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ORIGINAL PAPER

Seasonal dynamics of soil microbial biomass C and N of *Keteleeria fortunei* var. *cyclolepis* forests with different ages

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Abstract Soil microbial biomass is an important indicator to measure the dynamic changes of soil carbon pool. It is of great significance to understand the dynamics of soil microbial biomass in plantation for rational management and cultivation of plantation. In order to explore the temporal dynamics and influencing factors of soil microbial biomass of *Keteleeria fortunei* var. *cyclolepis* at different stand ages, the plantation of different ages (young forest, 5 years; middle-aged forest, 22 years; mature forest, 40 years) at the Guangxi Daguishan forest station of China were studied to examine the seasonal variation of their microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) by chloroform fumigation extraction method. It was found that among the forests of different age, MBC and MBN differed significantly in the 0–10 cm soil layer, and MBN differed significantly in the 10–20 cm soil layer, but there was no significant difference in MBC for the 10–20 cm soil layer or in either MBC or MBN for the 20–40 cm soil layer. With increasing maturity of the forest, MBC gradually decreased in the 0–10 cm soil layer and increased firstly and then decreased in the 10–20 cm and 20–40 cm soil layers, and MBN increased firstly and then decreased in all three soil layers. As the soil depth increased, both MBC and MBN gradually decreased for all three forests. The MBC and MBN basically had the same seasonal variation in all three soil layers of all three forests, i.e., high in the summer and low in the winter. Correlation analysis showed that MBC was significantly positively correlated with soil organic matter, total nitrogen, and soil moisture, whereas MBN was significantly positively correlated with soil total nitrogen. It showed that soil moisture content was the main factor determining the variation of soil microbial biomass by Redundancy analysis. The results showed that the soil properties changed continuously as the young forest grew into the middle-aged forest, which increased soil microbial biomass and enriched the soil nutrients. However, the soil microbial biomass declined as the middle-age forest continued to grow, and the soil nutrients were reduced in the mature forest.

Keywords Microbial biomass · Soil microbial nitrogen · Soil microbial carbon · Seasonal variation · Artificial forest · *Keteleeria fortunei* var. *cyclolepis*

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Introduction

The soil microbial biomass regulates the carbon and nitrogen cycle in terrestrial ecosystems and also plays a key role in the conversion and supply of nutrients (Berg and Smalla 2009). To ensure effective management and cultivation of plantation, it is imperative to thoroughly understand the dynamic changes of soil microbial biomass and its influencing factors during forest growth. The soil microbial biomass usually exhibits seasonal variation because it is strongly affected by water, temperature, soil physicochemical properties, nutrients, etc., all of which are in turn highly seasonal (Yao et al. 2011; Wang et al. 2016). Previous studies on the seasonal variation of soil microbial biomass in plantation mainly focused on the differences in land use pattern (Pandey et al. 2010; Li et al. 2018), forest type (Ravindran and Yang 2015), and forest age (Wen et al. 2014). For plantation of different age, existing studies on the soil microbial biomass have studied the surface soil layer (< 20 cm) of broad-leaved forests in tropical, subtropical, and temperate areas as well as of coniferous forests in subpolar areas (Yang et al. 2010; Vidyanagar 2010), but research is lacking on the soil microbial biomass of coniferous forests in subtropical regions or of deep soil layers.

In China, Guangxi is a major area for the production of high-valued tropical and subtropical tree species. Although the local geographical conditions are particularly suitable for the cultivation of high-valued tree species, the development of high-valued tree species is presently lacking. The three fast-growing tree species (*Pinaceae, Cunninghamia*, *Eucalyptus*) are the dominant species, but the potential of forest lands remains to be further exploited since other trees are largely neglected. For the sake of local economy in the mountainous areas of Guangxi, it is very important to make full use of the ecological advantages of Guangxi (in both tree species resources and geographical environment) and actively develop plantation of high-valued tree species to improve the productivity of forest land. *Keteleeria fortunei* var. *cyclolepis* is a high-valued native tree species in Guangxi that belongs to the *Keteleeria* genus of the Pinaceae family. It has very high economic value because it is an excellent species for both landscaping and the production of precious timber, and it is also an ideal species for reforestation in mountains (Liu et al. 2017). Present research on the artificial forest of *K. fortunei* var. *cyclolepis* focused mostly on the effects of topography, meteorology, and habitat on the forest growth and biomass, as well as the physiochemical properties of the forest soil (Huang et al. 2016), and there is currently no published work on the soil microbial biomass.

In this paper, seasonal dynamics of soil microbial biomass in *K. fortunei* var. *cyclolepis* plantation forests of different age at the Guangxi Daguishan forest station was studied to understand whether the changes of soil microbial biomass during the growth of *K. fortunei* var. *cyclolepis* plantation changed its soil properties. The soil fertility status was analyzed, and the relevant factors affecting the soil microbial biomass were discussed. The results provide a scientific basis for the rational management of *K. fortunei* var. *cyclolepis* plantation as well as cultivation of other coniferous forests in tropical and subtropical regions.

Materials and methods

Study site

The test sites (111° 20′ 5″ E, 24° 14′ 25″ N) are located at the Liupai branch of the Daguishan forest station in the northeast to the Hezhou city of Guangxi, China. The landform consists of low hills and low mountains, and the elevation is 80–1204 m. The test sites have humid subtropical monsoon climate with an average annual temperature of 19.3 °C, an average annual rainfall of 2056 mm, and an average relative humidity of 82% (Liu et al. 2017). The soil is developed from residual parent material and consists mainly of mountainous brow soil. The soil texture is clay. The soil thickness is about 90–120 cm. The selected test sites have essentially uniform environmental conditions and management measures. The age of the artificial *K. fortunei* var. *cyclolepis* forests varies from 5 years (young forest) to 22 years (middle-aged forest) and 40 years (mature forest). Additional information of the test sites is given in Table 1.

Methods

Three replicate plots (20 m × 20 m) were randomly selected in each forest site, and soil samples were taken at the nine plots from soil layers at 0–10, 10–20, and 20–40 cm in March, June, September, and December of 2016, hence each time 27 samples were brought back to the laboratory for analysis. Each fresh soil sample was divided into two parts. The first half was passed through a stainless steel filter (2 mm) and measured for soil microbial carbon (MBC) and soil microbial nitrogen (MBN), and the other half was air-dried, sifted, and measured for soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), pH, bulk density (BD), and soil water content (SWC) (Oksanen 2001; Binkley and Fisher 2012).The MBC and MBN were measured by chloroform fumigation (Vance et al. 1987). Soil was weighed (10.00 g × 4) to give four portions, which were placed in a vacuum dryer containing chloroform and incubated at 25 °C for 24 h. The soil samples before and after fumigation were mixed with aqueous K_2SO_4 (0.5 mol/L, 40 mL), shaken at 300 rpm for 30 min, and sifted. The filtrate was measured on a multi N/C 3100 (Analytik Jena AG, Germany) instrument.

Soil organic carbon was determined by combustion method and multi N/C 2100 analyzer (Analytik Jena AG, Jena German). Soil total nitrogen was determined by Kjeldahl method using Automatic kjeldahl apparatus (Kjeltec 8400, Foss, Hiller d, Danmark). The content of phosphorus was determined by molybdenum-antimony colorimeter using uv-2600 spectrophotometer. The soil pH was determined by the glass electrode method. The soil bulk density was

Table 1 Basic situation of the plantation of *K. fortunei* var. *cyclolepis* at different sites

Site	Age (a)	Altitude (m)	Slope aspect	Slope position	Slope degree	Mean height (m)	Mean *DBH* (cm)	Site density (N/hm^2)	Canopy density	Shrub compositions
YF	5	465	Southeast	Down slope	25°	1.7	3.5	1600	0.2	*Schisandra chinensis, Rubus corchorifolius, Schefflera octophylla, Phyllanthus cochinchinensis*
MAF	22	456	Southeast	Down slope	25°	18.6	20.3	850	0.5	*Ficus hirta, Rubus corchorifolius, Euonymus alatus, Psychotria rubra*
MF	40	437	Southeast	Down slope	15°	22.5	31.5	435	0.7	*Psychotria rubra, Cibotium barometz, Evodia lepta, Ficus hirta*

YF young forest, *MAF* middle-aged forest, *MF* mature forest. The same below

calculated by dividing the dry soil weight by the volume of the ring knife used for sampling (Binkley and Fisher 2012).

Data analysis

The seasonal variation of the soil physicochemical properties and microbial biomass of the artificial *K. fortunei* var. *cyclolepis* forests of different age was analyzed and plotted with Excel 2007. Variance analysis, Pearson correlation analysis, and Duncan's multiple range test were conducted with SPSS19.0. The significance level for all statistical analyses was set at $\alpha = 0.05$. In order to reflect the effect of soil physical and chemical properties on soil microbial biomass, the data of soil microbial biomass and soil physical and chemical properties were sequenced and made redundancy analysis (RDA) by Canoco 5.0 software.

Results

Vertical variation of soil microbial biomass and physicochemical properties

Figure 1 shows the vertical variation of the soil physicochemical properties and the microbial biomass. For the 0–10 cm soil layer, significant difference among forests of different age was found ($P < 0.05$) for TN, TP, MBC, and MBN, but not ($P > 0.05$) for SOC, pH, BD, SWC, and MBC/MBN. For the 10–20 cm soil layer, significant difference among forests of different age was found for SOC, TN, MBC, and MBC/MBN, but did not exist for MBN, TP, pH, BD, and SWC. For the 20–40 cm soil layer, there was no significant difference among forests of different age for MBC, MBN, MBC/MBN, SOC, TN, TP, pH, BD, and SWC.

In general, for the soil of all three forest sites, SOC, TN, TP, SWC, MBC, MBN gradually decreased with soil depth, BD increased with soil depth, and pH and MBC/MBN did not seem to clearly vary with soil depth. For the young forest, there was extremely significant difference among soil layers for SOC, TN, and TP but no significant difference for pH, BD, and SWC. For the middle-aged forest, there was extremely significant difference among soil layers for SOC, TN, and TP, significant difference for SWC, and no significant difference for pH and BD. For the mature forest, there was extremely significant difference among soil layers for SOC, TN, and TP, but no significant difference for pH, BD, and SWC.

Seasonal variation of soil microbial biomass

Figure 2 shows the seasonal variation of the soil microbial biomass for the artificial *K. fortunei* var. *cyclolepis* forests of different age. It can be noted that for all soil layers of all

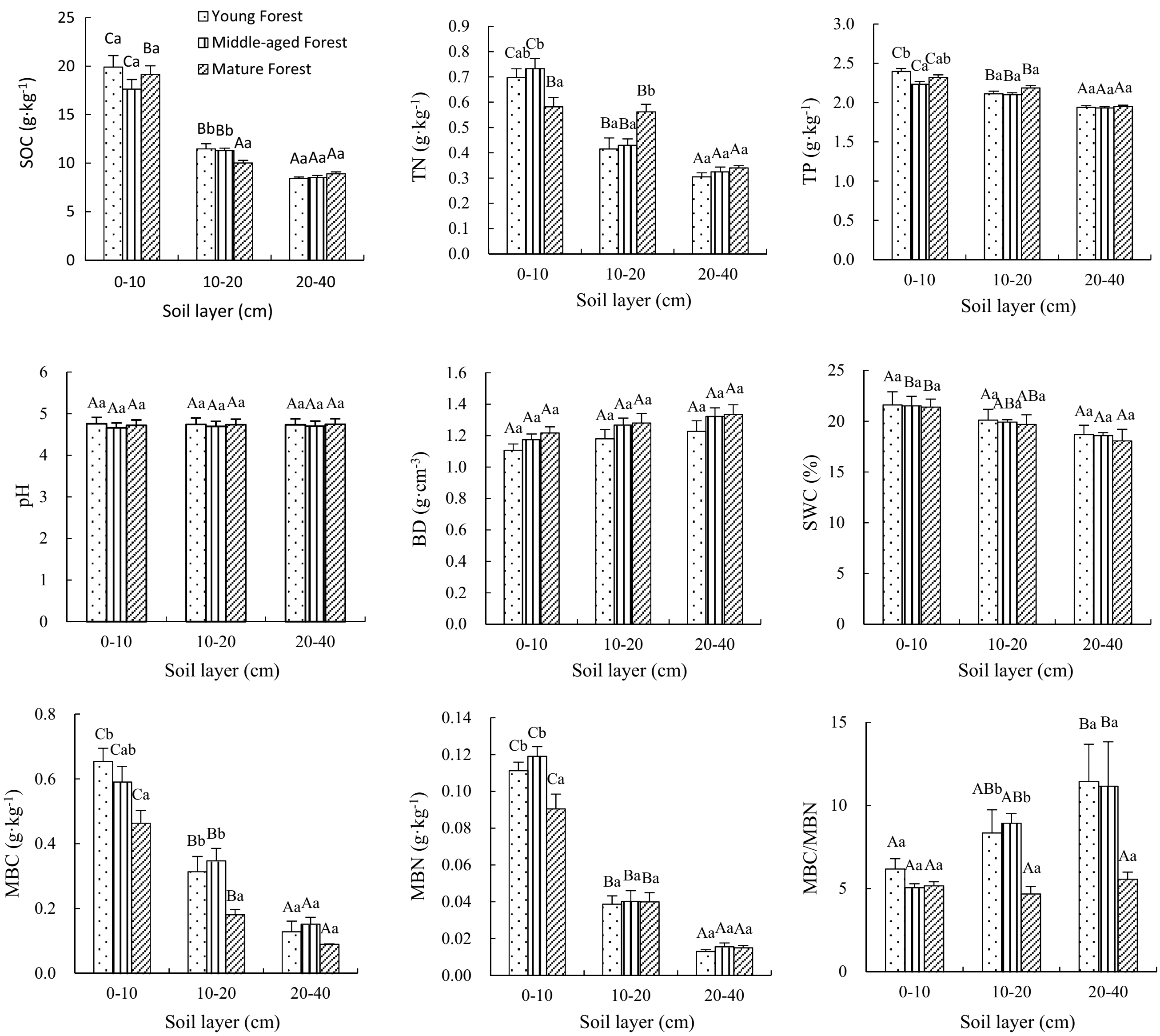

Fig. 1 The vertical changes in soil microbial biomass and physicochemical properties at different sites of artificial *K. fortunei* var. *cyclolepis* forests. Different capital letters of the same site indicate significant differences between soil layers, and different lower case letters of the same soil layer indicate significant differences across forest sites

forest sites, both MBC and MBN were the highest in summer and the lowest in winter. For the 0–10 cm soil, MBC and MBN fell in the order of summer > fall > spring > winter. For the 20–40 cm soil, MBC and MBN fell in the order of summer > spring > fall > winter. For the 10–20 cm soil, except the MBC of the young forest showed an order of summer > spring > fall > winter, the MBC of the middle-aged and mature forests, as well as the MBN of all three forest, all showed the order of summer > fall > spring > winter.

The seasonal variation of MBC/MBN was different. For the 0–10 cm soil, the MBC/MBN of all three forests fell in the order of spring > summer > winter > fall. For the 10–20 cm soil, the MBC/MBN of the young forest fell in the order of spring > winter > fall > summer, and the MBC/MBN of the middle-aged and mature forests fell in the order of spring > fall > winter > summer. For the 20–40 cm soil, the MBC/MBN of young and middle-aged forests fell in the order of spring > summer > winter > fall, and the MBC/MBN of the mature forest fell in the order of winter > spring > fall > summer.

Influence factors for the soil microbial biomass

Pearson correlation analysis (Table 2) showed that for the 0–10 cm soil, significant positive correlation existed between MBC and SOC, TN, SWC as well as between MBC/MBN

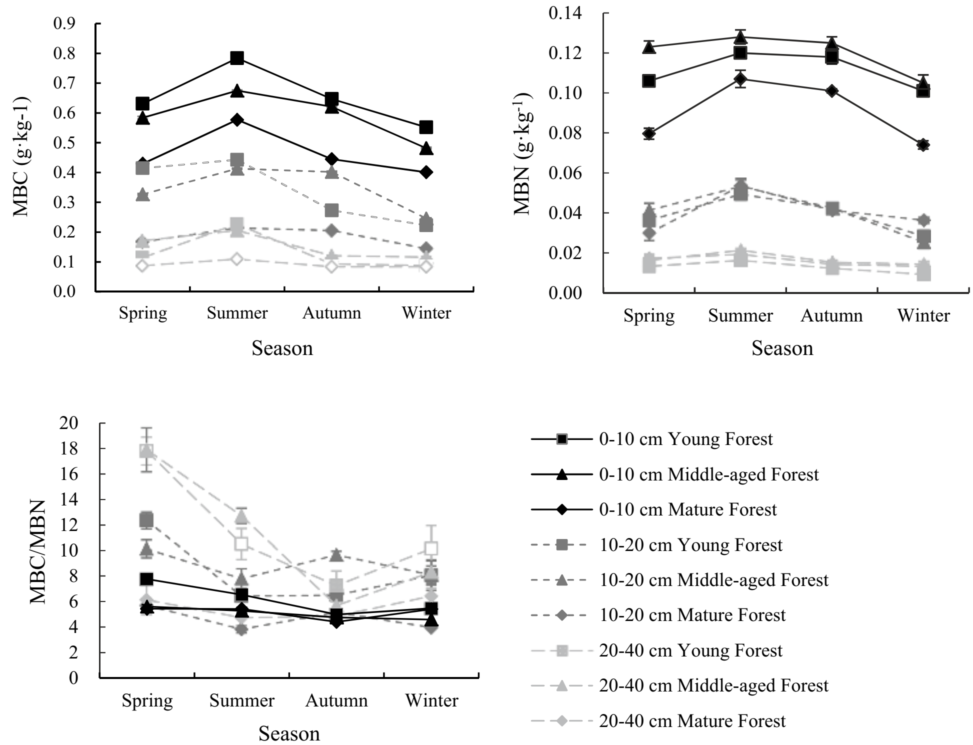

Fig. 2 The seasonal variation in soil microbial biomass at different forest sites of *K. fortunei* var. *cyclolepis* (Mean ± SE)

Table 2 Correlation between soil microbial biomass and influence factors

Soil layer (cm)	Microbial biomass	SOC	TN	TP	PH	BD	SWC
0–10	MBC	0.614*	0.685*	0.238	− 0.387	0.042	0.627*
	MBN	0.207	0.958**	− 0.066	− 0.212	0.160	0.381
	MBC/MBN	0.646*	0.079	0.345	− 0.344	− 0.095	0.500
10–20	MBC	0.781**	− 0.309	− 0.127	− 0.301	0.007	0.338
	MBN	0.292	0.595*	0.192	− 0.042	0.243	0.085
	MBC/MBN	0.618*	− 0.706*	− 0.232	− 0.279	−0.193	0.221
20–40	MBC	0.010	0.203	0.330	− 0.174	0.522	0.325
	MBN	0.469	0.676*	0.237	− 0.020	0.448	− 0.206
	MBC/MBN	− 0.029	− 0.469	0.006	0.056	− 0.114	0.444

*significant correlation at $p < 0.05$

and SOC, and there was also extremely significant positive correlation between MBN and TN. For the 10–20 cm soil, there were extremely significant positive correlation between MBC and SOC, significant positive correlation between MBN and TN, significant positive correlation between MBC/MBN and SOC, and significant negative correlation between MBC/MBN and TN. For the 20–40 cm soil, significant positive correlation existed between MBN and TN.

According to the RDA ordination diagram (Fig. 3), the total explanatory rate of soil physicochemical properties to microbial biomass change is 97.3%, the explanatory rates of axis I and axis II are 91.42% and 5.69%, respectively, and the cumulative explanatory rate is 97.11%, accounting for 98.8% of the total explanatory rate. It showed that the first two axes could reflect the relationship between soil microbial biomass and physicochemical properties well. After gradual selection of SWC, SOC, TN, pH, BD and TP, the cumulative values of explanatory variables were 75.6%, 94.2%, 95.3%, 96.5%, 97.2% and 97.3%, indicating that SWC was the main factor determining the variation of soil microbial biomass, and its explanatory rate was 75.6%.

Discussions

Difference in the soil microbial biomass of different forests

The results showed that there was significant differences in soil microbial biomass between different forest ages in the

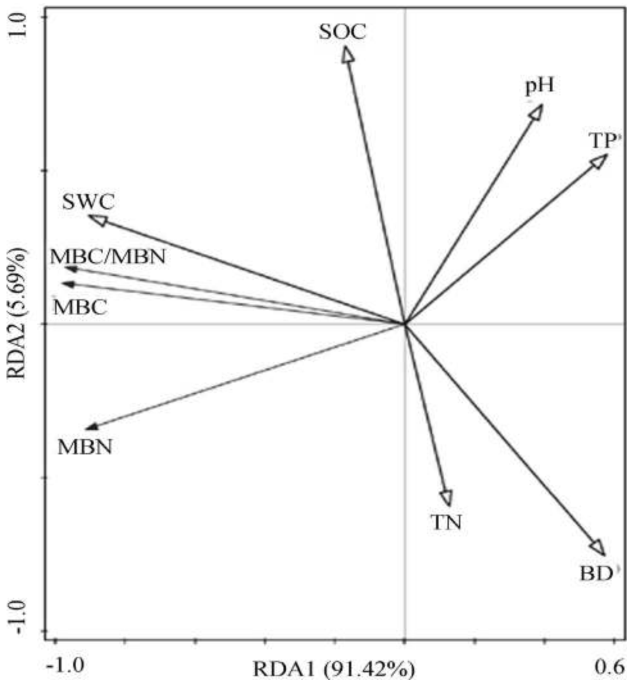

Fig. 3 RDA ordination diagram of soil microbial biomass and physicochemical properties

0–20 cm soil layer. With the increase of soil depth, there were no significant differences in soil microbial biomass between different forest ages, and both MBC and MBN gradually decreased. It indicated that the microbial biomass was more enriched and had greater diversity in the surface soil layers (0–10 and 10–20 cm) than in the deep soil layer (20–40 cm) for the *K. fortunei* var. *cyclolepis* plantations of different ages. It can be seen that the forest age strongly affected the soil microbial biomass in the surface layers. It is consistent with the research results of Xu et al. (2013) and Wen et al. (2014). However, the study of An et al. (2011) showed that the number of microorganisms was the highest in the soil layer of 10–40 cm, which maybe because the soil in the study area was sandy loam, and water and nutrients were easily diffused and leached to the deep layer, leading to the enrichment of microorganisms in the deep layer. The study area belongs to dark brown forest soil with more inputs of fine roots and litters and better ventilation and permeability in the surface soil, which was conducive to the growth and reproduction of microorganisms. On the other hand, as the soil layer deepened, soil water content decreased and the soil porosity declined which reduced the ion exchange in soil, impeded the diffusion of nutrients, and impaired the growth and distribution of soil microbes. There were no significant differences in the nutrients and physicochemical properties of soil among forests of different age, and thus their soil microbial biomass could not be well distinguished from each other (Fierer et al. 2003; Stockdale and Brookes 2006).

Generally speaking, as the age of the forest increased, the soil microbial biomass increased firstly and then decreased, in the order of middle-aged forest > young forest > mature forest. This was in line with the results reported by Wu et al. (2016) on the soil microbes of the *Larix principis-rupprechtii* Mayr forests at the Shanxi Taiyue mountain. It could be speculated that the young forest of *K. fortunei* var. *cyclolepis* had smaller trees, and forest floor light, humidity, and temperature were all favorable to the metabolism and growth of soil microbes. As the forest age increased, the amount of litter increased, which enriched the soil nutrients and provided additional energy source for microbial metabolism and synthesis. However, when the forest matured further, the canopy of the forests became denser, which reduced forest floor light and slowed decomposition of litter. Vegetations such as shrubs and herbs also declined consequently, which decreased soil nutrients and together lead to slower microbial growth (Kaiser et al. 2011; Song et al. 2015).

The ratio of soil microbial carbon to soil microbial nitrogen is often used as an important indicator of changes in the microbial community, and higher ratio indicates higher fungal biomass (Zhou and Wang 2015). In this study, there was no significant difference in MBC/MBN in soil layers of 0–10 cm and 20–40 cm between different forest ages, while MBC/MBN in soil layers of 10–20 cm was significantly different between different forest ages, and MBC/MBN in soil of young and medium forests was significantly greater than that in forest (Fig. 2). It indicated that *K. fortunei* var. *cyclolepis* plantations had little effect on the microbial community in the soil surface (0–10 cm) and deep (20–40 cm), but greater effect on the microbial community in the soil middle (10–20 cm). It may be that the soil organic matter was beneficial to the growth of soil fungal growth, while total nitrogen content was beneficial to the growth of the soil bacteria. There was significant differences between soil organic matter and total nitrogen content in 10–20 cm soil layer of different forest ages, which causes the difference of MBC/MBN in the soil layer of different forest ages. The specific i mechanism and reason of the impact still needed further in-depth study (Trap et al. 2011). With the deepening of soil layer, the MBC/MBN of *K. fortunei* var. *cyclolepis* plantation increased gradually, which similar to the previous research results (Fierer et al. 2003; An et al. 2011). It mainly because the soil surface nutrient limit was lower and it was more suitable for bacteria to survive.

Seasonal variation and influence factors of soil microbial biomass

The seasonal soil microbial biomass is strongly affected by humidity, temperature, soil physicochemical properties, and other environmental factors (Classen et al. 2007; Freppaz et al. 2014). In the current study of the artificial *K. fortunei* var. *cyclolepis* forests, the observed soil microbial carbon (MBC) and soil microbial nitrogen (MBN) were clearly high in the summer and low in the winter. That is, MBC and MBN were high in the growth season and low in the hibernation season. Similar trend was also observed by Devi and Yadava (2006) for the mixed forest of *Quercus Linn* in Manipur, India, by Ruan et al. (2004) for tropical rainforest, as well as by Li et al. (2014) for the mixed forest of *Cunninghamia lanceolata* Hook, *Pinus massoniana* Lamb and *Lithocarpus glaber* Nakai in the hilly areas of central Hunan in China. The higher temperature in the summer promotes the decomposition of litter, and nutrients and carbohydrates are brought into the soil from the earth surface by rain water, which accelerates the growth of soil microbes and increases both MBC and MBN. Conversely, since the winter is colder and drier, the decomposition of litter is slower and both microbial growth and activity are inhibited, leading to lower MBC and MBN (Lipson and Schmidt 2000). The seasonal variation of soil MBC/MBN in *K. fortunei* var. *cyclolepis* plantations of different forest ages also showed a certain rule, which was mainly manifested as that the soil MBC/MBN of the three forest ages was the largest in spring, and the soil MBC/MBN of the young and middle forests was greater than that of the mature forests, especially in the soil layer of 10–40 cm. We speculated that this may be caused by the increase of external temperature in spring, the smaller canopy density in young and middle forests, and the more sensitive response of surface soil to external temperature and the more light it received, which is conducive to fungus breeding and growth (Kaiser et al. 2014; Jefferies et al. 2010).

Soil microbial biomass is related to environmental factors such as temperature, humidity, soil moisture, soil nutrients, soil physicochemical properties, etc. (Devi and Yadava 2006; Liu et al. 2014). Current results showed that in the plantation of *K. fortunei* var. *cyclolepis*, MBC was significantly positively correlated with soil organic matter, total nitrogen, and soil moisture, while MBN was significantly positively correlated with soil total nitrogen. Therefore, soil organic matter and total nitrogen were important influence factors of MBC and MBN. High soil organic matter and total nitrogen can provide the soil microbes with sufficient carbon, nitrogen, and energy source for their synthesis and metabolism (Li et al. 2014). These results are consistent with previous findings. Yang et al. (2009) studied the seasonal changes of soil microbial biomass in the plantation of *Larix gmelinii* Kuzen and found significantly positive correlation between soil microbial biomass (both carbon and nitrogen) and soil organic matter and total nitrogen. The global analysis by Xu et al. (2013) on the soil microbial biomass (carbon, nitrogen, and phosphorus) in terrestrial ecosystems demonstrated that the spatial pattern of soil microbial biomass (carbon and nitrogen) was consistent with that of soil organic matter and total nitrogen.

In conclusion, the soil nutrients in middle age were more nutritious than young and mature forests of *K. fortunei* var. *cyclolepis* plantations, that is to say, the soil nutrients gradually increased with the development of the plantations, and reached the maximum at middle age. And then the soil nutrients gradually decreased with the increase of forest age, while the soil fertility declines also.

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