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Effects of Different Land Cover Types on Soil Microbial Biomass Carbon and Nitrogen in the Lower Reaches of Niyang River

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Abstract [**Objectives**] To comprehensively and deeply explore the effects of different land cover types in the lower reaches of Niyang River on soil microbial biomass carbon and nitrogen, and to provide a scientific basis for the rational use and sustainable management of land resources in this area. [**Methods**] Taking the 3 types of land cover (cultivated land, grass land and forest land) in the lower reaches of Niyang River in Tibet as the research object, the contents, distribution characteristics and relationships of soil organic carbon, organic nitrogen, microbial biomass carbon, microbial biomass nitrogen and readily oxidizable organic carbon, and their relationships were studied in 0 -10, 10 -20, 20 -40, 40 -60, and 60 -100 cm soil depth. [**Results**] The soil organic carbon content of forest land was higher than that of grass land and cultivated land; the vertical change trend of soil organic carbon content decreased with the increase of depth ($P<0.05$), and it was mainly concentrated in the soil with a depth of 0 -20 cm. The soil organic carbon content was significantly different among forest land, grass land and cultivated land ($P<0.05$), but there was no significant difference between cultivated land and grass land ($P>0.05$). The soil organic nitrogen content was significantly different among cultivated land, grass land, and forest land ($P<0.05$), but there was no significant difference between grass land and forest land ($P>0.05$). The readily oxidizable organic carbon, microbial biomass carbon and nitrogen in forest land were higher than that in cultivated land and grass land. The change trend of soil readily oxidizable organic carbon, microbial biomass carbon and microbial biomass nitrogen was similar to the change of soil organic carbon content, showing a significant positive correlation. In addition to being subject to land cover, soil microbial biomass carbon and nitrogen content were also subject to the interaction of factors such as soil temperature, humidity, pH and vegetation types. [**Conclusions**] Changes in land cover significantly affect soil organic carbon and nitrogen, readily oxidizable organic carbon, microbial biomass carbon and nitrogen content.

Key words Soil microbial biomass carbon and nitrogen, Land cover, Soil depths, Canonical correspondence analysis (CCA)

1 Introduction

As an important type of natural resources, land is the material basis for human survival and development. Land use and land coverage change (LUCC) is an important content of global environmental change and sustainable development research. Since International Geosphere-Biosphere Programme (IGBP) and International Human Dimensions Program (IHDP) jointly proposed the LUCC research program in 1995, LUCC research has always been the frontier and hot issue of global environmental change research[1]. Soil organic carbon (SOC) is the main component of the soil carbon pool, provides a lot of energy for the growth of plants and the life activities of soil microorganisms, and it also the core indicator characterizing soil fertility and basic soil fertility[2]. Soil microorganisms are an important source of soil living organic matter and soil nutrients. The number of soil microorganisms reflects the number of microorganisms involved in regulating the energy and nutrient cycles and the conversion of organic matter in the soil. Among them, soil microbial biomass carbon (MBC) and soil microbial biomass nitrogen (MBN) are its important indicators and can reflect changes in soil quality in a sensitive and timely manner[3-4]. Although soil microbial biomass carbon and nitrogen only account for 1% -4% of soil organic carbon (SOC) and total nitrogen 0.5% -8.0%[5], they are the most active carbon and nitrogen components in soil and directly participate in the mineralization of soil carbon and nitrogen, and regulate the carbon and nitrogen cycles of terrestrial ecosystems. MBC and MBN play an important role in the ecosystem carbon and nitrogen cycle[6]. Readily oxidizable carbon (ROC) is an active organic matter component in the SOC pool that has a certain degree of solubility, easily oxidized and easily decomposed by soil microorganisms. ROC can sensitively reflect changes in soil carbon, and is of great significance for regulating the soil carbon cycle and improving soil fertility[7]. Land cover change is one of the most important factors affecting the carbon cycle of terrestrial ecosystems, and the active organic carbon components in the soil under different land covers vary significantly[8-10]. Therefore, the research on the dynamic effects of different land use methods on soil organic carbon has become a hot spot in the current climate and environmental change research[11-12].

In recent years, due to the pressure of economic development and population growth in the Niyang River Basin, land cover has greatly changed, and the quantity and quality of organic carbon imported into the soil also have changed. However, it is still unclear what

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effect the different land cover in this area exerts on the soil MBC and MBN contents. Therefore, from the perspective of land cover, we compared the relationship between the MBC and MBN contents of different soil depths in three different land covers of more than 30 years of cultivated land, forest land and grass land in the Niyang River Basin. We intended to explore what are the effects of different land cover on soil MBC and MBN contents. The research results are expected to provide a certain basis for the rational use and sustainable management of land resources in the Niyang River Basin.

2 Materials and methods

2.1 Overview of sampling area The experiment was carried out in the lower reaches of the Niyang River in Nyingchi City, Tibet (29°44′ – 29°33′ N, 94°0915′ – 94°28′ E), with an altitude of 2 970 m and an annual average temperature of 7 – 16 ℃. The annual accumulated temperature > 10 ℃ is 2 272 ℃, the frost-free period is about 180 d, the annual average precipitation is about 650 – 750 mm, and the precipitation is mainly concentrated in June to September, with an average relative humidity of 63%, and the annual sunshine hours is 1 988.6 – 2 000.4 h. The native vegetation type belongs to the sub-region of the Niyang River coniferous forest in the temperature and humidity coniferous forest area in the middle reaches of the Yarlung Zangbo River[13].

2.2 Collection of soil samples In the experimental site, we selected 3 plots of cultivated land, forest land, and grass land as the research sample plots, each with an area of 20 m × 20 m. In early August 2019, we collected soil samples of 3 different land use types. We arranged 4 sample points in an "S" shape in each plot, with a spacing of 3 – 5 m between two adjacent sample points to determine the position of the soil profile. After removing the ground litter and herbaceous plants, we excavated 1.5 m long, 1.5 m wide, and 1 m deep soil profiles at the sampling point. Next, we divided them into 0 – 10, 10 – 20, 20 – 40, 40 – 60, and 60 – 100 cm soil layers, from bottom to top, and took 3 repetitions for each layer. We mixed the soil samples at the same level in each soil profile evenly, and removed impurities such as gravel and plant bodies in the soil samples. Then, we collected the mixed sample by the quarter method as the soil sample to be tested on this layer, put the soil sample to be tested into a sterile bag and brought to the laboratory, and stored in a refrigerator at 4 ℃. Finally, we completed the determination of SOC, organic nitrogen, soil ROC, MBC and MBN within one week (Table 1).

Table 1 Basic information of sampling points

Land use type	Coordinate of sampling point	Altitude//m	Soil texture	Vegetation type
CL	94°17′50.0172″ E 29°43′45.2568″ N	3 019	Loam	9, 10, 13, 14, 15, 16, 24
GL	94°17′49.9362″ E 29°43′45.3361″ N	3 019	Sandy soil	6, 7, 8, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 23, 25, 25
FL	94°17′49.8408″ E 29°43′43.2948″ N	3 020	Loam	1, 2, 3, 4, 5, 6, 13, 21, 23

Note: CL denotes cultivated land, GL denotes grass land, and FL denotes forest land; 1. *Picea likiangensis* var. *linzhensis*; 2. *Pinus densata*; 3. *Quercus aquifolioides*; 4. *Amygdalus mira*; 5. *Salix matsudana*; 6. *Rosa* sp.; 7. *Buddleja alternifolia*; 8. *Piptanthus concolor*; 9. *Artemisia sacrorum*; 10. *Artemisia sieversiana*; 11. *Aster souliei*; 12. *Rumex nepalensis* Spreng. var. *nepalensis*; 13. *Potentilla bifurca*; 14. *Elymus dahuricus*; 15. *Agropyron cristatum*; 16. *Plantago depressa*; 17. *Cynoglossum furcatum*; 18. *Erigeron breviscapus*; 19. *Polygonum polystachyum*; 20. *Sambucus williamsii*; 21. *Drynaria delavayi*; 22. *Verbascum thapsus*; 23. *Duchesnea indica*; 24. *Taraxacum mongolicum*; 25. *Rubus biflorus*.

2.3 Determination of soil physical and chemical properties We conducted this study in accordance with provisions of the forestry industry standard of the People's Republic of China *Observation Methodology for Long-term Forest Ecosystem Research* (LY/T 1952-2011). The contents of soil organic carbon and nitrogen were measured using the methods in soil agrochemical analysis[14]. The soil ROC was determined by the method proposed by Xu Minggang *et al.*[15]. The soil MBC and MBN were determined using chloroform fumigation extraction method[16–17]. Specific steps are as follows: accurately weighed 6 parts of dried soil samples equivalent to 25.0 g, and adjusted the water content of the soil samples to 50% of the water holding capacity. Among them, 3 soil samples were fumigated with absolute ethanol and chloroform, placed at 25 ℃ for 24 h, then took out, and vacuum was repeatedly applied to remove the chloroform; the other 3 samples were not fumigated as a control, and then leached with potassium sulfate solution, and the carbon in the leaching solution was determined by potassium dichromate oxidation method; the nitrogen in the extract was determined by potassium persulfate oxidation-ultraviolet spectrophotometry.

2.4 Data analysis We performed statistical analysis on the measurement results with the aid of Excel 10.0 and SPSS 20.0 statistical software to, and used Canon for 4.5 to plot chart.

3 Results and analysis

3.1 Characteristics of SOC, SON and ROC under different land cover As indicated in Table 2, the contents of SOC, SON and ROC in the 0 – 100 cm soil layer under different land cover were different. The average content of SOC in cultivated land (CL), grass land (GL) and forest land (FL) were 7.74, 8.46 and 9.16 g/kg, respectively, showing FL > GL > CL. The average content of SON in CL, GL and FL was 0.77, 0.87 and 0.86 g/kg, respectively, showing GL > FL > CL. The average soil ROC content of CL, GL and FL were 1.154, 1.148 and 1.718 g/kg, respectively, showing FL > CL > GL.

Table 2 Content of SOC and SON under different land use types

Land use type	Soil depth//cm	SOC//g/kg	SON//g/kg	ROC//mg/kg
CL	0 – 10	10.18 ± 2.31[c]	0.97 ± 0.32[b]	1.52 ± 0.31[b]
	10 – 20	10.72 ± 2.42[a]	1.07 ± 0.35[a]	1.59 ± 0.32[a]
	20 – 40	7.05 ± 1.76[a]	0.72 ± 0.31[a]	0.83 ± 0.17[b]
	40 – 60	5.63 ± 1.57[a]	0.57 ± 0.28[a]	0.91 ± 0.33[a]
	60 – 100	5.14 ± 0.89[a]	0.51 ± 0.22[a]	0.93 ± 0.29[a]
GL	0 – 10	17.68 ± 3.05[b]	1.76 ± 0.56[a]	2.43 ± 0.35[c]
	10 – 20	10.54 ± 2.66[a]	1.11 ± 0.68[a]	1.34 ± 0.46[b]
	20 – 40	4.62 ± 1.78[c]	0.51 ± 0.29[c]	0.76 ± 0.28[b]
	40 – 60	5.11 ± 1.47[b]	0.53 ± 0.25[b]	0.70 ± 0.32[b]
	60 – 100	4.36 ± 2.66[b]	0.46 ± 0.29[b]	0.51 ± 0.29[c]
FL	0 – 10	20.76 ± 2.89[a]	1.83 ± 0.47[a]	3.35 ± 0.72[a]
	10 – 20	9.56 ± 2.33[b]	0.99 ± 0.33[b]	1.59 ± 0.42[a]
	20 – 40	6.59 ± 1.36[b]	0.66 ± 0.44[b]	1.02 ± 0.48[a]
	40 – 60	5.20 ± 1.19[b]	0.50 ± 0.25[c]	0.95 ± 0.37[a]
	60 – 100	3.62 ± 0.47[c]	0.36 ± 0.17[c]	0.69 ± 0.33[b]

Note: The data are expressed as the mean ± standard error of each land use type. Different lowercase letters after the data indicate that the indicators are significantly different under different land use types ($P < 0.05$). The same as below.

Under different land cover, SOC was significantly different between FL, GL and CL ($P < 0.05$), SON was significantly different between CL, GL and FL ($P < 0.05$), but the difference between GL and FL was not significant ($P > 0.05$). The soil ROC was not significantly different between CL and GL ($P > 0.05$), while the difference between FL, CL and GL was significant ($P < 0.05$), and the contents of SOC and ROC of forest land were higher than that of CL and GL. In addition, from Table 2, it can be seen that under different land cover, the contents of SOC, SON and ROC all showed a significant decreasing trend with the increase of the soil depth ($P < 0.05$).

3.2 Characteristics of soil MBC and MBN under different land cover In this experiment, the contents of soil MBC and MBN under different land covers are shown in Table 3. From Table 3, it can be seen that there were differences in the MBC and MBN content of 0 – 100 cm soil under different land use types. Specifically, the average content of soil MBC in CL, GL and FL were 157.47, 161.63 and 176.13 mg/kg, respectively, showing FL > GL > CL. The average content of soil MBN in CL, GL and FL were 9.97, 15.09 and 36.78 mg/kg, respectively, showing FL > GL > CL.

Under different land use types, both soil MBC and MBN were significantly different between FL and CL and between FL and GL ($P < 0.05$), but there was no significant difference between CL and GL ($P > 0.05$). In addition, from Table 3, it can be seen that under different land use types, both soil MBC and MBN showed a significant decreasing trend with the increase of the soil depth ($P < 0.05$).

3.3 Canonical correspondence analysis (CCA) on environmental factors of different land cover and soil MBC and MBN The environmental factors discussed in this study include soil depth, SOC, SON, and ROC. Using the canonical correspondence analysis (CCA), we analyzed the relationship between environmental factors and MBC and MBN. We sorted different soil depths, SOC, SON, ROC, MBC, and MBN in different land cover into corresponding matrices and imported Canoco 4.5 software for processing. Using CCA method, we carried out ordination analysis, and obtained the two-dimensional ordination diagram of MBC and MBN (Fig. 1). The arrow in the figure represents the soil properties, and its quadrant represents the positive or negative of the correlation between the soil properties and the ordination axis; the length of the line indicates the correlation between soil properties and MBC and MBN, and the slope of the line on the ordination axis indicates the correlation between the soil properties and the ordination axis.

Table 3 Content of soil MBC and MBN under different land cover

Land use type	Soil depth//cm	MBC//mg/kg	MBN//mg/kg
CL	0 – 10	249.49 ± 0.57[a]	26.01 ± 0.43[b]
	10 – 20	147.86 ± 0.64[c]	9.99 ± 0.35[b]
	20 – 40	129.57 ± 0.74[b]	4.09 ± 0.63[c]
	40 – 60	137.93 ± 0.47[b]	5.61 ± 0.74[c]
	60 – 100	122.50 ± 0.32[c]	4.16 ± 0.46[b]
GL	0 – 10	166.60 ± 0.85[c]	22.89 ± 0.36[c]
	10 – 20	154.12 ± 0.51[b]	22.58 ± 0.32[a]
	20 – 40	151.75 ± 0.47[a]	10.29 ± 0.41[b]
	40 – 60	178.98 ± 0.62[a]	12.27 ± 0.54[a]
	60 – 100	156.68 ± 0.56[b]	7.45 ± 0.73[a]
FL	0 – 10	200.89 ± 0.87[b]	122.04 ± 0.64[a]
	10 – 20	174.36 ± 0.43[a]	20.65 ± 0.45[a]
	20 – 40	153.48 ± 0.42[a]	23.44 ± 0.63[a]
	40 – 60	178.34 ± 0.53[a]	9.62 ± 0.54[b]
	60 – 100	173.55 ± 0.41[a]	8.17 ± 0.72[a]

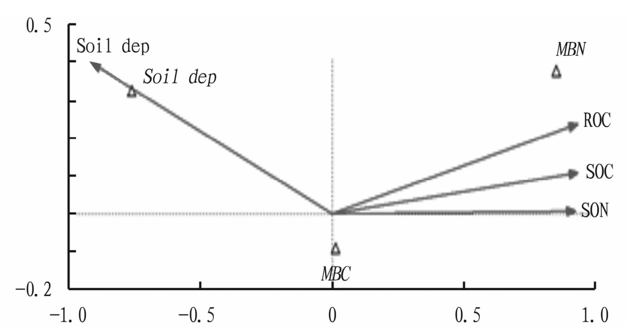

Fig. 1 CCA ordination diagram for environmental factors and soil MBC and MBN

From the CCA analysis (Fig. 1), according to the strength of the correlation, SOC, SON, ROC, MBC, MBN and the first axis of CCA all showed a very significant positive correlation ($P < 0.01$), the depth of the soil layer had a very significant negative correlation with the second axis ($P < 0.001$). In other words, MBC and MBN are mainly affected by the content of SOC and SON. If the content of SOC and SON is high, the content of soil MBC and MBN will be high, and vice versa. The content of soil SOC and SON is mainly affected by land use types. In other words, the content of soil MBC and MBN is also mainly restricted by land use types.

MBC and MBN are expressed in the form of triangles in the ordination diagram (Table 4). The eigenvalues of the first, second, third and fourth ordination axes were 0.141, 0.034, 0.004, and 0.002 respectively. The correlation coefficients of MBC and MBN with soil depth, SOC, SON and ROC were 0.991 and 0.955, respectively. The first axis of the CCA ordination explained 80.8% of the relationship between MBC, MBN and environment cumulatively, indicating that the first axis after CCA can better reflect the relationship between MBC and MBN and the environment in this area. In addition to the land use types, soil temperature and water content, pH, and vegetation type all affect the content of MBC and MBN.

Table 4 Summary of the CCA ordination axes

Item	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.141	0.034	0.004	0.002
Species-environment correlations	0.991	0.955	0.000	0.000
Cumulative percentage variance of species data	78.200	96.700	99.100	100.000
Cumulative percentage variance of species-environment // %	80.800	100.000	0.000	0.000

4 Discussion

4.1 Differences in SOC and SON content of different land cover The SOC is mainly affected by such factors as climate, vegetation, soil properties, and agricultural management measures. In this study, we found that the differences in land management measures under different land cover, the quality and quantity of litters, *etc.* are all important reasons for the differences in SOC content. Land use types directly change the surface cover, which is also the most important source of SOC. Vegetation coverage, root distribution, litter layer, *etc.* are all important factors influencing the SOC[18]. Through this study, we found the SOC content of FL was significantly higher than that of GL and CL. This was mainly due to the widespread distribution of woodland in this study area, often accompanied by a mixture of trees and shrubs (such as *Populus* sp. and *Rosa* sp.), and mixed coniferous and broad-leaved forests (such as *P. densata*, *P. likiangensis* var. *linzhensis*, and *Q. aquifolioides*) and other natural forests. The relatively stable stand structure accumulates abundant litter under the forest, which provides an important source for the accumulation of SOC. CL is strongly affected by human interference. Under the long-term selection of human activities, CL has less annual litter. CL fertilization is mainly by chemical fertilizer, and organic fertilizers are used less, and the above-ground part of the crop will be harvested after maturity, resulting in less external input of SOC in the cultivated land, which is consistent with findings of some studies[19]. Besides, farmers in the study area will not plow the land in time after harvesting the crops, but drive the livestock into the harvested land for feeding. This process will continue for 1 – 2 months, which will exacerbate the reduction of the external input of SOC in CL. Furthermore, GL in the study area has less management and protection. In addition, the area is a semi-agricultural and semi-pastoral area, the unreasonable grazing, livestock gnawing, and severe stamping, making the primary productivity of GL far lower than CL and FL. What's worse, the lack of scientific management of GL also restricts the accumulation of SOC.

The vertical distribution of SOC is closely related to factors such as litter, root distribution and external farming measures. In this study, we found that regardless of CL, GL or FL, the vertical change trend of SOC content decreased with the increase of soil depth ($P<0.05$). The SOC is mainly concentrated in the soil at a depth of 0 – 20 cm, and the SOC content at a depth of 20 cm or more is significantly higher than that at other soil layers, indicating that the degree of surface accumulation of SOC is relatively large, which is consistent with the results of previous studies[20]. For the FL, most of the branch and leaf residues and roots imported from the outside accumulate in the 0 – 10 cm soil, and release a large amount of nutrients into the soil after decomposition, which makes the surface SOC significantly higher than other soil layers, and the input of SOC in the deep soil is relatively small. On the other hand, root distribution will directly affect the vertical distribution of SOC content in the soil. For the FL, the root system is mainly distributed in the surface layer, which leads to higher surface SOC, and the plant root system in the deep soil layer is difficult to penetrate, and the distribution is less, so the SOC content gradually decreases from the surface layer to the deep layer. These indicate that the effects of litter, root distribution and soil disturbance on SOC are mainly in the surface soil, and have little effect on the middle and deep soils. The SOC content does not change significantly in the 0 – 20 cm soil of CL, mainly because 0 – 20 cm is the soil of the crop layer, and the organic matter entering the soil is more evenly distributed under the action of mechanical plowing, *etc.*, resulting in not obvious change of the SOC content in this layer, which is consistent with results of previous studies[10,21].

4.2 Effects of different land cover on ROC, MBC and MBN Under different land cover, the soil environment is different, and the degree of SOC decomposition and transformation is also different, so the soil ROC is also different[22]. The distribution of soil ROC in the 0 – 20 cm section is affected by factors such as soil vegetation environment, root distribution, biological activity, and artificial disturbance. There was no significant difference in soil ROC between CL and GL ($P>0.05$), while the difference between FL, CL, and GL was significant ($P<0.05$), and the soil ROC content of FL was higher than that of CL and GL. The CL is affected by human activities, and exposure of ROC components in the surface soil may reduce the stability of ROC and accelerate its oxidation process, manifested as the surface ROC lower than the deep soil[23]. However, in this study, we found that regardless of CL, GL, or FL, soil ROC content gradually decreases with the increase of soil depth, which may be related to total organic carbon, which is consistent with the results of Gong Yueyue *et al.*[10]. According to findings of Ma Heping *et al.*[24], the SOC content is an important factor affecting soil ROC, MBC and MBN content. If the SOC content is high, soil ROC, MBC and MBN content are also high. The change of soil total organic carbon content restricts the change of soil labile organic carbon (LOC) content. In this study, the change trend of soil ROC, MBC and MBN content is similar to the SOC content, which is consistent with the above research results.

4.3 Relationship between different land cover and MBC and MBN Soil organic matter is an important factor influencing the soil microbial biomass. The high content of organic matter can provide sufficient carbon, nitrogen and energy for the growth of soil microorganisms[25]. In this study, we found that the contents of MBC and MBN under different land cover are closely related to the contents of SOC and SON, showing a significant positive correlation. According to the CCA analysis, SOC, SON, ROC, MBC, MBN and the first axis of CCA all showed a very significant positive correlation ($P<0.01$). The eigenvalue of the first axis (0.141) was significantly greater than the eigenvalues of the second, third and fourth ordination axes (0.034, 0.004 and 0.002), indicating that the SOC content directly restricts the MBC content, that is, the soil MBC content is closely related to the soil SOC content, and there is a significant positive correlation, which is consistent with the research results of Zhou Chenni *et al*[26]. The change trend of soil MBN is consistent with the change trend of MBC. As explained above, the change of land use mode not only changes the vegetation structure and diversity on the ground, but also affects the change pattern and regulation mechanism of soil MBC due to changes in the input of above-ground and underground resources of plants. The content of soil MBC and MBN is mainly restricted by land use types, and also by the interaction of factors such as soil temperature, humidity, pH, and vegetation type, which is a very complex process.

5 Conclusions

(i) In this study area, the SOC content of FL is higher than that of GL and CL, and the vertical change trend of SOC content decreases with the increase of soil depth ($P<0.05$), and the SOC is mainly enriched in the soil at a depth of 0 – 20 cm. The SON also showed the same change trend as SOC. (ii) Under different land cover, the difference in soil ROC between CL and GL is not significant ($P>0.05$), while the difference between FL, CL and GL is significant ($P<0.05$), and the soil ROC content of FL is higher than CL and GL. The change trend of soil ROC, MBC and MBN content is similar to that of SOC. (iii) Under different land cover, the content of MBC and MBN is closely related to the content of SOC, showing a significant positive correlation. The change trend of soil MBN is consistent with the change trend of MBC. The soil MBC and MBN content are not only restricted by land use types, but also by the interaction of such factors as soil temperature, humidity, pH and vegetation types.

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