Enzyme stoichiometry indicates the variation of microbial nutrient requirements at different soil depths in subtropical forests

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Abstract:

Soil extracellular enzyme activities and associated enzymatic stoichiometry are considered indicators to nutrients availability and microbial substrate limitation. However, many of current studies have been focusing on upper most soil layer with a single enzyme as represent, leading to critical uncertainties in understanding the nutrient availability in deeper soils. In this study, we investigated C-, N- and P-acquiring enzyme activities and microbial C, N and P limitation across a range of soil layers (0 - 10, 10 - 20, 20 - 40 and 40 - 60 cm) in a natural secondary evergreen broad-leaved forest and a Chinese fir (*Cunninghamia lanceolata*) plantation forest in subtropical China. Our results showed that the two typical subtropical forests are commonly co-limited by C and P at all soil depths, but not N. The study found that microbial C and P limitation fluctuated along soil depth, and that higher N was demanded by microbes in soil under 20 cm in the two forests. Our results highlight the asymmetrical patterns of microbial nutrient limitation along the whole soil profile, and provide important information in understanding nutrient limitations in deeper soils. It is feasible to explicitly incorporate this vertical asymmetrical nutrient limitation patterns into future research priority.

Keywords: Deep soil; microbial nutrient limitation; soil enzyme; stoichiometry; subtropics.

1 Introduction

Forests in the tropical and subtropical area have the fastest growth rates, and exerts a more important role on sequestering atmospheric CO_2 when comparing with temperate areas in higher latitudes [1-3]. It is reported that tropical forest ecosystems were generally phosphorus limited, and additionally nitrogen limited in montane locations [4, 5]. Soil micro-organisms play a key role in releasing and maintaining soil nutrients. However, microbial processes are also limited by nutrients availability. Therefore, understanding the nutrient limitations of micro-organisms is essential for understanding the functions of ecosystems and for predicting ecosystem responses to global changes as well.

Soil enzymes, primarily produced by microbes, play a key role in decomposition, turnover and mineralization of soil organic matter, and the relative abundance of enzymes (named enzyme stoichiometry) reflects nutrient demands of microorganisms and nutrients availability in the environment [6-10]. Enzymes involved in the decomposition of cellulose (β-glucosidase (BG), cellobiosidase (CBH)), xylane (β-xylosidase, BX), chitin (β - 1,4-N-acetylglucosaminidase, NAG), polypeptides (leucine aminopeptidase, LAP) and phosphate (acid or alkaline phosphatase, AP), and have been widely recognized [e.g., 11-14]. However, carbon acquiring enzymes in forest soil were usually represented by only β-1,4-glucosidase (BG) and nitrogen acquiring enzymes were also represented by a single enzyme, β - 1,4-N-acetylglucosaminidase (NAG) because they are easier to study [15]. Thus, previous meta-analysis revealed that microbial C:N:P acquisition ratios converged on 1:1:1 when using ratios of ln(BG): ln(LAP + NAG): ln(AP) [15]. Polysaccharide degradation requires the interaction of various enzymes [16], using one single enzyme activity as an indicator of nutrient dynamics in soil may neglect synergistic effects of many other enzymes in forest ecosystem

38 [17, 18].

39 Recently, enzymatic stoichiometry has been suggested as an efficient method to indicate the relative
40 resource limitation of soil micro-organisms [19, 20]. The theory of enzymatic stoichiometry has been
41 widely used in different ecosystems [4, 15, 21-23], and a new vector analysis of eco-enzyme activities
42 based on this has been proposed by Moorhead et al. (2016) [24], who plotted the proportion of C:N
43 versus C:P acquiring enzyme activities to calculate the 'length' and 'angle' of vectors and quantify
44 relative C vs. nutrient acquisition and the relative P vs. N acquisition. The vector analysis has attracted
45 increasing attention [19, 23, 25, 26]. However, studies on enzyme activities together with enzymatic
46 stoichiometry utilizing whole C-acquiring enzymes as a group are rare in forest ecosystems. Paucity
47 of data on intact cognition of enzyme activities together with enzymatic stoichiometry constrains our
48 ability to accurately predict how soil nutrient cycle in forests will respond to climate change.

49 A large-scale study in North-South Transect of Eastern China (NSTEC) with a unique vegetation
50 belt ranging from boreal forest to tropical rain forest reported that microbial nutrient acquisition was
51 P-limited in tropical forests [27]. However, other researches indicated that karst and non-karst forests
52 in southwest China were P-limited, and microbial nutrient acquisition in karst ecosystem was more C-
53 and P-limited, rather than N-limited [23, 28]. To our best knowledge, however, no public enzymatic
54 stoichiometry studies have been conducted in the fast-growing forest ecosystem of Chinese fir
55 plantation (*Cunninghamia lanceolata*) which play a key role in regional and national carbon
56 sequestration.

57 Currently, most enzymatic stoichiometry studies focused on top soil (0-20 cm) mainly because
58 enzyme activity of surface soil is high and easy to change significantly with treatments [e.g., 15, 23,
59 27, 28]. Soils below 20 cm contribute over 50% of the global soil carbon stocks despite their low

carbon concentration [29]. Limited studies have found no consistent tendency of soil enzymes and enzymatic stoichiometry below 20 cm [13, 21, 30-32]. Besides, substrate limitation in deep soil is a major factor limiting microbial activity and controlling C turnover [33, 34]. Hence, exploring enzymatic stoichiometry patterns together with microbial nutriment limitation in deep soil can provide insights of biological mechanisms on nutrition cycling.

In this study, paired natural secondary forest (NSF) and fast-growing coniferous plantation forest (CPF) were selected to examine enzyme activities, enzymatic stoichiometry and microbial CNP limitation along the whole soil profile. Specifically, we addressed the following questions: (1) Will microbial C, N, and P acquisition be significantly higher in the Chinese-fir plantation than the secondary forest in subtropics? (2) How will soil depth affect enzyme activities and enzymatic stoichiometry in subtropical soils?

2 Materials and methods

2.1 Study sites

The study was conducted in Shunchang county (117° 29' ~ 118° 14' E, 26° 38' ~ 27° 12' N), northwest of Fujian province of China. The climate in the study area is subtropical marine monsoon with the annual mean temperature of 19.0 °C and the mean annual precipitation of 1629 mm. The predominant soil type is red soil, which is classified as Oxisol based on the USDA's Soil Taxonomy.

Evergreen broadleaf forests and coniferous plantation forests are the main forest types at the area. Natural secondary forest (NSF) and Chinese fir plantation forest (CPF) in the study originated from the same natural evergreen forests. In NSF and adjacent CFP, three blocks of 20 × 20 m size were

81 selected as pairing designs with similar slope and aspect (southern). The distance between paired NSF
82 and CPF in one block is about 50 meters. The distance between the three blocks is within 3 km from
83 each other.

84 The dominant tree species in NSF are *Castanopsis fargesii* Franch*, Castanopsis lamontii,*
85 *Castanopsis fargesii, Altingia gracilipes*. The main understory plant species in NSF included *Machilus*
86 *chrysotricha*, *Woodwardia japonica, Adinandra millettii, Ardisia crispa (*Thunb.*), Sarcandra glabra*
87 *(*Thunb.*) Nakai, Embelia rudis.* Canopy coverage is approximately 75%, the mean tree height and
88 stand density were 14.7 m and 1267 stem ha^{-1}, respectively. The CPF is dominated by Chinese fir
89 (*Cunninghamia lanceolata* (Lamb.) Hook (Taxodiaceae)) and dominant understory species are
90 *Machilus grijsii* Hance, *Vaccinium bracteatum* Thunb, *Cleredendrum cwtophyllum* Turcz,
91 *Dicranopteris dichotoma* (Thunb.) *Bernh*., *Rhizoma Cibotii.* Canopy coverage, mean tree height and
92 stand density in CPF were 90%, 18.6 m and 1725 stem ha^{-1}, respectively.

93 2.2 Soil sampling

94 Soil sampling was taken in August 2017. After removal of surface debris, soil samples were
95 collected randomly at four soil depths (0 - 10, 10 - 20, 20 - 40 and 40 - 60 cm) from five random
96 locations in each plot. In each sampling subplot, five soil cores at each layer were taken and mixed to
97 represent a composite sample. Samples were immediately placed in fresh-keeping container and
98 transported to laboratory for chemical analysis. Stones and root fragments were removed, and soils
99 were sieved with a mesh size of 2 mm before chemical analyses.
100

101 ## 2.3 Soil enzyme activities

102 Extracellular enzyme activities (EEA) were determined following the method of Saiya-Cork [35]
103 and German [36]. Fluorogenic methylumbelliferone-based (MUB) or methylcoumarin-based (MCA)
104 artificial substrates were used to estimate the activities of C-cycling [Xylanases (BX),
105 Cellobiohydrolase (CBH), beta-Glucosidase (BG)], N-cycling [beta -1,4-N-acetylglucosaminidase
106 (NAG), Leucine aminopeptidase (LAP)], and P-cycling [Acid phosphatase (AP)]. High substrate
107 concentration of each enzyme was adopted to ensure each enzyme to be assayed under saturation
108 conditions.

109 Briefly, suspensions of 1 g soil (dry weight equivalent) with 125 ml of 50 mM acetate buffer (pH
110 5.0) were dispersed using low-energy sonication (50 J s^{-1} output energy) [37]. The soil slurries and
111 substrate solutions were incubated for 180 min at 20 ± 0.5 °C. Fluorescence was measured in
112 microplates at an excitation wavelength of 365 nm and an emission wavelength of 450 nm with a
113 Synergy H4 Hybrid Microplate Reader (BioTek, USA). To ensure that all enzyme activities were
114 comparable, consistent parameters of incubation method and quench way were kept [7]. The
115 incubation plates were shaken at an interval of one every hour to ensure the complete reaction. The
116 maximum duration of keeping soil slurries in buffer was less than 30 min before adding MUB (MCA)-
117 linked substrate [38]. Fluorescence was conducted in 1 min following the addition of 10 µl 1.0 M
118 NaOH solution [36]. The time between the addition of substrate and the fluorescence reading was
119 standardized to 180 min to eliminate variation of enzyme activity.

120 Unit for enzyme activity was expressed as nmol activity g^{-1} dry soil h^{-1}. C acquisition was expressed
121 by the sum of Xylanases (BX), Cellobiohydrolase (CBH), and beta-Glucosidase (BG) activities. N
122 acquisition was represented by the sum of beta -1,4-N-acetylglucosaminidase (NAG) and Leucine

123 aminopeptidase (LAP), while P acquisition was showed as acid phosphatase (AP) activity. The
124 microbial C:N acquisition ratio was calculated as sums of hydrolytic enzymes using (BG + BX + CBH):
125 (NAG + LAP), while (BG + BX + CBH): (AP) and (NAG + LAP): (AP) were used for the microbial
126 C: P and N: P acquisition ratios, respectively.

127 2.4 Statistical analysis

128 All assayed parameters were reported as means ± standard errors (SE). One-way ANOVA followed
129 by LSD test was used to determine the significance of enzyme activities and soil enzyme stoichiometry
130 among the soil depths in each type of the two forests. Natural-log data transformations were used to
131 meet the assumption of normality and homoscedasticity. All statistical analyses were done by SPSS v
132 23.0 (IBM SPSS Statistics for Windows, ver. 23.0; IBM Corp., Armonk, NY, USA). Mixed linear
133 model analyses in SPSS v23.0 was selected to determine the effects of forest type, soil depth and their
134 interactions on soil enzyme activities and enzymatic stoichiometry.

135 The following three approaches were used to examine microbial resource acquisition. The first was
136 based on the method of Hill [22] to draw scatter plot of enzymatic stoichiometry, with Enzyme N/
137 Enzyme P as x axis and Enzyme C/ Enzyme N as y axis. Different resource constraints (N limitation,
138 P limitation, C&P limitation and N&P limitation) were shown in four district respectively. The second
139 approach was based on enzyme ratios of C:P and N:P. Lower enzyme C:P and N:P ratios are suggestive
140 of a degree of P limitation [4]. The third approach was vector analysis proposed by Moorhead [24].

141 Vector L (unitless) = $\sqrt{X^2 + Y^2}$ (1)

142 Vector A (degree) = Degrees (Atan2(X, Y)) (2)

143 Where

144 $X= \frac{Enzyme\ C}{Enzyme\ C + Enzyme\ P}$, $Y= \frac{Enzyme\ C}{Enzyme\ C + Enzyme\ N}$

145 A relatively longer vector L indicates greater C- acquisition, and the vector A < 45° and >45°

146 indicate relative degrees of N- and P-limitation, respectively [19, 28].

147 # 3 Results

148 ## 3.1 Soil enzyme activity

149 Results from mixed linear model showed that soil depth significantly influenced Enzyme C, N and

150 P acquisition, while the interactions of forest type × soil depth significantly affected enzyme C (Table

151 1; $P<0.05$). From the soil depths of 0–10 cm to 40-60 cm, the mean activities of C, N, and P acquisition

152 enzymes decreased by 64%, 48%, and 76%, respectively (Fig.1). There were no remarkable

153 differences of C-acquiring (BX+CBH+BG) and P-acquiring (AP) enzyme activities between NSF and

154 CPF at 0-10 cm, 10-20 cm, 20-40 cm, 40-60 cm depths. N-acquiring (NAG + LAP) enzyme activity

155 in NSF at 0-10 cm soil depth was significantly higher than that in CPF (Fig.1).

156 For NSF, C- and N-acquiring enzyme activities had the same distribution trend that surface soil (0-

157 10 cm) layer had remarkably higher activities than those at the depths of 10-20, 20-40 and 40-60 cm,

158 among which remarkable difference was not found (Fig.1). There was no significant difference in P-

159 acquiring enzyme activity between 0-10 cm and 10-20 cm depths, but P-acquiring enzyme activities

160 at the two soil depths were significantly higher than those at 20-40 and 40-60 cm depths. For CPF, C-

161 acquiring enzyme activity in 0-10 cm layer was remarkably higher than that in 20-40 and 40-60 cm

162 soil depths (Fig.1). Similarly, N-acquiring enzyme activity in topsoil (0-10 cm) was significantly

163 higher than in 10-20 and 40-60 cm soil depths. Enzyme P activity in 0-10 cm was almost 2-fold higher

164 than in 10-20 cm soil depth, and significantly higher than in 20-40 and 40-60 cm soil depths (Fig.1).

3.2 Soil enzymatic stoichiometry

Throughout the study area, mean soil enzymatic ratios of (BG+BX+CBH):(NAG+LAP), (BG+BX+CBH):AP and (NAG+LAP):AP were 3.88, 0.27 and 0.08, respectively. Enzyme activity ratio of C:P (0.30) in CPF was significantly higher than that of NSF (0.24) (Fig. 2), all of which were significantly affected by soil depth, forest type and their interactions (Table 1, $P<0.05$). While ratio of enzyme N:P was only affected notably by soil depth (Table 1, $P<0.05$). From 0–10 cm to 40-60 cm soil depth, mean soil enzymatic ratios of C:P and N:P increased by 59% and 119%, respectively (Fig. 2).

Enzymatic ratio of C:N in CPF located at 10-20 cm (5.91) was significantly higher than the other three soil layers (Fig. 3). At soil layers of 10-20 cm and 40-60 cm, enzymatic ratios of C:P in CPF were 0.30 and 0.40 respectively, which were significantly higher than those in NSF. The highest enzymatic ratio of C:P was observed at the soil layer of 20-40 cm in NSF (0.34) and 40 -60 cm in CPF (0.40), respectively (Fig. 3). Enzymatic ratios of C:P and N:P in CPF increased with soil depths. Enzymatic ratios of N: P at 0-10 cm and 10-20 cm depths in CPF were remarkably lower than those at 20-40 cm and 40-60 cm depths (Fig. 3)

3.3 Vector analysis

The plots of enzymatic stoichiometry indicated that four soil layers were co-C and P limited and not with N limited in NSF and CPF (Table 2, Fig. S1). Vector analysis showed that vector length and angle were not significantly different between NSF and CPF at 0-60 cm soil depth, indicating no differences of C-limited and P-limited between NSF and CPF at each soil layer (Table 2).

Vector length showed that there were no significant differences among the four soil layers in NSF,

and the third (20-40 cm) layer was notably lower than 0-10 and 10-20 cm soil layers in CPF (Table 2). Vector angle in CPF decreased significantly along the four soil layers, with angle greater than 45° in each layer. Although vector A varied significantly with depth in CPF, there was no coincident tendency of vector A in NSF. In NSF the angles at each soil layer were greater than 45°, and the largest and smallest angles occurred in the 10-20 cm and 20-40 cm soil depths, respectively. Further, the study did not find differences in N limitation between NSF and CPF (Table 2).

4 Discussion

4.1 Spatial variations of C-, N- and P- acquiring enzyme activities

Generally, hydrolase activities decreased gradually with the decrease of soil depth [15, 31, 32], we observed that C and P acquiring enzyme activities exhibited a decreasing trend with soil depth but N-acquiring enzyme activities had an increasing trend in NSF. The most fluctuated enzyme activity was at 10-20 cm soil layer, where C- acquiring enzyme activity in NSF and N- acquiring enzyme activity in NSF and CPF decreased sharply. The results indicated that microbial metabolisms were obviously higher at the 0-10 cm soil, which can explained by that fine root biomass of natural secondary forest and Chinese fir plantation were abundantly distributed at the top 0–10 cm soil layer [39] and active microbial activities due to rich easily-degradable weight organic substances in the topsoil [40, 41].

Despite no remarkable difference of C-acquiring enzyme activities was found between NSF and CPF at 0-10 cm soil layer such as BG enzyme, BX and CBH enzyme activities were significantly higher in NSF than in CPF at 0-10 cm (Table S1). Although enzymes are substrate specific and individual enzyme activity may not reflect total soil nutritional status [42], lots of studies utilized β-1,4-glucosidase (BG) as whole carbon acquiring enzyme because glucose released from cellulose is

the most abundant monomer in soil [17]. Oligo- and monosaccharides (termed sugars), including glucose, xylose, arabinose, galactose, mannose and rhamnose, come from decomposition of polysaccharides by microbial enzymes (cellulases, xylanases, glucosidases and chitinases etc.). These sugars are soluble and captured rapidly by microbes for metabolism and carbon storage [17]. Complicated belowground biochemical processes indicate that using a single enzyme to describe the mechanism of microbial nutrient demands may be biased and using more enzymes beyond β-1,4-glucosidase (BG) to reflect the mechanisms are needed.

In the present study, significant difference in N-acquiring enzyme was found at the surface soil between the two forests due to higher Leucine aminopeptidase (LAP) activity, which contributed almost 50% to the total N acquiring enzyme activity (Table S1). The result revealed LAP plays an important role in forest soil as found by Moorhead et al. (2016) [24]. Nitrogen -acquiring enzyme activity in CPF did not decrease gradually with soil depth, which was associated with LAP activity (Fig. 1). The distribution pattern of LAP showed a decreasing trend followed by an increasing trend with increasing soil depth (Table S1).

It was reported that Chinese fir might uptake phosphorus through soil acidification [43] and produce less acid phosphates [44], which resulted in high P demand for its rapid growth with relatively lower enzyme P activity at all soil depths in CPF compared to NSF .

4.2 Enzymatic stoichiometry and microbial nutrient limitations

The lower enzymatic ratios of N: P and C: P (<1) together with the results that vector angles were higher than 45° in both NSF and CPF at the 0-60 cm soil depth indicated P limitation in NSF and CPF [4, 45]. Besides, gradual decrease of vector A in CPF indicated that microbial P limitation gradually

228 reduced along soil depth (Table 2). While microbial P demand fluctuated according to soil depth in
229 NSF, with highest demand at 10-20 cm and the lowest demand at 20-40 cm soil layer. Former studies
230 revealed that P restrictions are quite common [5, 46, 47], the study tried to provide evidences of
231 microbial P limitation in middle-subtropical forests.

232 In the study, both the scatter plot of enzymatic stoichiometry (Fic. S1) and the vector analysis (Table
233 2) indicated that the two forests were commonly co-limited by C and P at all soil depths, which were
234 consistent with the study conducted at karst ecosystems in southwest China [28]. Meanwhile, higher
235 microbial C acquiring in CPF at 10-20 cm and 40-60 cm soil layers were found due to remarkably
236 higher enzyme C: P ratio at the two soil layers in CPF than in NSF. The results confirmed that forest
237 type, soil depth and their interactions have significant effects on the ratio of enzyme C: P (Table 1;
238 $P<0.05$).

239 It was also reported that many subtropical ecosystems are N-rich relative to other plant nutrients
240 [48-50], the study indicates that N was not limited with respect to microbial grow demand from the
241 results of enzymatic stoichiometry. That is, the C: N ratio may not reach the critical ratio of the two
242 elements for microbial growth. The sharply increasing trend of enzyme N:P ratio below 20 cm soil
243 layer indicated the higher N demand in deep soil, which was in line with the result of incubation
244 experiment of a subtropical mixed forest soil [51]. The higher enzyme C: N ratio in CPF at 0-20 cm
245 and 40-60 cm soil layers suggested that NSF was more N-limited compared to CPF.

246 Although the trends of enzyme C:N and C:P ratios remained unchanged at all soil layers between
247 the two forests when using single BG as whole C acquisition enzyme, enzyme C:P ratio at NSF
248 displayed a different trend along the soil depths. Enzyme C:P ratio between 20-40 cm and 40-60 cm
249 soil depths at NSF changed from significance to insignificance (Fig. 3;Fig. S2), which may

underestimate the C demand of NSF at the 20-40 cm soil depth. On the other hand, vector A in NSF at 40-60 cm soil depth become significantly higher than that in CPF when using single BG as whole C acquisition enzyme (Table S2), which may overestimate microbial P limitation in CPF at 40-60 cm soil depth. Thus, utilizing single BG as whole C acquisition enzyme may underestimate or overestimate microbial C and P demand in deep soils.

East Asian monsoon subtropical forests have one of the highest carbon uptakes of forests worldwide, which may be caused by young stand ages, and high nitrogen deposition [52, 53]. Nevertheless, research also indicated that soil total C concentration increased significantly and soil total P concentration decreased significantly across all soil depths in subtropical China during 1955 to 2016 [49]. To our best knowledge, this study pioneered in examining microbial nutrient demands along a whole soil profile in middle-subtropical forests, which should contribute to informational references for forest management in the subtropics.

5 Conclusion

The study utilized enzymatic stoichiometry to evaluate microbial C, N and P limitation in a natural secondary evergreen broad-leaved forest and a fast-growing plantation forest in the subtropics, where enzymatic stoichiometry is much less studied. We found that the two subtropical forests were co-limited by C and P at all soil depths (0-10, 10-20, 20-40 and 40-60 cm) and both forests were not N-limited. No significant differences of microbial C, N and P limitation between the two forests at all soil depths revealed the complicated belowground plant-microbe interactions. The results of enzyme stoichiometry indicate higher N demand by microbes in soils below 20 cm in both plantation forest and the secondary forest. The finding that C-acquiring (BX+CBH+BG), N-acquiring (NAG + LAP)

and P-acquiring enzyme activities decreased with increasing soil depth but there was no difference in enzyme activities along the whole soil profile indicates an imbalance of microbial nutrient demand at different soil depths. Utilizing single BG as whole C acquisition enzyme could miscalculate microbial C and P demands at different soil layers. This pioneer study in subtropical China in examining microbial nutrient demands in both top and deep soils provides important information for subtropical tree plantation and natural forest management, especially under future climate change.

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Table 1. Estimated fixed effects of soil depth, forest type and their interactions on soil enzyme, enzyme stoichiometry. C acquisition enzyme was represented by the sum of β-1,4-glucosidase (BG), cellobiohydrolase (CBH), β-xylosidase (BX). The sum of β-1,4-N-acetylglucosaminidase (NAG) and leucine aminopeptidase (LAP) was regarded as N acquisition, while the activity of acid phosphatase (AP) was considered as P acquisition enzyme. Enzymatic stoichiometry of C:N, C:P and N:P was calculated by the C, N and P acquisition enzyme respectively. Significant results (P<0.05) are indicated in bold.

Parameters	Intercept			Forest type			Soil depth			Forest type × Soil depth		
	DF	F	*P*	DF	F	*P*	DF	F	*P*	DF	F	*P*
Enzyme C	1	167.9	**< 0.001**	1	0.016	0.904	3	26.40	**< 0.001**	3	4.456	**0.026**
Enzyme N	1	963.7	**< 0.001**	1	6.458	0.060	3	14.31	**< 0.001**	3	1.118	0.388
Enzyme P	1	169.1	**< 0.001**	1	1.495	0.287	3	59.51	**< 0.001**	3	1.916	0.205
Enzyme C:N	1	141.1	**< 0.001**	1	1.098	0.362	3	3.183	0.087	3	1.595	0.268
Enzyme C:P	1	1994.6	**< 0.001**	1	23.29	**< 0.001**	3	21.68	**< 0.001**	3	5.491	**0.013**
Enzyme N:P	1	159.4	**< 0.001**	1	0.271	0.630	3	12.99	**0.001**	3	1.028	0.423

Table 2. Vector analysis between the natural secondary forest (NSF) and Chinese fir plantation forest (CPF) at different soil depth. Values represented mean ± standard error (n=3). Capital letters show the significant difference between the two forests at the same soil depth, and the different lower cases reflect the significant difference between soil depths within one forest.

Depth	Vector L		Vector A	
(cm)	NSF	CPF	NSF	CPF
0-10	0.813±0.02Aa	0.841±0.02Aa	77.64±0.71Aab	78.37±0.62Aa
10-20	0.78±0.06Aa	0.88±0.03Aa	79.14±1.20Aa	74.83±0.78Ab
20-40	0.81±0.04Aa	0.76±0.02Ab	71.75±1.87Ac	72.05±0.50Ac
40-60	0.75±0.02Aa	0.82±0.03Aab	74.54±0.95Abc	69.48±0.79Ad

FIGURE CAPTIONS

Fig. 1. Variations of C, N and P acquiring enzyme activities at different soil depths of the natural secondary forest (NSF) and Chinese fir plantation forest (CPF). Statistically significant differences were assumed at $P < 0.05$. All values are presented as mean ± standard error with 3 replicates. Capital letters show the significant difference between the two forests at the same soil depth, and the different lower cases reflect the significant difference between soil depths within one forest.

Fig. 2. Comparisons of enzymatic stoichiometry at the 0-60 cm soil depth of the natural secondary forest (NSF) and Chinese fir plantation forest (CPF). All values are presented as mean ± standard error. "*" indicates a significance at a $P < 0.05$ level.

Fig. 3. Variations of enzymatic stoichiometry at different soil depths of the natural secondary forest (NSF) and Chinese fir plantation forest (CPF). Capital letters mean the significant difference between the two forests at the same soil depth, and the different lower cases reflect the significant difference between soil depths within one forest. All values are presented as mean ± standard error with 3 replicates.

Fig. 1.

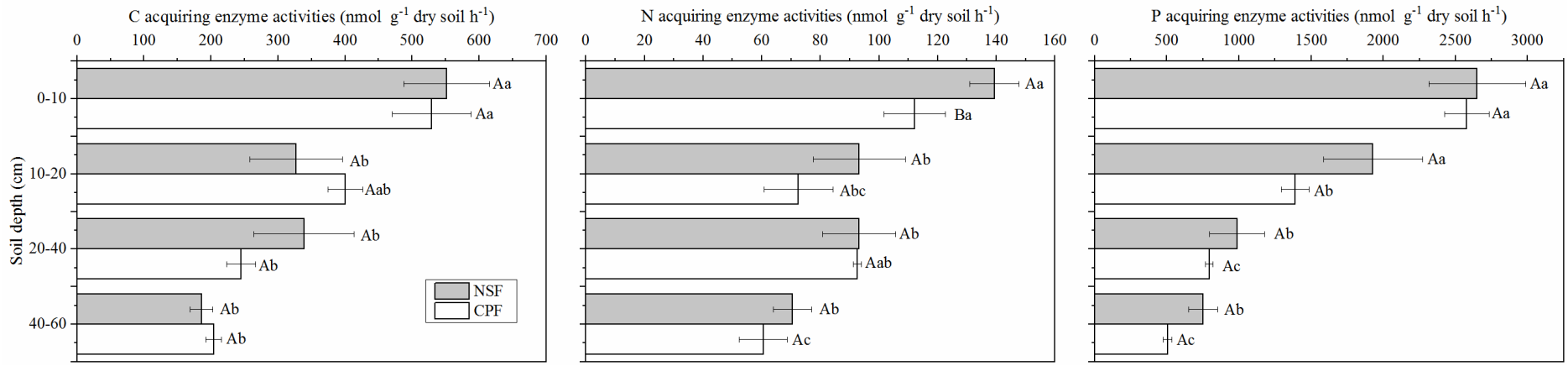

C acquiring enzyme activities (nmol g-1 dry soil h-1)
0
100
200
300
400
500
600
700
N acquiring enzyme activities (nmol g-1 dry soil h-1)
0
20
40
60
80
100
120
140
160
P acquiring enzyme activities (nmol g-1 dry soil h-1)
0
500
1000
1500
2000
2500
3000
Soil depth (cm)
0-10
10-20
20-40
40-60
Aa
Aa
Ab
Aab
Ab
Ab
Ab
Ab
Aa
Ba
Ab
Abc
Ab
Aab
Ab
Ac
Aa
Aa
Aa
Ab
Ab
Ac
NSF
CPF

Fig. 2.

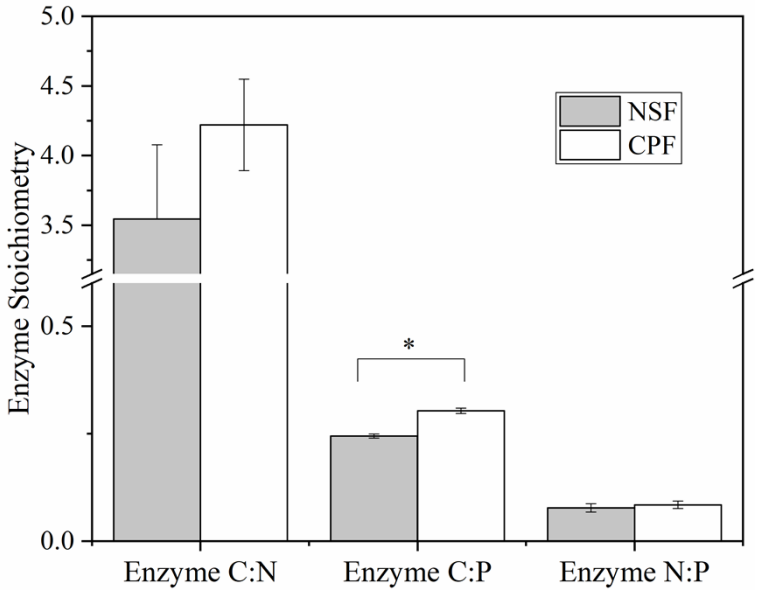
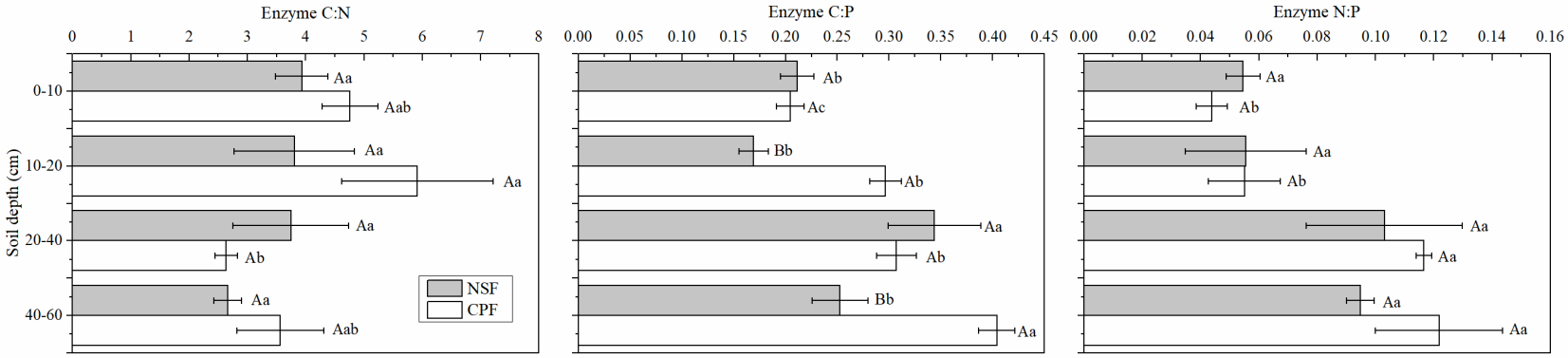

5.0
4.5
4.0
3.5
0.5
0.0
Enzyme Stoichiometry
NSF
CPF
*
Enzyme C:N
Enzyme C:P
Enzyme N:P

Fig. 3.


Enzyme C:N
Enzyme C:P
Enzyme N:P
Soil depth (cm)
0-10
10-20
20-40
40-60
NSF
CPF
Aa
Aab
Ab
Ac
Bb