# AVAILABILITY OF N AND P IN THE RHIZOSPHERE OF THREE SUBTROPICAL SPECIES

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GUAN X, WANG SL & ZHANG WD. 2016. Availability of N and P in the rhizosphere of three subtropical species. This study aimed to assess the variation in soil chemical properties in the rhizosphere soils between plantations of 25-year-old Chinese fir (*Cunninghamia lanceolata*), Chinese gugertree (*Schima superba*) and Masson pine (*Pinus massoniana*), which were all situated in a typical subtropical forest in southern China. Acid phosphatase activity, urease activity, nitrogen (N) mineralisation, nitrification rate, available phosphorus (P) and microbial biomass carbon (C), soil basal respiration, pH, total organic C, total N, total P and inorganic N concentrations were examined in the rhizosphere and bulk soils. Results indicated that total organic C, total N, total P, soil basal respiration, net N mineralisation, nitrification rate, urease activity and acid phosphatase activity significantly increased in the rhizosphere compared with bulk soil. This led to higher levels of available P,  $NH_4^+$ -N and  $NO_3^-$ -N concentrations in the rhizosphere soils. The magnitude of rhizosphere effects on N and P transformations were mostly greater in the Chinese fir plantation followed by the Chinese gugertree and Masson pine plantations. These findings implied that the three species had different capacities in terms of acquiring nutrients.

Keywords: Chinese gugertree, Chinese fir, Masson pine, N and P transformations, rhizosphere effect

#### **INTRODUCTION**

Rhizosphere processes play important roles in nutrient cycling. However, it is not clear how the effects of rhizosphere are influenced by tree species. The interaction between root exudation, nutrient uptake and microorganism has combined effects on rhizosphere nutrient cycling which are critical in maintaining productivity and stability of the ecosystem (Wang & Zabowski 1998). Rhizosphere processes have been wellstudied and described in agroecosystems and grass lands (Kuzyakov & Domanski 2000, Jones et al. 2004, Paterson 2003). However, little is known about the magnitude and ecological effects of rhizosphere in natural forest and subtropical ecosystems (Hobbie 1992, Phillips & Fahey 2006). It is difficult to quantify the rhizosphere effect, because rhizosphere processes are constantly changing in time and space and vary in extension and character (Smith 1990). Most studies of rhizosphere processes in trees have focused on seedlings. They provide limited and contradictory information about rhizosphere processes. For example, it was reported that available nitrogen (N) and phosphorus (P) in rhizosphere soil might be higher (Cardon et al. 2002, Turpault et al. 2005), lower (Chen et al. 2002) or equal (Ehrenfeld et al. 1997) to those in bulk soils. The rhizosphere nutrient cycling of trees in forest are different from seedlings in pots.

Different tree species have different nutrient uptake capacities which are determined by the characteristics and activities of the roots (Lovett & Rueth 1999, Lovett et al. 2004). Tree species may affect the nutrient cycle and ecosystem processes that occur in the rhizosphere (Calvaruso et al. 2011). It is important to know the differences in nutrient cycling in the rhizosphere so as to compare nutrient acquisition capacity of different tree species and to understand the process affecting the soil. However, studies on comparing nutrient acquisition capacity of different tree species are very limited (Phillips & Fahey 2006). Therefore, it is necessary to understand rhizosphere nutrient cycling of different tree species (Jones et al. 2004).

The main objective of this study was to compare and quantify rhizosphere effects of different tree species on transformation of soil N and P. Soil physical and chemical properties associated with N and P were compared in rhizosphere and bulk soils under 25-year-old Chinese fir, Chinese gugertree and Masson pine plantations in a typical subtropical area of south China. We hypothesised that rhizosphere effects of all three species vary with species and would lead to increased N and P transformations, but the magnitude of this effect would be different between the species. Specifically, this study was aimed at determining (1) if rhizosphere transformation of N and P significantly differed from bulk soil, and (2) how tree species differed in quantity and compositional structure of soil N and P.

# MATERIALS AND METHODS

#### Site description and experimental design

The study was conducted at the Huitong Experimental Station of Forest Ecology, Chinese Academy of Sciences (500 m above sea level), Hunan Province, China. The climate in this region is humid mid-subtropical monsoon with annual average temperature of 15.8 °C. Mean annual precipitation is 1200 mm and mean relative humidity during the duration of the study ranged from 34 to 93%. Textural composition is 10.6% sand, 42.6% silt and 46.8% clay in the 0–10 cm mineral soil layer with soil bulk density of 1.4 g cm<sup>-3</sup> (Wang et al. 2009).

We selected three 25-year-old plantations for this study: Chinese fir, Chinese gugertree and Masson pine. These species are the common tree species in subtropical region. Chinese fir, a coniferous species peculiar in China, is fast growing and has good quality timber of high value. Masson pine, also a fast-growing coniferous species, is highly adaptive to poor soil fertility. The deciduous Chinese gugertree is one of the dominant tree species in the subtropical evergreen broadleaf forest. The first generation Chinese fir trees were uprooted and removed in the autumn of 1986. In the early spring of 1987, Chinese fir, Chinese gugertree and Masson pine were adjacently planted on the same type of soil. All plantations were established with the same density of 3600 trees hm<sup>-2</sup>. At the time of study, the three plantations were 25 years old and had not received any intensive management practices including fertilisation and pruning.

# Soil sampling

Rhizosphere and bulk soils were collected in spring (April) of 2011. Four 10 m  $\times$  10 m plots were randomly established for soil sampling. Litter horizons were removed before soil sampling. Four to eight soil samples (depending on the number needed to collect rhizosphere soil for process-based assays) were randomly taken from the upper 10-20 cm layer using soil core of 5 cm in diameter from each plot to ensure that sufficient rhizosphere soil were collected. The collected soil cores were mixed into one sample for each plot. The soil was transported quickly to the lab after sampling and placed in a sorting basin where large aggregates were gently broken. Rhizosphere soil was the soil attached to the fine roots (< 2 mm diameter) after they were gently shaken, whereas the soil not attached to the roots was the bulk soil. Live fine roots were picked out. Each soil sample was divided into two subsamples. Field moist subsamples were immediately sieved through a 2-mm mesh and then stored at 4 °C until analyses for the estimation of microbial biomass carbon (C), NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, N mineralisation, acid phosphatase activity and urease activity. The second subsamples were air dried and divided into two subsamples. One subsample was sieved through a 2-mm mesh for the determination pH and basal respiration. The other subsample was sieved through a 0.25-mm mesh to determine total organic C, total N and total P.

# Laboratory analyses

Soil total organic C was determined by  $K_2Cr_2O_7$ - $H_2SO_4$  oxidation method and total N, by semimicro-Kjeldahl method. Soil total P was measured colorimetrically (Liu et al. 1996). Soil pH was determined in soil:water suspension (1:2.5) using glass electrode. Soil inorganic N (NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N) concentrations were

determined colorimetrically using indophenol blue spectrophotometric method and extracted with 2 M KCl extracting water solution. Available P was determined according to Liu et al. (1996) after acid extraction with 0.05 M HCI and 0.025 M H<sub>9</sub>SO<sub>4</sub>. Soil urease activity was determined using standard colorimetric method (Guan 1986) and expressed as mg of released NH<sub>3</sub>-N by 1 kg soil hour<sup>-1</sup> at 37 °C. Acid phosphatase activity was determined using the method of Wu et al (2006) and expressed as mg of hydrolysed phenol by 1 kg soil hour<sup>-1</sup> at 37 °C. Net N transformation rate (mineralisation and nitrification) was calculated as the difference in NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N concentrations between the incubated and initial samples.

Soil microbial biomass C was determined using the chloroform fumigation extraction method (Vance et al. 1987). Soil basal respiration was determined by measuring  $CO_2$  evolution (Chen et al. 2000). Field moist soil was aerobically incubated at 27 °C in 0.5 L-sealed glass jar. Carbon dioxide released from the soil was trapped in 0.1 N NaOH and measured by titration with 0.05 M HCl after 7 days. Microbial metabolic quotient was calculated by dividing the hourly basal respiration rate by the corresponding microbial biomass C. Microbial quotient was reported as percentage microbial biomass C over the corresponding total organic C.

#### Statistical analyses

All soil data are on oven (105 °C) dry weight basis and statistical analyses were conducted using SPSS 16.0. All data were tested for normality and homogeneity of error variances prior to comparing means. The magnitude of rhizosphere effect was calculated as the percentage difference between paired rhizosphere and bulk soil samples for a given response variable.

Rhizosphere effect  $\% = (V_{R} - V_{R}) \times 100 / V_{R}$ 

where V = soil variable, R = rhizosphere soil and B = bulk soil. A one-way analysis of variance (ANOVA) was carried out to examine the magnitude of rhizosphere effect. Paired samples *t*test was employed to compare the differences in each soil variable between rhizosphere and bulk soils for each tree species separately. Tukey's honestly significant difference (HSD) test was used for post hoc multiple comparisons. Significance level was set at  $\alpha = 0.05$  in all statistical analyses.

# RESULTS

#### Soil total organic C, total N and total P

Significant rhizosphere effect on soil total organic C and total N was observed in all plantations (Table 1), with the greatest effect in Chinese fir plantation (Table 2). There was no significant difference between rhizosphere and bulk soils for total P in Masson pine and Chinese gugertree, but total P content in the rhizosphere soil was significantly higher than in bulk soil for Chinese fir plantation. The pH value did not change significantly between rhizosphere and bulk soil in Masson pine and Chinese gugertree plantation. However, it significantly decreased by 8.1% in rhizosphere soil in comparison with bulk soil in Chinese fir plantation. The magnitude of the rhizosphere effect on total organic C, total N and total P differed significant among three species (Table 2).

## Microbial biomass C, basal respiration, microbial metabolic quotient and microbial quotient

There was little difference in microbial properties (microbial biomass C, soil basal respiration, microbial metabolic quotient and microbial quotient of the rhizosphere compared with bulk soil (Table 3). Similarly, microbial metabolic quotient and microbial quotient were not significant between rhizosphere and bulk soils. Basal respiration was 255.4, 55.6 and 155% greater in rhizosphere than in bulk soil of the Chinese fir, Chinese gugertree and Masson pine respectively (Table 3).

#### Transformations of N and P

 $\rm NH_4^{+}-N$  concentrations were similar in the rhizosphere and bulk soils for Chinese fir and Masson pine but, in Chinese gugertree plantation, it was significantly higher in the rhizosphere soil compared with bulk soil (Figure 1a). The  $\rm NO_3^{-}-N$  concentration significantly increased in the rhizosphere soil compared with bulk soil of Chinese fir and Chinese gugertree plantations.

Tree species	Soil	TOC (g kg <sup>-1</sup> )	TN (g kg <sup>-1</sup> )	TP (g kg <sup>-1</sup> )	pН
Chinese fir	Rhizosphere	42 (4.05) a	2.92 (0.17) a	0.27 (0.01) a	3.83 (0.07) b
	Bulk	11.17 (1.31) b	1.15 (0.12) b	0.18 (0.02) b	4.17 (0.06) a
Masson Pine	Rhizosphere	18.27 (0.67) a	1.64 (0.09) a	0.22 (0.01) a	4.12 (0.11) a
	Bulk	9.69 (0.59) b	1.09 (0.04) b	0.19 (0.01) a	4.14 (0.03) a
Chinese	Rhizosphere	33.46 (4.26) a	2.53 (0.23) a	0.25 (0.01) a	4.10 (0.03) a
gugertree	Bulk	10.38 (1.23) b	1.12 (0.06) b	0.19 (0.03) a	4.29 (0.43) a

**Table 1**Soil total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP) and pH in bulk and<br/>rhizosphere soils of three subtropical plantations

Values shown in tables are means  $\pm$  standard errors (n = 4), values with different letters are significantly different at p = 0.05 between rhizosphere and bulk soils for Chinese fir, Masson pine and Chinese gugertree plantations by paired-samples *t*-test

 Table 2
 Effects of rhizosphere of three subtropical species on percentage of soil variables

Tree species	TOC	TN	TP	APA
Chinese fir	292 (61.77) a	163 (35.81) a	57 (13.38) a	200 (58.83) a
Masson pine	92 (17.26) b	52 (13.16) b	15 (8.55) b	145 (27.26) b
Chinese gugertree	223 (16.99) a	125 (14.93) a	40 (20.60) a	179 (15.17) a

TOC = total organic carbon. TN = total nitrogen, TP = total phosphorus, APA = acid phosphatase activity; values shown in tables are means  $\pm$  standard errors (n = 4), values for microbial biomass carbon and urease were not significant, values in the same column with different superscript letters denote significant differences between tree species at p < 0.05, ns = not significant, all values for microbial biomass carbon, urease, net nitrification and N mineralisation were not significant

Table 3	Microbial biomass carbon (MBC), basal respiration (BR), microbial metabolic quotient (qCO <sub>2</sub> ),
	microbial quotient (MBC/TOC) in rhizosphere and bulk soils of three subtropical plantations

Tree species	Soil	BR (μg.g <sup>-1</sup> h <sup>-1</sup> )	MBC (mg C kg <sup>-1</sup> )	$\begin{array}{c} qCO_2 \\ (mg.g^{-1}h^{-1}) \end{array}$	MBC/TOC %
Chinese fir	Rhizosphere	2.48 (0.32) a	442.23 (88.58) a	0.61 (0.08) a	1.01 (0.14) a
	Bulk	0.69 (0.03) b	153.72 (47.49) a	0.57 (0.13) a	1.3 (0.25) a
Masson Pine	Rhizosphere	1.16 (0.09) a	220.09 (36.63) a	0.58 (0.1) a	1.19 (0.17) a
	Bulk	0.74 (0.03) b	146.43 (36.93) a	0.71 (0.29) a	1.57 (0.45) a
Chinese gugertree	Rhizosphere	2.04 (0.55) a	397.15 (130.11) a	0.55 (0.04) a	1.14 (0.27) a
	Bulk	0.8 (0.03) b	145.22 (23.69) a	0.62 (0.14) a	1.54 (0.43) a

TOC = total organic carbon, values shown in tables are means  $\pm$  standard errors (n = 4), values with different letters are significantly different at p = 0.05 between rhizosphere and bulk soils for Chinese fir, Masson pine and Chinese gugertree plantation by paired-samples *t*-test



**Figure 1**  $NH_4^+N$  and  $NO_3^-N$  concentrations, potential net N mineralisation, nitrification and urease in rhizosphere and bulk soils of three subtropical plantations, (mean ± SE, n = 4); different letters indicate significant differences between rhizosphere and bulk soils for each tree species by paired-samples *t*-test (p < 0.05)

Nitrogen mineralisation and nitrification rates were higher in rhizosphere soil compared with bulk soil in all plantations (Figures 1c and d). The magnitude of the rhizosphere effect on N mineralisation and nitrification rate was greatest in Chinese fir plantation and the least in Masson pine plantation. Urease activity was significantly higher in rhizosphere soil in all plantations.

Concentrations of available P and acid phosphatase activity were significantly enhanced in rhizosphere soil compared with bulk soil except for concentrations of available P in Chinese fir (Figure 2).

#### DISCUSSION

# Effects of rhizosphere transformation of N and P

Rhizosphere effect increased N and P transformations in all three plantations. Nitrogen mineralisation rate was significantly higher in rhizosphere soil compared with bulk soil for all plantations. Nitrification rate increased in rhizosphere soil of Chinese fir, Chinese gugertree and Masson pine with values at 167.5, 94.2 and 145.5% respectively (Figure 1d). Tree root stimulated gross N mineralisation and immobilisation. Enhanced N mineralisation may have resulted from rhizosphere priming effects on decomposition of soil organic matter (Colin-Belgrand et al. 2003) and grazing of rhizosphere

bacteria through microbial loop (Clarholm 1985, Jackson et al. 2008).

Greater N mineralisation and nitrification rates result in accumulation of total N and NO<sub>2</sub>-N for all plantations but only NH<sub>4</sub><sup>+</sup>-N in Masson pine plantation. Increases in soil N products were consistent with the observation by Turpault et al. (2005). N mineralisation and nitrification rates were higher in rhizosphere soil than in bulk soil. In general, enhanced N mineralisation in rhizosphere soil primarily results from rootinduced stimulation which may be due to positive rhizosphere priming effects on decomposition of soil organic matter (Phillips & Fahey 2005). Available N levels may accumulate, remain unchanged or deplete in the rhizosphere when compared with bulk soil (Ehrenfeld et al. 1997, Wang et al. 2001, Zhao et al. 2010, Koranda et al. 2011). This shows that trees have developed diverse strategies to obtain nutrients they need under limiting conditions (Carroll et al. 2003). Root exudates affect soil microbial activity and community composition in forest soil (Jackson et al. 2008). These different results between rhizosphere and bulk soils reflect the diversity of rhizosphere soil and morphological and physiological differences between tree species. They are also affected by other factors such as soil fertility and moisture as well as plant growth requirements (Phillips & Fahey 2006, Jackson et al. 2008).

As expected, urease and acid phosphatase activities were significantly increased in



Figure 2Availability of P and acid phosphatase activity (APA) in rhizosphere and bulk soils of<br/>three subtropical plantations (mean  $\pm$  SE, n = 4); different letters indicate significant<br/>differences between rhizosphere and bulk soils for each tree species by paired-samples<br/>*t*-test (p < 0.05)</th>

rhizosphere soil. Soil enzymes are primarily derived from plant roots and soil microorganisms (Chen 2003). Higher urease and acid phosphatase activity in the rhizosphere relative to bulk soil indicated that the former provided essential nutrients and labile C for the production of these enzymes. This finding was confirmed by the higher N products, total P, available P and total organic C concentrations in the rhizosphere soil compared with bulk soil. The rate of P supply is the most limiting factor to forest growth in many subtropical ecosystems (Chen 2003). A high acid phosphatase activity in the rhizosphere makes plant acquire more P. In a typical subtropical forest ecosystem with low P availability, high acid phosphatase activity can therefore result in accumulation of inorganic P in the rhizosphere. Enhanced available P and phosphatase activities in the rhizosphere of the three species in this study had been reported for slash pine and were attributed to increased root and microbial activities (Fox & Comerford 1992). Increased inorganic P suggested that the mineralisation rate of organic P was greater in rhizosphere soil than bulk soil.

# Species differences in rhizosphere effects

Consistent with our hypothesis, the magnitude of rhizosphere effects varied with tree species. Evidently, Chinese fir was effective in facilitating N and P transformations, whereas Masson pine had the lowest magnitude of rhizosphere effects. This finding suggested that the three tree species varied in their capacities to acquire the nutrients they needed. Although the magnitude of rhizosphere effect was different, the three tree species had similar patterns of rhizosphere effect on levels of soil N and available P as well as mineralisation. Rhizosphere effect mechanisms are primarily related to the difference between tree species in terms of root exudates, root traits, quantity and chemical property of root exudates to the rhizosphere and associated rhizosphere microbial processes (Yin et al. 2012).

In summary, the results suggested that rhizosphere effects were similar in the three tree species. The species had similar patterns of rhizosphere effects on N and P transformations. But they showed different magnitudes of rhizosphere effects on N and P transformations which suggested that the three species had different capacities to acquire the nutrients they need. Knowledge of the rhizosphere nutrient cycling for different tree species had important implications for forest nutrient dynamics and understanding of a more realistic rhizosphere process.

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