C:N:P stoichiometry of rhizosphere soils differed significantly among overstory trees and understory shrubs in plantations in subtropical China

Xiaoqin Dai[1,2*], Xiaoli Fu[1,2], Liang Kou[1,2], Huimin Wang[1,2,3*], Clinton C. Shock[4]

[1] Qianyanzhou Ecological Research Station, Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China

[2] Jiangxi Key Laboratory of Ecosystem Processes and Information, Ji'an, 343725, China

[3] College of Resources and Environment, University of Chinese Academy of Sciences, Beijing, 100190, China

[4] Malheur Experiment Station, Oregon State University, Ontario, OR 97914 USA

Email address: daixq@igsnrr.ac.cn (X.Q. Dai), fuxl@igsnrr.ac.cn (X.L. Fu), koul@igsnrr.ac.cn (L. Kou), wanghm@igsnrr.ac.cn (H.M. Wang), clinton.shock@oregonstate.edu (C.C. Shock)

[*]Corresponding author.

Tel.: +86 10 64889913; fax: +86 10 64889913

Email: daixq@igsnrr.ac.cn (X.Q. Dai), wanghm@igsnrr.ac.cn (H.M. Wang)

Abstract: Rhizosphere soil C:N:P stoichiometry is useful for identifying the linkage of plant species and soil nutrients, which can be particularly helpful for understory vegetation management of forest ecosystems. There has been limited research on rhizosphere soil stoichiometry, especially for co-existing overstory and understory plant species. We investigated the bulk and rhizosphere soil C:N:P stoichiometry of dominant overstory trees and understory shrubs (*Adinandra millettii, Eurya muricata* and *Loropetalum chinense*) in *Pinus massoniana, Pinus elliottii* and *Cunninghamia lanceolata* plantations in subtropical China. Rhizosphere soil C, N, and P concentrations and ratios increased significantly compared to bulk soil, and those of overstory trees also were higher than those of understory shrubs with exception of *L. chinense*. Rhizosphere soil C:N, C:P, and N:P of *L. chinense* were not significantly different with those of overstory trees, but higher than those of *A. millettii* and *E. muricata*. Soil pH significantly influenced the profiles produced by soil C, N, and P concentrations and their stoichiometries. This study indicated that the difference in nutrient status between overstory trees and understory shrubs was related to shrub species, in which soil pH was the dominant driving factor. Understory shrub species should be considered in plantation management to reduce resource competition among species.

Keywords: Biogeochemical niche, nutrient limitation, pure coniferous forests, plant species, red soil

1. Introduction

Plantations often consist of overstory trees, understory shrubs, and herbs (**Liu et al., 2017b**), which grow together and compete for nutrient resources, including nitrogen (N) and phosphorus (P) (**Wang et al., 2016**). Understory vegetation is commonly removed in plantation management to reduce competition for belowground resources and improve the productivity of target tree species (**Wagner et al., 2006**). The positive role of understory vegetation has been increasingly recognized in forest ecosystem processes and functions in recent years, which includes increasing soil carbon (C) and nutrients (**Qiao et al., 2014; Winsome et al., 2017**), alleviating soil acidification (**Fu et al., 2015**), and promoting litter decomposition (**Mao et al., 2016; Wang et al., 2016**). Therefore, retaining some understory vegetation can be advantageous for forest production and ecological functions in plantation management (**Fu et al., 2015**). Understanding which understory species should be removed or retained is still a significant challenge in forest ecosystem management.

Niche differentiation, including biogeochemical niche differentiation, has been studied extensively as a theoretical mechanism of species coexistence (**Peñuelas et al., 2008; Alvarez-Yepiz et al., 2017**). Biogeochemical niche differentiation reflects that the coexisting different species tend to use the nutrients in differing proportions, thus producing the different stoichiometry for coexisting different species (**Peñuelas et al., 2008; Urbina et al., 2017**). Different ecological stoichiometry implies the potential for different nutrient limitations (**Koerselman and Meuleman, 1996;**

Zechmeister-Boltenstern et al., 2015).

Differences in ecological stoichiometry among plant species can be used as criteria for choosing understory vegetation to coexist with overstory trees. In particular, rhizosphere soil stoichiometry can help assess the linkage of plant species and belowground nutrient balances (Bell et al., 2014). It is feasible to choose some understory species with divergent biogeochemical niche to coexist with target trees in forest ecosystems by using the rhizosphere soil stoichiometry of different species to infer their nutrient limitation. However, most studies about soil stoichiometry have been conducted on bulk soil (Li et al., 2012; Heuck et al., 2015; Chen et al., 2016). For example, Li et al. (2012) examined the variations in soil stoichiometry of C, N and P and the influencing factors based on 1069 soil samples at a depth of 0-20 cm from typical landscapes in southern subtropical China. A recent meta-analysis, based on 2,736 observations along soil profiles of 0-150 cm depth, evaluated temporal changes in soil C:N:P stoichiometry over the past 60 years across subtropical China (Yu et al., 2018). In the grassland, rhizosphere soil C:N:P stoichiometry also was referred by Bell et al. (2014) and Carrillo et al. (2017). However, limited evidence has been collected from rhizosphere soil occupied by different species within the same forests (Bell et al., 2014). Moreover, the differences in the nutrient limitations of co-existing species have rarely been considered from the perspective of rhizosphere stoichiometry.

Because of the rhizosphere's tight linkage between soil and plants, its processes are important in terrestrial C and nutrient cycles. Photosynthetically assimilated CO_2 released via rhizodeposition is the primary carbon source in soil (**Merbach et al., 1999**). A meta-analysis conducted by **Finzi et al. (2015)** showed that root-induced mineralization accounted for up to one-third of the total C and N mineralized in temperate forest soils. It is known that nearly all plant species depend on the symbiotic associations with mycorrhizal fungi to facilitate their nutrient acquisition (**van der Heijden et al., 2015**). The type of mycorrhizal association is specific for each plant species (**Toju et al., 2013**), which determine rhizosphere priming and ecosystem C, N, and P cycling (**Phillips and Fahey, 2006; Lin et al., 2016; Sulman et al., 2017**). There is emerging evidence that functional variations between arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi induce differences in soil C and nutrient cycling (**Cheeke et al., 2017**). For example, owing to greater root exudates rates, ECM-associated plants have higher effects on the microbial properties of rhizosphere soil than AM-associated plants (**Yin et al., 2014**). Variations in the rhizosphere processes among plant species are likely to influence the outcomes of their competition for nutrient resources (**Yin et al., 2012**).

Previous studies have emphasized nutrient limitations, inferred from leaf or soil stoichiometry, among overstory species from different forests (**Hume et al., 2016**); however, few studies have focused on nutrient partitioning between the overstory and understory species from the same forest ecosystem. The lack of such knowledge inhibited forest ecosystem management.

Pinus massonianas, *Pinus elliottii* and *Cunninghamia lanceolata* have been widely planted since the 1980s, and are the dominant plantation forests in mid-subtropical China.

P. massoniana and *P. elliottii* associate with ECM fungi, while *C. lanceolata* associates with AM fungi (**Lin et al., 2016**). The shrubland under the three pure coniferous forests is mainly dominated by *Adinandra millettii* and *Eurya muricata* (associated with AM fungi) and *Loropetalum chinense* (associated with ECM fungi) (**Su et al., 1992**).

We hypothesized that (1) rhizosphere soils have higher C:N and C:P compared to bulk soils because of more C rhizodeposition and N and P uptake by plant roots; (2) *P. massoniana* and *P. elliottii* that have higher rhizosphere soil C concentrations would induce greater C:N and C:P ratios compared to *C. lanceolata* due to their different fungal symbiosis; (3) overstory trees have different rhizosphere soil C:N:P ratios with some understory shrubs, thus inducing different nutrient biogeochemical niches. Examining the differences in soil C, N, and P stoichiometry, advantageous understory species could be selectively retained to coexist with tree species of interest in the plantation ecosystem. Accordingly, the objectives of this study are (1) to examine the differences in concentrations and their stoichiometries of C, N, and P between bulk and rhizosphere soils; (2) to determine how the plantations with different tree species affect rhizosphere soil C:N:P ratios; and (3) to clarify whether rhizosphere soil C:N:P ratios differ among co-existing species within a specific plantation or among three plantations.

2. Materials and Methods

2.1. Study site

The study was conducted at Qianyanzhou Ecological Research Station (26º44′ N, 115º03′ E) of the Chinese Academy of Sciences (CAS), located in Jiangxi Province in

Southern China. The site is a typical red soil, hilly region with a subtropical monsoon climate. Average annual temperature and precipitation vary between 17.4 and 18.9 ℃ and 945 and 2144 mm, respectively. The soils are weathered from red sandstone and mudstone and are classified as Typic Dystrudepts by the USDA system (Soil Survey Staff, 1975). The soil contains 17% sand, 68% silt, and 15% clay (**Wen et al., 2010**).

The zonal vegetation was evergreen broad-leaf forests that were largely destroyed due to human activities prior to the 1980s. Grassland and scattered shrubland became the dominant vegetation which induced severe soil degradation by 1983. Subsequent reforestation was initiated to prevent soil degradation in 1984-1986. The reforesting involved three main pure coniferous forests: *Pinus massoniana*s, *Pinus elliottii*, and *Cunninghamia lanceolata*. *P. massoniana* and *C. lanceolata* are native species, while *P. elliottii* was introduced from the southeastern United States. *P. massonian*as and *P. elliottii* were planted in the soils weathered from sandstone and *C. lanceolata* from mudstone. The properties of the soils for *P. massonian*as, *P. elliottii* and *C. lanceolata* were similar before the plantation establishment (**Fu et al., 2015**). By 2016, age of the three plantations was about 31 years old. The shrubland under the three pure coniferous forests was dominated primarily by *Adinandra millettii*, *Eurya muricata*, and *Loropetalum chinense*. The characteristics of overstory and understory vegetation in the three forest types are presented in Table 1.

2.2. Soil sampling

Soil samples were collected in mid-October of 2016. Five blocks were established on several spatially separate hilly slopes. Three forest types were randomly selected in each block, and a 20×20 m plot was established in each forest type. The distance between blocks was above 100 m and between plots was at least 50 m. No significant differences in soil properties of bulk soils were found among the five blocks ($P>0.05$). In the study site, the roots of overstory trees and shrubs mainly spread on the 0-20 cm soil depth (**Fu et al., 2016**), so bulk soil and rhizosphere soil were sampled from 0-20 cm soil depth. The mulch layer was removed before taking the cores. In each plot, 9 soil cores were randomly selected to acquire 0-10 and 10-20 cm bulk soil; then, these were combined and mixed thoroughly by soil layer. Meanwhile, the rhizosphere soils of overstory trees (*P. massoniana*, *P. elliottii* or *C. lanceolata*) and understory shrub species (*A. millettii*, *E. muricata,* and *L. chinense*) were sampled in each plot. Three to five plants of each species were randomly selected in each plot. Around the selected tree or shrub's trunk, the respective roots plus adhering soil at 0-20 cm soil depth were carefully dug out from the four sites of each plant. If some shrub species had a small root biomass, the plant was carefully dug to acquire its whole roots and adhering soils. The loosely adhering soil was gently shaken off the roots as rhizosphere soil, in which all visible portions of root were removed from the soil. Rhizosphere soil came from distances of less than 4 mm from the root surface (**Riley and Barber, 1970**). The rhizosphere soil was combined and mixed for the same species within the same plot. Each soil sample was collected in a plastic bag and placed in a cooler in the field, then carried to the laboratory. The soil was air-dried for subsequent total organic C, total N and P, and soil pH measurements.

2.3. Soil chemical analysis

Soil pH value was determined in a 1:2.5 soil:water ratio by a digital pH meter. Total organic C (SOC) and total soil nitrogen (TN) concentrations were measured using a Vario-Max N/CN elemental analyzer (Elementar Analysensysteme GmbH, Germany). Soil was digested with H_2SO_4-$HCLO_4$ and total P (TP) concentration was determined by ascorbic acid-molybdate blue colorimetry (Page et al. 1982). The ratios of SOC to TN (C:N), SOC to TP (C:P) and TN to TP (N:P) were calculated.

2.4. Data analysis

Soil C, N, and P concentrations and their ratios, and soil pH value were analyzed for rhizosphere soil for each species and 0-10 and 10-20 cm bulk soil from the same forest type, using linear mixed-effects models with soil sampling type fitted as a fixed effect and block fitted as a random effect. The differences in soil C, N, and P concentrations and their ratios between bulk and rhizosphere soils, and overstory tree and understory shrub, and among overstory species or shrub species were analyzed using linear mixed-effects models. All analyses of variance were conducted using IBM SPSS statistics 20. The standardized data matrix of C, N, and P, and their stoichiometries (TC, TN, TP, C:N, C:P, and N:P) were subjected to principal component analysis (PCA) using the rda function of the vegan package of R software. The first two principal components (PCs) were chosen to account for as much of the variability in the data as possible. The resulting PCs are latent variables and are uncorrelated each other.

3. Results

3.1 Soil pH and C, N and P concentrations

Bulk soils, rhizosphere soils of trees, and those of shrubs had significantly different soil pH value. Rhizosphere soil pH value was lower compared to bulk soils (Fig. 1). And the decrease in overstory rhizosphere soil was higher than that in understory rhizosphere soils (Fig. 1). The average rhizosphere soil C, N, and P concentrations were significantly higher than those of bulk soils (*P*=0.000), especially for overstory species and understory *L. chinense* (Fig. 2). The rhizosphere soil C, N, and P concentrations of overstory species were significantly higher than those of the average across the three understory species (*P*=0.000 for C and N, and *P*=0.01 for P), especially higher than those of *A. millettii* and *E. muricata* except for soil P concentrations in *P. elliottii* forests (Fig. 2). Among understory shrub species, rhizosphere soil C, N, and P were also significantly different (*P*=0.001 for C, *P*=0.000 for N, and *P*=0.003 for P). Rhizosphere soil C, N, and P concenrations of *L. chinense* respectively increased by 33-310%, 31-200%, and 1-73% compared to those of *A. millettii* and *E. muricata*. The differences were greatest in the *P. massoniana* forest and least in the *P. elliottii* forest.

3.2 Soil C, N and P stoichiometry

Bulk and rhizosphere soil C:N ratios were respectively 9.6-19.6:1 and 13.3-28.9:1, C:P ratios were 51.1-132:1 and 114-499:1, and N:P ratios were 4.8-7.7:1 and 7.2-17.3:1. Generally, soil C:N, C:P, and N:P of rhizosphere soil were significantly higher than those of bulk soil (*P*=0.000, Fig. 3). Soil C:N at the 0-10 cm soil depth was significantly higher than that at the 10-20 cm soil depth, but C:P and N:P were not significantly different between the 0-10 and 10-20 cm soil depths in the three plantations. Among overstory species, the rhizosphere soil C:N and C:P of *P. massoniana* and *P. elliottii* were higher than those of *C. lanceolata* (*P*=0.001), but no significant difference of rhizopshere soil N:P was found among them (*P*>0.05). Overstory rhizosphere soil C:N, C:P, and N:P were significantly higher than those of the average across the three shrub rhizosphere soils (*P*=0.000 for C:N and C:P, and *P*=0.001 for N:P), and especially higher than those of *A. millettii* and *E. muricata* (Fig. 3). In most cases, the rhizosphere soil C:N, C:P, and N:P of *L. chinense* was not significantly different with those of overstory rhizosphere soil (Fig. 3).

Soil C, N, and P, and their stoichiometries differed significantly among bulk soils, rhizosphere soils of trees, and those of shrubs: PC1 and PC2 respectively explained 86.1% and 13.3% of the total variance (Fig. 4a). Overstory species and understory species *L. chinense* grouped to the right had higher C, N, and P and stoichiometries, while bulk soil and understory species *A. millettii* and *E. muricata* grouped to the left had lower C, N, and P and stoichiometries along with PC1 (Fig. 4b). PC1 scores were significantly negatively correlated with soil pH (Fig. 5).

4. Discussion

4.1 Effects of bulk and rhizosphere soils on C:N:P stoichiometry

Plants allocate C via their roots to rhizosphere soil that drives microbes to mineralize N and P from organic matter in the soil (**Meier et al., 2015**). As a result, soil nutrients and microbial biomass in the rhizosphere are generally much more abundant than those in bulk soil (**Finzi et al., 2015; Kuzyakov and Blagodatskaya, 2015**). This process is consistent with the result that rhizosphere soil C, N, and P concentrations were significantly greater than the bulk soil (Fig. 2). Rhizosphere soil C, N, and P concentrations were 3.5 times, 1.9 times, and 34% greater respectively compared to bulk soil. The imbalanced difference induced higher soil C:N, C:P, and N:P ratios in rhizosphere soil compared to bulk soil (Fig. 3). The high C:N and C:P ratios implied that rhizosphere soil microbes had relatively more nutrient (e.g. N and P) limitations than energy (C) limitations compared to bulk soil. This could be because approximately 11-20% of the photosynthate is released to the soil as root exudate, in which the most abundant components are carbohydrates and organic acids (**Merbach et al., 1999**). A large amount of rhizodeposited C makes rhizosphere soil microorganisms proliferate quickly and mineralize N and P for their needs from the organic matter in the soil (**Mwafulirwa et al., 2017; Huo et al., 2017**). Although soil N and P concentrations were greater in rhizosphere soil compared to bulk soils, the soil C concentration was 46 g kg^{-1} greater compared to the bulk soil. Theoretically, an extra 6 g N kg^{-1} and 1 g P kg^{-1} should be needed to meet the demand of soil microbial biomass (C:N:P 42:6:1) in rhizosphere soil (**Spohn, 2016**). However, only 1.63 g N kg^{-1} and 0.05 g P kg^{-1} more were present in rhizosphere soil compared to the bulk soil. It is obvious that rhizosphere soil microbes had greater N and P limitations compared to the bulk soil. Net nutrient mineralization also occurs at high C:nutrient ratios due to nutrient turnover in the microbial biomass (**Spohn, 2016)**, in that microbial N and P remineralization is taken up by plants and microbes, or loss from ecosystems.

Previous studies reported that *P. massoniana* forests with 4.4:1 soil N:P ratios were mainly limited by the availability of P in the southern subtropics of China **(Liu et al., 2016)**. Here, the bulk soil N:P ratio averaged to 6.3:1 while that of the rhizosphere soil was 12.2:1 (Fig. 3), indicating that P limitation also occurred at the study site. The P limitation was relatively more severe in rhizosphere soil although higher soil P concentration was found in rhizosphere soil compared to the bulk soil, mainly due to the stoichiometric relationship between microbial biomass and its substrate controls the element cycling of ecosystems (**Spohn, 2016**). Generally, the N:P ratio in microbes is 6-7:1 (**Zechmeister-Boltenstern et al., 2015; Spohn, 2016**), thus microbes will mine out more N to meet their P requirement in the rhizosphere soil (12.2 N coupled by 1 P) compared to bulk soil (6.3 N coupled by 1 P). Only 34% increase in soil P concentration was not enough to meet rhizosphere soil P requirement where abundant soil microbial and crowded root masses exist.

Moreover, roots and rhizosphere microbes may reduce P availability in acid soils through acidification of the rhizosphere (**Hinsinger, 2001**). **Liu et al.** (**2017a**) reported that compared to N, soil P is deficient for plant demands in subtropical China. As a hotspot of root uptake, it is possible that rhizosphere soil P deficiency would be more severe compared to bulk soil due to the intense competition of plants and soil microbes, especially for acidic soil where most P is bound in insoluble complexes **(Zhao and Wu, 2014)**.

4.2 Effects of overstory and understory species on C:N:P stoichiometry

Due to different quantities and qualities of root exudate and rhizodeposits from different plant species (**Ohta and Hiura, 2016**), rhizosphere soil environments and microbial communities change with plant species (**Zhang et al., 2011**), further influencing soil nutrient cycling and its stoichiometry. **Zhao et al., (2014) and Di Palo and Fornara (2015)** showed that plant composition was more important in explaining plant C:nutrient stoichiometric variation than was soil nutrient contents, e.g. SOC, TN, TP, available N and P. In the present study, the rhizosphere soil C:N and C:P of *P. massoniana* and *P. elliottii* were higher than those of *C. lanceolata* (Fig.3). This result indicated that C deposition in roots was likely higher for *P. massoniana* and *P. elliottii* than for *C. lanceolata*. This finding is mainly because *C. lanceolata* roots are colonized by arbuscular mycorrhizal (AM) fungi, while *P. massoniana* and *P. elliottii* are colonized by ectomycorrhizal (ECM) fungi (**Lin et al., 2016**). Previous investigations indicated that ECM trees would exude more C from roots than AM trees (**Philips and Fahey, 2005**). **Yin et al. (2014)** indicated that exudation rates of ECM tree species were nearly two times greater compared to those of AM tree species. Because the two mycorrhizal types exhibit striking differences in morphology, C sink strength, and hyphae spatial extent (**Finlay and Soderstrom, 1992**), ECM tree species must invest more C to build their hyphae network. Thus, it is possible that microbes in the rhizosphere soil of *C. lanceolata* were more limited by energy than by nutrition compared to those of *P. massoniana* and *P. elliottii*. Under C limitation, microbes will mineralize C to meet their energy requirement, while extra N and P are released to the soil for plant uptake (**Schimel and Weintraub, 2003**). The released excess N and P could favor the formation of larger volumes of timber for *C. lanceolata*.

It is known that trees, shrubs, and herbs have significantly different leaf stoichiometry (**Pan et al.**, **2015**). **Fan et al., (2015)** showed higher leaf C:P and N:P ratios for overstory trees than for understory plants. They also found that soil and plant N:P ratio was strongly related. As we hypothesized, overstory rhizosphere soil C:N, C:P, and N:P ratios were significantly higher than those of shrub rhizosphere soil (Fig.3). This is because rhizosphere soil C, N, and P concentrations of overstory trees respectively increased by 54 g kg^{-1} (119%), 1.7 g kg^{-1} (77%), and 0.04 g kg^{-1} (20%) compared to those of understory shrubs (Fig. 2). Firstly, overstory trees may have greater C deposition to rhizosphere soil than do understory shrubs (Fig. 2), which induced higher rhizosphere soil C:N and C:P ratios of overstory trees. C allocation to roots is closely linked with photosynthesis (**Brzostek et al., 2015**). Overstory trees generally have a higher photosynthetic capacity than understory shrubs (**Sakai et al., 2005**), implying the higher root exudation rate of overstory trees. It is well documented that the root exudation rate varies among herbaceous plants, crops, and tree seedlings (**Badri and Vivanco, 2009**). Secondly, rhizosphere soil microbes of overstory trees would mineralize excess N accompanying with soil P mineralization according to the average soil microbial biomass C:N:P ratio (42:6:1) compared to those of understory shrubs. Thirdly, foliar N:P ratio of overstory trees was 16:1 and that of understory shrubs was 20:1 in the study site, implying relatively less N and more P uptake of overstory trees compared to understory shrubs (Unpublished results).

In most cases, the rhizosphere soil C:N, C:P, and N:P of *L. chinense* were similar with those of overstory rhizosphere soil (Fig. 3, Fig. 4a). The variations of the root exudation among co-existing species relate to their functional traits and demand for N and P (**Sun et al., 2017**). Root morphology has a strong effect on the C flux from roots to soil. For example, increased root branching can increase root exudation rates (**Groleau-Renaud et al., 1998**). **Yin et al., (2012)** indicated that differences in the altered morphological and functional characteristics of the roots could be largely responsible for rhizosphere N transformations. It has been verified that *L. chinense* fine root morphological plasticity is higher than that of *C. lanceolata*, which increases the fine root nutrient absorption rate according to the study of **Wang et al. (2015)**.

Among the shrub species, higher rhizosphere soil C, N, and P and their ratios were found for *L. chinense* compared to those of *A. millettii* and *E. muricata*, possibly because *L. chinense* roots are colonized by ECM fungi, while *A. millettii* and *E. muricata* are colonized by AM fungi (**Su et al., 1992**), which suggests a higher exudation rate for *L. chinense* compared to *A. millettii* and *E. muricata*. Moreover, *L. chinense* fine root morphological plasticity was greater, which induced its roots to grow widely into the litter layer (**Wang et al., 2016**). In the current study the roots of *L. chinense* occasionally also grew into the litter layers, but the roots of *A. millettii* and *E. muricata* tend to extend into deeper soil. Although the rhizosphere soil came from the roots of 0-20 cm soil depth, it is possible that the parts of rhizosphere soil of *L. chinense* came from surface soil while those of *A. millettii* and *E. muricata* were from deeper soil. Thus the rhizosphere soil from the roots growing into the litter layer will have the characteristics like surface soil, while the rhizosphere soil from the roots growing to the deeper soil will have the characteristics like subsurface soil. This also is a possible reason for the higher C:N, C:P, and N:P ratios for *L. chinense* and the lower C:N, C:P, and N:P ratios for *A. millettii* and *E. muricata*. The difference in soil C, N and P concentrations and stoichiometries among understory species may not be the direct results of the presence of these species, but the root morphological plasticity lead the roots to the different soil depths, which indirectly induced the different rhizosphere soil C, N and P concentrations and stoichiometries. Further study concerning the fine root morphological plasticity of *A. millettii* and *E. muricata* is required to confirm this result.

4.3 Effects of soil pH on soil C:N:P stoichiometry

Low-molecular-mass organic acids (LMMOAs), such citric acid and oxalic acid, are the main components of root exudate (**Tyler and Ström, 1995**) and vary significantly among tree species (**Aoki et al., 2012; Ohta and Hiura, 2016**). The profiles produced by soil C, N, and P and their stoichiometries of bulk soil and rhizosphere soil of different species were significantly correlated with soil pH (Fig. 5). The C:N, C:P, and N:P ratios were higher when the pH was lower (Fig. 4), indicating that lower pH values had higher N and P nutrition limitations, especially P nutrition limitations. **Suo et al. (2016)** also showed that soil pH was the most important predictor of soil C:N and N:P rather than tree composition or leaf litter input in temperate forests. Soil pH is an important determinant of microbial community composition and activity in forest ecosystems (**Baath and Anderson, 2003; Zhou et al., 2017**), that, in turn, affects ecological processes such as microbial C use efficiency, soil respiration, and N and P mineralization (**Kuzyakov and Blagodatskaya, 2016**). In acid soils, amendment of soil pH can improve the soil available nutrient concentrations (**Carrino-Kyker et al., 2016**), thus promote the growth of target trees. The understory could alleviate soil acidification in subtropical China **(Fu et al., 2015)**, but the understory also influences the growth of overstory trees. In plantation management, retaining a part of understory vegetation is desirable for forest production and the maintenance of ecological function (**Fu et al., 2015**). In the three plantations, the relative dominance of *L. chinense* was up to about 30% (Table 1). Meanwhile, biogeochemical niche of *L. chinense* was similar to the overstory trees, which possibly induce competition for nutrients between *L. chinense* and the overstory trees. Thus our study suggested that *L. chinense* of the three main understory shrubs could be considered for removal in plantation management to achieve higher productivity and ecological function.

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Figure captions

Fig. 1. Bulk and rhizosphere soil pH of overstory and understory species in (a) *P. massoniana*, (b) *P. elliottii*, and (c) *C. lanceolata* plantations in subtropical China. $P<0.05$ indicates significant difference among six soil sampling types.

Fig. 2. Bulk and rhizosphere soil C, N, and P concentrations of overstory and understory species in *P. massoniana* (a, d, h), *P. elliottii* (b, e, i), and *C. lanceolata* (c, f, g) plantations in subtropical China. Different letters above the mean± SE indicate significant differences among bulk soils, rhizosphere soils of the tree species and rhizosphere soils of the shrub species in each plantation at $P<0.05$.

Fig. 3. Bulk and rhizosphere soil C:N:P stoichiometry of overstory and understory species in *P. massoniana* (a, d, h), *P. elliottii* (b, e, i), and *C. lanceolata* (c, f, g) plantations in subtropical China. Different letters above the mean± SE values indicate significant differences among bulk soils, rhizosphere soils of the tree species and rhizosphere soils of the shrub species in each plantation at $P<0.05$.

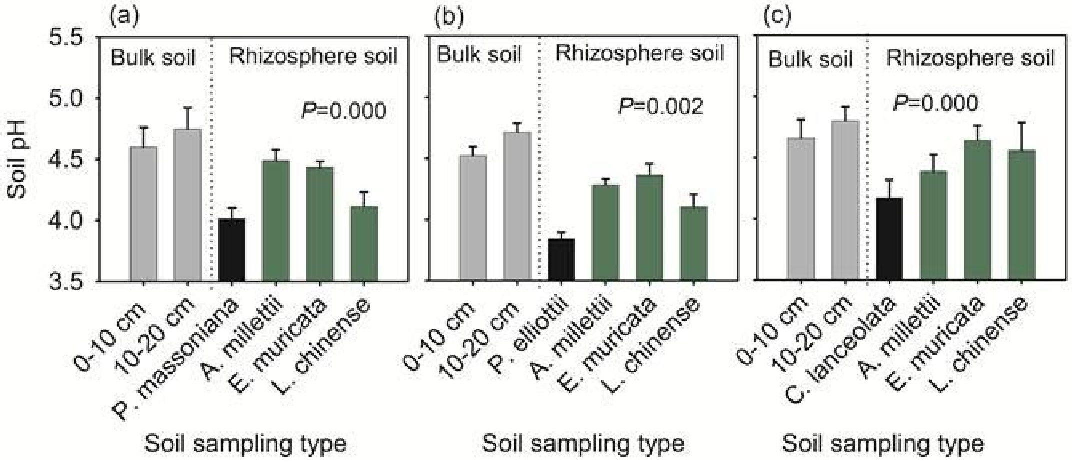
Fig. 4. Principal component analysis of soil C, N, P, and their stoichiometries of the bulk soil and rhizosphere soil of overstory and understory species. a) Separation of soil samples by bulk soil (BS) and rhizosphere soil (RS) of plant species. The gray symbols are 0-10 cm depth of BS, the white symbols are 10-20 cm depth of BS, the red symbols are the RS of *A. millettii*, the purple symbols are the RS of *E. muricata*, the blue symbols are the RS of *L. chinense*, and the green symbols are the RS of overstory trees. b) Distribution of soil C, N, P, and their ratios among different bulk soils, rhizosphere soils

of trees and those of shrubs.

Fig. 5. Relationship of the first axis (PC1) scores of principal component analysis with soil pH.

Table 1 Stand characteristics

Plantation	Diameter at breast height (cm)	Tree height (m)	Stand density (trees ha^{-1})	Canopy density (cm^2 cm^{-2})	Important value of shrub species (%)		
					A. millettii	*E. muricata*	*L. chinense*
P. massoniana	19. 6±1.4	20.2±0.6	1960±211	0.79±0.05	14.7	8.00	33.7
P. elliottii	24.6±1.2	22.0±0.8	2060±309	0.75±0.06	25.3	21.3	36.0
C. lanceolata	20.5±2.1	17.2±3.0	2440±357	0.77±0.04	37.9	29.7	22.2



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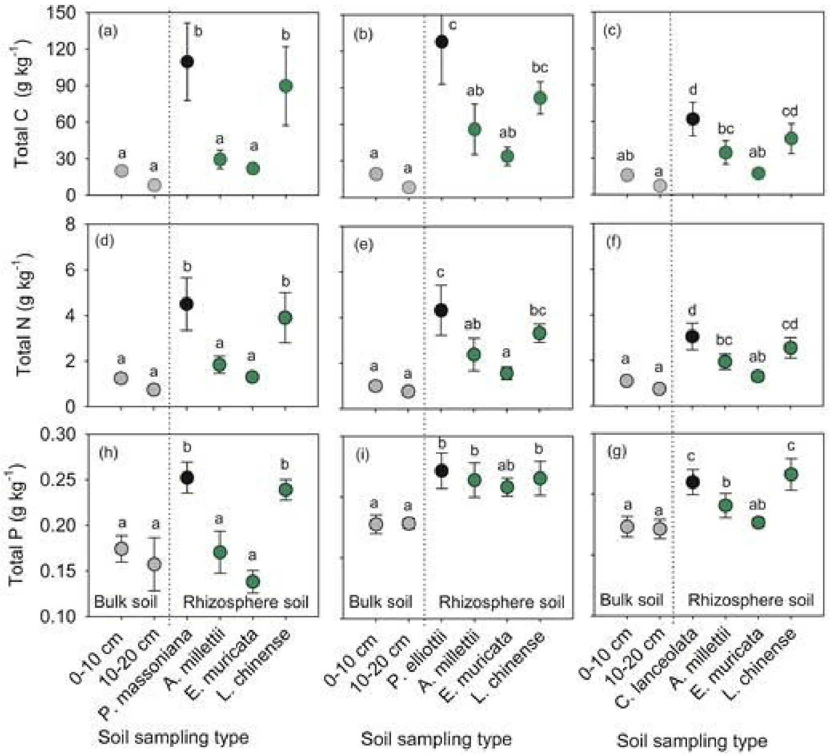
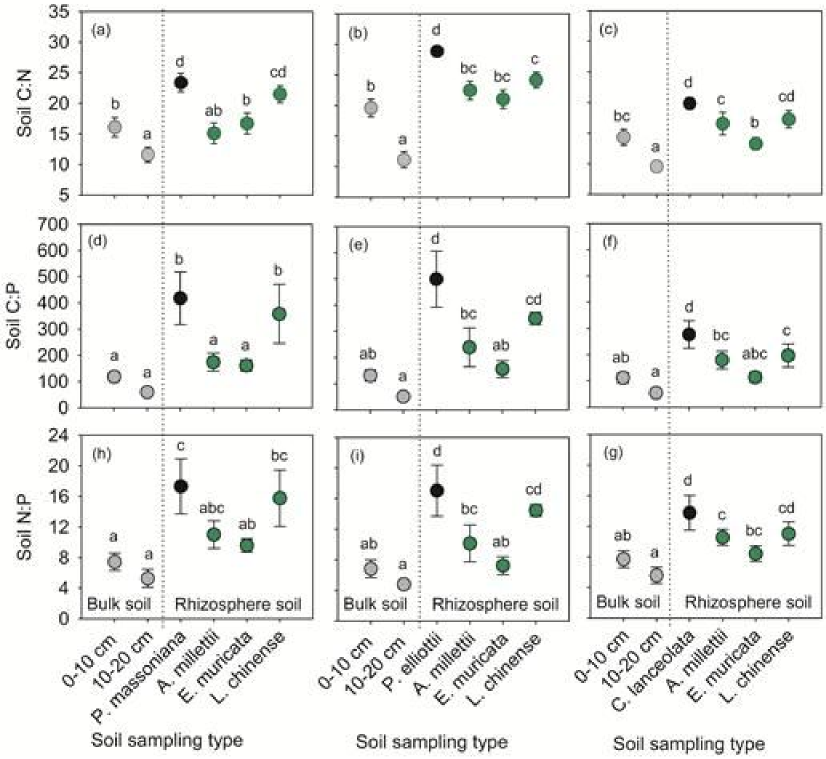
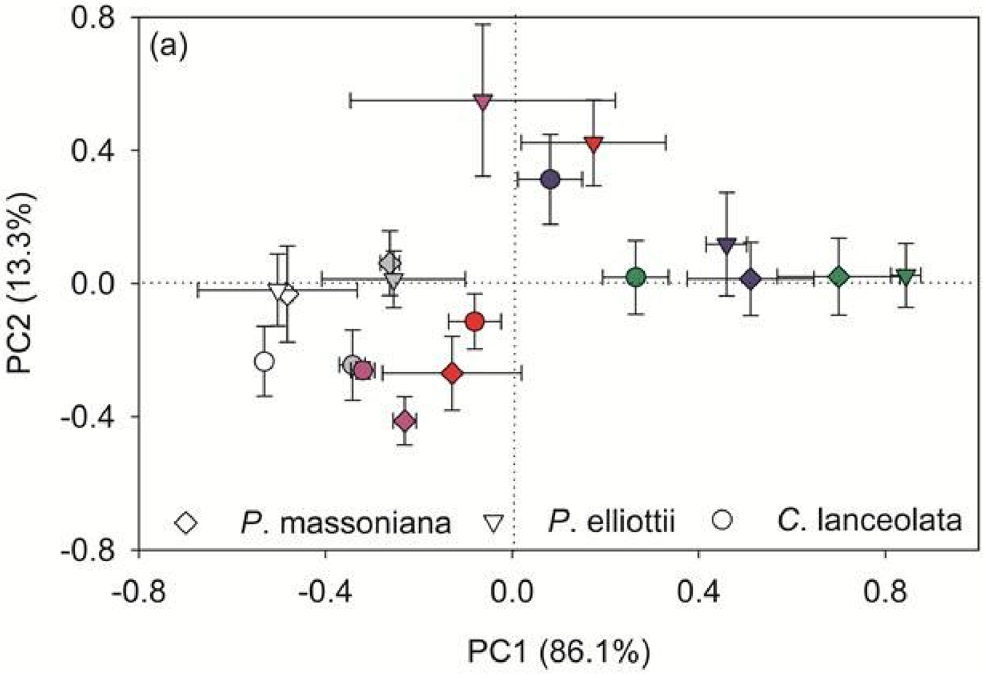
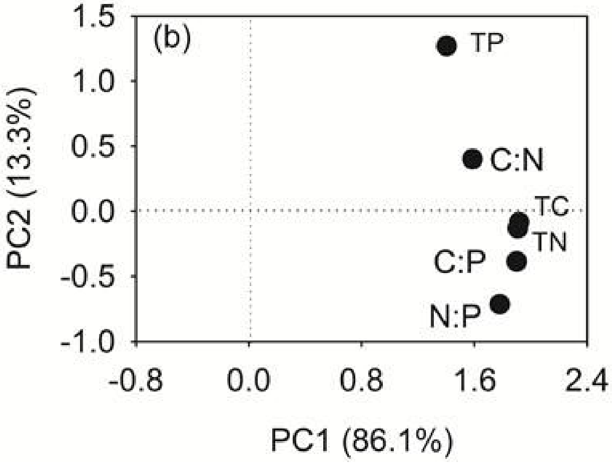


Fig. 2. Bulk and rhizosphere soil C, N, and P concentrations of overstory and understory species in *P. massoniana* (a, d, h), *P. elliottii* (b, e, i), and *C. lanceolata* (c, f, g) plantations in subtropical China. Different letters above the mean±SE indicate significant differences among bulk soils, rhizosphere soils of the tree species and rhizosphere soils of the shrub species in each plantation at $P<0.05$.

613


614 Fig. 3. Bulk and rhizosphere soil C:N:P stoichiometry of overstory and understory 615 species in *P. massoniana* (a, d, h), *P. elliottii* (b, e, i), and *C. lanceolata* (c, f, g) 616 plantations in subtropical China. Different letters above the mean ± SE values indicate 617 significant differences among bulk soils, rhizosphere soils of the tree species and 618 rhizosphere soils of the shrub species in each plantation at $P<0.05$.





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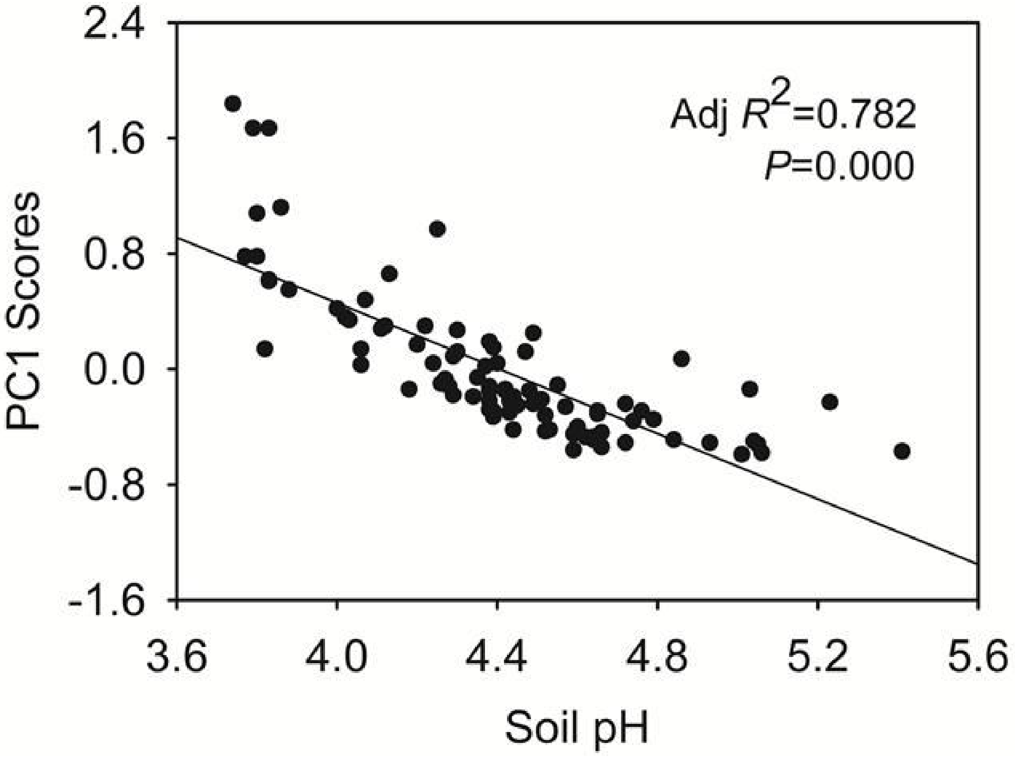

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