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Improved nutrient status affects soil microbial biomass, respiration, and functional diversity in a Lei bamboo plantation under intensive management

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Abstract

Purpose Intensive management, such as fertilization and organic mulching, is applied frequently in Lei bamboo (*Phyllostachys praecox*) plantations to achieve higher production in subtropical China. However, responses as well as key impact factors of soil microbial properties under such management remain uncertain. We analyzed the relationships between nutrient changes and microbial properties and assessed the main factors determining microbial biomass, activity, and functional diversity in soils under intensive management in a Lei bamboo plantation.

Materials and methods Soil samples of treatments of no fertilization (control), chemical fertilization (CF), and chemical and organic fertilization combined with organic mulching (CFOM + M) were taken before mulching. The soil organic carbon (SOC), dissolved organic carbon, and total and available nitrogen (N), phosphorus (P), and potassium (K) were measured. Microbial biomass carbon (MBC), basal respiration, and mineralization were also analyzed. Community level of physiological profile (CLPP) of microorganisms was analyzed by BIOLOG method to estimate the functional diversity and carbon (C) source utilization patterns of microbes. Principal component analysis (PCA), principal response curve (PRC), correlation analysis, regression analysis, and redundancy analysis (RDA) were performed to clarify changes in variables and determine the factors influencing microbial properties.

Results and discussion SOC and total and available N, P, and K increased as follows: CFOM + M > CF > control. However, C/P and N/P ratios showed an opposite trend. MBC and respiration were not affected, but microbial quotient and metabolic quotient declined under intensive management. McIntosh diversity index was much higher in CFOM + M. The PCA showed that microorganisms in CFOM + M had a stronger ability to use most C sources. Weaker utilization of serine indicated an alleviation of nutrient deficiency in CFOM + M. PRC of CLPP showed a significant treatment effect and that utilization of serine sensitively responded to nutrient status over the whole incubation time. RDA showed that total and available N, total K, and C/P were the main factors influencing utilization of C sources by microbial communities.

Conclusions Fertilization combined with organic mulching increased soil nutrients, microbial biomass, and respiration in a Lei bamboo plantation. Abundant nutrients also increased C source use efficiency of microorganisms under intensive management. Changes of N and K and C/P might have led to a shift in microorganisms toward a different life strategy and determined the change in C source utilization patterns of microbial communities.

Keywords Community level physiological profile · McIntosh index · Microbial quotient · Metabolic quotient · Organic mulching · *Phyllostachys praecox*

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1 Introduction

The total global bamboo area is estimated to be 31.5 M ha and represents about 1% of the total global forest area (Kuehl

2015). Most bamboo forest is in tropical and subtropical areas of the world. Bamboo resources are increasing, and global bamboo resources increased by approximately 2 M ha during 1990–2010 (Kuehl 2015). Since 1980, the bamboo area in China has increased by 3% annually (Cao et al. 2011) and the area was recently estimated to be 6.72 M ha (Li et al. 2015). Intensive management such as fertilization, organic mulching, understory removal, and tillage has increased production and profit of bamboo in China. However, the effects of intensive management on soil nutrient cycling, microbial properties, microbial diversity, and community shifts in bamboo plantations remain unclear.

The influences of land-use conversion (Zhang et al. 2013a) and different intensive management (Li et al. 2010; Zhuang et al. 2011; Li et al. 2013) have been studied in relation to changes in soil organic carbon (SOC), labile carbon (C), C chemical composition, and microbial biomass C (MBC) of bamboo ecosystem. Variations in microbial activity, such as carbon dioxide (CO_2) efflux from soils under land-use change or different management, have also been discussed (Jiang et al. 2006, Liu et al. 2011, Zhang et al. 2013b). Soil microbial community structure and diversity of bamboo ecosystem were also addressed in previous research. For example, Chang et al. (2016) analyzed the effect of elevation gradient on microbial communities in Moso bamboo plantations by phospholipid-derived fatty acid analysis and denaturing gradient gel electrophoresis profiles. Qin et al. (2014) studied the response of the soil fungal community to intensive management by denaturing gradient gel electrophoresis in a bamboo forest. Pyro-sequencing techniques were also applied to measure the changes of the soil bacterial community after Moso bamboo invasion (Lin et al. 2014). However, the effect of intensive management on soil microbial properties, especially microbial diversity and community, is very uncertain.

Lei bamboo (*Phyllostachys praecox*) is a species mainly distributed in southeast China. Because of its delicious shoot, Lei bamboo has high economic value for local farmers. Obtaining a high yield of Lei bamboo shoots requires intensive management, such as understory removal, fertilization, and tillage. To produce more shoots and harvest them earlier, winter organic mulching combined with fertilization is used by local farmers (Zhuang et al. 2011; Zhang et al. 2013a). This technique involves placing an abundant mixture of rice straw and rice husk in the bamboo plantation to maintain suitable temperature and moisture in winter. Intensive management, particularly fertilization combined with organic mulching, provides abundant nutrients to the bamboo ecosystem and subsequently affects soil physico-chemical or biological properties. Changes in soil C pool, nutrient status, and CO_2 efflux in bamboo ecosystems with fertilization and organic mulching have been widely studied (Jiang et al. 2009; Li et al. 2010; Zhuang et al. 2011; Zhang et al. 2013a). Community level physiological profiles (CLPP) provide a measure of C metabolic abilities of the microbial community (Garland and Mills 1991). The BIOLOG method, a classic technique for CLPP analysis, has been used to assess the response of microbial functional diversity under intensive management in bamboo plantations (Xu et al. 2008). Nevertheless, few researchers have focused on the changes of microbial functional diversity in bamboo ecosystems with fertilization combined with organic mulching. Furthermore, whether there are microbial species that are sensitive to intensive management is unknown. Additionally, the pivotal factor affecting microbial functional diversity remains undefined. Probing the above questions will help to better estimate the effect of intensive management on bamboo ecosystem processes and stability from the perspective of microbial functional diversity. The aim of the present study is to clarify the effect of nutrient improvement on soil microbial properties and determine the key factors influencing soil microbial biomass, respiration, functional diversity, and C utilization patterns of the microbial community under intensive management in a Lei bamboo plantation.

2 Materials and methods

2.1 Site description

The sampling site was located in Taihuyuan, Lin'an City (30° 17′ N, 119° 34′ E) in the northwest of Zhejiang Province, China (Fig. 1). There is a typical subtropical monsoon climate with annual mean precipitation of 1614 mm, annual mean temperature of 16.0 °C, and a frost-free period of 237 days at the site. The soil was yellow-red soil derived from sandstone. It was classified as a Ferrisol according to the US Department of Agriculture Classification System (USDA 1999). A plot experiment had been carried out in the Lei bamboo (*P. praecox*) plantation for 5 years when soil samples were taken in 2012. The area of each plot was 100 m^2 and the density of the bamboo stands was 15,000 plants ha^{-1}. Three treatments with three replicates were designed in the plot experiment: no fertilizer control (control), chemical fertilizer application (CF), and chemical and organic fertilizer application combined with winter organic mulching (CFOM + M). In CF treatment, a total of 2.3 t ha^{-1} $year^{-1}$ nitrogen, phosphorus, and potassium (N, P, and K, respectively) compound fertilizer ($N/P_2O_5/K_2O$ = 15:15:15) plus additional 1.1 t ha^{-1} $year^{-1}$ urea (N content of 46%) were split applied in May, September, and before winter mulching. In the CFOM + M treatment, except for the above chemical fertilizer, 11.3 t ha^{-1} $year^{-1}$ of poultry manure (N 2.10%, P_2O_5 3.50%, and K_2O 2.40%) was also applied. Organic material including total 40 t ha^{-1} $year^{-1}$ of rice straw and 55 t ha^{-1} $year^{-1}$ of rice husk covered the soil surface from November or December to March.

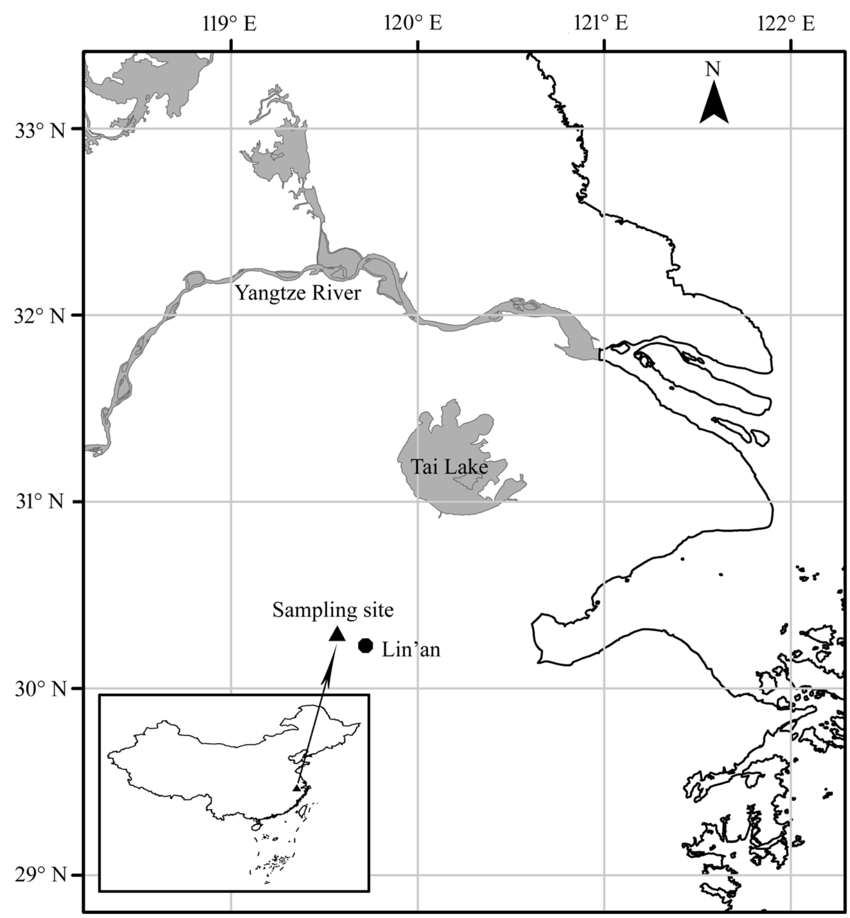

Fig. 1 Location of study area and sampling site (*black up-pointing triangle*)

2.2 Soil sampling and measurement

Five soil subsamples (depth of 0–20 cm) were randomly collected in each plot before mulching in December 2012. The subsamples were mixed and sieved through a 2-mm sieve to remove visible plant debris and small stones. Part of the sample was air-dried for subsequent physico-chemical property measurements. The rest of the sample was stored at 4 °C to assess biological characters. SOC was determined by Tyurin method. Briefly, this was digested by 0.8 M $K_2Cr_2O_4/H_2SO_4$ (1:1) and titrated with 0.2 M ferrous sulfate. Total N (TN) was detected by the Kjeldahl method. Available N (AN) was measured by the alkali-hydrolysis and diffusion method. Total P (TP) was melted by sodium carbonate and available P (AP) was extracted by 0.5 M sodium bicarbonate, respectively, and determined by molybdenum-blue colorimetry. Total K (TK) was digested by $HF–HClO_4$, and available K (AK) was extracted by 1 M ammonium acetate, respectively, and detected by flame photometry (Lu 1999).

MBC was determined using the fumigation–extraction method (Vance et al. 1987). C which was extracted from unfumigated soil was defined as dissolved organic carbon (DOC). Basal respiration and mineralization were determined from the CO_2 production rate using the alkali absorption and titration method (Lu 1999).

Soil microbial functional diversity and C source utilization patterns of the microbial community were assessed by CLPP analysis using the BIOLOG method (Garland and Mills 1991). Of soil, 5 g was suspended in 50 mL of 0.85% sodium chloride solution. The mixture was shaken for 30 min and settled for another 30 min at 25 °C. The supernatant was diluted and 100-μl aliquots were inoculated into a 96-well Eco-plate (Biolog Inc., Hayward, CA, USA) and were incubated at 25 °C. Light absorbance of each well was measured at regular 24-h intervals.

2.3 Data analysis

Cumulative C mineralization was fitted with a first-order kinetic equation:

$$C_t = C_0\left(1-e^{-kt}\right) \quad (1)$$

where C_t is the cumulative C mineralization after time t, C_0 is potentially mineralizable C, k is the mineralization rate constant, and t is the incubation time. For CLPP analysis, average well color development (AWCD) was estimated and a principal response curve (PRC) used to analyze the treatment effect over the whole incubation time (Wang et al. 2012). Microbial functional diversity indexes—Shannon (H′), Simpson (D), and McIntosh (U) indexes—were calculated according to Magurran (Magurran 1988) using the following formulae:

$$H' = -\sum p_i \ln p_i \quad (2)$$

$$\frac{1}{D} = \sum \frac{(n_i(n_i\text{-}1))}{(N(N\text{-}1))} \quad (3)$$

$$U = \sqrt{\left(\sum n_i^2\right)} \quad (4)$$

where p_i is the proportional color development of the ith well on total color development of all wells of a plate, n_i is the color development of the ith well, and N is the total color development of the whole wells.

Ordination analysis of CLPP including principal component analysis (PCA) and redundancy analysis (RDA) were performed to show the changes of C utilization patterns of microbial community and their relationships with environmental factors. Significant differences among treatments were determined by one-way ANOVA with Duncan's multiple range test at $p < 0.05$. Relationships between individual C sources and scores of principal component 1 (PC1) or 2 (PC2) were determined by Pearson's correlation analysis. Relationships between environmental factors and microbial properties were determined by stepwise regression analysis using SPSS 13.0 for Windows (SPSS, Chicago, IL, USA). PRC and ordination analysis were carried out using R (version 3.2.3; R Development Core Team) with the vegan package.

3 Results

3.1 Soil nutrient changes under intensive management

SOC, TN, AN, TP, AP, TK, and AK increased as follows: CFOM + M > CF > control (Table 1). Chemical and organic fertilization combined with mulching remarkably increased soil nutrients. For instance, SOC, TN, TP, and TK in CFOM + M were 55.06, 46.62, 119.23, and 15.78% higher than corresponding values in control. Similar to total nutrients, AN, AP, and AK in CFOM + M were 35.59, 1203.81, and 784.15% higher than those in control. There was no significant difference in DOC among the different treatments. C/P and N/P ratios in CF and CFOM + M were 10.94–29.55 and 3.63–36.78% lower than those in control, respectively; however, there was no obvious difference in C/N among treatments (Table 1).

3.2 Microbial biomass and respiration under intensive management

There were no significant differences in MBC and respiration among treatments (Table 2). During 28 days of incubation, the

Table 1 Nutrient state in soil of a Lei bamboo plantation under different treatments

	SOC (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	TK (g kg^{-1})	AN (mg kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})	DOC (mg kg^{-1})	C/N	C/P	N/P
Control	12.15 ± 0.51c	1.48 ± 0.12b	0.43 ± 0.02c	20.90 ± 0.05c	137.67 ± 23.30b	14.66 ± 4.22c	68.33 ± 3.82b	168.70 ± 7.86a	9.60 ± 0.72a	72.88 ± 1.59a	7.63 ± 0.70a
CF	13.77 ± 0.86b	1.83 ± 0.15a	0.55 ± 0.04b	23.62 ± 0.31b	158.67 ± 7.29ab	58.21 ± 5.86b	74.17 ± 8.78b	180.33 ± 7.21a	8.85 ± 1.05a	64.91 ± 8.00a	7.35 ± 0.76a
CFOM + M	18.85 ± 0.41a	2.17 ± 0.23a	0.95 ± 0.05a	24.19 ± 0.28a	186.67 ± 2.02a	191.07 ± 7.19a	604.17 ± 64.15a	167.94 ± 8.07a	10.22 ± 1.25a	51.34 ± 3.49b	5.05 ± 0.34b

Mean values ± S.D. (n = 3). Different lower case letters in each column indicate a significant difference at $p < 0.05$

Control no fertilization control, *CF* chemical fertilization, *CFOM + M* chemical and organic fertilization combined with organic mulching, *SOC* soil organic carbon, *TN* total nitrogen, *TP* total phosphorus, *TK* total potassium, *AN* available nitrogen, *AP* available phosphorus, *AK* available potassium, *DOC* dissolved organic carbon, *C/N* carbon to nitrogen ratio, *C/P* carbon to phosphorus ratio, *N/P* nitrogen to phosphorus ratio

Table 2 Microbial properties in soil of a Lei bamboo plantation under different treatments

	MBC (mg kg^{-1})	Respiration (mg C–CO_2 g^{-1})	Cumulative mineralization (mg C–CO_2 g^{-1})	C_0 (g kg^{-1})	k ($day^{-1)}$)	R^2	qMBC	qCO_2
Control	340.94 ± 19.59a	113.89 ± 6.68a	807.93 ± 107.06a	0.982	0.060	0.9783	0.028 ± 0.001a	0.334 ± 0.000a
CF	360.36 ± 50.74a	117.64 ± 19.77a	988.82 ± 320.98a	1.376	0.044	0.9803	0.025 ± 0.002ab	0.326 ± 0.009a
CFOM + M	407.33 ± 28.33a	118.12 ± 8.07a	1311.7 ± 209.a	11.11	0.004	0.9933	0.022 ± 0.000b	0.290 ± 0.000b

Mean values ± S.D. (n = 3). Different lower case letters on each column indicate a significant difference at $p < 0.05$

Control no fertilization control, *CF* chemical fertilization, *CFOM + M* chemical and organic fertilization combined with organic mulching, *MBC* microbial biomass carbon, C_0 potentially mineralizable carbon, *qMBC* microbial quotient, qCO_2 metabolic quotient

cumulative production of CO_2–C of soils in control, CF, and CFOM + M was 807.93, 988.82, and 1311.70 mg kg^{-1}, respectively. The results of first-order kinetics models indicated that potential mineralizable C of different treatments was as follows: CFOM + M > CF > control. Nevertheless, the trend of mineralization rate was the opposite: control > CF > CFOM + M (Table 2). The CFOM + M treatment also had a significantly decreased microbial quotient (i.e., MBC/SOC ratio, qMBC) and a dramatically reduced metabolic quotient (i.e., basal respiration/MBC, qCO_2) compared to control (Table 2). Stepwise regression analysis demonstrated that cumulative mineralization was affected by SOC (Table 3). qMBC was closely related to C/P and qCO_2 was respectively related to C/P, SOC, and AP (Table 3).

3.3 CLPP of microorganisms under intensive management

AWCD of all treatments increased quickly with incubation time after 48 h (Fig. 2). AWCD among different treatments did not change significantly during the whole incubation time. AWCD at 144 h was selected to calculate the functional diversity indexes for the different treatments. The results showed no obvious differences in H′ and D indexes among treatments (Table 4). Furthermore, U index in CFOM + M was 48.32% and greatly higher than that in control ($p < 0.05$, Table 4). Regression analysis showed that AWCD (144 h) and U were closely related to AN (Table 3).

Table 3 Relationships among environmental variables and microbial properties analyzed by stepwise regression

	Independent variables	R^2	p
Cumulative mineralization	SOC	0.786	<0.05
qMBC	C/P	0.933	<0.05
qCO_2	C/P, SOC, AP	0.987	<0.01
AWCD	AN	0.624	<0.05
U index	AN	0.676	<0.05

qMBC microbial quotient, qCO_2 metabolic quotient, *AWCD* average well color development, *U index* McIntosh index, *SOC* soil organic carbon, *AN* available nitrogen, *AP* available phosphorus, *C/P* carbon to phosphorus ratio

PCA indicated that different treatments also influenced C source utilization patterns of the microbial community. PC1 explained 40.6% of the variation and separated CFOM + M from control and CF along the horizontal axis. PC2 accounted for 17.2% of the total variation and further distributed the treatments along a diagonal line between PC1 and PC2 (Fig. 3). PCA results also revealed that microorganisms of CFOM + M had a stronger ability to utilize most C sources; however, microbial utilization ability for C sources was weak in control (Fig. 3). Utilization of D-malic acid and L-serine is closely related to the separation of treatments along PC1 and PC2, respectively (Fig. 3). Correlation analysis showed that 13 types of C sources (four carboxylic acids, three carbohydrates, three amino acids, one amide, one phenolic acid, and one polymer) were significantly related to PC1; three types of carbon sources (one carboxylic acid, one amino acid, and one phenolic acid) were related to PC2 (Table 5). The results demonstrated that the main C source types that contributed to the discrimination of CLPP in different treatments were carboxylic acids, amino acids, and carbohydrates.

PRC can clarify the treatment effect over time and infer the response of species to stressors with multi-variables (Brink and Braak 1999). It can also be used to highlight the difference in CLPP of microorganisms (Wang et al. 2012). The PRC results demonstrated different CLPP between the CFOM + M, CF, and control in the whole incubation time (Fig. 4). The deviation of CFOM + M and CF from control increased with incubation time after 48 h and stabilized after 144 h (Fig. 4). PRC also indicated changes of D-malic acid in response to variation in CFOM + M, but changes of L-serine were opposite to the variation in CFOM + M during the whole incubation time (Fig. 4).

RDA uncovered the effects of environmental factors on microbial CLPP for the different treatments. Similar to PCA, the treatments were roughly separated according to patterns of microbial community level C source utilization (Fig. 5). Redundancy analysis axis 1 (RDA1) and 2 (RDA2) explained 34.1 and 13.9%, respectively, of the total variation of C source utilization patterns of microbial community because of the constrained effect

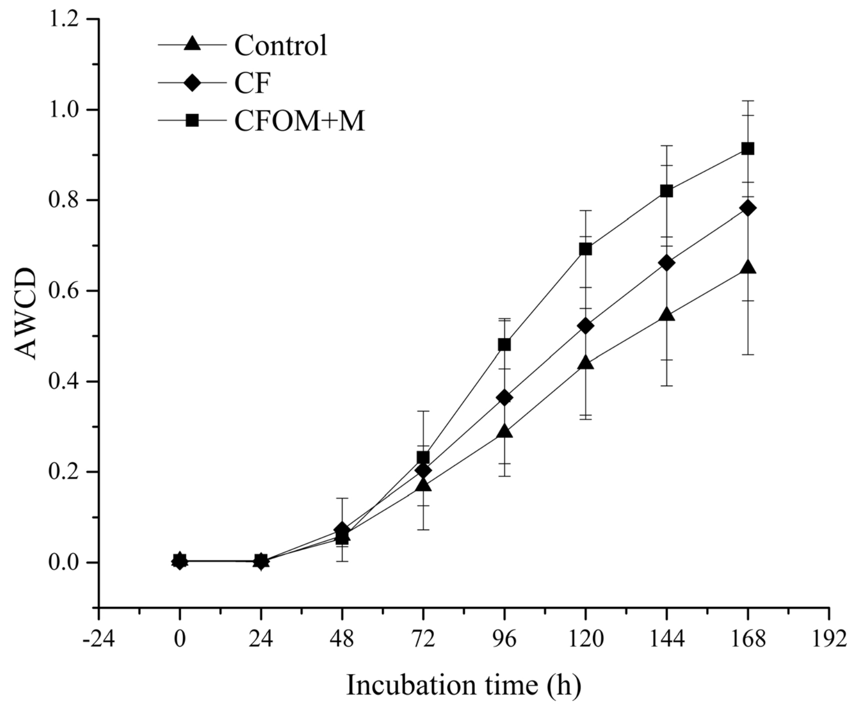

Fig. 2 Average well color development (AWCD) representing carbon utilization ability of microbial communities under different treatments. *Control* no fertilization control, *CF* chemical fertilization, *CFOM + M* chemical and organic fertilization combined with organic mulching. The *error bar* indicates standard deviation

of environmental factors. AN, TN, TK, and C/P significantly affected the C source use patterns of microbes for the different treatments ($p < 0.05$).

4 Discussion

4.1 Effects of fertilization combined with mulching on soil nutrients

Positive, negative, or negligible effects of intensive management on soil C of bamboo ecosystems have been reported previously. For instance, several researchers found that long-term intensive management decreased the SOC in bamboo ecosystems (Zhou et al. 2006; Li et al. 2013). However, SOC increased when fertilization and organic mulching were applied in a bamboo plantation (Li et al. 2010; Zhuang et al. 2011). In the present study, SOC was much higher in CF and CFOM + M compared to control. On the one hand, fertilization improves growth of bamboo, increases root exudate, and subsequently increases SOC (Li et al. 2010; Li et al. 2013). On the other hand, decomposition and incorporation of mulched organic material further enlarge the soil C pool in bamboo forest (Li et al. 2010; Zhuang et al. 2011). It was reported that soil labile C increased with organic mulching (Huang et al. 2008a; Li et al. 2010); however, a decline in labile C was observed under intensive management without organic mulching practice (Zhou et al. 2006; Li et al. 2013). In the present study, there was no significant difference in DOC among treatments. As an active organic C fraction, DOC could be influenced by local climate, soil type, organic residue management, duration of experiment, as well as microbial consumption. Such differences may have led to our results differing from previous studies.

Table 4 Microbial functional diversity indexes in soil of a Lei bamboo forest under different treatments

	AWCD	Shannon H′	McIntosh U	Simpson D
Control	0.54 ± 0.15a	3.12 ± 0.08a	3.69 ± 0.88b	0.95 ± 0.00a
CF	0.66 ± 0.21a	3.12 ± 0.13a	4.51 ± 1.11ab	0.95 ± 0.01a
CFOM + M	0.82 ± 0.10a	3.17 ± 0.07a	5.48 ± 0.41a	0.95 ± 0.00a

Mean values ± S.D. (n = 3). Different lower case letters on each column indicate a significant difference at $p < 0.05$

Control no fertilization control, *CF* chemical fertilization, *CFOM + M* chemical and organic fertilization combined with organic mulching, *AWCD* average well color development

In addition to C, fertilization and organic mulching can incorporate N, P, and K nutrients into the soil of the bamboo ecosystem. Zhang et al. (2013a) discussed that when a paddy field was converted to a Lei bamboo forest, soil P and K availability would be increased by fertilization, decomposition of mulched organic matter, and bamboo litter degradation. Tu et al. (2011) reported that N deposition led to accumulation of soil C, N, and P in a plantation of bitter bamboo (*Pleioblastus amarus*). Consistent with previous research, soil TN, TP, TK, AN, AP, and AK increased in CF and CFOM + M due to intensive management in the present study. Dramatically low C/P and N/P also indicated a P enrichment under fertilization combined with organic mulching.

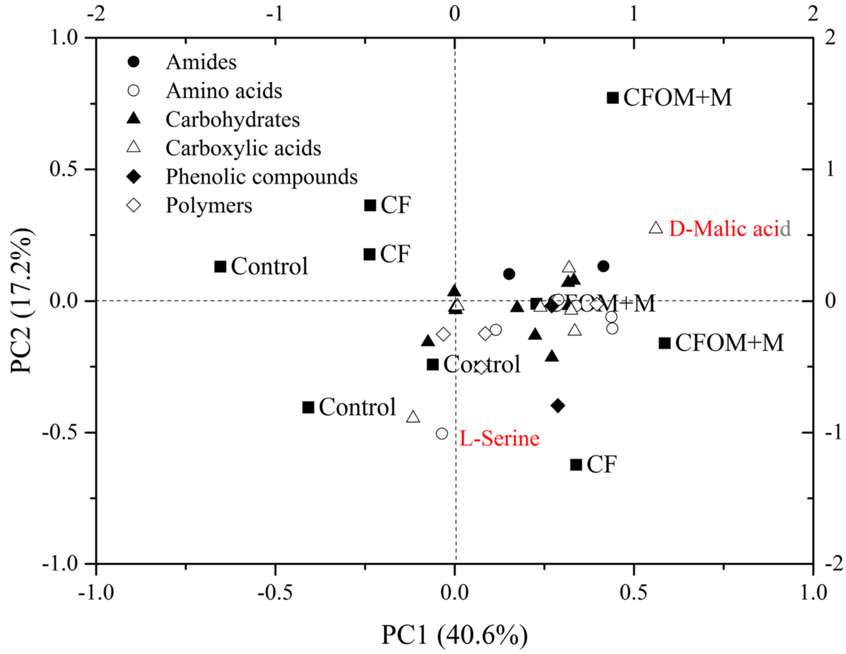

Fig. 3 Principal component analysis of carbon source utilization patterns of microbial community under different treatments. *Control* no fertilization control, *CF* chemical fertilization, *CFOM + M* chemical and organic fertilization combined with organic mulching

4.2 Effects of fertilization combined with organic mulching on soil microbial biomass and respiration

Jiang et al. (2006) reported that MBC increased with combined application of organic and mineral fertilizer. Compared to no mulching, MBC was significantly higher for a mulching treatment (Huang et al. 2008b). Heavy winter mulching and high rates of fertilization significantly enhanced soil CO_2 efflux in a Lei bamboo forest (Jiang et al. 2009). Dissimilar to previous reports, intensive management had no significant impact on MBC and soil basal respiration in the present study. However, decrease of qMBC under intensive management indicated when abundant exogenous C was supplied, only a limited amount of C was incorporated into microbial biomass. There was also a significant decline in qCO_2 in the CFOM + M treatment. Usually, higher qCO_2 indicates that microorganisms have a lower C use efficiency, since they divert energy from growth to maintenance (Killham 1985). Thus, lower qCO_2 indicated that nutrient increases have a positive effect on C utilization efficiency of microorganisms in the present study (Ma et al. 2015). Total SOC is closely related to changes of cumulative mineralization in bamboo forest (Zhuang et al. 2011). This may explain the increase of cumulative mineralization and potentially mineralizable C under intensive management in the present study.

The regression analysis showed that C/P was related to soil qMBC and qCO_2. Zheng et al. (2015) reported that soil MBC increased but the metabolic quotient of heat (similar to the relationship of respiration to MBC, metabolic quotient of heat represents output of total heat of microbes as it relates to MBC during the metabolic process) decreased when soil P was also increased with N fertilization. They also pointed out that long-term fertilization of P coupled with N, especially combined with organic fertilizer, greatly improved soil fertility and microbial activity. We also concluded that chemical and organic fertilization combined with mulching led to nutrient enrichment and subsequently enhanced the microbial C use efficiency.

4.3 Effects of fertilization combined with organic mulching on CLPP of microorganisms

The soil microbial community plays an essential role in nutrient cycling and organic C turnover (Doran and Zeiss 2000; Burton et al. 2010). Previous research has focused on microbial diversity and community changes under bamboo invasion (Lin et al. 2014), land-use conversion (Guo et al. 2016), elevation gradient (Chang et al. 2016; Lin and Chiu 2016) as well as intensive management in bamboo plantations (Xu et al. 2008; Qin et al. 2014). Soil bacterial functional diversity is also considered a sensitive index that is influenced by management (Gomez et al. 2006). In the present study, changes in AWCD were not significant among treatments. However, regression analysis showed that AWCD was tightly related to AN—indicating promotion of microbial activities with nutrient enrichment. The AWCD at 144 h was selected for analyzing microbial functional diversity indexes and C use patterns. Dissimilar to previous study (Xu et al. 2008), there was a dramatic increase of U index in treatment of fertilization combined with organic mulching in the present work. The U index depends on the number of individuals and their distribution among species in a population (McIntosh 1967), and a higher index indicates that more C sources could be utilized evenly under fertilization combined with organic mulching.

Table 5 Correlation coefficients of 31 carbon sources with principal components 1 and 2 (PC1 and PC2, respectively)

Substrate category	Carbon source	PC1	PC2
Amides	Phenyl-ethylamine	0.711*	NS
	Putrescine	NS	NS
Amino acids	L-Arginine	NS	NS
	L-Asparagine	0.868**	NS
	L-Phenylalanine	0.954**	NS
	L-Serine	NS	−0.789*
	L-Threonine	0.855**	NS
	Glycyl-L-glutamic acid	NS	NS
Carbohydrates	β-Methyl-D-glucoside	0.683*	NS
	D-Galactonic acid γ-lactone	NS	NS
	D-Xylose	NS	NS
	i-Erythritol	NS	NS
	D-Mannitol	0.693*	NS
	N-Acetyl-D-glucosamine	NS	NS
	D-Cellobiose	0.746*	NS
	Glucose-1-phosphate	NS	NS
	α-D-Lactose	NS	NS
	D, L-α-Glycerol phosphate	NS	NS
Carboxylic acids	Pyruvic acid methyl ester	NS	NS
	D-Galactonic acid	0.810**	NS
	γ-Hydroxybutyric acid	NS	−0.675*
	D-Glucosaminic acid	0.870**	NS
	Itaconic acid	NS	NS
	α-Ketobutyric acid	0.772*	NS
	D-Malic acid	0.761*	NS
Phenolic compounds	2-Hydroxy benzoic acid	NS	−0.770*
	4-Hydroxy benzoic acid	0.799**	NS
Polymers	Tween 40	NS	NS
	Tween 80	NS	NS
	α-Cyclodextrin	0.808**	NS
	Glycogen	NS	NS

*, ** Correlation significant at $p < 0.05$ (2-tailed) and $p < 0.01$ (2-tailed), respectively

PCA results demonstrated that microorganisms of the CFOM + M treatment were better able to use most of the C sources, as shown by a separation of CFOM + M from control. Correlation analysis indicated that utilization of carboxylic acid, carbohydrates, and amino acids influenced the differentiation of C source use patterns of microbial communities in the different treatments. Furthermore, the use of D-malic acid was positively, but the use of L-serine was negatively, related to CFOM + M. The results of PRC also showed that the deviation of intensive management from control increased over the whole incubation time. The weighted scores (canonical coefficient) of C sources demonstrated that D-malic acid and L-serine were the most sensitive indicators of changes in different treatments. Anaerobic metabolism can occur and malate can accumulate in plants under stress (Kozlowski 1997); thus, we propose that organic mulching might have led to oxygen deficiency and accumulation of malic acid in plant and soil, and finally affected the C source utilization of microorganisms. Serine is a common component of plant root exudates (Griffiths et al. 1999). Ruamrungsri et al. (1996) found that total amino acids including serine were higher in plant roots under N, P, or K deficiency stress. Therefore, we suggest that lower serine utilization indicated an alleviation of stress from nutrient limitation under intensive management.

RDA results showed that TN, TK, AN, and C/P were the main factors influencing C source utilization of microbial communities for the different treatments. *Oligotrophic* microorganisms grow under very low nutrient conditions, but *copiotrophic* microorganisms grow rapidly at high nutrient concentrations (Giovannoni et al. 2014). For instance, it was reported that N and P nutrient elevation increased the relative abundance of *copiotrophic* bacterial taxa (Leff et al. 2015). Therefore, we suggest that transformation of *oligotrophic* to *copiotrophic* microbes might have led to a shift in the microbial community when environmental nutrients were increased in the present study. N or P excess or limitation also plays key roles in organic C dynamics and microorganism changes

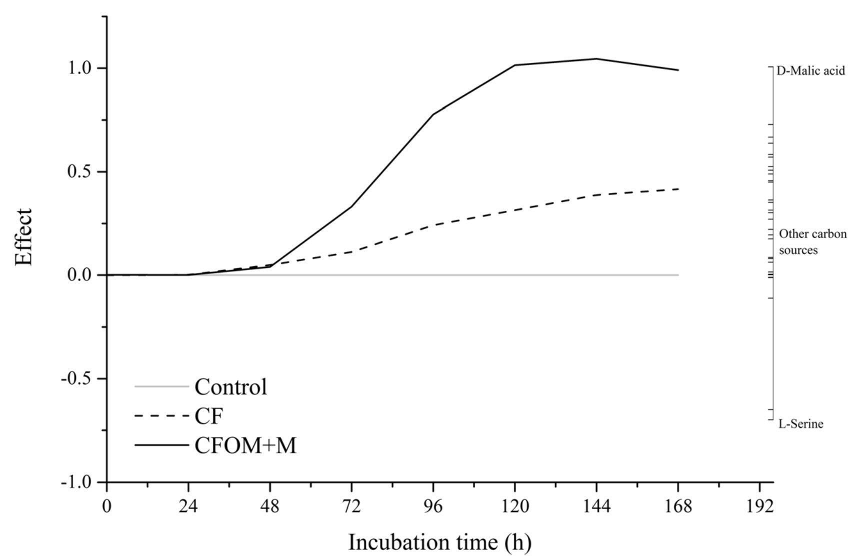

Fig. 4 Principal response curve representing treatment effect under different treatments. The *vertical axis* on the *right* represents the canonical coefficient of carbon sources. Higher absolute values indicate positive or negative response of carbon source to treatments. Values close to zero indicate weak relationships between carbon sources and treatments. *Control* no fertilization control, *CF* chemical fertilization, *CFOM + M* chemical and organic fertilization combined with organic mulching

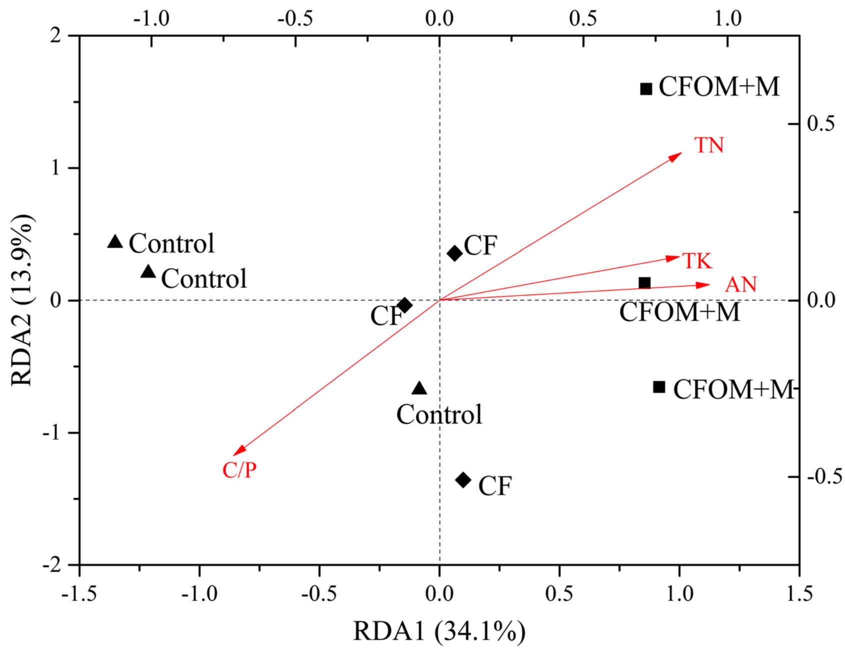

Fig. 5 Redundancy analysis of carbon source utilization patterns of microbial communities under different treatments. *Control* no fertilization control, *CF* chemical fertilization, *CFOM + M* chemical and organic fertilization combined with organic mulching, *TN* total nitrogen, *TK* total potassium, *AN* available nitrogen, *C/P* carbon to phosphorus ratio

(Griffiths et al. 2012). Fanin et al. (2013) illustrated that C/nutrient ratios of dissolved organic matter in litter were closely related to the soil microbial community. Ma et al. (2016) concluded that changes of soil microbial community composition were associated with increased P and C/P and N/P. Elemental ratios (i.e., resource stoichiometry) may affect microbial community composition through r or K life strategies (Kaiser et al. 2014). Microbes of r-strategy grow rapidly when environmental resources are sufficient, but K-strategy microbes survive when resources are deficient. Therefore, we propose that abundant nutrients favored the growth of fast-growing microorganisms and changed the C use pattern of the whole community under intensive management.

Abundant nutrients may have increased soil microbial functional diversity and led to enhancement of C utilization of fast-growing microorganism (i.e., r-strategy). However, r-strategy microorganisms also have higher extinction rates when the environment changes dramatically. Therefore, the potentially negative effect of fertilization combined with organic mulching on soil microbial properties, particularly on microbial diversity should be considered in the long run, if nutrient accumulation exceeds the tolerance of the bamboo ecosystem.

5 Conclusions

Intensive management increased SOC and soil total and available nutrients in a Lei bamboo plantation. The qMBC decreased, but qCO_2 clearly declined, indicating a higher C utilizing efficiency of microorganisms under fertilization combined with organic mulching. Fertilization combined with organic mulching also increased functional diversity of microorganisms. Response of L-serine indicated an alleviation of nutrient deficiency under intensive management. TN, TK, AN, and C/P were the main factors influencing C source utilization patterns of microbial communities. Sufficient nutrients benefited the C source utilization of rapid-growing microorganisms and led to shifts in the microbial community. The long-term effect of fertilization combined with organic mulching on soil microbial properties, functional diversity, and stability of bamboo ecosystems should be further considered.

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