

# Accumulation of residual soil microbial carbon in Chinese fir plantation soils after nitrogen and phosphorus additions

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**Abstract** Nitrogen (N) and phosphorus (P) additions can affect soil microbial carbon (C) accumulation. However, the mechanisms that drive the changes in residual microbial C that occur after N and P additions have not been well-defined for Chinese fir plantations in subtropical China. We set up six different treatments, viz. a control (CK), two N treatments (N<sub>1</sub>: 50 kg ha<sup>-1</sup> a<sup>-1</sup>; N<sub>2</sub>: 100 kg ha<sup>-1</sup> a<sup>-1</sup>), one P treatment (P: 50 kg ha<sup>-1</sup> a<sup>-1</sup>), and two combined N and P treatments (N<sub>1</sub>P: 50 kg ha<sup>-1</sup> a<sup>-1</sup> of N + 50 kg ha<sup>-1</sup> a<sup>-1</sup> of P; N<sub>2</sub>P:

100 kg ha<sup>-1</sup> a<sup>-1</sup> of N + 50 kg ha<sup>-1</sup> a<sup>-1</sup> of P). We then investigated the influences of N and P additions on residual microbial C. The results showed that soil pH and microbial biomass decreased after N additions, while microbial biomass increased after P additions. Soil organic carbon (SOC) and residual microbial C contents increased in the N and P treatments but not in the control. Residual microbial C accumulation varied according to treatment and declined in the order: N<sub>2</sub>P > N<sub>1</sub>P > N<sub>2</sub> > N<sub>1</sub> > P > CK. Residual microbial C contents were positively correlated with available N, P, and SOC contents, but were negatively correlated with soil pH. The ratio of residual fungal C to residual bacterial C increased under P additions, but declined under combined N + P additions. The ratio of residual microbial C to SOC increased from 11 to 14% under the N<sub>1</sub>P and N<sub>2</sub>P treatments, respectively. Our results suggest that the concentrations of residual microbial C and the stability of SOC would increase under combined applications of N and P fertilizers in subtropical Chinese fir plantation soils.

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**Keywords** Amino sugar · Chinese fir plantation · N and P additions · Residual microbial carbon · Soil environment variable

## Introduction

Residual microbial carbon (C) accounts for between 25 and 40% of total soil organic carbon (SOC) and is mainly derived from microbial metabolites and residues (Ding et al. 2013; Khan et al. 2016). Amino sugars, including glucosamine (GluN), muramic acid (MurN), and galactosamine (GalN), are important biomarkers of residual microbial C (Amelung 2001). For example, GluN and

MurN have been used as biomarkers for residual fungal C and residual bacterial C, respectively (Amelung 2001; Liang et al. 2011). The residual fungal C to residual bacterial C ratio has been used to illustrate the stability of residual microbial C and SOC (Amelung 2001; Six et al. 2006). The origin of GalN, however, is controversial (Engelking et al. 2007; Ding et al. 2013). Soil microbial biomass carbon (MBC) and phospholipid fatty acids (PLFA) degrade rapidly after cell death (Zelles 1999). Soil MBC is one component of the organic C that living microorganisms provide, and accounts for about 1% of total SOC (Wallenstein et al. 2006). As biomarkers of living microorganisms, PLFAs have concentrations of approximately 9–12 nmol g<sup>-1</sup> in soil (Dong et al. 2015). Residual microbial C is more stable, and makes a higher contribution to SOC, than do MBC or PLFA (Khan et al. 2016).

To date, studies of residual microbial C have mostly focused on agricultural ecosystems. For example, MurN and residual bacterial C contents increased after exogenous nitrogen (N) additions, but MurN degraded when soil N was deficient (He et al. 2011). Some authors have reported positive correlations (Kindler et al. 2009; Ding et al. 2013), while others have reported no relationship (Chantigny et al. 1997) between the accumulation of residual microbial C and soil living microbial biomass. It is thought that N and P additions might affect PLFA and MBC contents in subtropical forest soils. Nitrogen additions might cause MBC to either decrease (Wallenstein et al. 2006; Treseder 2008) or increase (Dong et al. 2015; Wu et al. 2015). Other studies reported that additions of P only and combined additions of N and P resulted in increased PLFAs (Wang et al. 2014; Dong et al. 2015) and promoted MBC (Geiseler and Scow 2014; Huang et al. 2016). However, the relationship between residual microbial C and PLFAs is poorly defined. Further, few studies have addressed the effects of N and P additions on residual microbial C in subtropical forests.

Chinese fir (*Cunninghamia lanceolata*), the main coniferous forest species in subtropical China, covers around  $9.11 \times 10^6$  hectares and accounts for about 18% of China's plantations (Dong et al. 2015). During recent decades, soil fertility and timber production have declined sharply because of continuous cropping, short rotation periods, and clear cutting (Yang et al. 2005). Fertilization has been recommended as the most effective and feasible method to reverse the downward trend in timber production (Liao et al. 2014). The aim of this study was to assess the impacts of N and P fertilization on residual microbial C and to identify and quantify the factors that drive variations in residual microbial C. In our previous study, soil nutrient content increased after N and P additions (Dong et al. 2015). We therefore hypothesize that N and P additions

will be conducive to the accumulation of residual microbial C, especially when N and P are applied together.

## Materials and methods

### Site description

Our study site was in the Shixi Forest Plantation, Jiangxi Province, southern China (26°44'52"N, 115°04'13"E, elevation 100 m a.s.l.). The Chinese fir plantation was planted in 2000. The site is characterized by a subtropical monsoon climate, with mean annual temperature of 17.9 °C and mean annual precipitation of 1471.2 mm, respectively. Soils were classified as Ultisols using the USDA-NRCS soil taxonomy (1996). Mean soil temperatures in November, April, and July were 14.5, 17.5, and 26.2 °C, respectively, and soil moisture content was 23, 27, 23%, respectively. Soil bulk density was 1.31 g cm<sup>-3</sup>, soil pH was 4.6, and SOC, total N, and total P concentrations were 17.16, 1.20, and 0.16 g kg<sup>-1</sup>, respectively.

### Experimental design

Eighteen 400 m<sup>2</sup> (20 × 20 m) sampling plots were demarcated in November 2011. We separated plots by distances of at least 10 m. Six different treatments with three randomly distributed replicates were used, as follows: (1) control (CK): with no fertilization; (2) N addition N<sub>1</sub>: 50 kg ha<sup>-1</sup> a<sup>-1</sup> of N; (3) N addition N<sub>2</sub>: 100 kg ha<sup>-1</sup> a<sup>-1</sup> of N; (4) P addition P: 50 kg ha<sup>-1</sup> a<sup>-1</sup> of P; (5) NP addition N<sub>1</sub>P: 50 kg ha<sup>-1</sup> a<sup>-1</sup> of N + 50 kg ha<sup>-1</sup> a<sup>-1</sup> of P; (6) NP addition N<sub>2</sub>P: 100 kg ha<sup>-1</sup> year<sup>-1</sup> of N + 50 kg ha<sup>-1</sup> a<sup>-1</sup> of P.

Nitrogen was added as NH<sub>4</sub>NO<sub>3</sub> and P was added as NaH<sub>2</sub>PO<sub>4</sub> (Dong et al. 2015). Fertilizers were mixed with sand and were hand-scattered at 3-month intervals from March 2012 until December 2015. Up to July 2015, 550 and 1100 kg of N were added to the N<sub>1</sub> and N<sub>2</sub> treatments, respectively; 550 kg of P were added to the P treatment; 550 kg of N and 550 kg of P were added to the N<sub>1</sub>P treatment, and 1100 kg of N and 550 kg of P were added to the N<sub>2</sub>P treatment. Application rates varied by season: the applications in March and June (growing season) each accounted for 30% of the total annual application, while the applications in September and December (non-growing season) each accounted for 20% of the total annual application. To avoid potential impacts on soil organisms, understory plants were removed manually at regular intervals but no herbicide was applied.

## Soil sampling and analysis

Soils were sampled in November 2014 and in April and July 2015. Before sampling, the litter layer was carefully removed. Five soil cores (with an inner diameter of 5 cm) were collected randomly from the 0–10 cm soil layer of each plot and composited uniformly to reduce within-plot variability. All fresh soil samples were sieved through a 2-mm mesh and stored at 4 °C until analysis for soil pH and PLFAs.

Soil pH was measured in a soil–water suspension at a ratio of 1:2.5 using a digital pH meter. Soil organic carbon and total nitrogen (TN) were measured with an elemental analyzer (Elementar, Vario Max, Germany). Available N (AN), available P (AP), and total phosphorus (TP) concentrations were quantified using a continuous flow analyzer (BranLubbe AA3, Germany) following extraction with 1 mol L<sup>-1</sup> KCl, extraction with 0.03 mol L<sup>-1</sup> NH<sub>4</sub>F and 0.025 mol L<sup>-1</sup> HCl, and digestion with H<sub>2</sub>SO<sub>4</sub>–HClO<sub>4</sub>, respectively.

The soil microbial community was characterized using PLFA analysis. Samples were dissolved in hexane and analyzed by gas chromatography (Agilent 7890 B) with MIDI peak identification software (Version 4.5; MIDI Inc., Newark, DE). Bacterial biomarkers were represented by i14:0, i15:0, a15:0, i16:0, a16:0, i17:0, a17:0, 16:1ω9c, cy17:0, 18:1ω5c, 18:1ω7c, and cy19:0; fungal biomarkers were represented by 18:3ω6c, 18:1ω9c and 16:1ω5c. Total PLFAs were the sum of bacterial PLFAs, fungal PLFAs, 10Me16:0, 10Me17:0, and 10Me18:0. The internal standard was 19:0.

The amino sugars (MurN, GluN, GalN) were determined using the method of Appuhn et al. (2001), with O-phthalaldehyde (OPA) derivatization, as described by Indorf et al. (2011). Fresh samples of 0.5 g soil were hydrolysed for 6 h at 105 °C with 10 mL 6 mol L<sup>-1</sup> HCl. The derivatives were separated by a high-performance liquid chromatographer (HPLC) (Dionex Ultimate 3000) equipped with an octadecylsilylated silica (ODS) gel column (Acclaim120 C18; 4.6 × 150 mm, 3 μm), and detected using a fluorescence detector with an emission wavelength of 445 nm and an excitation wavelength of 330 nm. Amino sugars were identified and quantified with reference to chromatograms of standard solutions containing mixed amino sugars.

## Calculations and statistical analysis

Residual fungal C was calculated by subtracting bacterial GluN from total GluN as an index for fungal residues, assuming that MurN and GluN were present at a ratio of 1:2 in bacterial cells (Engelking et al. 2007), as follows:

$$\text{Residual fungal C (mg g}^{-1} \text{ dry weight)} = (\text{mmol g}^{-1} \text{ GluN} - 2 \times \text{mmol g}^{-1} \text{ MurN}) \times 179.2 \text{ g mol}^{-1} \times 9,$$

where the molecular weight of GluN was 179.2, and a value of 9 was used to convert fungal GluN to residual fungal C.

Residual bacterial C served as an index of bacterial residues and was calculated by multiplying the concentration of MurN by 45 (Appuhn et al. 2006), as follows:

$$\text{Residual bacterial C (mg g}^{-1} \text{ dry weight)} = \text{mmol g}^{-1} \text{ MurN} \times 251.2 \text{ g mol}^{-1} \times 45,$$

where the molecular weight of MurN was 251.2, and a value of 45 was used to convert bacterial MurN to residual bacterial C.

Residual microbial C was represented by the sum of residual fungal C and residual bacterial C.

All results were reported as means ± standard errors (SEs) (n = 3). We used two-way analysis of variance to evaluate the effects of treatments and seasonal variations on soil environment variables and residual microbial C. We used principal component analysis (PCA) to evaluate the effects of all treatments on soil environment variables, microbial biomass, and residual microbial C. SPSS 18.0 for Windows (SPSS 18.0 Inc., Chicago, IL, USA) was used for both of these analyses. The correlations between residual microbial C and soil environment variables were evaluated using CANOCO software versions 4.5. Sigmaplot 10.0 was used for drawing graphs. The level of significance was P < 0.05.

## Results

### Effects of N and P additions on soil properties

Compared with the control, AP, bacterial biomass (B), and soil organic carbon (SOC), increased by about 4.5 times, 28, and 18%, respectively, after P additions. Concentrations of SOC in the N<sub>2</sub> and NP treatments were increased by about 43 and 33%, respectively. Nitrogen additions led to soil acidification, and caused bacterial biomass (B), fungal biomass (F), and total microbial biomass (total PLFAs) to decrease (Table 1). The ratios of fungal biomass to bacterial biomass (F/B) were only affected by the N<sub>2</sub> treatment.

There were significant seasonal variations in soil available nutrients, microbial biomass, and pH (Table 2). Soil available nutrients (NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>3-</sup>), and F and B were higher in the growing season (April, July), and lower in the non-growing season (November). Conversely, soil pH was lower in the growing season (April, July), and higher in the non-growing season (November). Soil organic carbon was higher in the growing season (April), and lower in the non-growing season (November). There were no significant seasonal variations in TN, TP, and the F/B ratio.

**Table 1** Responses of soil properties to N and P additions

Treatment	pH	F/B	SOC (mg g <sup>-1</sup> )	TN (mg g <sup>-1</sup> )	TP (mg g <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> (mg kg <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> (mg kg <sup>-1</sup> )	PO <sub>4</sub> <sup>3-</sup> (mg kg <sup>-1</sup> )	Total PLFA (nmol g <sup>-1</sup> )	F (nmol g <sup>-1</sup> )	B (nmol g <sup>-1</sup> )
CK	4.7 ± 0.04a	0.24 ± 0.01b	17.1 ± 0.6c	1.2 ± 0.03d	0.16 ± 0.01b	3.3 ± 0.6d	18.1 ± 1.3c	4.1 ± 0.9c	9.6 ± 0.4b	1.6 ± 0.08ab	6.4 ± 0.3bc
N <sub>1</sub>	4.5 ± 0.04b	0.26 ± 0.01ab	18.3 ± 0.6c	1.2 ± 0.03 cd	0.15 ± 0.01b	8.5 ± 0.6c	26.3 ± 1.3b	4.6 ± 0.9c	7.6 ± 0.4c	1.3 ± 0.08cd	5.1 ± 0.3d
N <sub>2</sub>	4.3 ± 0.04b	0.28 ± 0.01a	23.1 ± 0.6a	1.4 ± 0.03a	0.17 ± 0.01b	16.1 ± 0.6a	34.0 ± 1.3a	5.0 ± 0.9c	6.1 ± 0.4d	1.1 ± 0.08d	4.2 ± 0.3e
P	4.6 ± 0.04a	0.22 ± 0.01b	20.1 ± 0.6b	1.3 ± 0.03bc	0.26 ± 0.01a	3.4 ± 0.6d	20.1 ± 1.3c	22.8 ± 0.9b	11.6 ± 0.4a	1.7 ± 0.08a	8.2 ± 0.3a
N <sub>1</sub> P	4.4 ± 0.04b	0.23 ± 0.01b	22.4 ± 0.6a	1.4 ± 0.03ab	0.28 ± 0.01a	7.7 ± 0.6c	20.7 ± 1.3c	21.4 ± 0.9b	9.7 ± 0.4b	1.5 ± 0.08bc	6.7 ± 0.3b
N <sub>2</sub> P	4.4 ± 0.04b	0.25 ± 0.01ab	23.0 ± 0.6a	1.4 ± 0.03ab	0.25 ± 0.01a	13.9 ± 0.6b	24.7 ± 1.3b	25.7 ± 0.9a	8.7 ± 0.4bc	1.4 ± 0.08bc	5.7 ± 0.3 cd

Means ± SEs (n = 3). The same letter for each row was not significantly different at  $P < 0.05$

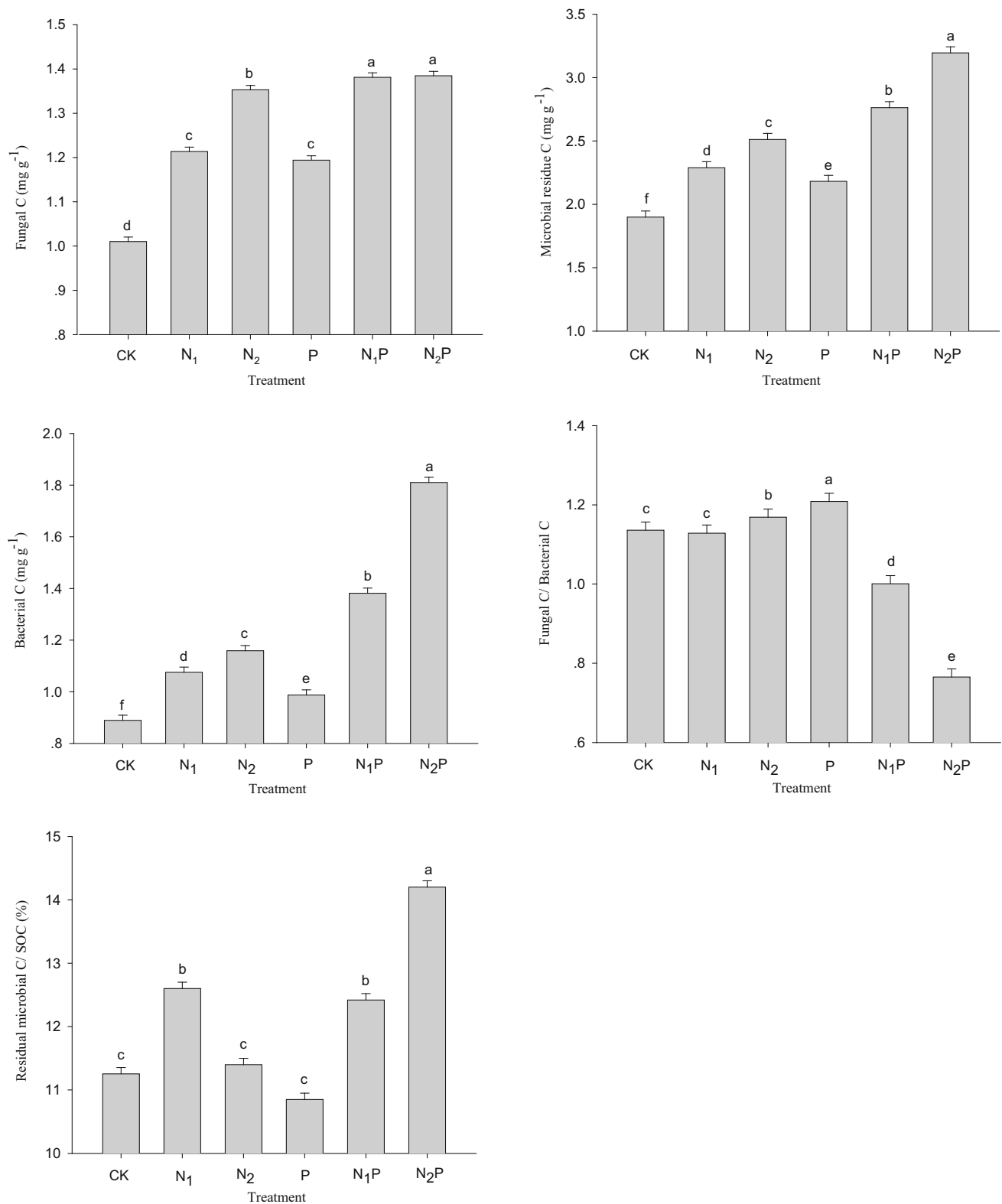
SOC soil organic carbon, TN total nitrogen, TP total phosphorus, NO<sub>3</sub><sup>-</sup> nitrate N, NH<sub>4</sub><sup>+</sup> ammonia N, PO<sub>4</sub><sup>3-</sup> available phosphorus, Total PLFA total microbial biomass, F fungal biomass, B bacterial biomass, F/B fungal biomass/bacterial biomass

**Table 2** Seasonal variation in soil properties

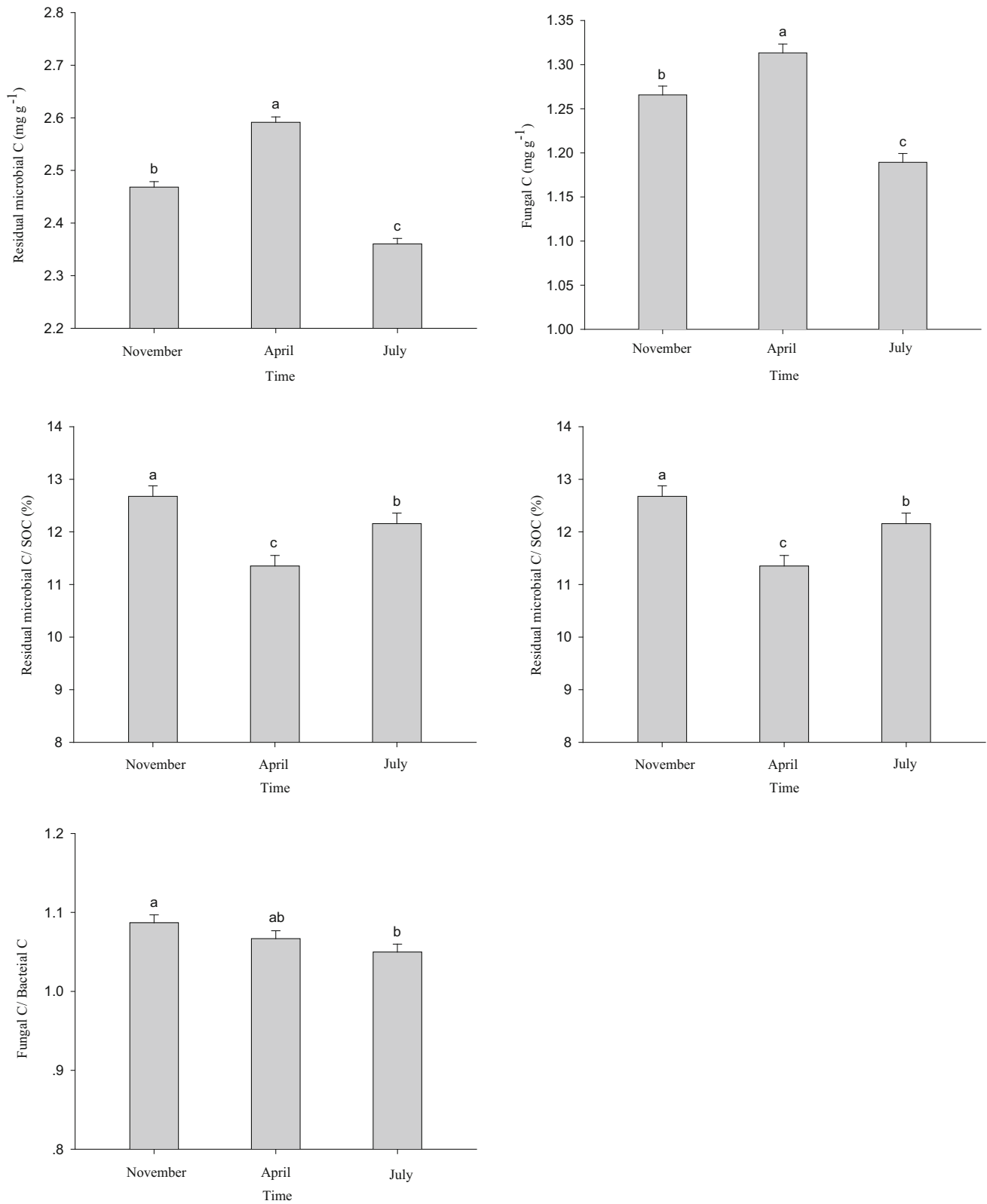
Time	pH	F/B	SOC (mg g <sup>-1</sup> )	TN (mg g <sup>-1</sup> )	TP (mg g <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> (mg kg <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> (mg kg <sup>-1</sup> )	PO <sub>4</sub> <sup>3-</sup> (mg kg <sup>-1</sup> )	Total PLFA (nmol g <sup>-1</sup> )	F (nmol g <sup>-1</sup> )	B (nmol g <sup>-1</sup> )
November 2014	4.6 ± 0.03a	0.24 ± 0.01a	19.5 ± 0.2b	1.3 ± 0.02a	0.2 ± 0.01a	7.4 ± 0.4c	14.4 ± 0.9c	11.3 ± 0.04c	4.0 ± 0.2c	0.6 ± 0.06b	2.8 ± 0.1c
April 2015	4.4 ± 0.03b	0.25 ± 0.01a	22.9 ± 0.2a	1.4 ± 0.02a	0.2 ± 0.01a	8.3 ± 0.4b	30.7 ± 0.9a	14.3 ± 0.04b	10.3 ± 0.2b	1.8 ± 0.06a	7.0 ± 0.1b
July 2015	4.5 ± 0.03b	0.23 ± 0.01a	19.6 ± 0.2b	1.3 ± 0.02a	0.2 ± 0.01a	10.8 ± 0.4a	26.9 ± 0.9b	16.2 ± 0.04a	12.3 ± 0.2a	1.9 ± 0.06a	8.3 ± 0.1a

Means ± SEs (n = 3). The same letter for each row was not significantly different at  $P < 0.05$

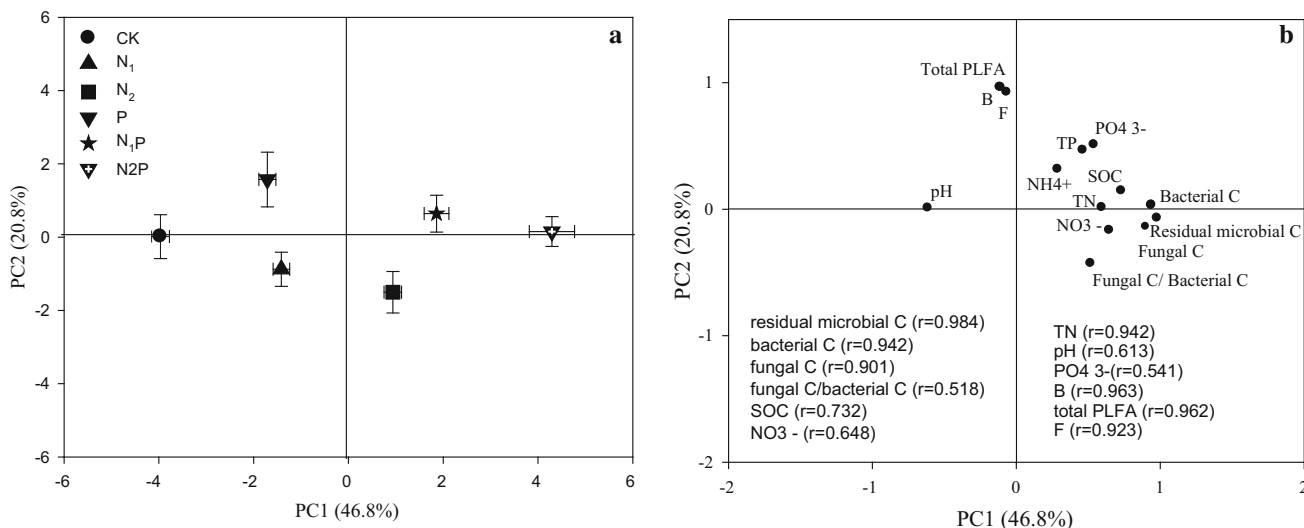
SOC soil organic carbon, TN total nitrogen, TP total phosphorus, NO<sub>3</sub><sup>-</sup> nitrate N, NH<sub>4</sub><sup>+</sup> ammonia N, PO<sub>4</sub><sup>3-</sup> available phosphorus, Total PLFA total microbial biomass, F fungal biomass, B bacterial biomass, F/B fungal biomass/bacterial biomass



**Fig. 1** Responses of residual microbial carbon to N and P additions

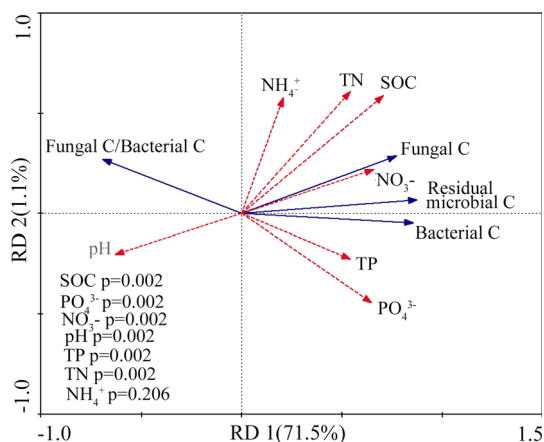


**Fig. 2** Seasonal variation in residual microbial carbon



**Fig. 3** Principal component analyses (PCA) of the PLFAs, residual microbial carbon, and environmental variables in the soil samples from different N and P treatments. **a** Score plot of the different N and P treatments. **b** Loading values of the PLFAs, soil properties, and residual microbial carbon. Bars represent standard error. Fungal

C = residual fungal carbon; Bacterial C = residual bacterial carbon; Residual microbial C = residual microbial carbon; SOC = soil organic carbon;  $\text{NO}_3^-$  = nitrate N;  $\text{NH}_4^+$  = ammonia N;  $\text{PO}_4^{3-}$  = available phosphorus; TN = total nitrogen; TP = total phosphorus



**Fig. 4** Redundancy analysis (RDA) of residual microbial carbon and environmental variables in the soil samples. The explanatory variables are indicated by different arrows: residual microbial carbon by black arrows: Fungal C = residual fungal carbon; Bacterial C = residual bacterial carbon; Residual microbial C = residual microbial carbon, environmental variables by dashed arrow: SOC = soil organic carbon;  $\text{NO}_3^-$  = nitrate N;  $\text{NH}_4^+$  = ammonia N;  $\text{PO}_4^{3-}$  = available phosphorus; TN = total nitrogen; TP = total phosphorus

**Effects of N and P additions on residual microbial carbon content**

Additions of N and P, and particularly combined N + P additions, resulted in increased residual fungal and bacterial C contents (Fig. 1). Relative to the control, concentrations of residual fungal C, residual bacterial C, and residual microbial C increased by 38–39, 55–103, and 45–68% in the N + P treatments. The residual fungal C to

residual bacterial C ratio improved with P additions, while the residual fungal C to residual bacterial C ratio decreased with combined additions of N + P.

Residual microbial C contents accounted for about 11–14% of SOC after N or P additions. The ratios of residual microbial C to SOC ratios were 7–27% higher in the  $\text{N}_1$  and NP treatments than in the control.

Residual fungal C, residual bacterial C, and residual microbial C were higher in the growing season than in the non-growing season (Fig. 2). The ratio of residual fungal C to residual bacterial C was higher in November (1.08), and lower in July (1.05). The ratio of residual microbial C to SOC was higher in the non-growing season (November) than in the growing season (April, July).

**Principal component analysis**

The first (PC1) and second (PC2) principal components explained 46.8 and 20.8% of the total variance, respectively (Fig. 3a). The effects of the various N and P treatments on microbial biomass, residual microbial C contents, and environmental variables in PC1 differed significantly, and were ranked in the order  $\text{N}_2\text{P} > \text{N}_1\text{P} > \text{N}_2 > \text{N}_1 > \text{P} > \text{CK}$ . The  $\text{N}_2\text{P}$  treatment, to the right of PC1, had the highest score, and the CK treatment, to the left of PC1, had the lowest score. The P treatment had the highest score in PC2.

PC1 was mainly associated with residual microbial C, soil nutrients, and bacterial, fungal, and total PLFA contents (Fig. 3b). Residual microbial C and soil nutrient contents were affected by the  $\text{N}_1\text{P}$  and  $\text{N}_2\text{P}$  treatments. Soil



pH was affected most by N additions. B, F, and total microbial biomass were influenced by P additions.

### Redundancy analysis

The first (RD1) and second canonical axes explained 71.5 and 1.1% of the total variance (Fig. 4). RD1 was positively correlated with soil nutrients (SOC,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ , TP, TN) and soil pH, while RD2 was positively correlated with  $\text{NH}_4^+$ . Residual fungal C, residual bacterial C, and residual microbial C were positively correlated with SOC,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ , TP, and TN, and were negatively correlated with soil pH. The ratio of the residual fungal C to residual bacterial C was positively correlated with soil pH.

### Discussion

Compared with the control, P additions caused an increase in microbial biomass. Phosphorus was limited at the experimental site, and long-term P additions improved soil available nutrients for microbial organisms, which then increased the living microbial biomass, as also reported by others (Chen et al. 2010; Wang et al. 2014; Huang et al. 2016). Nitrogen additions resulted in reduced microbial biomass, which is consistent with the reports by Wallenstein et al. (2006) and Yang et al. (2005), but contrasts with the results reported by Dong et al. (2015). One or two years of N additions can increase the growth rate of forest trees and the accumulation of litter (Chen et al. 2010). Short-term N additions can cause an increase in microbial biomass (Wu et al. 2015; Dong et al. 2015). In this study, N additions over three to four years led to deteriorations in soil environmental properties, such as acidification, increases in soil osmotic potential, and deficiencies in  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , which could result in decreased microbial biomass (Treseder 2008).

This study demonstrates that N and/or P additions, and particularly N + P additions, promoted residual fungal C, residual bacterial C, and residual microbial C, all results consistent with our hypothesis. Comparison with the control showed that addition of N reduced microbial biomass while the contents of residual microbial C increased. This agrees with previous reports of no relationship between residual microbial C and microbial biomass (Ding et al. 2013), but contrasts with the results of Kindler et al. (2009) and Liang et al. (2011), who found that the increase in residual microbial C resulted from improvements in the living microbial biomass. Soil pH and microbial biomass decreased after N additions, and residual microbial C was negatively correlated with soil pH (Fig. 4). Second, microorganisms struggle to decompose residual microbial C under excessive N additions, which means that N

accumulates (Fontaine et al. 2011); this may explain why residual microbial C increased more under N additions than under P additions. Third, various authors reported that N additions accelerated soil nitrification in acid forest soils, promoting death of microorganisms (Dail et al. 2001). Most residual microbial C (> 90%) occurs in dead microbial cells (Amelung 2001). Residual microbial C contents were positively correlated with AP and SOC contents in this study (Fig. 4). The increases in AP and SOC contents might promote reproduction and death of microorganisms, and this might cause increase in residual microbial C content (Joergensen et al. 2010). However, while microbial biomass did not change in the N + P treatment, residual microbial C increased dramatically because of the balance between the growth and death of microorganisms.

In this study, residual microbial C contents were not related to soil moisture or temperature (data not shown), but were positively correlated with SOC contents (Fig. 4). The contents of SOC were higher in April, indicating greater accumulation of residual microbial C in April. The SOC contents in July and November were similar, but the residual microbial C contents were lower in July than November, and most likely reflect the fact that the increased microbial biomass and plants absorbed large quantities of residual microbial C for growth during July (Table 2).

The ratio of residual fungal C to residual bacterial C decreased in the N + P treatment, but increased with addition of P and  $\text{N}_2$  (Fig. 1). This possibly reflected the differing availability of soil N fractions ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) between fertilizer treatments (Fig. 4). The increase in available N (especially  $\text{NH}_4^+$ ) promoted metabolism of bacteria, and accelerated the formation of MurN and residual bacterial C (Yevdokimov et al. 2008), thereby causing a decrease in the ratio of MurN and residual bacterial C, and an increase in the stability of residual microbial C in SOC. On the other hand, fungus had priority in nutrient acquisitions and MurN had a faster turnover rate than GluN when there were shortages of available N (Liang et al. 2011). Therefore, GluN and residual fungal C increased rapidly. MurN was either decomposed or used for fungal metabolism when available N and SOC were in short supply (Amelung 2001; Wallenstein et al. 2006; Ding et al. 2013). We found that N and P fertilizer additions did not affect F/B ratios. Previous studies showed that the F/B ratio was correlated with the ratio of residual fungal C to residual bacterial C (Kindler et al. 2009), which agreed with our earlier study (Dong et al. 2015) but disagreed with this study. The inconsistency may reflect differences between the ecosystems studied and the components of the different fertilizer products. Further, while the F/B ratio is known to be strongly affected by soil moisture (Wardle and



Parkinson 1990), our results showed that soil moisture was similar after N and P additions (data not shown).

## Conclusions

Through this study, we have demonstrated that the SOC contents and the residual microbial C to SOC fertilization ratios improved after N and/or P additions, with particular improvements observed after combined N + P additions. Soil pH decreased and soil became acidified after N additions. Living microbial biomass increased after P additions. Residual microbial C contents increased in the different treatments after N and P additions, with most increase in the N<sub>2</sub>P treatment and least increase in the CK (N<sub>2</sub>-P > N<sub>1</sub>P > N<sub>2</sub> > N<sub>1</sub> > P > CK). The residual microbial C contents were positively correlated with available N, P, and SOC, but were negatively correlated with soil pH. There were no significant correlations between residual microbial C and living microbial biomass.

The ratio of residual fungal C to residual bacterial C decreased after N + P additions, but increased after P additions, which indicates that N and P additions decreased, but P additions on their own increased, the stability of residual microbial C.

Combined N and P additions were beneficial for accumulation of residual microbial C and SOC, while P additions increased the stability of residual microbial C and SOC. We can therefore conclude that P and N plus P combined additions can improve soil quality in Chinese fir plantations in the subtropics.

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