RESEARCH ARTICLE

Effects of different litter treatments on soil microbial biomass carbon and nitrogen in temperate grassland

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Soil microbial biomass carbon (SMBC) and soil microbial biomass nitrogen (SMBN) are important indicators of ecological function. In this study, the surface layers of the temperate grassland 0-10, 10-20, 20-30, and 30-40 cm were selected as the research objects. The relationships between soil microbial biomass (SMB) and the different treatments as well as the soil depth were discussed to provide a scientific basis for the sustainable development of grassland ecosystems. This study took Changling Grassland Agriculture and Animal Husbandry Ecological Experiment Station as the research sites. Experiments were conducted with different litter treatments at different soil depths on SMBC and SMBN contents including (1) keeping the litter and biomass untouched when vegetation falls in autumn (WL); (2) not supplying Leymus chinensis and harvesting around biomass, *i.e.* harvesting the litter on the ground without retaining the litter and biomass on the ground and without adding Leymus chinensis (WG); (3) retaining over ground biomass and supplying Leymus chinensis, which was to retain the litter and add Leymus chinensis to the ground when the original litter remains unchanged (ZL); (4) grounding biomass supplementation with seeded Leymus chinensis by adding Leymus in the soil while retaining the original litter (ZG). The results showed that the contents of SMBC, SMBN, and the carbon (C)/nitrogen (N) ratio demonstrated decreasing trends with the increase of soil depth. ZL had the highest SMBC and SMBN with the highest concentrations of 133.87 and 309.69 mg/kg, respectively. At profile depths of 0-10 cm, SMBC and SMBN decreased with increasing soil depth in various treatments except in WG. When soil depth increased, the C/N ratio decreased with the highest in WG and the lowest in WL.

Keywords: soil microbial biomass; carbon; nitrogen; carbon/nitrogen ratio; temperate grasslands; grassland.

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Introduction

Soil microbial biomass (SMB) is active component of soil organic matter (SOM) formation and directly contributes to terrestrial ecological transformations as well as carbon (C) and nitrogen (N) cycling [1]. Soil microbial biomass carbon (SMBC) is an important component of SOM, which reflects the fluctuation of soil microbial activities and soil organic C emissions. Soil microbial biomass nitrogen (SMBN) is a significant source of N nutrition for plants and a reservoir that releases readily available reactive N into soil for plant uptake [2]. Therefore, SMBC and SMBN are the key factors that contribute to a complex chain of multiple benefits to the soil health and fertility. For agricultural lands, SOM is decreasing while SMBC and SMBN are not high. However, both SMBC and SMBN are the active components of soil organic nutrients that might reflect the availability of nutrients and microbial activity in the soil. They are also essential indicators for assessing the soil microbial diversity and soil fertility as a habitat for plant growth and sustainability. Furthermore, they have ability to be delicate and precise, and can be utilized as preliminary biological indicators to detect changes in soil nutrient stocks as well as C and N sources [3]. They can also serve as the measurements of soil exposure to diverse abiotic factors as well as the key indicators for the degree of physical and chemical influence. The C/N ratio has an impact on SMB fixation, releasing of elements from organic fertilizers, affecting soil health and C and N cycles. Therefore, it contributes to environmental protection and sustainability [4].

Grassland ecosystem is important for the decomposition and transformation of organic matter. Soil microorganisms is important in this ecological process [5]. Soil microorganisms play an important role in carbon cycling, nutrient cycling, regulation, and maintenance of plant diversity [6]. As an important decomposer of ecosystems, soil microorganisms are directly involved in the mineralization of organic matter, humus formation, and soil nutrient transformation and cycling. The most active components in Grassland ecosystems are SMBC and SMBN, which may represent the soil ecosystem and the spectrum of soil nutrient variations, that is important for the C and N cycles in grassland ecosystems. At present, the grassland SMBC and SMBN research has gained a momentum and steadily attention of academics and produced certain outcomes [2, 4]. Previous research revealed that the SMBC and SMBN of various Grassland types decreased as altitude and the degree of grassland degradation decrease [7]. Several studies reported that SMBC and SMBN in alpine grassland, in particular, decreased as soil depth increased [2]. Studies also showed that different management practices such as grazing, reseeding, mowing, and fencing could influent the decomposition of litter, whereas the amount and quality of available C and N delivered to soil microorganisms might enhance the microbial community structure (MCS) and activity to affect SMBC and SMBN levels [8]. Moreover, the focus on the coupling relationship between C and N is mostly on the interaction between SMB and C and N levels. Several researchers looked into quantitative N studies as well as qualitative C/N ratio factors [9]. However, there are few publications on the spatial variation of C/N ratio, its affecting factors at regional scales, and the effects of different litter treatments on SMBC and SMBN. In order to investigate the characteristics of SMBC and SMBN contents in typical temperate Leymus chinensis grassland, this study selected Changling grassland animal husbandry, ecological experimental station of typical sample area as the research sites to investigate the influences of different litter treatments at different soil depth on the soil microbial biomass carbon, nitrogen, and carbon and nitrogen ratio to provide the scientific basis for the study of the terrestrial ecosystem carbon cycle parameters.

Materials and methods

Experimental location

Sample site was situated in Changling Grassland Agro-pastoral Ecology Experimental Station (123°31’18”E, 44°33’29”N) (Figure 1), which is in the temperate sub-humid and semi-arid zone. Summer is hot and rainy in the continental monsoon zone, while winter is bitterly cold and dry, and spring is windy. The average annual wind speed is 4.0 m/s with 26.3 days of high wind. The annual sunshine time is about 2,600 – 2,900 hours and the frost-free season is about 100 - 130 days. The majority of the landforms is fixed sand dunes. The location is situated in the zone where agriculture and animal husbandry are intertwined. The non-zonal soil types are Aeolian soil, meadow soil, swamp soil, and saline-alkali soil with a pH of 7.5-10.0, while the zonal soil type is Chernozem soil. The low plain has meadow vegetation with scattered woods on the sand dunes. The main establishment or dominant species in the area includes *L. chinensis*, *Chloris virgata*, *Suacda salsa*, *Setaria luescens*, and

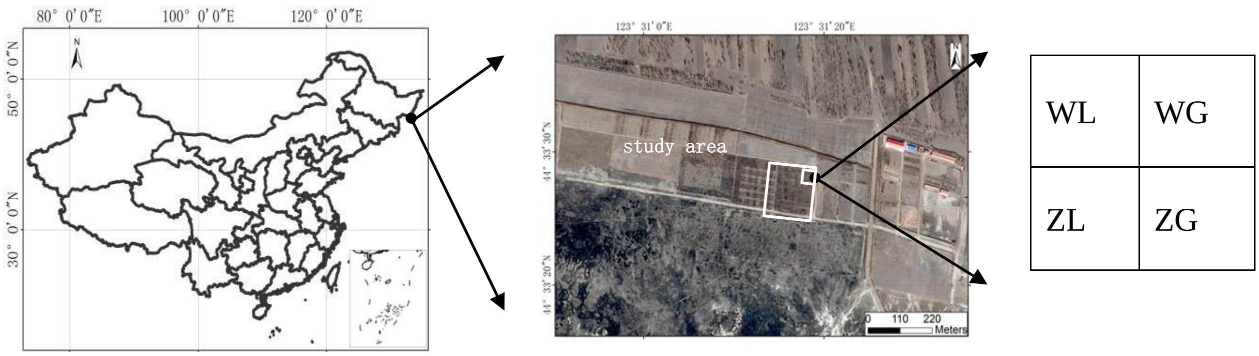


Figure 1. The location of the study area and the layout of the experimental plot.

Puccinellia tenuiflora.

Experimental design

A split-plot design was used in the test plot with a total of four repeating blocks (A, B, C, and D). These four subplots were arranged in the way shown in Figure 1. Among them, "seeding" referred as reseeding Leymus chinensis before the addition of litter in early May 2010 with the reseeding seeds of 2,000 grains/m^2, while "unplanted" referred as not reseeding Leymus chinensis, and "staying" referred as the aboveground biomass of the current year that was retained in autumn every year, as well as "cut" referred as the aboveground biomass of the current year that was mowed every autumn with the stubble height of 3 cm. The four alphabets "Z", "W", "L", and "G" denoted the four seeding treatments.

Sample collection

A liner-type straight pressure undisturbed soil borrower (Jilin Kuai Agricultural Technology, Siping, Jilin, China) was used to collect 0 - 40 cm soil samples (2.5 cm in diameter of the borehole) from the 0 g/m^2 litter plot from selected representative lot to prepare samples. Four groups of sampling points were arranged in the field study area. The sampling depths were 0 - 10, 10 - 20, 20 - 30, and 30 - 40 cm soil depths, respectively. Sampling was done at four different points in each plot. Samples were collected on November 8, 2015.

Determination of soil microbial biomass carbon (SMBC)

The method of fumigation extraction-C automated analyzer was used to determine SMBC. Briefly, two samples of cultivated soil (each was equivalent to a drying basis weight of 25 g) were included in this test. One part of the soil was fumigated with ethanol-free chloroform for 24 hours. Then, the chloroform was removed from the soil before 0.5 M K_2SO_4 leaching agent (soil-water ratio 1:4, mass concentration) was added. The leaching was carried out by shaking for 30 minutes at 300 rpm with medium-speed quantitative filter paper. The other part of soil was not fumigated with the same extraction method. A Phoenix 8000 C-automatic analyzer (Zhuhai Tianchuang Instrument, Zhuhai, Guangdong, China) was used to determine the amount of C in the extract.

Determination of soil microbial biomass nitrogen (SMBN)

Fumigation extraction-flow injection analyzer method was used to determine SMBN. Briefly, 15 mL of the above extract was placed in a 250 mL digestion tube. 0.3 mL of 0.19 M copper sulfate solution, 5 mL of analytically pure concentrated sulfuric acid, and a small amount of anti-boiling particulate matter were added. The mixture was digested until it became clear, and then, refluxed for 3 hours. The volume was then adjusted to a 100 mL in a volumetric flask. The FIAstar 5000 flow injection analyzer (Ruixuan Electronic

Technology, Shanghai, China) was used for SMBN determination [9].

Calculation of SMBC and SMBN

The sketched C standard curve was near to 1 after examination indicating great reliability. The SMBC content was obtained through the SMBC calculation formula, and the SMBN calculation method was the same as follow:

$$SMBC = (C_g - C_0) \times V \times 1000 / (\mathrm{m} \times k_{EC})$$

Where C_g and C_0 were the C concentrations of the fumigated soil sample and the unfumigated soil sample in mg/L, respectively. 1,000 was the factor to convert kg. V was the volume of 0.5 M K_2SO_4 solution in L. m was the dry mass of the soil sample in g. k_{EC} was the ratio of carbon leached out of the microorganisms killed during the culture and took the value of 0.38.

Data processing and statistical analysis

Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA) and SPSS 13.0 (IBM, Armonk, New York, USA) were used for data processing and statistical analysis. The Duncan technique was employed for multiple comparisons [10].

Results

Effects of different treatments on microbial biomass carbon in soil

The SMBC efflux in soil demonstrated a distinct pattern with peak activity among the different treatments (WL, WG, ZL, and ZG). SMBC was 105.92 mg/kg on average in WL (Figure 2) where the largest SMBC was 269.95 mg/kg at 0-10 cm depth and the smallest SMBC was 21.11 mg/kg at 30-40 cm depth with the difference of 248.84 mg/kg. The average SMBC in WG was 89.19 mg/kg, where the highest SMBC was 206.74 mg/kg at 0-10 cm depth and the lowest SMBC was at the 20-30 cm depth with the SMBC amount range as 0-10 > 30-40 > 10-20 > 20-30 cm depths. SMBC in ZL was the highest one among the others ($P < 0.05$). The average SMBC in ZL was 133.87 mg/kg, where the highest SMBC was up to 309.69 mg/kg at the depth of 0-10 cm and the lowest SMBC was 49.63 mg/kg at the depth of 30-40 cm with a difference of 260.06 mg/kg. The average SMBC in ZG was 130.47 mg/kg, where the highest concentration of SMBC was 272.49 mg/kg at 0-10 cm depth and the lowest concentration was at 30-40 cm depth. Except for WG, all other treatments demonstrated that the SMBC gradually dropped as the depth of soil increased.

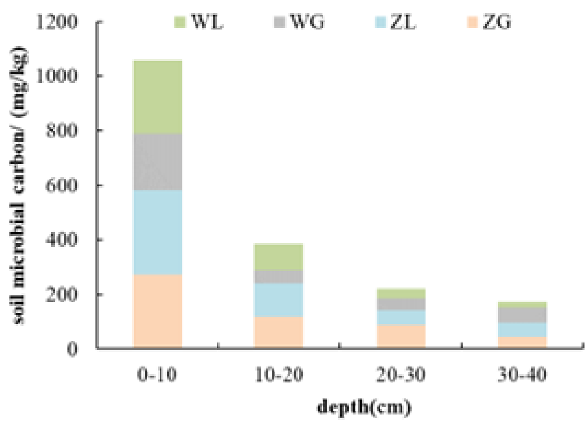


Figure 2. Characteristics of SMBC under different treatments.

Characteristics of microbial biomass carbon in soil at different depth zones

At different depth zones, there were significant changes in SMBC ($P < 0.05$). The SMBC usually decreased as soil depth increased. At a depth of 0-10 cm in the soil, the average SMBC was 264.72 mg/kg. The SMBC in ZL was the highest one (309.69 mg/kg), while the SMBC in WG was the lowest one. The general trend of SMBC with different treatments at the soil depth of 0-10 cm was as ZL > ZG > WL > WG. The average SMBC was 96.04 mg/kg at the depth of 10-20 cm in the soil with the highest one of 121.93 mg/kg in ZL and the lowest level was in WG. The SMBC concentrations of various treatments in soil depth 10-20 cm were ZL > ZG > WL > WG. The average SMBC was 55.59 mg/kg at the depth of 20-30 cm in the soil, where the highest SMBC content was 86.97 mg/kg in ZG and the lowest one was 37.28 mg/kg in WL with a difference of 49.69 mg/kg. The SMBC concentrations with the different treatments were notes as ZG > ZL > WG > WL at the soli depth of 20-30 cm. The average SMBC was 43.11 mg/kg at the soil depth of 30-40 cm, where the highest SMBC was 56.34 mg/kg in WG and the lowest one was the lowest in WL. The SMBC with different treatments at the soil depth of 30-40 cm were as WG > ZL > ZG > WL. (Figure 3)

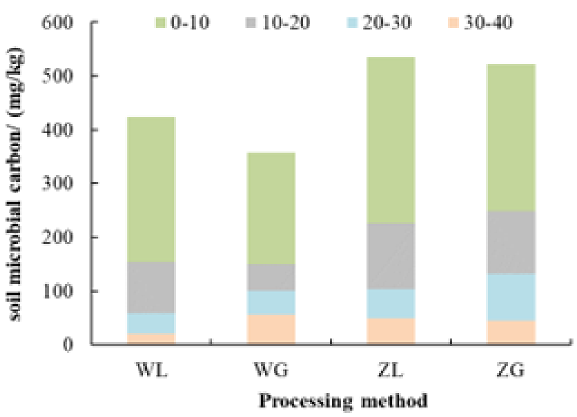


Figure 3. Vertical distributions of SMBC at different depths.

Effects of different treatments on microbial biomass nitrogen in the soil

SMBN efflux in soil demonstrated a distinct pattern with peak activity among different treatments. SMBN in ZL was higher than that of the other three treatments (Figure 4). SMBN was 17.73 mg/kg on average in WL, where the maximum SMBN was 28.66 mg/kg in the 0-10 cm and the minimum SMBN was 52.64 mg/kg in 30-40 cm with a difference of 26.02 mg/kg. The average SMBN in WG was 11.54 mg/kg, where the highest SMBN was 22.75 mg/kg at 10-20 cm depth and the lowest SMBN was at 0-10 cm depth with the SMBN trend as 10-20 > 20-30 > 30-40 > 0-10 cm at different soil depths. The average SMBN in ZL was 18.45 mg/kg, where the highest SMBN was 27.29 mg/kg at the soil depth of 0-10 cm and the lowest SMBN was 7.9 mg/kg at the soil depth of 30-40 cm with a difference of 19.39 mg/kg. In ZG treatment, the average SMBN was 18.47 mg/kg. The highest SMBN concentration was 36.01 mg/kg at 0-10 cm soil depth and the lowest concentration was at 30-40 cm soil depth. Except for the WG, all other treatments demonstrated that the SMBN gradually dropped as the depth of soil increased.

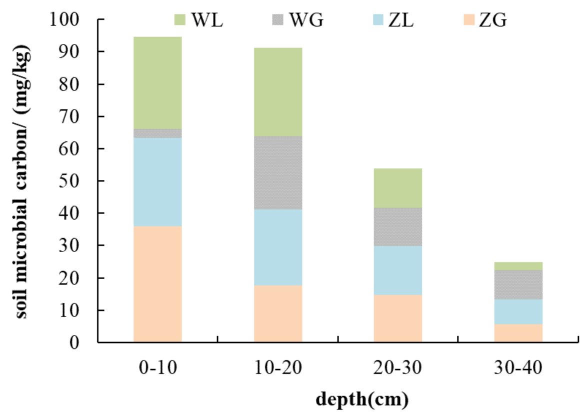


Figure 4. Characteristics of SMBN under different treatments.

Characteristics of microbial biomass nitrogen in soil at different depth zones

At different soil depth zones, there were significant changes in SMBN ($P < 0.05$). At the depth of 0-10 cm in the soil, the average SMBN was 23.67 mg/kg. The SMBN in ZG was the highest (36.01 mg/kg), while the SMBN in WG was the lowest. The general trend in SMBN with different treatments was found as ZG > WL > ZL > WG at 0-10 cm soil depth. The average SMBN was 22.82 mg/kg at depth of 10-20 cm in the soil, where the highest SMBN concentration was 27.41 mg/kg in WL and the lowest SMBN level was 17.55 mg/kg in ZG with a clear difference of 9.86 mg/kg. The SMBC of various treatments were WL > ZL > WG > ZG at 10-20 cm soil depth. The average SMBN was 13.43 mg/kg at depth of 20-30 cm in the soil, where the highest SMBN content was 21.06 mg/kg in ZL and the lowest SMBN content was 17.79 mg/kg in WG with a difference of 3.27 mg/kg. The SMBN with different treatments were as ZL > ZG > WL > WG at 20-30 cm depth in the soil. The average SMBN was 6.25 mg/kg at the soil depth of 30-40 cm, where the highest SMBC was 8.92 mg/kg in WG and the lowest SMBN content was in WL. The

SMBN concentrations with different treatments were as WG > ZL > ZG > WL at the soil depth of 30-40 cm (Figure 5).

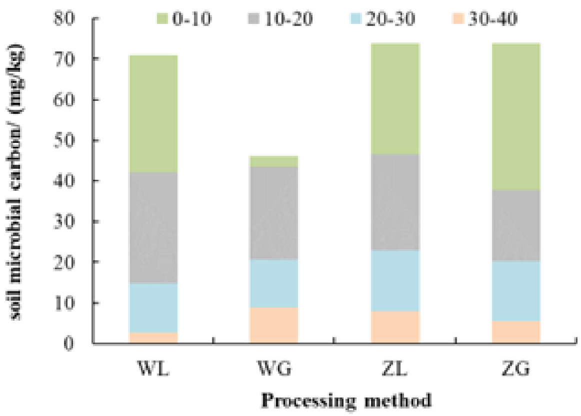
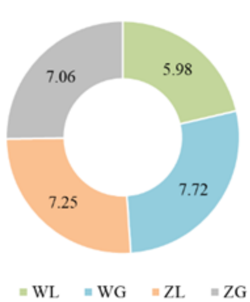


Figure 5. Vertical distributions of SMBN at different depths.

Effects of different treatments on C/N ratio

Soil C/N specific efflux of different treatments demonstrated the different patterns and peak activity. ZG showed the lowest C/N ratio while WG had the highest C/N ratio (Figure 6A). The C/N ratio at the depth of 0-10 cm soil depth showed the largest value of 9.42 in WG treatment mode with the trend as WG > ZL > WL > ZG (Figure 6B). The C/N ratio was the smallest (3.05) at the depth of 10-20 cm. The difference between the highest and lowest C/N ratio was 6.37 with WG had the smallest proportion. The trend of C/N ratio at the soil depth of 10-20 cm was ZG > ZL > WL > WG. In addition, at the depth of 20-30 cm, the lowest C/N ratio was 5.89 in WL treatment. The trend of C/N ratio was ZG > WG > ZL > WL. At the depth of 30-40 cm, the highest C/N ratio was 8.20 in ZG.

A.



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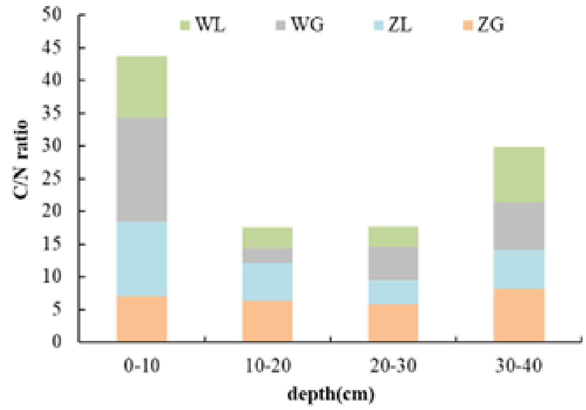


Figure 6. Different treatments on C/N ratio. **A.** C/N ratio in different treatments. **B.** C/N ratio in different treatments at the same depth.

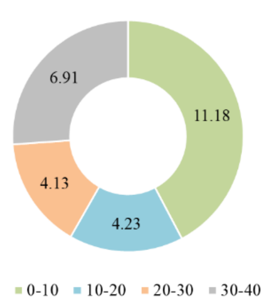
Characteristics of C/N ratio at different depths

The C/N ratio of the surface layer was the highest in the soil depth of 0-10 cm (Figure 7A). When the soil depth increased, the C/N ratio fluctuated and decreased (Figure 7B). The average C/N ratio of the 0-10 cm soil depth was the highest (11.18), while the average C/N ratio of the 10-20 cm soil depth was the lowest (4.23) with the trend as 0-10 > 30-40 > 20-30 > 10-20 cm. The C/N ratio in WG was the highest at different depths, while WL had the lowest C/N ratio. The trends of C/N ratio for all four treatments at the different soil depths followed the same sequence described above.

Nutrient content under different treatments

Under different treatment modes, SMBC in ZL treatment reached the maximum value of 535.5 mg/kg (Figure 8). The SMBN in ZG treatment reached the maximum value of 73.86 mg/kg. C/N ratio in WG treatment reached the maximum value of 30.35 mg/kg.

A.



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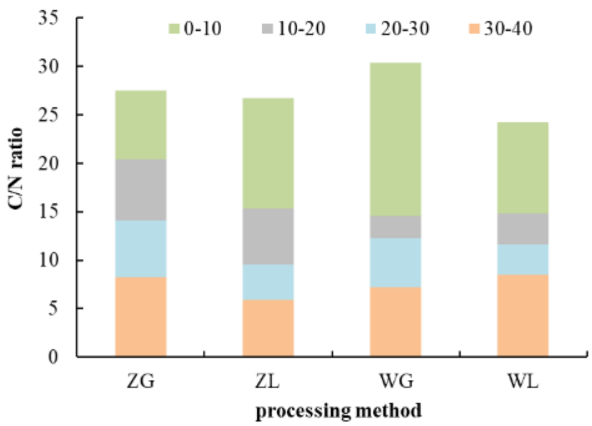

Figure 7. C/N ratio at different depths. **A.** C/N ratio at different depths. **B.** C/N ratio at different depths in the same treatment.

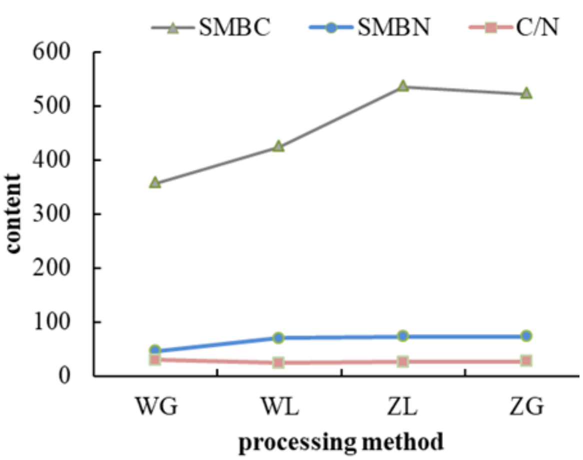

Figure 8. Nutrient content under different treatments.

Discussion

Characteristics of soil microbial biomass carbon and soil microbial biomass nitrogen

SMBC and SMBN are the metrics for the amount of C and N in soil organic matter. SMBC and SMBN are the major source and sink of nutrients that facilitate mineralization for plant growth and development [11]. The results of this study found that, at the 0-40 cm, SMBC in ZL was 49.63-309.69 mg/kg with an upper range that was somewhat lower or similar to that of moderately degraded grassland [9]. However, it was greater than that of moderately and severely degraded grassland as swell as an alpine meadow [8]. The SMBN of 0-40 cm in ZL was only 7.9-27.29 mg/kg with the upper limit lower than that of lightly degraded grassland [12]. The microbial biomass C/N of 0-40 cm soil depth was 3.05-9.41, which was higher than that of degraded grassland and alpine meadow [12] and might be the result of the comprehensive impact of climate, soil, and vegetation in the study area. Climate change has caused a large increase in global warming and precipitation in recent years, resulting in a "greening" tendency throughout the whole grassland ecosystem [10]. Above-ground plants and soil microbes both get benefit from this transformation [9]. On the other hand, the breakdown of litter in WL will progressively liberate soil C [3], making C more readily taken and consumed by microorganisms, probably leading to a rise in the SMBC ratio in the soil. The vertical distribution pattern of the SMB C/N ratio clearly indicated that it was decreased as soil depth was increased [8]. As soil depth increases, microbial activity reduces and the degree of mineralization of soil nutrients by microorganisms decreases as well.

Influencing factors of soil microbial biomass carbon and soil microbial biomass nitrogen

There are a number of variables that influence the dynamics of SMB [3]. SMBC and SMBN are effective nutrient reservoirs for soil delivery of plant nutrients and may reflect small changes in organic C and N limitations in the soil environment [7]. They are also directly engaged in soil biological and chemical transformation processes [13], and are important in evaluating environmental changes, soil fertility, and plant nutrition [11]. This study showed that SMBC and SMBN of Leymus chinensis grassland in temperate zone demonstrated approximate distribution with the change of soil depth with the top layer (0-10 cm) was the highest and decreased significantly with the increase of soil depth [13]. At the same time, the surface soil provides good hydrothermal and aeration conditions [9]. A large amount of plant root residues has been accumulated in the surface layer of the soil, which is influenced by aboveground plants and accumulates more organic matter (dead leaves, humus, *etc*.), and has more C input (root secretions, litter decomposition) and C transfer (deep to shallow) [7]. Furthermore, due to root growth and development, the surface soil provides good hydrothermal and aeration conditions [13]. All of these factors contribute to the development and reproduction of surface microbes, therefore, raising the SMBC and SMBN, which are required for microorganisms to fix additional C and N supplements. Therefore, practical management actions such as replanting paddocks can efficiently increase soil C and N contents as well as plant uptake and CO_2 fixation, resulting in increased SMBC and SMBN.

This study showed that SMBC and SMBN could be used as biological indicators to judge the fertility status of soil and provided a basis for improving soil fertility level and soil fertilization effect [14]. The highly significant positive correlations between SMBC and SMBN and C/N ratio at different treatments and soil depths were observed because the carbon and nitrogen sequestration by soil microorganisms depended mainly on the biomass size of soil microorganisms [15]. Reasonable management measures can effectively increase soil organic carbon and microbial biomass carbon, improve the amount of CO_2 absorbed and fixed by plants, and then, increase soil microbial biomass carbon content. Therefore, different litter addition and management measures can cause changes in microbial biomass at different depths of soil, resulting in differences in soil microbial biomass carbon and nutrients, which will affect grassland soil function and structure, and play an important role in global carbon cycling.

Conclusion

The highest SMBC in ZL was 105.92 mg/kg on average. The SMBC content reached the largest of 269.95 mg/kg at the soil depth of 0-10 cm. Except for the WG, the SMBC of all other treatments decreased significantly with the increase of soil depth. Similarly, the average SMBN in ZL was 18.45 mg/kg with the highest amount of 28.66 mg/kg also at the soil depth of 0-10 cm. Except for the WG, the SMBN of all the other treatments decreased with increasing soil depth. The highest C/N ration was 7.72 in WG. The C/N ratio fluctuated and decreased significantly with increasing soil depth among the treatments.

Acknowledgements

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