

**EFFECTS OF *LEYMUS CHINENSIS* (TRIN.) TZVELEV GRASSLAND LITTER RETURN ON SOIL MICROBIAL BIOMASS CARBON OF THE SONGNEN SANDY LAND IN CHINA**

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**Abstract**

Effects of litter return of *Leymus chinensis* grassland on soil microbial biomass carbon of the Songnen sandy land were investigated. Characteristics of soil microbial biomass carbon (SMBC) from subhumid area of *L. chinensis* grassland ecosystem at different content of litter returns (0, 200 and 400 g/m<sup>2</sup>) and soil depths (0-10, 10-20, 20-30 and 30-40 cm) were evaluated. Results showed that the content of SMBC increased with the increase in litter return at the same soil depth. The content was highest (426.17 mg/kg) at 400 g/m<sup>2</sup> litter return, which was 1.61 and 1.50 times higher than at 0 and 200 g/m<sup>2</sup>, respectively at the depth of 0-10 cm, and decreased sharply at 10-40 cm. These results showed that increasing the amount of litter return and suitable management measures can effectively enhance the carbon content of soil microbial biomass and improve soil microbial activity and fertility to ensure the balance of grassland ecosystem.

**Introduction**

Terrestrial carbon cycle is a crucial component of the global carbon cycle because it is related to the formation of terrestrial ecosystem productivity and affects the energy balance of the entire ecosystem (Ge *et al.* 2014, Luo *et al.* 2015). Microorganisms are the main decomposers of terrestrial ecosystems, driving the flow of ecosystem materials and energy while acquiring resources to build their own biomass. They also regulate the cycling of carbon and nutrients across the soil-plant-atmosphere continuum, thus affecting the structure and function of ecosystems (Luo *et al.* 2015, Guo *et al.* 2017, Adingo *et al.* 2021). Soil microbial biomass carbon is the sum of carbon in living and dead microorganisms with a volume less than 5000  $\mu\text{m}^3$  in the soil. Grassland is a vital component of the terrestrial ecosystem. Notably, soil microorganisms play a dominant role in the decomposition and transformation of organic matter in the grassland ecosystem (Lin *et al.* 2017). Microbial biomass carbon in grassland is an active component of soil organic matter and is sensitive to small changes in the level of the soil ecosystem (He *et al.* 2010). Microbial carbon in grassland is influenced by several factors in the ecosystem. For example, soil microbial biomass carbon of different grassland types has been shown to decrease with the decrease in altitude and aggravation of grassland desertification (Welemariam *et al.* 2018). Also, soil microbial biomass carbon decreases with the increase soil depth in alpine grasslands (Nie *et al.* 2021). Different management measures have also been shown to affect litter decomposition in grasslands (Zhang *et al.* 2014, Dietrich *et al.* 2017, Nusse *et al.* 2017, Meng and Liu 2021, Wang and Du 2021). Moreover, the quantity and quality of available carbon provided to soil microorganisms can stimulate community activity and affect soil microbial carbon content (Zhang *et al.* 2015). However, effects of different litter returns on microbial biomass carbon has not been fully explored. Therefore, the present study was aimed to investigate effects of different litter returns on SMBC at different depths in temperate *L. chinensis* grassland of the songnen sandy land. The findings of this study would provide new insights into the study of carbon cycling in terrestrial ecosystems.

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### Materials and Methods

The sample site was located at the Changling Grassland Agro-pastoral Ecology Experimental Station (123°31'18"E, 44°33'29"N) of the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences in Changling County, Jilin Province, China (Fig. 1a). The experimental station was established in 2009. It has a low plain terrain with an elevation of 145 m and a distribution of banded sand dunes. The climate of the area is semi-arid and semi-humid monsoon in the middle temperate zone, with an average annual temperature of 4.9 °C and effective cumulative temperature  $\geq 10$  °C is 2919 °C. The area receives an average annual precipitation of 310-480 mm, which is mainly concentrated between June and September. It is located in the agriculture and animal husbandry staggered belt and has diverse soil types, including windy sandy, meadow, alkaline, and salty, with a pH of 7.5-10.0. The low plain vegetation is meadow; sand dunes are distributed with sparse forest. The main establishment or dominant species in the area includes *L. chinensis*, *Chloris virgata*, *Suaeda salsa*, *Setaria luescens* and *Puccinellia tenuiflora* and so on.

The experimental sample plot was structured in a split-zone design with five replicate blocks (A, B, C, D, E). Each block contained five plots measuring 16 × 16 m each (Fig. 1b). Five litter restitution treatments, including two 0 restitution treatments, 200 g/m<sup>2</sup>, 400 g/m<sup>2</sup>, and 600 g/m<sup>2</sup> litter restitution, were randomly arranged in the plots. Litter was added in early May 2010.

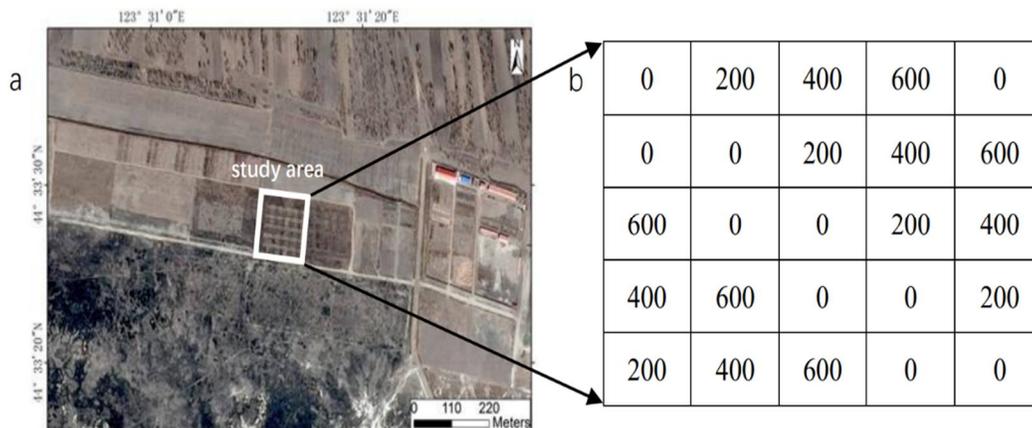


Fig. 1. Location of study area and description of different treatments (a. Location of the study area; b. Layout of the experimental plots).

Soil samples from 0-40 cm were collected at 0, 200, and 400 g/m<sup>2</sup> litter sample plots (borehole diameter 2.5 cm) using a tube-lined direct-pressure in situ soil collector. The samples were collected at four points in each plot from November 8 to 9, 2015.

Chloroform fumigation leaching method was used to measure the soil microbial biomass carbon. Briefly, the soil samples were passed through a 2 mm sieve after removing visible plant residues. The soil water content was adjusted to 40% of field water holding capacity and incubated at 25°C for 4-7 days. Six parts of wet soil (each containing 50 g of dry soil) were added to a 100 ml glass vial and extracted with 200 ml of 0.5 mol/l K<sub>2</sub>SO<sub>4</sub>. The other soil samples were placed in a desiccator with ethanol-free chloroform and wet filter paper strips. The soil samples were then vacuumed until the chloroform boiled violently for more than 2 min. The samples were then placed in a dark place at 25°C for 24 hrs to allow the chloroform to evaporate. Then, 200 ml 0.5M

$K_2SO_4$  was added, and the mixture was shaken for 30 min and then filtered. TC and TN in the filtrate of fumigated and unfumigated soil samples were determined by combustion at 850 °C using a total CN analyzer.

The sketched carbon standard curve is near to 1 with high confidence after examination. The soil biomass microbial carbon was determined using the formula (Lee *et al.* 2015):

$$(MB-C)=(C_E-C_0)\times V\times 1000/(m\times K_{EC}) \quad (1)$$

where  $MB-C$  is the microbial biomass carbon in  $mg/kg$ ;  $C_0$  is the total C concentration of the unfumigated soil sample in  $mg/l$ ;  $C_E$  is the total C concentration of the fumigated soil sample; 1000 is the coefficient of conversion;  $V$  is the volume of 0.5 mol/l  $K_2SO_4$  solution in  $l$ ;  $m$  is the drying mass of the soil sample in  $g$ ;  $K_{EC}$  is the proportion of carbon extracted from the killed microorganism during culture, taken as 0.38.

Data were analyzed using SPSS 17.0. Analysis of variance (ANOVA) was performed to compare the differences in microbial biomass carbon between different litter returns and different depths. Statistical significance level was set at  $p < 0.05$ .

## Results and Discussion

The content of soil microbial biomass carbon increased with the increase in litter return (Fig. 2). At 0-10 cm depth, the soil microbial carbon content was the highest at 264.72  $mg/kg$  under 0  $g/m^2$  of litter return. However, the content of soil microbial biomass carbon decreased continuously with the increase in depth; the concentrations at 10-20, 20-30 and 30-40 cm were 96.04, 55.59 and 43.11  $mg/kg$ , respectively. With 200  $g/m^2$  of litter return, the content of soil microbial biomass carbon was the highest at 0-10 cm depth (283.89  $mg/kg$ ), and decreased with increasing depth to 97.41, 58.55 and 37.86  $mg/kg$  at 10-20, 20-30 and 30-40 cm, respectively. The maximum level of soil microbial carbon was 426.17  $mg/kg$  at a depth of 0-10 cm with 400  $g/m^2$  of litter return, and it decreased continuously with increasing depth to 101.46, 68.04 and 46.77  $mg/kg$  at 10-20, 20-30 and 30-40 cm, respectively.



Fig. 2. Characteristics of soil microbial biomass carbon with different litter returns.

The content of soil microbial biomass carbon decreased with the increase of depth under different amounts of litter return; significant differences were observed at 0-10 cm depth ( $P < 0.05$ ), and decreased sharply below 10 cm depth. At 0-10 cm depth, the content of soil microbial biomass carbon at 400 g/m<sup>2</sup> litter return was significantly higher than at 0 g/m<sup>2</sup> and 200 g/m<sup>2</sup> returns ( $P < 0.05$ ), 1.61 and 1.50 times higher than at 0 and 200 g/m<sup>2</sup> litter returns, respectively. Although the content of soil microbial biomass carbon decreased with increasing depth, no significant differences were observed among different litter returns at the depths of 10-20, 20-30 and 30-40 cm ( $P > 0.05$ ).

Soil microbial biomass carbon reflects small changes in the soil environment and directly participates in biological and chemical transformations of the soil. Also, it is an effective reservoir of plant nutrients and thus acts as an indicator of environmental changes, soil fertility, and plant nutrition (Xu *et al.* 2018, Chagas *et al.* 2022). The results of this study showed that the content of soil microbial biomass carbon of *L. chinensis* grassland in Songnen sandy land increased with the increase in litter returning amount. The content of soil microbial biomass carbon in the surface soil was higher at 400 g/m<sup>2</sup> than that at 0 and 200 g/m<sup>2</sup>. However, the content decreased with the increase in soil depth. Also, soil microbial biomass carbon had obvious surface aggregation and was significantly higher at 0-10 cm than at 10-40 cm ( $P < 0.05$ ). This observation is consistent with the results reported by Zhao *et al.* (2014) and Qi *et al.* (2018).

Soil properties such as surface litter, temperature, moisture, and nutrients influence soil microbial biomass carbon (Li *et al.* 2014). The accumulation of litter on the soil surface regulates soil temperature and moisture, thus affecting the living environment of soil microorganisms and determining their number and activity. This, in turn, affects the decomposition of organic matter and the release, transfer, and utilization of nutrients (Marie *et al.* 2016). Meanwhile, optimum hydrothermal and aeration conditions of the surface soil coupled with the accumulation of plant root residues provide richer nutrient conditions for microbial activities (Zhao *et al.* 2014). Therefore, higher litter return improved the soil nutrient conditions, increasing the carbon content of soil microbial load.

The content of soil microbial biomass carbon responded differently to different management measures. Soil microbial population was positively correlated with soil nutrient content and above- and below-ground plant biomass (Lin *et al.* 2017). In the present study, the above and below-ground biomass was significantly increased by litter return treatments. Also, the treatments improved the soil environment, thus increasing the content of microbial biomass carbon. These results show that the quantity of litter return can influence microbial biomass at different depths of the soil profile. This can further affect soil microbial biomass carbon and nutrients, which in turn influence the function and structure of grassland soils.

The main findings of the present study are as follows: The content of soil microbial biomass carbon was significantly different at different soil depths under the same litter return amount. The content was highest at 0-10 cm depth, while no significant differences were observed among 10-20, 20-30 and 30-40 cm soil depths.

Under the same depth condition, the content of soil microbial biomass carbon increased with the increase in litter return. At 0-10 cm soil depth, the content was significantly higher at 400 g/m<sup>2</sup> litter return than at 0 and 200 g/m<sup>2</sup> return. However, no significant difference was found between 10-20, 20-30 and 30-40 cm soil layers at different litter returns. Altogether, these results showed that litter return is crucial in boosting and maintaining soil microbial biomass carbon, which is vital for maintaining grassland ecosystems.

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