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RESEARCH ARTICLE

Huaqin XU, Runlin XIAO, Tongqing SONG

Effects of different fertilization on microbial biomass carbon from the red soil in tea garden

Abstract The present study examined the influence of the different fertilization on the dynamic of soil microbial biomass carbon (SMBC) of red soil in tea gardens. The results showed that straw mulching, intercropping, chemical fertilizer could all improve the amount of the soil microbial biomass C. The annual variation of microbial biomass C showed the tendency of ''low–high–low–high'', and the influences were variable with the time. For the annual average of soil microbial biomass C, Treatment 1(T_1) (straw mulching + 100% organic manure), Treatment 2 (T_2) (straw mulching + 75% organic manure + 25% fertilizer), Treatment 3 (T_3) (straw mulching + 50% organic manure + 50% fertilizer), Treatment 4 (T_4) (straw mulching + 25% organic manure + 75% fertilizer), Treatment 5 (T_5) (100% fertilizer),Treatment 6 (T_6) (intercropping white clover) were 17.05%, 32.38%, 32.05%, 24.30%, 26.23%, 24.63% higher, respectively, than CK, and the differences among all the treatments were significant ($P < 0.05$). The correlation of the SMBC with the active organic matter, the total nitrogen, the microbial biomass N, the microbial biomass P were remarkable, but no significant correlation was found with available nitrogen, total phosphorus, total potassium and moisture. Compared with other treatments, those mixed with organic matter and chemical fertilizer were more advantageous to enhance the soil fertility.

Keywords tea garden, different fertilization, soil microbial biomass carbon

1 Introduction

Subtropical hilly region is one of the major tea production regions in China. Most of the tea garden soil there is of red soil with low content of nitrogen and organic matter. How to effectively improve the status of soil nutrients and realize safe and high-efficient production and sustainable development of tea has become a concerned issue. Undoubtedly, applying organic fertilizer is the effective way for increasing content of soil nutrients, enhancing output of tea and improving quality of tea. Dong et al. (2000) demonstrated that after shading treatment and applying organic fertilizers in tea gardens, the free-form aromas in summer tea including linalool and geraniol increased significantly, which is helpful for improving the aroma quality of summer tea. Tang et al. (2006) found that applying organic fertilizers to covered tea trees could greatly strengthen the activity of β-glucosidase, and release bound alcohol aroma's main enzymes, thus enhance the aroma of tea. Song et al. (2006) and Xiao et al. (2005, 2006) applied the ecological control technologies including interplanting clover, covering tea trees with sun-shading net or with straws, which had great effects on improving soil physical and chemical properties of tea gardens and the tea quality. However, the reports concerning the effect of fertilizer application on tea garden soil microorganisms are still rarely seen at present.

Soil microorganism is the motive power for soil organic matter and soil nutrient transformation and cycling. The soil microbial biomass does not only play a key role in the cycling and transforming process of C, N, P, etc., but also serves as the most important ''warehouse'' and ''source'' of these nutrient elements. The soil microbial biomass carbon, as the active warehouse or source for soil nutrient transformation and the media for carbon cycling and turnover, has the significant positive correlation with the potential soil applicable nitrogen, thus being regarded as the most active part among soil organic matters, which can also show the effective status of soil nutrients and the change of biological activity after the soil is affected by the external world. Generally the soil microorganism's car-

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Huaqin XU, Runlin XIAO (✉), Tongqing SONG
Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China
E-mail: xiaorl@isa.ac.cn

Huaqin XU
Hunan Agricultural University, Changsha 410128, China

bon content is relatively constant, accounting for 40% to 50% of the dry matters. Voroney (1983) found that the organic carbon increment and soil microbial biomass extracted from the fumigated and unfumigated soils were highly correlated. The factors affecting soil microbial biomass carbon content include climate and environmental conditions, soil fertility, fertilizer application and crop planting patterns. The soil microbial biomass carbon transforms rapidly, which can reflect the change of soil organic matters before the change of total soil carbon is detected, and it is the soil quality index with more sensitivity. As the biological index for evaluating the soundness of soil, it has already been shown more and more concern by the scholars worldwide.

The aim of this paper was to describe the effects of straw mulching and intercropping clover on soil nutrient, dynamic changes of soil microbial biomass carbon, in order to provide an evaluation basis for soil soundness.

2 Methods

2.1 Experimental tea garden

Our experiment was conducted in Dongxishan Tea Plantation (113°19′E, 28°33′N, elevation 135 m), Changsha county, Hunan Province, China. The garden enjoys a subtropical monsoon climate, with mean annual temperature of 16.5°C–20.5°C, mean temperature of 11.9°C in January, 27.9°C in June, and the extreme low/high temperature in this region was −5.2°C/39.1°C. The accumulated effective temperature ($\geqslant 10$°C) is 6539°C. Annual mean precipitation is 1389 mm, 76% of which is in April to June. The red soil there was derived from granite. The tea cultivar was *Fuyun Zaohao Cha*, planted in February, 2001. Rows were arranged from east to west. Row spacing was 160 cm. Green manure crop was white clover (*Trifolium repens* Linn.), provided by Grass Scientific Department, Agricultural Academy of Hunan Agricultural University, and sowed in March, 2002, with the seed amount of 4.5 $kg\cdot hm^{-2}$. Straws to cover tea plants were from the local late rice, with a coverage on the tea rows of 15000 $kg\cdot hm^{-2}$ evenly. It was designed large scale with small areas contrasted experiments. Each area was 2 hm^2 and was arranged orderly. The ecological environment and management in each treatment were at the same level. The seven treatments, T_1, T_2, T_3, T_4, T_5, T_6 and CK, are listed in Table 1.

2.2 Investigation and analysis

Soil organic matter was measured with the K_2CrO_4 oxidation and $FeSO_4$ titration method. Water-soluble N was measured with alkali absorption method. Available P was extracted by $NaHCO_3$, and measured by colorimetric estimation. Available K was extracted by 1 $mol\cdot L^{-1}$ KCl and measured by flame photometer. Soil water content was measured with oven-dry method (Bao, 2000).

Microbial biomass carbon (MBC) was determined by a fumigation–extraction method (Vance et al., 1987). Fumigated and non-fumigated soils were extracted with 0.5 $mol\cdot L^{-1}$ K_2SO_4 for 30 min (1:5 soil: extractant ratio), filtered and the aliquot was analyzed for organic C by acid-dichromate oxidation. The additional oxidisable C obtained from the fumigated soils represents the microbial C flush and was converted to MBC using the formula: Microbial C=C flush/0.35.

All data collected were processed with Excel-2003, and ANOVA analysis was processed with SPSS.12.0

3 Results

3.1 Effects of different treatments on soil nutrient contents

Different treatments of fertility betterment enhanced the content of the major soil nutrients (Table 2). Except single fertilizer, the growth rates of the remaining organic matters were found ranged from 0.77% to 17.69%, the growth rate of total nitrogen ranged from 3.08% to 15.88%, the growth rate of total phosphorous ranged from 5.06% to 20.62%, the growth rate of total potassium ranged from 12.66% to 13.75%, and the growth rate of available

Table 1 Design of the experiment

treatment	straw mulching/ $kg\cdot hm^{-2}$	intercropping white clover/ $kg\cdot hm^{-2}$	organic manure/ $kg\cdot hm^{-2}$	fertilizer			
				carbamide/ $kg\cdot hm^{-2}$	calcium superphosphate/ $kg\cdot hm^{-2}$	KCl/ $kg\cdot hm^{-2}$	ternary compound fertilizer/ $kg\cdot hm^{-2}$
T_1	15000	0	6000	0	0	0	0
T_2	15000	0	3930	240	570	19.95	0
T_3	15000	0	1830	480	690	82.8	0
T_4	15000	0	1050	570	738	99	0
T_5	0	0	0	570	738	99	600
T_6	0	4.5	0	0	0	0	0
CK	0	0	0	0	0	0	0

Table 2 Effects of different treatments on soil nutrient contents

treatment	total N/g·kg^{-1}	available N/mg·kg^{-1}	organic matter/g·kg^{-1}	total P/g·kg^{-1}	total K/g·kg^{-1}
T_1	1.11 (0.18)	16.41 (0.17)	12.4 (0.40)	0.68 (0.47)	21.82 (0.41)
T_2	1.24 (0.51)	21.69 (0.63)	12.13 (0.33)	0.62 (0.19)	21.68 (0.39)
T_3	1.4 (0.92)	21.79 (0.64)	13.77 (0.73)	0.61 (0.11)	21.84 (0.42)
T_4	1.12 (0.23)	19.97 (0.37)	11.79(0.24)	0.64 (0.29)	21.71 (0.39)
T_5	1.14 (0.25)	24.98 (1.12)	11.71 (0.20)	0.65 (0.33)	21.63 (0.78)
T_6	1.13 (0.22)	18.98 (0.21)	11.89 (0.27)	0.75 (0.790	21.67 (0.59)
CK	1.07 (0.07)	12.31 (0.79)	11.7 (0.11)	0.61 (0.15)	19.2 (0.05)

Note: The figures in brackets are standard deviations.

nitrogen ranged from 33.3% to 102.92%. After covering the plantation with rice straws, the contents of organic matters and total potassium were effectively increased, owing to the fact that after the straw was decomposed, the elements of straws entered into the soil layer, providing abundant carbon and potassium for the soil. In the high-temperature drying season, some of the white clover plants will die, and form a coverage on the soil surface of tea garden. Being similar to the case of covering straws, the xylem and protein compound of white clover could not be easily decomposed, while the same remain in soil, and decomposed into soil organic matters and strengthened the content of organic matters in top soils. The nitrogen fixation of its roots also contributed a certain amount of increase to the content of total nitrogen in soil. However, although a single fertilizer also increased the content of total nitrogen and available nitrogen in soil, the increase of soil organic matters was found insufficient, the ratio of soil C/N was reduced and the large quantity of N element accumulated in soil made the N metabolism of tea plant enhanced, which is unfavorable for producing tea with good quality.

3.2 Impacts of different measures for fertility betterment on SMBC

3.2.1 Dynamic changes of SMBC

When comparing the changes of SMBC being treated in different ways in the whole year (Table 3), it could be found out that except in October, all T_5 SMBC values were higher than CK, and in May, June, July and August, all the differences reached the significant levels ($P < 0.05$). In May, the SMBC value was maximum, showed that after applying fertilizer, the increase of a large quantity of available N promoted the growth of microorganism. In addition, as the weather getting warmer, the activity of microorganism increased greatly. However, the ratio of C/N needed by microorganism had a certain scope. Due to the fact that the carbon source in soil was limited and the available nitrogen was sufficient, the decomposing of original organic carbon in soil was accelerated, resulting in the reduction of the total quantity of organic carbon accumulated in soil and the ratio of C/N of soil. The above situation also restrained the reproduction of microorganism, especially in the peak period (in June, July and August) for growth of tea, there was a contradiction between microorganism and tea, which was focused on the fighting for available carbon; therefore, the content of SMBC decreased gradually.

Except in April, all the SMBC values in T_1, T_2, T_3 and T_4 during the period from May to November were higher than CK. In May, June, July and August, the difference between T_1 and CK reached the significant level ($P < 0.05$). During the period from May to November, the difference between T_2 and CK reached the significant level. SMBC values in T_3 and T_4 in the whole year were higher than CK. The difference between T_3 and CK in April, May, June, July, August, October and November also reached the significant level. And the difference between T_4 and CK in May, June, July, August, October and November was significant.

When comparing with CK, the difference between T_6 and CK in May, June, July, October and November

Table 3 Effects of different treatments on SMBC community (mg·kg^{-1})

treatment month	T_1	T_2	T_3	T_4	T_5	T_6	CK
4	155.1b (7.3)	146.81a (4.2)	205.2d (2.9)	172.46c (6.7)	180.1c (5.0)	139.1a (3.5)	166.3c (17.3)
5	225.7b (19.2)	289.6c (5.7)	243.17b (4.9)	314.13d (17.1)	322.9d (10.3)	233.71b (14.8)	185.13a (27.3)
6	207.0b (40.5)	274.2d (4.5)	240.63c (20.3)	287.5d (13.1)	240.3c (26.8)	230.3c (17.7)	140.2a (3.7)
7	207.1b (6.1)	233.89d (21.8)	215.9c (10.5)	215.5c (1.78)	200.8b (28.5)	228.0d (11.1)	130.6a (9.2)
8	183.4b (14.7)	207.95d (10.4)	207.36d (32.9)	191.1c (21.8)	172.5b (6.6)	179.9b (35.5)	139.58a (4.3)
9	149.7a (25.1)	206.23d (24.8)	172.28b (5.6)	151.33a (16.5)	169.6b (3.3)	180.6b (1.6)	148.08a (23.4)
10	164.2a (22.2)	211.6d (6.3)	205.69c (18.4)	187.9b (14.4)	152.6a (35.1)	207.3c (4.6)	156.3a (17.5)
11	187.23b (24.1)	231.1b (33.3)	227.31d (11.9)	199.7c (7.8)	186.3b (4.3)	223.6d (21.1)	160.42a (5.9)

Note: The same letter in a column means no significant differences between treatments at $P < 0.05$. Standard deviations were in brackets.

reached the significant level ($P < 0.05$). Taking all figures in the whole year into consideration, in May, as the weather getting warmer, the activity of microorganism increased and the SMBC value increased as well. In May, June and July, the activity of microorganism and the SMBC value kept stable; while in August and September when it was drought, the clovers gradually died and the withered branches and leaves covered the soil, resulting in the increase of organic matters in soil and the ratio of C/N. The fertilizer was not applied and the clover's nitrogen fixation function became weak, therefore, the quantity of N in soil was reduced, which further restrained the reproduction of microorganism and made the value of SMBC reduced. In October, the weather got colder, making the drought of soil eased. Under this circumstance, the clover started to regain growth; therefore, the microorganism became active again and the value of SMBC gradually increased as well.

3.2.2 Comprehensive evaluation of SMBC

When comprehensively comparing the average value of soil microbial biomass carbon in the whole year, we found out that T_5 was 26.23% higher than CK and the difference between T_5 and Ck reached the significant level ($P < 0.05$). It is thus evident that fertilizer application greatly stimulated the proliferation of tea garden's soil microorganism, which was coincident with the result in the ecological system of farmland with low fertility, fertilizer could significantly enhance the activity of soil microorganism (Hu et al., 2006). However, Li et al. (2004) found that fertilizer application played no apparent role in stimulating and activating black soil microorganism. According to Cao et al. (2006), in the high-input and intensive agroecosystem, the fertility betterment of single fertilizer application beared the bad effect, which even restrained the microorganism; therefore, it is evident that its effect on soil microorganism exerted by single fertilizer application was related with the fertility of soil.

When comparing with CK, the average values of SMBC in T_1, T_2, T_3, T_4 for the whole year increased by 17.05%, 32.38%, 32.05% and 24.30% respectively, with all differences reaching the significant level ($P < 0.05$). This fact demonstrates that covering land with straws and applying organic and inorganic fertilizers at different ratios can greatly stimulate the proliferation of microorganism. There were no apparent differences among the average value of SMBC in treatments of T_2, T_3 and T_4, but when comparing them with T_1 and CK, we can find some apparent differences. The T_2 average value of SMBC was the highest, while T_1 beared the lowest figures. This situation may be caused by the fact that, in the experiment, the straw was directly applied in the farmland where crops were growing and the straw's ratio of C/N was higher. The soil accepted a large quantity of fresh organic carbon source; therefore, if the nitrogen element was not timely replenished in a comparatively long time, the competition for available nitrogen between crops and microorganism would occur, due to the fact that the C source was in the sufficient state, while the N source was in the insufficient state. Under this circumstance, the growth of microorganism would be restrained. Appropriately applying urea in the straw decomposing process, the ratio of C/N was reduced, which is always favorable for promoting the activity of microorganism. In July, August and September, the drought and high temperature season in Hunan Province, the T_2 SMBC value maintained high, and the microorganism remained relatively active in this period, which was favorable for supplying nutrients to tea in summer.

T_6 average value of soil microbial biomass carbon in the whole year was 24.63% higher than CK, with the difference reaching the significant level ($P < 0.05$). Intercropping white clover in tea garden effectively increased the microbial biomass carbon content in surface soil and enhanced soil fertility, which was coincident with the results by Peng et al. (2005) and Shen et al. (2006).

3.3 Correlation between SMBC and major fertility factors

Through conducting relevant analysis (Table 4) on soil microbial biomass carbon and major fertility factors, we can understand that soil microbial biomass carbon, active organic matters, soil total nitrogen, microbial biomass carbon and microbial biomass phosphorous had a significant positive correlation ($P < 0.05$), while they had no obvious correlations with the available nitrogen, soil total phosphorous, soil total potassium and soil moisture. According to Li et al. (2004) and Song et al. (2003), the microbial biomass carbon was very sensitive to different kinds of fertility betterment measures, and it could not be directly affected by inorganic nitrogen. This fact is one major advantage for microbial biomass carbon when it is used as an evaluation index in soil biology. The results of these studies further demonstrate that in the unique soil ecological environment of tea gardens, the soil microbial biomass carbon still enjoyed the high sensitivity, which

Table 4 The correlation coefficient between soil microbial biomass C and influencing factors

factor	correlation coefficient
microbial biomass N	0.7523^*
microbial biomass P	0.6524^*
available N	0.4275
organic matter	0.7024^*
total N	0.7682^*
total P	−0.2112
total K	0.3154
water content	0.3543

Note: All the figures in the table are the averages in growing season; * represents $P < 0.05$, $n = 7$.

can be taken as the effective index for evaluating the changes of a tea garden's fertility.

4 Discussion

Wang et al. (2006) found that the soil microbial biomass carbon in subtropical red soil hilly forest land and drought land mainly ranged from 100 mg·kg^{-1} to 500 mg·kg^{-1}. In our experiment, the microbial biomass carbon in the tea garden, which was treated for fertility betterment for four years, ranged from 100 mg·kg^{-1} to 250 mg·kg^{-1}, indicating that such soil's fertility was relatively low and may be attributed to the many years of tea planting, where the acid soil was formed in the tea garden, making the microorganism's growth and metabolism became slower under such acid environment and its ability for mineralizing organic carbon became lower as well (Xue et al., 2005).

Comparing the application of single fertilizer with the application of adequate amount of organic materials, the combined application of organic materials and fertilizers is more favorable for improving the fertility of soil; for this reason the straws, covering the garden land, provided a good environment and energies for soil microorganism during its decomposing process, and replenished an appropriate amount of available nitrogen and adjusted the soil C/N ratio, and then provided rich carbon and nitrogen sources to soil microorganism and substantially enhanced the activity of soil microbial biomass.

Interplanting clover in tea gardens, an ecological measure for improving soil fertility, has already been shown more and more concern by researchers and tea growers. The economical application of nitrogen in legume and non-legume's interplanting is a hot issue worldwide. The nitrogen application's niche separation is the main mechanism in legume and non-legume's interplanting system, while the mutual benefit of interspecific nitrogen becomes more apparent under the low productivity of the system. Our study showed that, without applying fertilizers, the soil microbial biomass carbon content in the tea garden interplanted with white clover was still increased significantly, and white clover plants effectively adjusted the tea garden's ecological system (Song et al., 2006; Xiao et al., 2005) in terms of water content, temperature and pest control. Undoubtedly, intercroppping white clover in tea garden is a completely new thought for establishing a safe mode for tea garden in the subtropical hilly regions. However, how to further adjust the activity of soil microorganism, reasonably apply fertilizers and realize the virtuous cycling of nutrients in tea garden's ecological system using the white clover's nitrogen fixation function still requires further studies.

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