EFFECTS OF CONSECUTIVE TURNOVER OF GREEN MANURE AND N FERTILIZER ON SOIL MICROBIAL BIOMASS AND ENZYME ACTIVITY IN TOBACCO-PLANTED FIELD

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ABSTRACT

The objective of this study is to investigate the effects of consecutive turnover of green manure (ryegrass (Lolium perenne L.)) on soil microbial biomass C and N and enzyme activities. The results showed that the incorporation of green manure in tobacco-planted fields generally significantly enhanced enzyme activities (soil para nitrophenol (PNP), urease, catalase, and sucrase) and soil microbial biomass contents. The extent of enhancement increased with the increase in consecutive turnover. The C and N contents in microbial biomass were significantly lower in conventionally cultivated soil than in soils from the green manure-incorporated field. This finding indicated that incorporation of green manure could stimulate the activity of soil microorganisms. The average improvement gained from the T3 treatment (three-year consecutive cultivation of green manure since the harvest of tobacco) relative to the T0 treatment (conventional cultivation without any green manure) over the whole growth period of tobacco was 1.34 to 1.52 times for urease activity, 1.11 to 1.19 times for PNP activity, 1.58 to 1.71 times for sucrase activity, and 1.24 to 1.50 times for catalase activity. Overall, the activities of soil enzymes correlated well with the soil microbial biomass C and N. This study provides guidance on the measurement of green manure to manage the activation of soil microbial biomass and improve the quality of tobacco-planted soil.

Key words: Enzyme activity, Green manure, Soil microbial biomass, Tobacco.

INTRODUCTION

Soil compaction, declining soil fertility, and decreasing soil microbial biomass diversity are increasingly more serious in tobacco-planted fields because of the long-term application of chemical fertilizers (Zhang, 2010). The phenomena (soil compaction, declining soil fertility, et al.) are important limiting factors for further improvement of tobacco quality in China (Liu et al., 2006). The application of green manure to soil is considered a good management practice in tobacco agricultural production systems (Li, 2013). It increases cropping system sustainability by reducing soil erosion and ameliorating soil physical properties (Smith et al., 1987) by increasing soil microbial biomass C and N (Tejada, 2008), increasing enzyme activities (Li, 2011), and stimulating soil microbial growth and activity (Eriksen, 2005). Planting and incorporation of green manure during winter are important to restore and improve soil fertility and achieve the sustainable development of tobacco production (Liu, 2006).

Soil microbial biomass is not only used as an indicator of soil quality, it is also one of the main agents that controls the cycle of important nutrient elements in terrestrial ecosystems (Zahir Shah, 2010). Soil microbial biomass C and N are important parameters in nutrient conversion and circulation of soil C and N, they reflect the levels of soil microbes

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and fertility (Wang, 2004). Soil enzymes are metabolic products from animal and plant residue decomposition, plant root exudates, and soil microbes, which are special components of biochemical activity and involved in many important biochemical processes in soil (Burns, 1978). Soil enzyme activity is an important indicator of soil fertility, it represents the degree of material metabolism in soil and reflects the status of crop nutrient uptake and utilization and crop growth and development (Mijangos, 2006). In recent years, research on soil microbial and enzyme activity has gained increased interest. Several methods have been used to study soil microbial and enzyme activity including conservation tillage practices (Zhao, 2009; Yang, 2010), land use patterns (Yang, 2005; Yu, 2008; Xu, 2009), application of fertilizers (Ma, 2007), and incorporation of crop residues (Song, 2002; Cao, 2006). Few studies have investigated the effects of incorporation of green manure on soil microorganisms and enzyme activities in tobacco fields. In particular, the dynamic changes in soil microbial biomass and soil enzyme activity have not been reported throughout the growth period of tobacco after the consecutive turnover of green manure. Green manures, also referred to as fertility building crops, may be broadly defined as crops grown for the benefit of the soil. They can improve microbial biomass and soil organic fertility (Zahir, 2010) and enhance tobacco quality (Shi, 2002; Si, 2011).

The aims of this study are as follow: (1) to examine the quantitative relationship between the returning ages of the incorporation of green manure and soil microbial biomass C and N; (2) to analyze the quantitative relationship between the returning ages of the incorporation of green manure and soil enzyme activities; and (3) to describe the quantitative variation trend of soil enzyme activities and soil microbial biomass C and N at different treatment soils in different growth periods of tobacco.

MATERIALS AND METHODS

The field experiment was conducted from 2005 to 2008 at Wu Long County (E107°33.5882, N29°16.5932, Altitude 1036 m) in Chongqing of China. The experiment commenced with the planting of ryegrass (Lolium perenne L.) soon after the tobacco harvest in October. After a 150 d growth period, ryegrass was incorporated into the soil receiving 171 kg ha⁻¹ N (NO₃⁻) as fertilizer along with a basal dose of P (P₂O₅) and K (K₂O). The soil chemical properties in the experimental site were as follow: pH, 5.4 - 5.5; soil organic matter, 24.1 g kg⁻¹ to 24.2 g kg⁻¹; available nitrogen, 124.6 mg kg⁻¹ to 124.7 mg kg⁻¹; available phosphorus, 21.8 mg kg⁻¹ to 21.9 mg kg⁻¹; and available potassium, 150.7 mg kg⁻¹ to 150.9 mg kg⁻¹. The experimental tobacco variety, Yunyan87, was transplanted on 5 May, three replications for per treatment.

The experiment was designed with four treatments and three consecutive turnovers of green manure: T0 (conventional cultivation without any green manure), T1 (one-year cultivation of green manure since the harvest of tobacco), T2 (two-year consecutive cultivation of green manure since the harvest of tobacco), and T3 (three-year consecutive cultivation of green manure since the harvest of tobacco). The green manure biomass was incorporated 22,500 kg ha⁻¹ annually.

Soil cores were taken from each plot between two randomly selected tobacco plants at a depth of 0 cm to 20 cm 10 d (planting period), 30 (rosette stage), 45 d (vigorous growth stage), 60 d (budding stage), 75 d (toping stage), and 90 d (rip stage) after the tobacco was planted. Soil cores from each plot were bulked, transported in a cooler to the laboratory, stored at 5 °C and then analyzed for soil microbial biomass C and N and enzymatic activities.

Microbial biomass C and N were estimated using the chloroform fumigation incubation method of Brookes (1985) and Vance (1987). The activities of urease and phosphatase were determined by colorimetry (Guan, 1986). Catalase activity was measured quantitatively by titration with potassium permanganate (Paterson, 1984). Sucrase activity was measured using the 3, 5-dinitrosalicylic acid method of Sumner (1935).

RESULTS AND DISCUSSION

Effects of consecutive turnover of green manure on soil microbial biomass C and N

Microbial biomass C is a sensitive indicator (Bradley, 1944) and important component of soil organic matter. The effective C pool of soil modulates C elements and nutrient flow, whereas the static C pool only affects soil properties (Blair, 1995). The
soil samples were analyzed for microbial biomass C after three years of experimentation. Soil microbial biomass C was significantly affected by the turnover times of green manure (Table 1). Microbial biomass C was highest in T3 and lowest in T0. The content of soil biomass C was highest at 30 d after transplantation of green manure (T1, T2, T3) and increased by 31.0% to 67.1% after the three-year consecutive turnover of green manure compared with the control. The amount of microbial biomass C increased with increasing age of the incorporated green manure. This result is partly due to the fact that most incorporated green manure materials decompose and mineralize to release nutrients usually between 3 and 6 wks (Arakeri, 1962; Lalljee, 1995). Differences in the microbial biomass C content suggest that it is affected by different management practices of green manure as described by Zahir (2010).

Microbial biomass N indicates the N pool in the soil and is important in the regulation of soil N cycling and transformation processes (Schnure, 1987). Table 2 presents a summary of the descriptive statistics of microbial biomass N from the four treatments. The table shows that the microbial biomass N was about twice as high in T3 as in T0. The concentrations of microbial biomass N in the four treatments were significantly different and showed rapid fluctuations; N contents were highest 30 d and 75 d after transplantation of manure. Green manure decomposes rapidly and releases N quickly. The results showed that the soil microbial biomass N increases with increasing age of the incorporated green manure. In comparison with the control group, the contents of soil microbial biomass N increased by 23.0% to 145.1% after a three-year consecutive turnover of green manure. Soil microbes can assimilate available N in soil to multiply and a large amount of N is fixed by microbes. N is subsequently consumed and released from soil microbes for tobacco growth and development. From the vigorous growth stage to the topping stage during the rainy season, the remnants of organic materials are further decomposed by soil organisms and the excess N is again fixed. Therefore, the maximum microbial biomass N was observed at the resettlement stage and topping stages; low contents were observed at the vigorous growth and budding stages. This finding shows that incorporation of green manure adds an organic C source and improves the physical-chemical properties of soil. It also stimulates the activity of soil microorganisms, as described by Yadvinder (2005), and helps fulfill the nutrient requirements of tobacco.

### Effects of consecutive turnover of green Manure on soil PNP

Phosphatases are enzymes that produce nutrient mineralization and liberation of products that are important in plant nutrition (Dick, 1996). These enzymes are involved in the P cycle in soil. In particular, acid phosphatase provides a potential index of mineralization of soil organic P (Eivazi, 1977). The level of phosphatase activity can reflect the status of soil available P (Guan, 1986). Numerous studies show that the activity of soil phosphatases improves by incorporation of green manure (Shah, 2010; Gajda, 2005; Tejada, 2008). The present study observed the effect of green

### TABLE 1: Effect of turnover of green manure on microbial biomass C of the soil in different days after transplanting (mg kg⁻¹).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>10 (d)</th>
<th>30 (d)</th>
<th>45 (d)</th>
<th>60 (d)</th>
<th>75 (d)</th>
<th>90 (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3</td>
<td>137.34 a</td>
<td>194.74 a</td>
<td>127.78 a</td>
<td>166.32 a</td>
<td>144.78 a</td>
<td>156.27 a</td>
</tr>
<tr>
<td>T2</td>
<td>110.53 b</td>
<td>163.62 b</td>
<td>123.34 a</td>
<td>146.51 b</td>
<td>122.65 b</td>
<td>126.32 b</td>
</tr>
<tr>
<td>T1</td>
<td>105.89 bc</td>
<td>130.79 c</td>
<td>105.62 b</td>
<td>133.34 b</td>
<td>112.67 c</td>
<td>121.67 b</td>
</tr>
<tr>
<td>T0</td>
<td>102.15 c</td>
<td>116.52 d</td>
<td>90.53 c</td>
<td>110.94 c</td>
<td>110.55 c</td>
<td>105.21 c</td>
</tr>
</tbody>
</table>

Mean values in each column with the same letter(s) are not significantly different using Duncan tests at 5% of probability.

### TABLE 2: Effect of turnover of green manure on microbial biomass N of the soil in different days after transplanting (mg kg⁻¹).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>10 (d)</th>
<th>30 (d)</th>
<th>45 (d)</th>
<th>60 (d)</th>
<th>75 (d)</th>
<th>90 (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3</td>
<td>40.49 a</td>
<td>60.25 a</td>
<td>36.34 a</td>
<td>27.56 a</td>
<td>67.32 a</td>
<td>49.27 a</td>
</tr>
<tr>
<td>T2</td>
<td>37.56 b</td>
<td>54.39 a</td>
<td>23.68 b</td>
<td>22.77 ab</td>
<td>53.66 b</td>
<td>26.83 b</td>
</tr>
<tr>
<td>T1</td>
<td>35.65 b</td>
<td>45.24 b</td>
<td>18.17 c</td>
<td>15.67 bc</td>
<td>41.37 c</td>
<td>21.65 c</td>
</tr>
<tr>
<td>T0</td>
<td>32.93 c</td>
<td>37.50 c</td>
<td>14.83 d</td>
<td>11.88 c</td>
<td>31.17 d</td>
<td>20.34 c</td>
</tr>
</tbody>
</table>

Mean values in each column with the same letter(s) are not significantly different using Duncan tests at 5% of probability.
manure on the PNP activity of soil as shown in Fig. 1. The figure shows that soil PNP activity is significantly enhanced by incorporation of green manure, and increases and decreases in the tobacco growth were observed. The maximum PNP activity was observed at the vigorous growth stage (45 d). The PNP activity increased as the age of the incorporated green manure increased. Green manure increased the PNP activity of T1, T2, and T3 by 1.06, 1.20, and 2.14 times, respectively, compared with T0. The dynamics of PNP activity showed the P demand of tobacco and the available P supply from the soil. During the vigorous growth stage of tobacco, the demand for P increases. The peak of PNP activity appearing at different treatments shows that the amount of P is enhanced by incorporation of green manure, especially over several years of consecutive turnover of green manure. Similar results of the close relation between the content of P, PNP activity, and incorporated green manure have been presented by Xian (2011) and Xu (2011).

**Effects of consecutive turnover of green manure on soil urease**

Urease is a hydrolytic enzyme that attacks the N and C bond in amide compounds (Guillermina, 2007). Urease is directly involved in the conversion of N compounds. The availability of the soil N supply is reflected by the urease activity to a certain extent, the level of urease activity in response to a certain event, and the actual soil N supply (Guan, 1986). Urease activity was significantly increased in the green manure-treated fields compared with the conventional cultivation plots (Fig. 2). In our field study, urease activity increased in a similar way as the PNP activity.
trial, the highest urease activities were obtained over the three-year green manure treatment, and the lowest, values were observed in the conventional monocultured soil. The dynamic variation trend of urease activity was similar in all treatments over the whole growth period of tobacco. The highest urease activities occurred in the vigorous growth stage in different treatments. Urease activity were 1.19, 1.34, and 1.52 times greater in the T1, T2, and T3 plots, respectively, than in the T0 plots. Soil enzymes were released from microbial activity, root exudates, and decomposing plant and animal residues (Guan, 1986). The returning age of the incorporated green manure enhanced the increases in soil microbial biomass and urease activity. This result is similar to those found by Bolton (1985) and Thind (2000). Therefore, coordination between soil N transformation, accumulation, usability and soil nutrient preserving capability can be reflected by the urease activity.

**Effects of consecutive turnover of green manure on soil catalase**

Catalase which is widely present in soil and in vivo, is directly involved in respiration. It also removes the toxic hydrogen peroxide from the respiration process (Guan, 1986). Measurements of catalase activity were significantly lower in T0 than in T1, T2, and T3 in tobacco field, and the catalase activity increased with increasing age of the incorporated green manure (Fig. 3). The activity of catalase was increased to 1.24 to 1.50 times that of the control after three consecutive year of green manure turnover. This enhancement in activity is due to the increased nutrition brought about by microbial activity in soil as stimulated by the green manure. A similar variation trend of soil catalase activity was observed in all four treatments. Catalase activity initially decreased, then increased, decreased again, increased, and then decreased again with the growth of tobacco. The study of Han (2010; 2010) found a similar relation between the catalase activity and growth stages of tobacco.

**Effects of consecutive turnover of green manure on soil sucrase**

Sucrase is the name given to a number of enzymes that catalyze the hydrolysis of sucrose to fructose and glucose. Sucrase activity can be used to reflect the level of soil fertility (Zhang, 2001), and it is an important index of biological activity. In our experiment, the activity of sucrase was respectively lowest and highest in the budding and rip stages of tobacco growth in all treatments. This result is similar to that found by Zhang (2010). In comparison with conventionally treated soil, sucrase activity markedly increased by incorporation of green manure and with increasing returning age (Fig. 4). The average improvement in sucrase activity gained by T3 relative to T0 was 1.58 to 2.73 times over the whole growth period of tobacco. Liu (2010) suggested the positive effects of incorporation of green manure on soil sucrase activity. The increase in sucrase activity by incorporation of green manure treatment could be due to manure residues that are subject to rapid degradation. The presence of more organic matter is likely to provide a large amount of microbial biomass because organic matter serves as a source of energy for soil microorganisms.

**CONCLUSIONS**

A field experiment was conducted for three consecutive years to study the effects of incorporation of green manure on soil microbial biomass C and N and soil enzyme activities in paddy soils from Wulong County, Chongqiong, China. The results showed that microbial biomass C and N and soil enzyme activities are sensitive to green manure application on a short-term basis. Soil microbial biomass C and N and enzymatic activities were also closely related. Similar variation trends were observed with the increase in returning age of the incorporated green manure. During the whole growth period of tobacco, the contents of soil microbial biomass C and N increased by 31.0% to 67.1% and 23.0% to 145.1%, respectively, compared with the control after a three-year consecutive turnover of green manure. Soil microbial biomass C and N were significantly affected by the turnover times of green manure. The average improvements gained by the soil urease, PNP, sucrase, and catalase activities in the T3 treatment relative to the T0 treatment were 1.34 to 1.52, 1.11 to 1.19, 1.58 to 1.17, and 1.24 to 1.50 times, respectively, over the whole growth period of tobacco. In conclusion, incorporation of green manure can be a useful management practice to enhance microbial C and N and soil enzyme activity, improve the physical-chemical properties of soil and provide a suitable soil environment for tobacco. Our results indicate that the microbiological attributes
are highly responsive and sensitive to the beneficial influence of the incorporated green manure. Therefore, crop rotation between green manure and tobacco should be done in tobacco-planted fields. Future research should focus on the comprehensive evaluation of tobacco quality, including the influences of returning ages of incorporation of green manure, soil microbial biomass dynamics, and soil enzyme activities in the field.

**REFERENCES**


