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Effect of Grazing Intensity on Microorganisms Quantity and Microbial Biomass of Soil in Grassland under Protection Forest of Songnen Plain

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Abstract: Although, grazing is a common utilization pattern of grassland, little information is available about the effect of grazing intensity on microorganism quantity and microbial biomass of soil in grassland under protection forest. The present study was conducted to investigate the effect of grazing intensity on microorganisms and microbial biomass of soil in grassland under protective forest of Songnen plain. The grassland was divided into different part treated with Non-Grazing (NG), Light Grazing (LG), Moderate Grazing (MG), Severe Grazing (SG) and Extreme Grazing (EG), respectively. Three sampling points were selected in each grazing treatment site and soil samples (0-10 cm depth) were collected five times at each sampling points. Plate-counting technique was used to analyses soil microorganism quantity. MBC and MBN of soil were determined using the Chloroform-Fumigatione Extraction Method. The results showed that LG and MG could significantly increase quantity of microorganism, MBC and MBN of soil while SG and EG significantly decrease them. These results demonstrated the importance of moderate grazing in grassland under protection forest of Songnen plain.

Key words: Grazing intensity, microorganisms quantity, microbial biomass, songnen plain, China

INTRODUCTION

Grazing as a most simple and economic utilization pattern of grassland plays important role in maintaining cycling of important nutrient elements such as C, N, P, S and other nutrients in grassland ecosystems and stability of soil ecosystem (Tracy and Frank, 1998; Hamilton and Frank, 2001). However, it has been widely accepted that overgrazing not only leads to increasing of soil nutrients output and decreasing of soil fertility but also loss of function of the whole grassland ecosystem (Czegledi and Radacsi, 2005; TianWen and WeiGuo, 2009). Therefore, employing a reasonable grazing intensity is very important for the grassland ecosystem.

Microorganisms and microbial biomass of soil play a leading role in the formation and evolution process of soil (Bardgett *et al*., 1997; Wardle, 1992). Soil microbial biomass is the primary catalyst of biogeochemical processes as well as an energy and nutrient reservoir. It has been proved that Microbial Biomass C (MBC) and N (MBN) are often significantly correlated with chemical characteristics of soils (Gajda and Martyniuk, 2005). Recently, soil microbial biomass is considered as an important early indicator of changes of soil quality and soil total organic matter since, it is extremely sensitive to environmental change and can response early to the function change of ecosystem (Qi *et al*., 2011; Fua *et al*., 2012). For these reasons, studies have been conducted to investigate the effect of grazing on soil microbial processes associated with maintaining ecosystem stability. However, these studies have produced contradictory results. It had been shown that increasing grazing intensity could increase microbial biomass and cessation of grazing decreases it (Bardgett *et al*., 1997). There were also some studies which showed that grazing could decrease the soil microbial biomass or had no effects on soil microbial biomass (Moussa *et al*., 2007; Wu *et al*., 2010). These contradictory results may result from inherent soil properties, soil interactions with vegetation, environmental factors and grazing intensity. Therefore, it is essential to examine the response of the soil microbial biomass to grazing under different soil conditions.

Songnen plain, located in the central section of Northeast China is one of the high production grasslands in China with good habitat conditions plenty of hydrothermal resources and high quality of grass. Songnen plain grassland ecosystem has been deteriorated seriously in association with soil alkalization and grassland community succession from perennial grass to one year grass due to long term of overgrazing. Grassland under protection forest is one of the most important grazing spaces of the ecotone between agriculture and animal husbandry in Songnen plain. To the knowledge,

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there is lack of information on response of soil microbial biomass to grazing in grassland under protection forest of Songnen plain. The objective of this study was to investigate the effect of grazing intensity on microorganisms and microbial biomass of soil in grassland under protective forest of Songnen plain.

MATERIALS AND METHODS

Experimental grassland: The research site (123°44' West longitude; 44°40' North latitude) was located at Jilin province horse breeding farm North of ecotone between agriculture and animal husbandry of Songnen plain in China. The annual precipitation is form 310-580 mm and 70% of the annual precipitation occurring in the period from June to August. Annual evaporation is 1135-1565 mm and average temperature is 4.9°C. The protection forest consists of poplars. The grassland under protection forest has been used as one of the main grazing sites in local area.

Experimental design: Earlier study has shown that poplar leaves are the main food of local livestock and residual quantity of intact leaves on the ground decreases as the grazing intensity increases because of food intake and trample of the livestock during grazing. Therefore, in this study, residual quantity and integrity of leaves on the ground was used as a criterion to divide the grassland into Non-Grazing (NG), Light Grazing (LG), Moderate Grazing (MG), Severe Grazing (SG) and Extreme Grazing (EG). The detailed information of this criterion is shown in Table 1. Three sampling points were selected in each grazing treatment site and soil samples (0-10 cm depth) were collected five times at each sampling points. The soil samples were stored in an ice box and then transferred to laboratory until processing for soil microorganism quantity and microbial biomass analysis.

Soil microorganism quantity analyses: Plate-counting technique was used to analyses soil microorganism quantity. Briefly, bacteria, actinomycetes and fungus were cultured in Beef extract peptone medium, Gause's No. 1 synthetic medium and Rose Bengal medium, respectively. For each kind of microorganism, three replicates and dilutions were conducted to culture for counting. Before the microorganism count was carried out, bacteria were cultured 2 days at 30°C, actinomycetes and fungus were cultured 4 and 7 days at 28°C, respectively.

Soil Microbial biomass (C and N) analyses: MBC and MBN of soil were determined using the Chloroform-Fumigatione Extraction Method (Vance *et al*., 1987).

Table 1: Criterion of the grazing intensity

Item	NG	LG	MG	SG	EG
Biomass of intact leaves (g/m^2)	>50	20~50	5~20	2~5	<2

Briefly, the fumigated and unfumigated soil samples (20 g) were both extracted using 100 mL of 0.5 M K_2SO_4 then extracts were filtered through 0.45 mm filter membrane. Both fumigated and unfumigated soil samples were incubated in the presence of NaOH solution. The amount of CO_2 and mineral N were measured both in fumigated and unfumigated samples. The amount of MBC and MBN were calculated as follows:

$$MBC = \frac{(Fc-Ufc)}{kc}$$

Where:
Fc = CO_2 from fumigated soil
Ufc = CO_2 from unfumigated soil
Kc = 0.45

$$MBN = \frac{(Fn-Ufn)}{kn}$$

Where:
Fn = The flush of NH_4-N from fumigated soil
Ufn = The NH_4-N mineralized from unfumigated soil
Kn = 0.54

Statistical analysis: The microorganism quantity, MBC and MBN were expressed as mean±SD. The data were analyzed using SPSS16.0 Software (Version 16.0; SPSS Inc., Chicago). One-way ANOVA was utilized to determine the significance of differences of microorganism quantity, MBC and MBN of soil in different grazing intensity treatments. In general, $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Results of numbers of soil microorganisms in different grazing intensity are shown in Table 2. For the microbial community structure of grassland soil in different grazing intensity treatments, quantity of bacteria is the highest and that of actinomycetes is the second-highest followed with fungus. Although, grazing intensity didn't change soil microbial community structure however, it significantly changed the quantity of bacteria, actinomycetes and fungus in soil. As shown in Table 2, compared to NG, LG and MG significantly increased the number of soil microorganisms while SG and EG significantly decreased it. The number of soil microorganisms in EG treatment was the smallest among different grazing treatments.

Table 2: Numbers of soil microorganisms in different grazing intensity (individual/g dry soil)

Grazing intensity	Bacteria ($\times 10^7$)	Actinomycetes ($\times 10^5$)	Fungus ($\times 10^4$)
NG	8.14 ± 0.82^{bA}	6.14 ± 1.04^{B}	9.65 ± 1.15^{B}
LG	9.75 ± 1.21^{aA}	8.75 ± 1.13^{A}	11.67 ± 1.36^{A}
MG	9.63 ± 0.96^{aA}	9.53 ± 0.84^{A}	11.88 ± 0.97^{A}
SG	4.26 ± 1.09^{bB}	3.96 ± 0.76^{aC}	5.39 ± 0.82^{C}
EG	2.21 ± 1.13^{cB}	2.42 ± 0.88^{bC}	2.83 ± 1.02^{D}

Values in the same column with different lowercase letters and capital letter indicate significant differences at $p<0.05$ and 0.001 levels, respectively

Results of soil MBC and MBN in different grazing intensity are shown in Table 3. The results showed that MBC and MBN of soil were affected significantly by grazing intensity. Except for LG and MG, MBC and MBN of soil decreased as grazing intensity increased. Compared to NG, MBC and MBN of soil treated with LG and MG increased significantly ($p<0.01$) while those with SG and EG decreased significantly ($p<0.01$). Among the different treatments, MBC and MBN of soil treated with LG and MG were the highest and those with EG was the lowest.

Grassland under protection forest of Songnen plain is a special grassland type which locates in the ecotone between agriculture and animal husbandry. Grazing livestock is the most common activity on this place. Soil microorganism is an important part of grassland ecosystem and its activities are closely relevant to vegetation types, soil fertility, grazing intensity, etc. Although, a lot of research had been conducted to investigate the effect of grazing intensity on grassland soil microorganism (Song *et al*., 2008; Wu *et al*., 2010; Fua *et al*., 2012) to the knowledge, there is lack of the information on the effect of grazing intensity on soil microorganism of grassland under protection forest. Results of the present study showed that LG and MG could significantly increase quantity of microorganism, MBC and MBN of soil in grassland under protection forest of Songnen plain however, these data decreased as grazing intensity increased.

Results of this study showed that quantity of soil microorganism decreased in SG and EG treatments while it increased in LG and MG treatments. The decrease of soil microorganism in LG and MG treatments could be due to reducing of aboveground biomass litter amount and root exudates of vegetation and content of soil organic matter caused by over-grazing animals which resulted in the lack of nutrition for microorganism and ultimately decreased microorganism number (Wendu *et al*., 2010). For LG and MG treatments, the increase of soil microorganism may be caused by the special condition of grassland under protection forest. Grassland under protection forest was formed under protection forest and its vegetation structure significantly changed due to the change of light and water resource, so it is different with those classical grasslands. Leaves of the protection forest have been the main pasture resources of livestock. Vast of leaves may suppress the growth of grassland and even will destroy grassland if the leaves can not be utilized. Therefore, moderate grazing could benefit to the grassland and increase the quantity of soil microorganism. The earlier study had shown that LG and MG can increase yield and quality of grass in grassland under protection forest of Songnen plain (He *et al*., 2004).

Table 3: MBC and MBN in different grazing intensity ($\mu g\ g^{-1}$ dry soil)

Grazing intensity	MBC	MBN
NG	342.54 ± 17.36^{B}	48.25 ± 4.63^{B}
LG	471.62 ± 32.19^{A}	68.18 ± 5.52^{A}
MG	446.27 ± 9.570^{A}	57.36 ± 2.48^{A}
SG	194.68 ± 8.340^{C}	19.49 ± 2.13^{C}
EG	105.42 ± 8.420^{D}	17.26 ± 1.89^{C}

Values in the same column with different capital letter indicate significant differences at $p<0.001$ levels

Soil microbial biomass is an important indicator of soil quality and total organic matter. Results of the present study also indicated that MBC and MBN of soil decreased in LG and MG treatments and increased in SG and EG treatments. Earlier studies also have shown that MBC and MBN of soil decreases as grazing intensity increases. The change of MBC and MBN was in line with that of microorganism quantity in different grazing intensity. It had been shown that the numbers of the soil microorganisms and the soil microbial biomass were in significant or very significant positive correlation (Song *et al*., 2008). These results indicated that soil microbial biomass could reflect change of microorganism number to some extent.

CONCLUSION

To the knowledge, this study is the first conducted in grassland under protection forest of Songnen plain to assess the effect of grazing intensity on soil microorganism. The results showed that LG and MG could significantly increase quantity of microorganism, MBC and MBN of soil while SG and EG decrease them. The present study also indicated the importance of moderate grazing in grassland under protection forest.

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