± 0.22 b	3.10 ± 0.41 a	2.00 ± 0.10 b
Soil C:N		17.38 ± 3.15	13.88 ± 2.13	11.09 ± 0.26	11.18 ± 0.13
Sand	%	46.98 ± 2.80 a	88.42 ± 5.74 b	56.87 ± 6.02	62.05 ± 3.59
Silt	%	19.62 ± 6.13 a	4.80 ± 3.24 b	16.68 ± 2.11	14.43 ± 1.71
Clay	%	33.40 ± 5.83 a	6.78 ± 3.23 b	26.45 ± 3.96	23.52 ± 2.03
Avail. P	mg/kg	27.13 ± 10.96	32.17 ± 8.96	39.21 ± 6.34	32.34 ± 7.38
<b>5-15 cm</b>					
Moisture	%	20.88 ± 1.60 a	2.72 ± 0.36 b	7.52 ± 0.34	6.68 ± 0.94
pH (H <sub>2</sub> O)		9.50 ± 0.47	9.38 ± 0.23	7.17 ± 0.15	7.11 ± 0.14
EC	mS/m	67.04 ± 38.77 a	9.85 ± 2.91 b	8.21 ± 3.39 a	4.47 ± 0.62 b
$C_{org}$	g/kg	13.30 ± 2.50 a	1.95 ± 0.51 b	20.51 ± 2.20 a	14.53 ± 2.98 b
TN	g/kg	2.20 ± 1.06 a	0.41 ± 0.10 b	2.24 ± 0.37 a	1.55 ± 0.31 b
Soil C:N		19.81 ± 4.02 a	11.82 ± 5.19 b	11.51 ± 0.26 a	10.93 ± 0.34 b
Sand	%	53.16 ± 6.58 a	92.64 ± 4.62 b	53.81 ± 10.14	62.52 ± 3.48
Silt	%	20.54 ± 12.17 a	1.99 ± 3.01 b	18.40 ± 4.11 a	13.66 ± 1.60 b
Clay	%	26.30 ± 8.29 a	5.37 ± 2.12 b	27.79 ± 6.18	23.82 ± 1.97
Avail. P	mg/kg	13.98 ± 1.63	17.20 ± 3.68	30.23 ± 3.99 a	21.48 ± 2.21 b

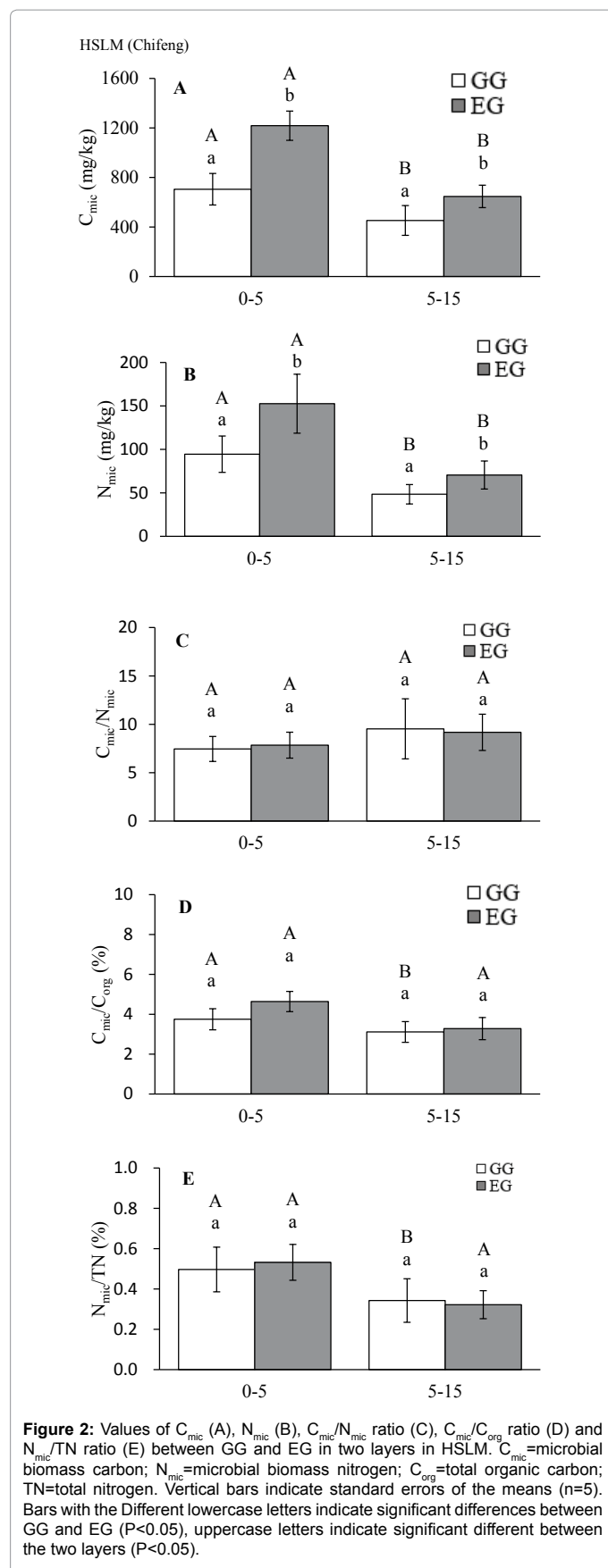
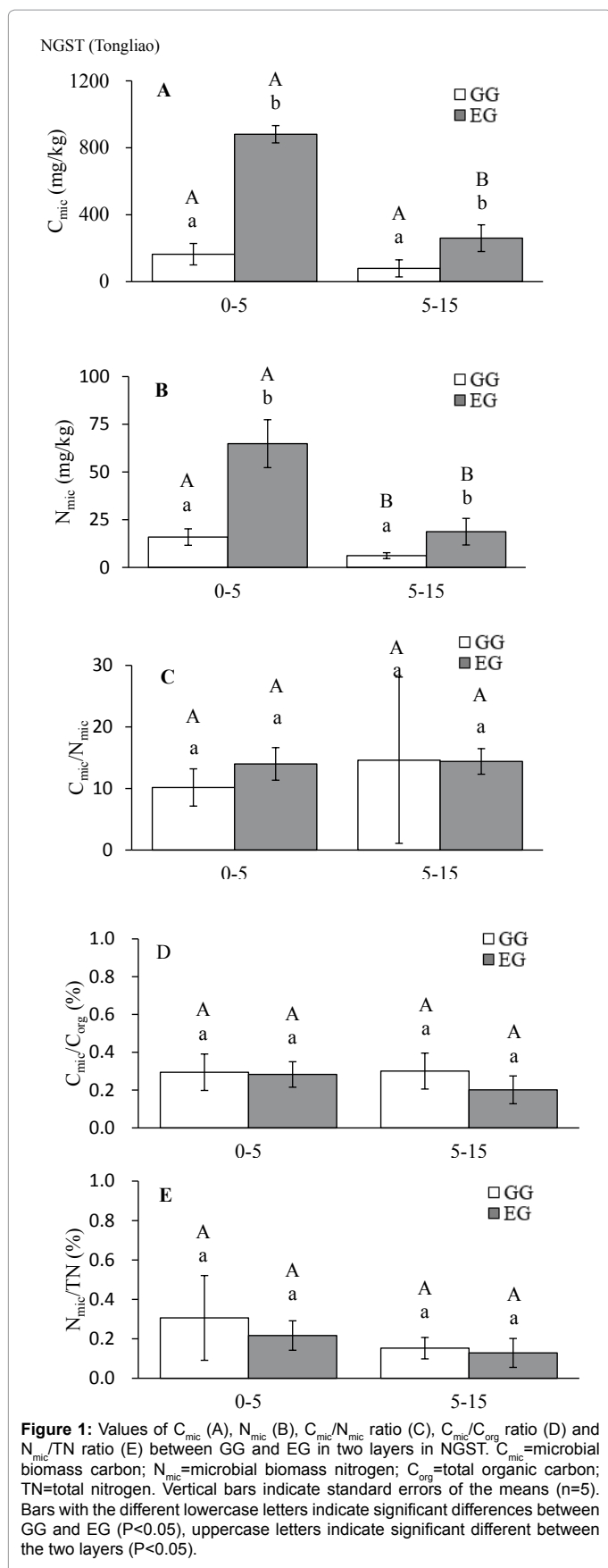
Means (± SE) for each variable followed by different letter indicate significant differences between EG and GG within the same sites ( $P < 0.05$ )

**Table 2:** Physico-chemical characteristics of soil in the study sites in 0-5 and 5-15 cm layers.

	$C_{mic}$	$N_{mic}$	Moisture	pH	EC	$C_{org}$	TN	TC/TN	Sand	Silt	Clay
$N_{mic}$	0.967**										
Moisture	0.818**	0.724**									
pH	0.472*	0.397	0.55**								
EC	0.75**	0.638**	0.89**	0.732**							
$C_{org}$	0.932**	0.85**	0.92**	0.446*	0.803**						
TN	0.828**	0.764**	0.874**	0.613**	0.851**	0.865**					
TC/TN	0.33	0.303	0.485**	0.056	0.279	0.336	0.296				
Sand	-0.774**	-0.708**	-0.93**	-0.504*	-0.812**	-0.828**	-0.882**	-0.645**			
Silt	0.553**	0.477*	0.778**	0.263	0.564**	0.699**	0.716**	0.603**	-0.854**		
Clay	0.79**	0.745**	0.858**	0.593*	0.842**	0.757**	0.831**	0.544**	-0.908**	0.557**	
Avail. P	0.234	0.16	0.038	-0.354	0.07	0.302	0.12	-0.198	0.033	0.054	-0.096

Pearson's correlation coefficient, n=20, \* $P < 0.05$ , \*\* $P < 0.01$

**Table 3:** Correlation matrix (r-values) for  $C_{mic}$  and  $N_{mic}$  and physical-chemical characteristics of soils in NGST.



	C <sub>mic</sub>	N <sub>mic</sub>	Moisture	pH	EC	C <sub>org</sub>	TN	TC/TN	Sand	Silt	Clay
N <sub>mic</sub>	0.9**										
Moisture	0.904**	0.793**									
pH	-0.663**	-0.6**	-0.476*								
EC	0.574**	0.52*	0.587**	-0.001							
C <sub>org</sub>	0.879**	0.755**	0.873**	-0.504*	0.708**						
TN	0.904**	0.815**	0.867**	-0.443*	0.796**	0.94**					
TC/TN	0.033	0.024	0.026	0.07	0.151	0.177	0.101				
Sand	-0.164	-0.034	-0.356	0.1	-0.293	-0.474*	-0.299	-0.427*			
Silt	0.225	0.115	0.402	-0.052	0.442*	0.563**	0.41	0.475*	-0.973**		
Clay	0.113	-0.027	0.309	-0.133	0.171	0.391	0.205	0.375	-0.985**	0.919**	
Avail. P	0.696**	0.645**	0.468*	-0.549**	0.437*	0.689**	0.659**	0.184	-0.073	0.107	0.044

Pearson's correlation coefficient, n=20, \*P<0.05, \*\*P<0.01

**Table 4:** Correlation matrix (r-values) for C<sub>mic</sub> and N<sub>mic</sub> and physical-chemical characteristics of soils in HSLM.

C<sub>mic</sub>:N<sub>mic</sub>, C<sub>mic</sub>:C<sub>org</sub> and N<sub>mic</sub>:TN ratios in NGST fluctuated from 10.2 to 14.6, from 0.2 to 0.4 and from 0.1 to 0.3, respectively (Figures 1c, 1d and 1e). In HSLM, C<sub>mic</sub>:N<sub>mic</sub>, C<sub>mic</sub>:C<sub>org</sub> and N<sub>mic</sub>:TN ratios fluctuated from 7.4 to 9.5, from 3.1 to 4.6 and from 0.3 to 0.5, respectively (Figures 2c, 2d and 2e). C<sub>mic</sub>:N<sub>mic</sub> ratio in 0-5 cm was lower than 5-15 cm and in 0-5 cm C<sub>mic</sub>:N<sub>mic</sub> ratio in EG was higher than in GG while in 5-15 cm the one in GG was higher than in EG in both NGST and HSLM (Figures 1 and 2c), and the difference was not significant (P>0.05). In NGST, C<sub>mic</sub>:C<sub>org</sub> and N<sub>mic</sub>:TN ratios in GG was higher than in EG in both two layers, but in contrast, in HSLM, the ones in EG were higher than GG except for N<sub>mic</sub>:TN ratio in 5-15 cm. The difference between the GG and EG as well as between the two layers were not significant (P>0.05).

## Discussion

### Soil physico-chemical properties

Our results show higher sand content and significantly lower moisture, C<sub>org</sub>, EC, TN and silt content in NGST and HSLM with GG compared to EG (Table 2). Fencing increased soil moisture compared to grazing (Table 2); consistent with the previous studies [46-51] which shows that soil water content significantly increased after long-term fencing. And in this study, fencing also increased the above-ground biomass and coverage (Table 2), which could decrease evaporation. In addition, bare ground caused by grazing could become hotter than covered soil, which caused a decreased in soil moisture and increase soil erosion risk [51,52]. In HSLM, a slight soil pH in GG compared with in EG in 0-5 cm layer (Table 2), which may be due to the urine deposition in GG. The animal urine inputs in GG could increase due to the hydrolysis of urine-urea [53-55]. Moreover, Grassland restoration has improved the content of phospholipid fatty acid (PLFA) in soils [56], and PLFA had significant negative correlation with soil pH (P<0.05) [57], meaning that vegetation recovery reduced soil pH. But in NGST, soil pH decreased after grazing (Table 2) and the effect was significant in 0-5 cm layer (P<0.05). Soil pH correlated positively with Mg<sup>2+</sup>/Ca<sup>2+</sup> ratio and exchangeable Mg<sup>2+</sup> in the alkalinized soil [58]. And in this study, Mg<sup>2+</sup>/Ca<sup>2+</sup> ratio and exchangeable Mg<sup>2+</sup> significantly decreased after grazing compared with fencing (data not show). Soil EC was highest in 0-5 cm layer in EG of NGST and soil EC significantly decreased after grazing in both NGST and HSLM (Table 2). Soil EC was high in NGST was caused the natural and geographical reasons: Tongliao located in the middle Horqin Sandy Land and is low in precipitation and high in evaporation which causes the salt accumulation in the soil especially in the surface layer. C<sub>org</sub> and TN in EG were significantly higher than in GG in both NGST and HSLM and decreased with depth (Table 2). Similarly, C<sub>org</sub> and TN levels increased with grazing exclosure in other

arid and semi-arid rangelands [59,60]. This may be due to aboveground litter accumulation on surface soil by fencing which contribute organic matter returned to soil [60-64]. In addition, underground root also significantly influenced soil C and N levels [65,66] and it has been reported that after fencing, vegetation grows better and developed better root system compared to grazing plots which is conducive to soil organic matter formation and accumulation [67-69].

### Microbial biomass C and N

In the present study, the C<sub>mic</sub> and N<sub>mic</sub> were found to significantly decrease in GG compared to EG and decreased in the deeper layers, which indicated that long-time free grazing was deleterious for microbial growth. The C<sub>mic</sub> and N<sub>mic</sub> are generally related to the soil organic matter content in forest soils [70-72]. Soil organic carbon is the major source of energy for the soil microorganisms [73]. And in this study, the C<sub>mic</sub> and N<sub>mic</sub> were also positive correlations with soil moisture, C<sub>org</sub> and TN content which were significantly decreased in grazing than exclosure (Figure 2; Table 1). This may be caused by the disturbance of livestock grazing and trampling. In 0-5 cm layer, the soil moisture, C<sub>org</sub> and TN content were all higher in NGST than in HSLM in EG, but the C<sub>mic</sub> and N<sub>mic</sub> in NGST were significantly lower than in HSLM. And the soil pH in GG and EG in NGST was 8.83 and 9.62, respectively. Soil pH is also important as microbial growth declines under conditions that are too acid or too alkaline. The previous study indicated that the concentration of soil microbial biomass C is the greatest at pH 7.00 [74].

Soil C<sub>mic</sub>:N<sub>mic</sub> ratio was an important index reflecting N supply ability. And the C<sub>mic</sub>:N<sub>mic</sub> ratio is often used to describe the structure and state of the microbial community. A high C<sub>mic</sub>:N<sub>mic</sub> ratio indicates that the microbial biomass contains a high proportion of fungi, whereas a low value suggests that bacteria predominate in the microbial populations [75]. Jenkinson (1976) and Anderson and Domsch (1980) reported that bacterial dominant soil had a C<sub>mic</sub>:N<sub>mic</sub> ratio between 3 and 6, whereas a C<sub>mic</sub>:N<sub>mic</sub> ratio between 7 and 12 indicated the dominance of fungi [76,77]. In 0-5 cm, the C<sub>mic</sub>:N<sub>mic</sub> ratios of the soils from NGST and HSLM were, on average, 10.2 and 7.5 in grazing, and 14.0 and 7.8 in EG, respectively. And in 5-15 cm, the data were 14.6 and 9.5 in grazing, and 14.4 and 9.2 in EG, respectively (Figure 2). It indicated that both in NGST and HSLM, the microorganism community was in the dominance of fungi. The C<sub>mic</sub>:N<sub>mic</sub> ratios in EG was higher than in GG in the surface layer but the results in subsurface layer showed opposite. The difference between GG and EG was not significant. In grazing site, the C<sub>mic</sub>:N<sub>mic</sub> ratios in NGST was higher than in HSLM in two layers, and in EG, it showed significantly higher in NGST than in

HSLM. The  $C_{mic}:N_{mic}$  ratio is affected by soil properties such as moisture content, texture, pH,  $C_{mic}:C_{org}$  and  $N_{mic}:N_{tot}$  ratios (i.e., the substrate availability), N incorporation in fungi and the ratio of active to dormant microorganisms [76,77]. In this study, the soil moisture,  $C_{org}$  and silt and clay content were all higher in NGST than in HSLM in EG which can explain the  $C_{mic}:N_{mic}$  ratios was significantly higher in NGST than in HSLM. Results indicated that the difference soil physical-chemical properties between the two experimental sites had a particular impact on this ratio rather than the effect of livestock grazing.

$C_{mic}:C_{org}$  ratio has been proposed that the biomass C is more sensitive to changes in soil quality than the total organic C and therefore the ratio of  $C_{mic}$  to  $C_{org}$  may provide an early warning system for changes in organic matter dynamics, e.g., forest soil degradation in terms of soil organic matter loss. It is an index of the mineralization rate of soil microbes on organic matter, the higher value represents higher mineralization rate and could induce higher soil nutrient utilization rate. Furthermore, the higher ratio represents that the maintenance of the same amount of microorganisms required relatively less energy, suggesting higher soil quality for the growth of soil microorganisms. The ratio of  $N_{mic}:NT$  has the same significant as the ratio of  $C_{mic}:C_{org}$ . As shown in Figure 2, the  $C_{mic}:C_{org}$  ratio in EG was significantly higher than in grazing in HSLM in 0-5 cm but in 5-15 cm the difference was not significant. The  $N_{mic}:TN$  ratio was higher in EG in 0-5 cm in HSLM but in 5-15 cm the result was opposite and the difference was not significant. It indicated that in 0-5 cm there was more accumulation of degradable organic compounds. But the opposite results showed in NGST site, the  $C_{mic}:N_{mic}$  was higher than in GG than EG. Furthermore, the  $C_{mic}:N_{mic}$  in HSLM was significantly higher than in NGST in both GG and EG. These results suggested that the soil quality in NGST was low and low efficiency in the conversion of  $C_{org}$  into  $C_{mic}$  not only in GG but also in EG.

## Conclusion

Our results showed that, in semi-arid grassland in southeast part of Inner Mongolia, grazing decreased the soil microbial C and N both in NGST and HSLM. And we found  $C_{mic}$  and  $N_{mic}$  and  $C_{mic}$  to  $C_{org}$  ratio in NGST was lower than in HSLM. This indicated that high soil pH affected microbial growth and also utilization of soil organic matter. Our data suggest that fencing is an appropriate strategy in these grassland ecosystems and these findings are important for assessing effect of grazing on grassland ecosystems.

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