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*Full Length Article*

# Soil Microbial Biomass Characteristics and Activity at different Stages of Forest Succession in Mid-subtropical Region of China

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## Abstract

The soil samples of 0 to 10 cm layer and 10 to 20 cm layer were compared between a pine (*Pinus massoniana*) forest (PF), a pine and broadleaf mixed forest (MF) and an evergreen broadleaf forest (BF), representing different succession stages of evergreen broad-leaf forest, to investigated the effects of forest succession on soil microbial biomass characteristics. From PF to BF there was an increase in soil organic carbon (SOC), total nitrogen (TN), and microbial biomass carbon ($C_{mic}$). At the 0–10 cm layer from PF to BF, SOC, TN, $C_{mic}$ and microbial biomass nitrogen ($N_{mic}$) increased. The correlation analysis showed that: $C_{mic}$ and $N_{mic}$ were strongly correlated to SOC and TN. In addition, contribution of $C_{mic}$/SOC was the lowest in PF, and was significantly and negatively correlated to SOC TN and strongly correlated to $C_{mic}$ and $C_{mic}/N_{mic}$. Soil respiration was strongly correlated with SOC, TN and $C_{mic}$. The $qCO_2$ was significantly and negatively correlated to SOC, $C_{mic}$ and $N_{mic}$. Succession from PF to MF to BF leaded to an increase in soil microbial biomass and soil nutrients. The quality of litter and the improvement of soil fertility are the main cause of microbial biomass increases with the succession process gradually. 

**Keywords:** Evergreen broad-leaf forest; Soil microbial biomass; Microbial activity; Mid-subtropical region
**Abbreviations:** pine (*Pinus massoniana*) forest (PF), pine and broadleaf mixed forest (MF), evergreen broadleaf forest (BF), microbial biomass carbon ($C_{mic}$), nitrogen ($N_{mic}$), soil organic carbon (SOC) and total nitrogen (TN), Metabolic quotient ($qCO_2$)

## Introduction

Soil microbial biomass can be regarded as the momentum for the alteration and circulation of soil organic matter and soil nutrients, and can be reserve storage of plant available nutrients in the soil (Sicardi *et al*. 2004). It is very sensitive to changes of biotic factors and abiotic factors. Despite different site conditions, there are large differences in soil microbial biomass in forests with different species composition (Wardle 1992). Soil microorganisms is a moderator of soil material cycle as well as a part of soil organic carbon (SOC) pool and available nutrients, and is functionally important in the soil ecological process (Wang *et al*. 2006). Soil microbial activity regulates and controls soil carbon acquisition capacity, carbon mineralization process, nutrients cycling and ecosystem productivity (Han *et al.* 2007).

Evergreen broad-leaved forest in central subtropical region of China has suffered from great damages, leaving few natural forests, and most remaining forests are secondary and man-made forests. Although it is generally believed that soil microorganisms are instrumental in ecosystem, study on how soil microbial biomass and microbial activity change during succession stages of evergreen broad-leaved forests and what impacts will it bring to the carbon cycle of regional forests soil is still lacking **(**Wang and Wang 2011**)**. The study on the change law of soil microbial biomass and microbial activity in the succession stages has theoretical and practical importance in revealing the natural recovery law and soil carbon sequestration of evergreen broadleaf forests in central subtropical region.

We studied the soil microbial biomass and activity of three forest types representing the forest restoration stage



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which include pine forest (PF), mixed pine and broad-leaved forest (MF), and evergreen broad-leaved forest(BF) (Table 1) to explore the effect of forest succession on soil microbial biomass characteristics and provide scientific evidence for the recovery of natural secondary forests.

## Materials and Methods

### Site description

The study area is located in the Yingzuijie nature reserve of Hunan Province (26°46′–26°59′N, 109°48′–109°58′E). The reserve is located in the transitional zone between the Yunnan Guizhou Plateau and the mountainous area on the South Bank of the Yangtze River. The altitude of the reserve is between 270 and 938 m above mean sea level. The climate in this area is humid subtropical monsoon climate, with an average annual temperature of 15.9°C from 1990 to 2010. The annual average precipitation was 1400mm, 76% of which occurs between April and August (Zeng *et al.* 2013, 2015).

There are three types of natural vegetation in the reserve: PF, MF and BF, aged between 25 and 70. They represent the series from pioneer to climax. The soil texture is classified as clay loam. The soil mainly comes from slate and shale, and is classified as Oxisol under the USDA taxonomy. From May to July 2010, three plots of 20 m × 20 m were established for each forest type (Zeng *et al.* 2015).

### Soil sampling and analysis

Soil samples were collected at depths of 0–10 and 10–20 cm for each plot. Each sample is divided into two parts. One of them was screened immediately through a 2 mm sieve and then stored at 4°C until analysis of microbial biomass carbon and nitrogen and basal respiration for up to 3 days. The second part was to air drying the sample and then grinding (Zeng *et al.* 2015).

Soil organic carbon (SOC) and total nitrogen (TN) were determined using Vario-MAX C/N auto-analyzer (made in Germany; Elementar) as described by Liu (1996). Chloroform fumigation-extraction method was used to estimate soil microbial biomass C, N ($C_{mic}$, $N_{mic}$) as reported earlier (Joergensen and Brookes 1990; Lin *et al.* 1999). Basal respiration was determined by measuring $CO_2$ evolution. Metabolic quotient or $qCO_2$ (μg $CO_2$-Creleased $mg^{-1}$ biomass C $h^{-1}$) was calculated as the ratio of basal respiration and $C_{mic}$. The microbial quotient was calculated as the ratio of $C_{mic}$ to SOC (Zeng *et al.* 2015).

### Statistical analysis

Experimental design was completely randomized. One-way analyses of variance (ANOVA) were used to test for significant differences in SOC and TN and microbial properties between PF, MF and BF. The least significant differences (LSD) were calculated when treatments were significantly different. The relationships between soil microbial properties and nutrients were analyzed. Analyses were performed with SPSS release 13.0, and the significant level was fixed at 0.05(Zeng *et al.* 2015).

## Results

### Soil carbon and nitrogen in succession stages

As is shown in Table 2, the average SOC and TN content of three forests in the soil layer of 0–10 cm was higher than that in the soil layer of 10–20 cm. When it came to the same soil layer, the SOC average content decreased from BF to PF. In the soil layer of 0–10 cm, the SOC average content of PF indicated significant ($P<0.05$) difference from that of BF and PF. In the soil layer of 10–20 cm, the SOC average content of BF showed significant ($P<0.05$) differences with that of PF and MF.

As shown in Table 4 TN and SOC showed a significant positive correlation ($r=0.989$, $P<0.01$). In the same soil layer, the average TN content increased from PF to BF. In the soil layer of 0–10 cm, the average TN content of PF revealed significant ($P<0.05$) difference as compared to BF and MF. In the 10–20 cm soil layer, there was significant ($P<0.05$) difference of TN average content between PF and BF. For different soil layers in different succession stages, the soil carbon and nitrogen ratio of MF was higher than that of other forests, and there are significant ($P<0.05$) differences among three forests.

### Soil microbial biomass carbon and nitrogen in succession stages

The $C_{mic}/N_{mic}$ reflects the ratio of bacteria and fungi in the soil. In this study, average content of $C_{mic}$ and $N_{mic}$ of forests changed in accordance with the change of the average content of SOC, and decreased with the soil depth. In the same soil layer, the variation pattern of $C_{mic}$ and $N_{mic}$ increase from PF to BF. There were significant ($P<0.05$) differences in $C_{mic}$ and $N_{mic}$ between soil layers. In the soil layer of both 0–10 and 10–20 cm, the $C_{mic}$/ $N_{mic}$ increased from BF to PF, and there were significant ($P<0.05$) differences among the forests (Fig. 1).

### Soil microorganism's activity in succession stages

In this study, there were significant ($P<0.05$) differences among three forests about the microbial quotient (Table 4). Besides, the microbial quotient had a significant negative correlation with SOC and TN ($r = -0.619$, $r = -0.635$, $P<0.05$), and had significant positive correlation with $C_{mic}$ and C/N ($r = 0.685$, $r =0.624$, $P<0.05$) (Table 3).

As is shown in Table 3, in the soil layer of 0–10 cm, the soil basal respiration increased from PF to BF, but there were no significant($P>0.05$) differences among the forests.

**Table 1:** The stand characteristics under different succession stages of the evergreen broad-leaved forest (mean ± SE; n=3)

| Forest type | Stand age (a) | Average DBH (cm) | Average height (m) | Wood plant density ($plan.hm^{-2}$) | Slope aspect | Slope gradient/ (°) | Bulk density of soil ($g.cm^{-3}$) |
|---|---|---|---|---|---|---|---|
| PF | 25~30 | 16.35±0.51 | 13.5±0.31 | 1100±51 | Southeast | 15 | 1.36±0.04 |
| MF | 45~50 | 17.23±0.45 | 13.9±0.25 | 1325±55 | Southeast | 15 | 1.32±0.03 |
| BF | 65~70 | 19.2±0.68 | 14.8±0.32 | 1150±52 | Southeast | 15 | 1.29±0.04 |

PF, a pine forest; MF, a pine and broadleaf mixed forest; BF, an evergreen broadleaf forest

**Table 2:** Concentrations of soil nutrients under the pine forest (PF), the pine and broadleaf mixed forest (MF) and the evergreen broadleaf forest (BF)

| Forest | Depth (cm) | SOC (g/kg) | Total N (g/kg) | C/N | pH |
|---|---|---|---|---|---|
| PF | 0–10 | 20.29(1.41) b | 1.18(0.07) b | 17.19(0.49) a | 4.19(0.08) a |
| MF | | 23.97(1.29) b | 1.78(0.16) a | 13.46(0.29) b | 4.25(0.06) a |
| BF | | 41.96(4.16) a | 2.33(0.2) a | 18.01(0.36) a | 4.24(0.03) a |
| PF | 10–20 | 13.43(0.89) b | 0.90(0.05) b | 14.92(0.22) a | 4.24(0.1) a |
| MF | | 15.14(1.42) b | 1.49(0.21) a | 10.16(0.17) c | 4.33(0.03) a |
| BF | | 21.87(1.29) a | 1.80(0.13) a | 12.15(0.21) b | 4.38(0.08) a |

Note: Means in the same column with different letters differ significantly at $P$<0.05 level

**Table 3:** Pearson's correction coefficients of soil properties under different succession stages of the evergreen broad-leaved

| Variables | SOC | $qCO_2$ | Basal respiration | $C_{mic}$ /SOC | $C_{mic}/N_{mic}$ | $N_{mic}$ | $C_{mic}$ |
|---|---|---|---|---|---|---|---|
| TN | 0.989** | 0.138 | 0.612* | -0.635* | 0.141 | 0.762** | 0.987** |
| SOC | | -0.846** | 0.651* | -0.619* | 0.273 | 0.725** | 0.996** |
| $qCO_2$ | | | 0.213 | 0.109 | 0.153 | -0.857** | -0.859** |
| Basal respiration | | | | 0.218 | 0.146 | 0.191 | 0.643* |
| $C_{mic}$ /SOC | | | | | 0.624* | 0.109 | 0.685* |
| $C_{mic}/N_{mic}$ | | | | | | -0.732** | 0.357 |
| $C_{mic}$ | | | | | | | 0.756* |

(Note): *$P$< 0.05; **$P$<0.01

In the soil layer of 10–20 cm, the soil basal respiration increases from PF to MF, but there are no significant ($P$>0.05) differences among the forests (Table 3). In this study, the soil basal respiration has a significant positive correlation with SOC, TN and $C_{mic}$ (r > 0.55, P<0.05) (Table 3). In the same soil layer, $qCO_2$ increases from BF to PF, and there are significant differences between PF and BF (P<0.05) (Table 4). The $qCO_2$ has a significant negative correlation with SOC, $C_{mic}$ and $N_{mic}$ (r< -0.54, P<0.01) (Table 3).

## Discussion

The soil TN concentration in PF is significantly lower than that of other forests, which indicates that N is likely to be a limiting factor for the growth of pines (Zeng *et al.* 2013). The SOC and TN concentration in MF and BF is higher than that of PF, which is likely to be the result of the increase in litter input and the decrease in surface litter decomposition products infiltration. As is indicated in the study, litter production of secondary broadleaved forest was large with relatively high SOC and TN content, while pure *Cunninghamia lanceolata* plantation was relatively low (Wang and Wang 2011). The litter of pine decomposes at a slow speed and leaves a large amount of phenolic compounds and lignin (Scholes and Nowicki 1998). In the stage of MF and BF, the invasion of broadleaf tree species improves the quality of the litter (Zhang *et al.* 2008), which gives back higher nutrients to the soil through litter composition than PF do and acquires higher supply of soil organic carbon.

In this study, the average $C_{mic}$ and $N_{mic}$ content in BF was higher than that of PF and MF. The forest litter content increased with the community succession, and the increased soil porosity and reduced soil bulk density are beneficial for the accumulation of SOC and microbial biomass (Huang *et al.* 2013). For BF, the litter C/N was relatively low with nutrients easy to release and many available microbial components, and $C_{mic}$ (1021.95 $mg.kg^{-1}$) was large. For MF, the litter C/N was relatively higher with nutrients difficult to decompose and low soil microbial biomass, and MF increase soil nutrients and microbial activity (Hu *et al.* 2005). Microbial biomass is closely related to ground plant productivity in many ecosystems (Zak *et al.* 1994), because microbial biomass is dependent on the input of the soil carbon. As indicated by Diaz-Ravina *et al.* (1988), the microbial biomass of low SOC will decrease, and vice versa. In this study, more microbial biomass existed in BF and MF which displayed large biomass (1021.95 $mg.kg^{-1}$ in BF, 569.12 $mg.kg^{-1}$ in MF). Also, SOC indicated significant correlation with $C_{mic}$ and $N_{mic}$ and $C_{mic}$ changed in the same manner as SOC in the succession process. BF and MF produce more root exudates and litter, and the increase of SOC will lead to increased microbial biomass (1021.95 $mg.kg^{-1}$ in BF, 569.12 $mg.kg^{-1}$ in MF).

**Table 4:** Soil microbial activity under different succession stages of the evergreen broad-leaved forest (mean ±SE n=3)

| Forest type | Soil layer/cm | Basal respiration [mg/(kg.h)] | $qCO_2$ [mg/(g.h)] | $C_{mic}$/SOC /% |
|---|---|---|---|---|
| PF | 0~10 | 1.12±0.05a | 2.51±0.12a | 2.22±0.11b |
| MF | | 1.26±0.04a | 2.22±0.05ab | 2.38±0.05a |
| BF | | 1.36±0.08a | 1.33±0.07b | 2.44±0.05a |
| PF | 10~20 | 0.57±0.06a | 2.97±0.11a | 1.44±0.07b |
| MF | | 0.85±0.03a | 2.31±0.03ab | 2.17±0.04a |
| BF | | 0.74±0.02a | 1.43±0.05b | 2.42±0.02a |

Note: Means in the same column with different letters differ significantly at $P<0.05$ level

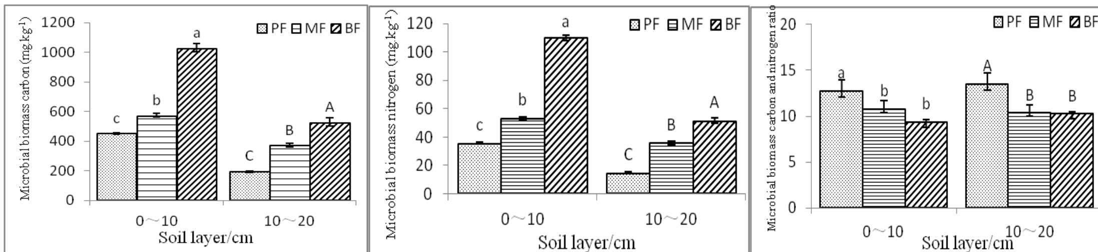


**Fig. 1:** Soil microbial biomass carbon、nitrogen and $C_{mic}$ / $N_{mic}$ under different succession stages of the evergreen broad-leaved forest (n=3). Letters mean significant difference in the same soil layer between different stand (P<0.05)

The soil basal respiration rate depends on soil microbial biomass and the utilization efficiency of the ground substance (Islam and Weil 2000). In this study, the soil basal respiration indicated a positive correlation with SOC, TN and $C_{mic}$ (r=0.651,r=0.612, r=0.643, $P<0.05$). Compared with BF, the litter of PF was difficult to decompose with low soil basal respiration. Compared with MF and BF, the litter of PF was also difficult to decompose, so the differences of the soil respiration in the early stage of succession were non-significant. With the process of succession, the increased soil microbial biomass and soil basal respiration rates indicated that the changes in the vegetation litter input improved soil fertility and enhanced the biological activity of the soil.

Metabolic quotient ($qCO_2$) reflects the utilization efficiency of organic ingredients by soil microbial population, represents the microbial biomass and activity, and indicates the changing tendency of the soil quality and the maturity of the soil ecosystem (Wardle and Ghani 1995). It has a low value in a relatively stable and mature ecosystem. In this study, $qCO_2$ decrease significantly with the succession process, and there was a negative correlation between $qCO_2$ and SOC, $C_{mic}$ and $N_{mic}$ (r=-0.846, r = -0.859, r = -0.857, P<0.01). In the early stage of the succession, the utilization efficiency of the ground substance by the soil microorganisms decreases and $qCO_2$ increases as result of human disturbances and the quality of the litter (Liao and Boutton 2008). With the invasion of the broadleaf tree species, the external interruption decreases, the microbial community structure changes, the utilization efficiency of the microbial ground substance increases and $qCO_2$ reduces gradually (Liao and Boutton 2008). Chen and Yang (2013) in their studies on the soil microbial features of the purple soil hilly slope in different recovery stages found that $qCO_2$ in the grass community stage was significantly higher than that in the latter three recovery stages. Microorganisms convert the substrate C into microbial carbon effectively, and little C will be released through respiration with decreased $qCO_2$ (Behera and Sahani 2003). Therefore, the high content of $qCO_2$ in PF reflected the decreasing utilization efficiency of the soil microbial community substrate which is consistent the report that the released quantity of soil $CO_2$ in the mixed forests is significantly lower than that of deciduous forest and coniferous forest (Mou 2004).

In this study, microbial quotient of BF was higher than that of PF and MF, because the carbon input of plant litter and microbial quotient increase. In the succession process, microbial quotient can be used to effectively predicate the change of the quantity and quality of SOC, which can represent the effects on the soil carbon pool from the succession. Fan *et al.* (2013) reported that microbial quotient in the mid- and final stage of the mid-subtropical evergreen broadleaf forests succession was higher than that in the early stage. In the early stage of the succession, soil carbon accumulation increases and soil effective carbon pool is enhanced. It has been reported that microbial quotient of broadleaf forests is higher than that of pine and broadleaf mixed forests, and that broadleaf forests are more capable of sustaining soil microbial biomass than pine and broadleaf mixed forests with stronger accumulation of the soil carbon (Li *et al.* 2014).

## Conclusion

In this study area, the vegetation succession led to increased soil microbial biomass and improved soil fertility. Improved input quality of vegetation litter and increased soil fertility will enhance the biological activity of the soil, which is primary cause of the gradually increasing $C_{mic}$ and $N_{mic}$ and microbial quotient in the succession process. Some management measures like closing hillsides to facilitate afforestation and selective cutting can be adopted to promote the transformation of pine and broadleaf mixed forests into evergreen broadleaf forests and the recovery of forest soil fertility.

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## Author Contributions

Zhang-quan Zeng designed this experiment and wrote this paper. Tang Hong measured the soil microbial biomass and gave some advice when writing the paper, Minghong Li measured soil respiration, Si-long Wang analyzed these data. Rui Yang, Yandong Niu, Jia Luo and Can-ming Zhang participated in field investigation and analyzed samples.

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