



Latitudinal patterns of soil extracellular enzyme activities and their controlling factors in *Pinus massoniana* plantations in subtropical China

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ARTICLE INFO

Keywords:

Extracellular enzyme activity
Enzymatic C:N:P acquisition ratio
Latitudinal gradients
Planted forest
Climatic factors

ABSTRACT

Soil extracellular enzyme activities (EEAs) and enzymatic acquisition ratios are significantly affected by both abiotic and biotic factors across various scales and ecosystems. However, what determines the soil EEAs and their acquisition ratios in planted forests remains poorly understood. In this study, we measured the activities of β -1,4-glucosidase (BG), β -1,4-N-acetylglucosaminidase (NAG) and acid phosphatase (AP) and explored their key controlling factors in *Pinus massoniana* plantations along a 1100 km latitudinal gradient in subtropical China. The three enzyme activities increased with an increase in latitude and were significantly and negatively affected by mean annual temperature (MAT) and mean annual precipitation (MAP), but were positively correlated with soil available P content. From south to north, the enzymatic C:N ($\ln(\text{BG}):\ln(\text{NAG})$) acquisition ratio decreased significantly, while the enzymatic C:P ($\ln(\text{BG}):\ln(\text{AP})$) and N:P ($\ln(\text{NAG}):\ln(\text{AP})$) acquisition ratios increased significantly. The latitudinal patterns of the enzymatic C:P and N:P acquisition ratios were significantly and negatively affected by MAT and MAP, but were positively affected by soil available P content. In total, climatic factors (e.g., MAT and MAP) accounted for more (14.2% and 25.5%, respectively) of the latitudinal variations in soil EEAs and their acquisition ratios than soil (e.g., available P) and stand (e.g., diameter at breast height). Therefore, these results illustrate that planted forests are susceptible to variations in soil EEAs and their acquisition ratios along a latitudinal gradient and that such changes are mainly driven by climate. Moreover, the similar average enzymatic C:P and N:P acquisition ratios in this pine plantation with the literature values reported for tropical and subtropical natural forests indicate that the pine plantations in subtropical China may be strongly P limited.

1. Introduction

Soil extracellular enzymes, as the “proximate agents of soil organic matter and litter decomposition” (Sinsabaugh et al., 2008), impose effective regulation on nutrient cycling, such as carbon (C), nitrogen (N) and phosphorus (P), in terrestrial ecosystems (Burns et al., 2013; German et al., 2011; Nannipieri et al., 2018). Based on resource allocation theory, microbes produce more extracellular enzymes to mine the limited amounts of elements from soils (Allison and Vitousek, 2005;

Bloom, et al., 1985; Bowles et al., 2014; Fujita et al., 2019). As a result, the acquisition ratios of soil extracellular enzyme activities (EEAs) reflect the biogeochemical equilibrium between the nutrient requirements of microbial assemblages and the nutrient availability of environment (Allison et al., 2007; Sinsabaugh et al. 2009). Soil EEAs and their acquisition ratios have been widely used to explore nutrient cycling and resource constraints of microbes in terrestrial ecosystems (Fujita et al., 2019; Sinsabaugh et al., 2009; Waring et al., 2014; Xu et al., 2017; Zhou et al., 2020).

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<https://doi.org/10.1016/j.foreco.2021.119358>

Received 2 February 2021; Received in revised form 7 May 2021; Accepted 9 May 2021

Available online 27 May 2021

0378-1127/© 2021 Published by Elsevier B.V.

To promote specific ecosystem services by manipulating enzyme activities, it is necessary to identify the key controlling factors of EEAs (Burns et al., 2013). Previous studies have indicated that soil EEAs and enzymatic acquisition ratios are significantly affected by both abiotic (e.g., climate and soil properties) and biotic (e.g., plant nutrient and vegetation types) factors from local to global scales (Cui et al., 2018; He et al., 2020; Jones et al., 2019; Mayor et al., 2016; Sinsabaugh et al., 2008; Waring et al., 2014; Xu et al., 2017; Zhou et al., 2020). However, the effects of individual factors on soil EEAs are complex. For instance, the relationship between soil β -glucosidase activity and soil pH varied from nonsignificant (Sinsabaugh et al., 2008) and positive (Xu et al., 2017) to negative (Peng and Wang, 2016; Stark et al., 2014; Wang and Lu, 2006). One possible explanation is that these studies were conducted at different scales and in different ecosystems. Generally, spatial patterns are particularly valuable for identifying the controlling factors of soil EEAs (Burns et al., 2013) because they can provide important information on the limitations of microbial biomass stoichiometry for organic matter composition under changing environmental conditions (Xu et al., 2017; Zhou et al., 2020). Any generalization of the abiotic and biotic controls on soil EEAs and enzymatic acquisition ratios across space and ecosystem types is largely constrained by the fact that most previous studies were either conducted on single sites or by means of meta-analyses (Jones et al., 2019; Mayor et al., 2016; Sinsabaugh et al., 2008, 2009; Waring et al., 2014; Xiao et al., 2018; Zheng et al., 2018). Large-scale experimental studies on this topic are currently lacking, which creates a knowledge gap in our understanding of the spatial-temporal dynamics and controls of soil EEAs and their acquisition ratios.

China's terrestrial ecosystem is characterized by the diverse vegetation types and the high environmental heterogeneity and thus contributes to a good platform for the exploring spatial patterns of soil EEAs and enzymatic acquisition ratios as well as their controlling factors. For example, the soil EEAs and enzymatic acquisition ratios along the longitudinal gradients of temperate grasslands (Peng and Wang, 2016) and

along the latitudinal gradients of farmlands in northern China (Liu et al., 2008) are largely controlled by soil nutrient availabilities, while those along the latitudinal gradients of natural forests in eastern China are significantly affected by climatic factors (Xu et al., 2017; Zhou et al., 2020). Indeed, soil EEAs and their acquisition ratios did not exhibit significant latitudinal patterns in the natural forests of eastern China (Xu et al., 2017; Zhou et al., 2020) due to differences in vegetation types between sampling sites. As determined in previous studies, vegetation types modulated the variations in soil EEAs and enzymatic stoichiometry (Cui et al., 2018; He et al., 2020). Planted forests, which account for 7% of total global forest areas (FAO, 2020), differ from natural systems in several ways, such as consisting of only one species and having poor soil nutrient levels (Guo and Ren, 2014). Therefore, the spatial patterns of soil EEAs and enzymatic acquisition ratios and their drivers in plantation ecosystems may differ from those in natural forests. However, the effects of abiotic and biotic factors on soil EEAs and their acquisition ratios in plantation ecosystems have only been explored at single sites (Cui et al., 2018; Zhao et al., 2018). Moreover, a national meta-analysis, which covered 57 experimental sites in China's forest ecosystems, indicated that only 16% of the 561 valid data points of soil EEAs were from planted forests (Zheng et al., 2018). It is unclear which factors affect soil EEAs and enzymatic acquisition ratios along spatial gradients in planted forests.

China has the largest area of planted forests (FAO, 2020), with >50% distributed in subtropical China (National Forestry and Grassland Administration, 2019). To address the issues outlined above, we investigated the soil EEAs and enzymatic acquisition ratios in forests planted with *Pinus massoniana* (hereafter referred to as pine) along a 1100 km latitudinal transect from south to north in subtropical China (Fig. 1). This species has been widely planted or aerially seeded across subtropical China, with a total area of approximately 8 million hectares (National Forestry and Grassland Administration, 2019) that covers a wide range of environmental gradients (such as climate, stand characteristics and soil properties; Tables 1 and S1). This large-scale species

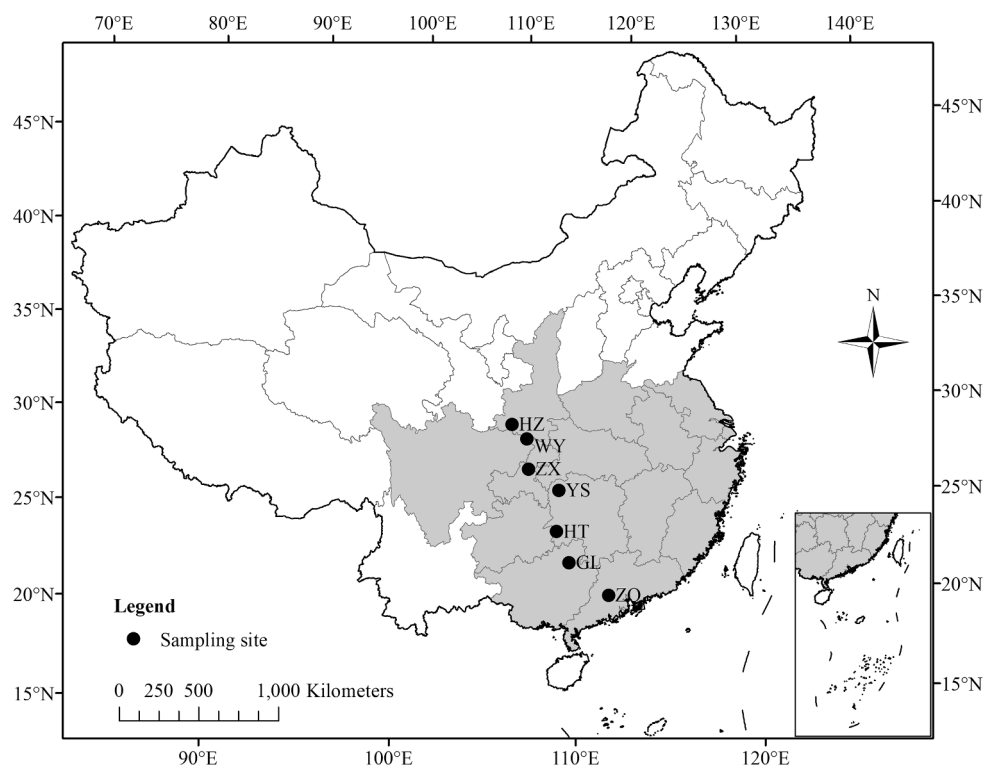


Fig. 1. Locations of seven sites of pure pine plantations along a 1100 km transect in subtropical China. The shaded part of the map represents the geographical location of the study area and distribution area of *P. massoniana* in China. ZQ, Zhaoqing; GL, Guilin; HT, Huitong; YS, Yongshun; ZX, Zhongxian; WY, Wanyuan; and HZ, Hanzhong.

Table 1

Information for the seven sites of pure pine plantations along a 1100 km transect in subtropical China.

Variables	Zhaoqing	Guilin	Huitong	Yongshun	Zhongxian	Wanyuan	Hanzhong
Longitude (°E)	112.5010	110.3070	109.6502	109.9517	108.0645	108.032	107.0654
Latitude (°N)	23.0940	25.0583	26.8280	29.1385	30.4282	32.1587	33.0118
Altitude (m a.s.l.)	65 ± 25	158 ± 6	326 ± 20	427 ± 9	917 ± 24	967 ± 78	586 ± 31
MAT (°C)	22.0 ± 0.0	19.2 ± 0.0	16.8 ± 0.0	16.2 ± 0.1	16.9 ± 0.0	15.5 ± 0.0	13.8 ± 0.1
MAP (mm)	1747 ± 2	1648 ± 3	1330 ± 2	1395 ± 4	1211 ± 0	1091 ± 1	917 ± 1
Stand age (year)	24 ± 2	29 ± 1	27 ± 2	22 ± 2	38 ± 7	41 ± 7	35 ± 6
Stand density (tree ha ⁻¹)	1200 ± 208	833 ± 212	2384 ± 42	1122 ± 256	1963 ± 268	2079 ± 614	2022 ± 55
DBH (cm)	16.6 ± 1.6	21.7 ± 2.2	14.8 ± 1.5	16.2 ± 1.2	15.5 ± 0.8	16.4 ± 2.2	12.9 ± 1.1
Soil type	Red soil	Red soil	Red soil	Yellow soil	Yellow soil	Yellow soil	Yellow soil

distribution allows us to investigate the latitudinal patterns of soil EEAs and their acquisition ratios and the relative importance of climatic factors, edaphic variables and stand characteristics. This study was conducted in subtropical pine plantations, in which the community structures (pure vs. mixed) are different from those of natural forests (Guo and Ren, 2014; Xu et al. 2017; Zhou et al. 2020). Therefore, we hypothesized that the soil EEAs and enzymatic acquisition ratios in subtropical pine plantations would significantly increase from south to north (Hypothesis 1) to meet the nutrient demands due to the small effect of vegetation composition in afforested ecosystems (Zhao et al., 2018). Additionally, climatic factors other than edaphic variables and stand characteristics showed obvious latitudinal patterns in the subtropical pine plantations (Tables 1 and S1). As a result, climate determines the latitudinal patterns of soil EEAs and their acquisition ratios along a latitudinal gradient (Hypothesis 2).

Longitude, latitude and altitude data were measured using a handheld GPS (UniStrong G138, China). MAT, mean annual temperature; and MAP, mean annual precipitation. The climatic data were obtained by Kriging interpolation (1 km × 1 km resolution) in ArcGIS 10.2 (<https://www.esri.com>) based to the average data (1980–2012) from the National Meteorological Information Center of China (<http://www.data.cma.cn>). All values are reported as means ± standard errors (n = 3).

2. Materials and methods

2.1. Transect and study sites

Along a latitudinal gradient from south to north in subtropical China, we selected seven sites (latitude 23.0944°–33.0118°N, longitude 107.0654°–112.5010°E) with pure pine plantations for the present study (Fig. 1): Zhaoqing (ZQ), Guilin (GL), Huitong (HT), Yongshun (YS), Zhongxian (ZX), Wanyuan (WY) and Hanzhong (HZ). The 1100 km south-north transect spans approximately 10 degrees of latitude from 23°N to 33°N. The MAT and MAP ranged from 22.0 °C (south) to 13.8 °C (north) and from 1747 mm (south) to 917 mm (north), respectively (Table 1). The soil types were defined as red soil (e.g., ZQ, GL and HT) and yellow soil (e.g., YS, ZX, WY and HZ) according to the Chinese soil taxonomic classification and belong to Ultisols in the USDA soil taxonomic system (Zhang et al., 2005). These soil types are highly weathered (Waring et al., 2014; Vitousek et al., 2010).

2.2. Soil sampling and processing

At each site, three 600 m² (mostly 20 m × 30 m) plots in pure pine plantations, with a minimum distance of 1 km but <10 km between any two plots (i.e., spatially separated stands), were randomly selected and considered to be independent replicates (Peng and Wang, 2016; Zhao et al., 2018; Zhou et al., 2020). After the forest floor materials (e.g., litters, herbs and humus) were removed, surface soil samples (0–10 cm) were collected from 10 to 15 sampling points by using a soil auger with a diameter of 5 cm along an S-shaped line in each plot. The samples were hand mixed, homogenized and used as the soil sample for a plot. After visible roots and stones were removed from the fresh soil, the composite

soil samples were divided into two subsamples. One subsample was sieved through a 2 mm mesh and transported in an ice box to the laboratory as soon as possible, and was then stored until enzyme analysis at −20 °C to prevent microbial activity and minimize changes in the enzymatic community during storage (DeForest, 2009; Lee et al., 2007). Although the freezing process may have some effects on soil enzyme activities (DeForest, 2009; Lee et al., 2007), we assumed that it was unlikely to have altered the experimental outcome among plots/sites since all of our soil samples were subjected to freezing at the same temperature and to the same process (Stone et al., 2012). Another subsample was air-dried indoors and then sieved through a 2 mm mesh to measure soil chemical properties. Meanwhile, the species of all individuals with DBH (diameter at breast height of 1.3 m) ≥ 5 cm in the entire plot were recorded to estimate the stand density and then the cores of five to seven individuals (close to the average of all individuals within the plot) at the DBH were collected to determine the stand age (Table 1).

2.3. Soil chemistry measurements

Soil pH was measured at a soil-to-water ratio of 1:2.5 (v:v) with a pH meter (HI2221, Hanna, Italy) (Liu et al., 2008). Soil organic C and total N contents were determined with an elemental analyser (Euro EA, Hekatech Gabh, Germany) (He et al., 2020; Zhou et al., 2020). Soil total P content was determined by plasma emission spectroscopy (IRIS Intrepid II XSP, Thermo Fisher Scientific, USA) following digestion with $\text{HNO}_3\text{-HClO}_4\text{-HF}$ (He et al., 2020). Soil available N content was determined by conversion to NH_4^+ under NaOH conditions (1.8 mol L⁻¹), collection in an H_3BO_3 solution (2%), and subsequent titration with a standard HCl solution (0.01 mol L⁻¹) (Wang and Lu, 2006). Soil available P content was analyzed using a continuous flow analyser (Analytical AA3 Auto Analyser, SEAL, Germany) after extraction with $\text{HCl-H}_2\text{SO}_4$ solution (Cui et al., 2018). The average values of the soil chemical properties and their differences among sampling sites can be found in Table S1.

2.4. Fluorometric enzyme assays

The activities of β -1,4-glucosidase (BG, EC 3.2.1.21), β -1,4-N-acetylglucosaminidase (NAG, EC 3.2.1.14) and acid phosphatase (AP, EC 3.1.3.1) were measured using a modified version of the standard fluorometric protocol (German et al., 2011), which has been widely used in previous studies (Jing et al., 2020; Peng and Wang, 2016; Stone et al., 2012; Xu et al., 2017; Zhou et al., 2020). These three enzymes (i.e., BG, NAG and AP) usually represent C-, N- and organic P-acquiring enzymes, respectively (Cui et al., 2018; He et al., 2020; Waring et al., 2014; Zhao et al., 2018). In the present study, the three enzymes were selected for the following three reasons. First, the potential activities of BG, NAG and AP enzymes are usually associated with the rates of microbial metabolism and biogeochemical processes of soil C, N and P, respectively, and generally represent the microbial nutrient demands (Sinsabaugh et al., 2008, 2009). Second, the enzyme activities of soil stored at −20 °C were not significantly different from those of soil stored at 4 °C

(DeForest, 2009; Lee et al., 2007). Consequently, we can make comparisons with previous studies of the natural forests of eastern China (Xu et al., 2017; Zhou et al., 2020). Last, the activities of these three enzymes can provide both an indication of the sampling history and a snapshot of the sampling time due to their being less susceptible to denaturation and having long half-life (Mayor et al., 2016).

Briefly, after the frozen soil samples were thawed in a 4 °C refrigerator, we performed assays using 96-well microplates, with eight replicate wells per sample per assay. Therefore, the analysis consisted of eight replicate wells for each blank, a negative control, and a quench standard. Soil slurries were prepared as equivalents to 1 g of dry soil in 125 ml of sodium acetate buffer (50 mmol L⁻¹) at the ambient pH (5.3) of the soil samples and were then stirred for 1 min. A mixture solution of soil slurry (800 µl) and 200 µmol L⁻¹ of a specific substrate (i.e., 4-methylumbelliferone, 200 µl; Peng and Wang, 2016; Zhou et al., 2020) was injected into each well of the 96-well microplates. The microplates were incubated in the dark at 25 °C for 3 h. To stop the reaction, a 10 µl aliquot of 1 mol L⁻¹ NaOH was added to each well. Fluorescence was measured using a microplate fluorometer (SpectraMax i3x, Molecular Devices, Beckman Coulter, CA, USA) with 365 nm excitation and 450 nm emission filters. After correcting for negative controls and quench standards, the soil enzyme activities were expressed in units of nmol h⁻¹ g⁻¹ soil (German et al., 2011). The soil enzymatic C:N, C:P and N:P acquisition ratios were calculated by using $\ln(\text{BG}):\ln(\text{NAG})$, $\ln(\text{BG}):\ln(\text{AP})$ and $\ln(\text{NAG}):\ln(\text{AP})$, respectively (Cui et al., 2018; He et al., 2020; Waring et al., 2014; Zhao et al., 2018). Information about soil EEAs and enzymatic acquisition ratios and their differences among sampling sites is shown in Table S2.

2.5. Statistical analysis

After normality test of enzyme data (Kolmogorov-Smirnov test, $P > 0.05$), natural logarithm transformations of the enzyme data were applied to fulfill the assumptions of normality and homoscedasticity (Peng and Wang, 2016; Xu et al., 2017). One-way analysis of variance (ANOVA) and Duncan's multiple range tests (Xu et al., 2017) were used to determine the differences in all data among different sites. Type II standard major axis (SMA) regression was used to test the strength of the relationships between BG, NAG and AP activities across the dataset (Waring et al., 2014). A simple Pearson's correlation analysis was used to determine the relationships of soil EEAs and their acquisition ratios with a single factor and to find the main drivers according to the significance (P value) (Liu et al., 2008; Peng and Wang, 2016; Zhou et al., 2020). Additional relationships among the significant variables, soil EEAs and enzymatic acquisition ratios were identified by multiple linear regression analysis (Sinsabaugh et al., 2008; Xu et al., 2017). Variation partitioning analysis was performed to examine the relative contributions of the significant variables (Peng and Wang, 2016; Zhou et al., 2020). In the linear regression and variation partitioning analyses, we included DBH rather than stand density because DBH was significantly and negatively correlated with stand density (Table S3) and represented an indicator of growth capacity and biomass to some extent (Bloom et al., 1985; Vitousek et al., 2010). All statistical analyses were performed with SPSS 19.0 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA). Variation partitioning analysis was conducted using Canoco 5.0 (Biometris-Plant Research International, Wageningen, the Netherlands). Plots were generated using Origin 9.0 (Origin Lab Corporation, Northampton, MA, USA).

3. Results

3.1. Latitudinal patterns of soil EEAs and their acquisition ratios

Soil BG, NAG and AP enzyme activities ranged from 11.88 ± 2.41 (Guilin) to 72.01 ± 46.14 nmol h⁻¹ g⁻¹ dry soil (Hanzhong) with an average value of 41.27 ± 8.63 nmol h⁻¹ g⁻¹ dry soil, from 11.26 ± 4.85

(Zhaoqing) to 79.92 ± 24.56 nmol h⁻¹ g⁻¹ dry soil (Hanzhong) with an average value of 41.33 ± 7.07 nmol h⁻¹ g⁻¹ dry soil, and from 154.03 ± 34.89 (Guilin) to 428.09 ± 168.27 nmol h⁻¹ g⁻¹ dry soil (Hanzhong) with an average value of 281.58 ± 30.24 nmol h⁻¹ g⁻¹ dry soil, respectively (Table S2). Overall, these three soil EEAs significantly and linearly increased with increasing latitude (all $P < 0.05$, with a marginally significant level of $P = 0.062$ for AP activity; Fig. 2A-C) and exhibited the highest values at the highest latitude (Hanzhong; Table S2).

The soil enzymatic C:N, C:P and N:P acquisition ratios ranged from 0.87 ± 0.13 (Hanzhong) to 1.79 ± 0.45 (Zhaoqing) with an average value of 1.02 ± 0.10 , from 0.49 ± 0.05 (Guilin) to 0.72 ± 0.04 (Wanyuan) with an average value of 0.58 ± 0.04 , and from 0.37 ± 0.10 (Zhaoqing) to 0.73 ± 0.04 (Hanzhong) with an average value of 0.60 ± 0.04 , respectively. The enzymatic C:N acquisition ratio significantly decreased with increasing latitude ($P = 0.007$; Fig. 2D), and thus, the highest ratio was at the lowest latitude site (Zhaoqing; Table S2). The enzymatic C:P and N:P acquisition ratios significantly and linearly increased with increasing latitude (all $P < 0.05$; Fig. 2E, F) and exhibited higher values at the higher latitudes (C:P in Wanyuan and N:P in Hanzhong; Table S2). The relationships between $\ln(\text{BG})$ and $\ln(\text{NAG})$ seemed to be close to 1:1 (Fig. 3A), while the paired relationship between $\ln(\text{BG})$ and $\ln(\text{AP})$ (Fig. 3B) and that between $\ln(\text{NAG})$ and $\ln(\text{AP})$ (Fig. 3C) significantly deviated from 1:1.

3.2. Environmental controls on soil EEAs and their acquisition ratios

The BG, NAG, and AP activities as well as enzymatic C:P and N:P acquisition ratios were significantly and negatively related to both MAT and MAP, and were positively related to soil available P (all $P < 0.05$; Table 2). The AP activity showed significant and negative relationships with DBH ($P < 0.05$; Table 2). The enzymatic C:N acquisition ratio was significantly and positively correlated with both MAT and MAP (all $P < 0.05$), and was negatively correlated with stand density ($P < 0.05$; Table 2). Regression analysis showed that MAT, MAP, soil available P and DBH individually explained 20.2–60.6%, 19.4–56.6%, 19.3–31.6%, and 23.7–32.2% of the variations in soil EEAs and their acquisition ratios, respectively (Table 3). The combined effects of MAT and MAP explained 61.0% of the variation in NAG activity and 54.2% of the variation in enzymatic N:P acquisition ratio (Table 3). Climate (MAT and/or MAP) combined with soil available P and/or DBH explained high proportions (29.1–69.3%) of the variations in soil EEAs and their acquisition ratios (Table 3). Furthermore, variation partitioning analysis showed that climatic factors could explain 14.2% and 25.5% of the variances in soil EEAs and their acquisition ratios, respectively (Fig. 4). Climatic factors combined with soil available P explained 14.4% of the variances in soil EEAs (Fig. 4A), and they combined with DBH to explain 10.5% of the variances in soil enzymatic acquisition ratios (Fig. 4B). Similarly, the total effects of climatic factors (20.5–61.0%) accounted for higher variations in individual soil EEAs and their acquisition ratios than did soil available P (4.4–31.5%) and DBH (3.6–32.2%) (Table S4).

4. Discussion

4.1. Latitudinal patterns of soil EEAs and their acquisition ratios in pure pine plantations

We found that, except for the enzymatic C:N acquisition ratio, soil BG, NAG and AP activities as well as the enzymatic C:P and N:P acquisition ratios in pure pine plantations significantly and linearly increased with latitude from south to north (Fig. 2). These results support the first hypothesis, which assumes gradual increases in soil EEAs and their acquisition ratios along a latitudinal gradient. Soil properties affect the variations in soil EEAs via their influences on soil microbial functions (Allison et al., 2007; Sinsabaugh et al., 2008; Stark et al., 2014). For example, soil properties (e.g., pH and bulk density) indirectly affected

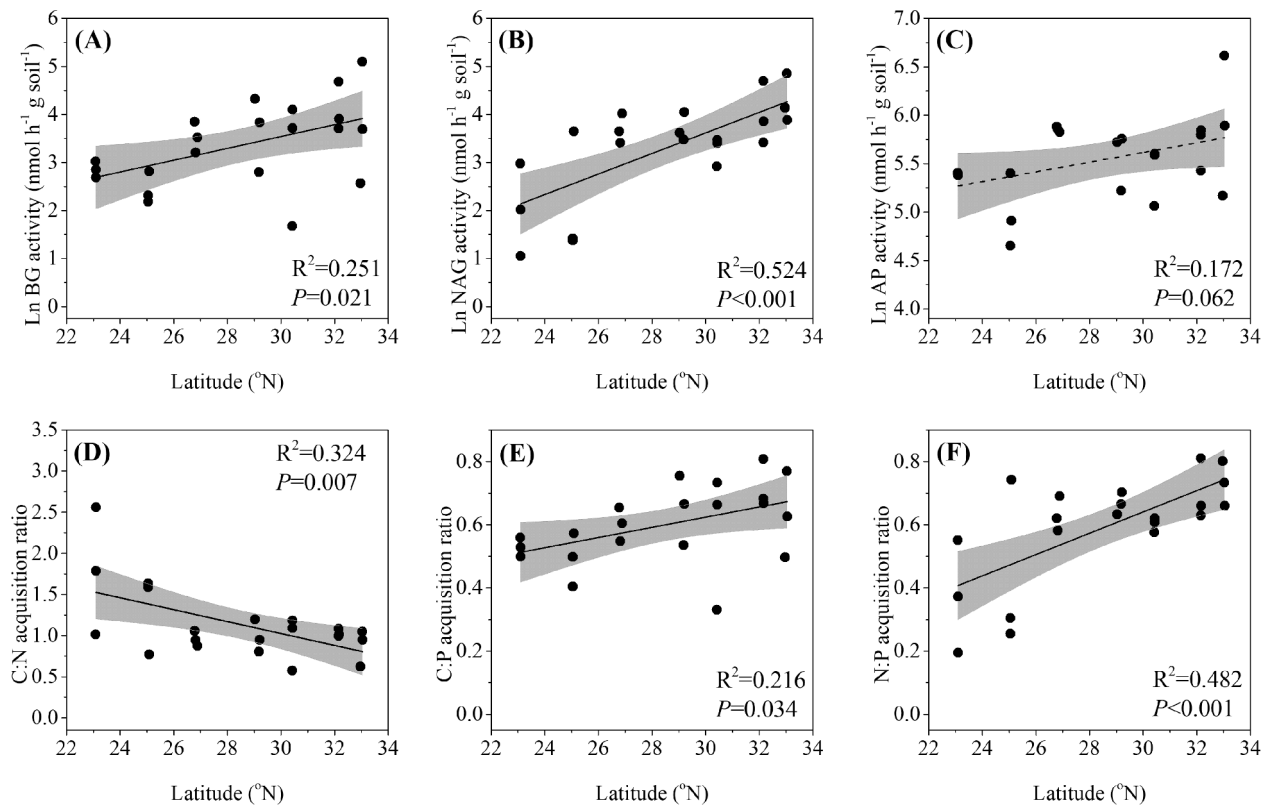


Fig. 2. Latitudinal patterns of soil extracellular enzyme activities and enzymatic acquisition ratios at depths of 0–10 cm in pure pine plantations from south to north in subtropical China ($n = 21$). (A) BG, β -1,4-glucosidase; (B) NAG, β -1,4-N-acetylglucosaminidase; (C) AP, acid phosphatase; (D) enzymatic C:N acquisition ratio; (E) enzymatic C:P acquisition ratio; and (F) enzymatic N:P acquisition ratio. All data of enzyme activities (i.e., BG, NAG, and AP) were transformed by using natural logarithms, and the enzymatic C:N:P acquisition ratios were calculated by using the enzyme activities after the ln-transformations. The solid line was significant at a level of 0.05, and the dotted line was not significant at a level of 0.05. Shading represents the 95% confidence interval.

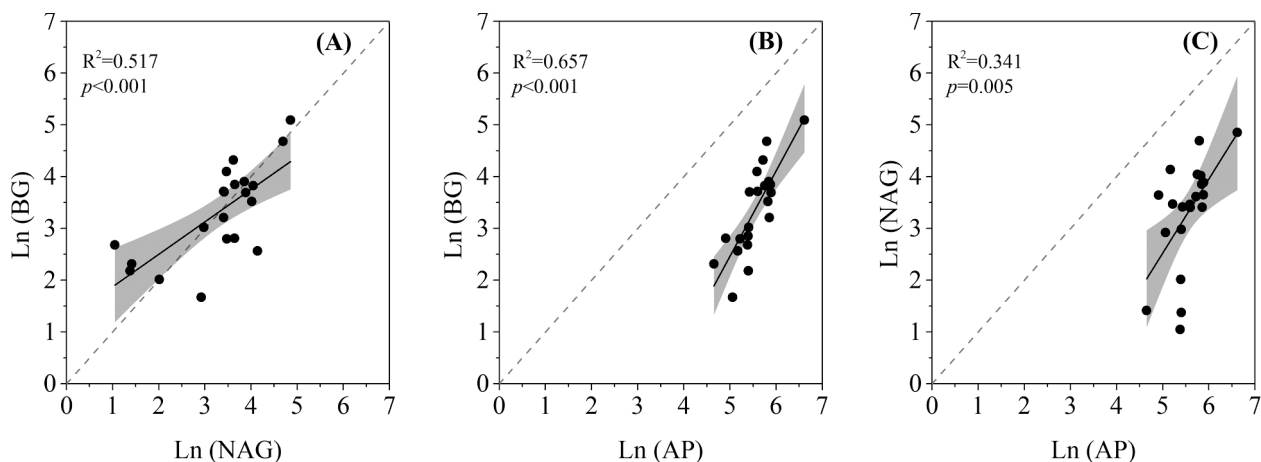


Fig. 3. Standard major axis (SMA) regressions between the natural logarithms of (A) BG and NAG, (B) BG and AP, and (C) NAG and AP ($n = 21$). BG, β -1,4-glucosidase; NAG, β -1,4-N-acetylglucosaminidase; and AP, acid phosphatase. The solid line was significant at a level of 0.05 (black line). Reference lines with slopes of 1.0 are shown on the graphs (grey line). Shading represents the 95% confidence interval.

soil BG and NAG enzyme activities through soil microbial biomass C in eastern natural forests (Wang et al., 2018) and in subtropical degraded forests (Feng et al., 2019) in China. Unfortunately, microbial properties were not determined from the sampling sites in the present study. Except for indirect effects, most studies have shown that soil properties can directly affect soil EEAs (Peng and Wang, 2016; Wang et al., 2018; Xu et al., 2017; Zhou et al., 2020). When soil nutrient availability was low, microorganisms secreted more enzymes to obtain the corresponding nutrients (Allison and Vitousek, 2005; Sinsabaugh et al., 2009). In this

study, soil organic C, total N, and available N contents decreased from south to north (Table S1). Therefore, soil BG and NAG activities significantly increased from south to north (Fig. 2A, B), while the enzymatic C:N acquisition ratio showed the opposite trend (Fig. 2D). These results did not agree with previous findings in natural forests in eastern China (Xu et al., 2017; Zhou et al., 2020) due to different stand origins (planted vs. natural) and compositions (pure vs. mixed). Additionally, these two studies showed that soil AP activity did not change (Xu et al., 2017) or declined (Zhou et al., 2020) with increasing latitude. In the present

Table 2

Pearson's correlation coefficients between environmental factors and soil extracellular enzyme activities and their acquisition ratios (n = 21).

Variables	Enzyme activities			Enzymatic acquisition ratios		
	BG	NAG	AP	C:N	C:P	N:P
MAT	−0.511*	−0.679**	−0.493*	0.646*	−0.450*	−0.736**
MAP	−0.526*	−0.681**	−0.512*	0.580**	−0.440*	−0.700**
pH	0.270	0.403	0.147	−0.269	0.213	0.339
Organic C	−0.029	−0.179	−0.016	0.085	0.197	−0.025
Total N	−0.141	−0.259	−0.117	0.173	0.006	−0.157
Total P	−0.072	−0.262	−0.164	0.068	0.077	−0.058
Available N	0.056	−0.052	0.007	−0.099	0.313	0.161
Available P	0.651*	0.749*	0.463*	−0.208	0.525*	0.494*
Soil C:N	0.281	0.156	0.186	−0.129	0.434*	0.248
Soil C:P	−0.029	−0.101	0.023	0.042	0.171	0.015
Soil N:P	−0.157	−0.209	−0.103	0.152	−0.039	−0.148
Age	0.138	0.218	0.032	−0.165	0.280	0.335
Density	0.108	0.204	0.342	−0.481*	0.092	0.415
DBH	−0.211	−0.251	−0.469**	0.414	−0.192	−0.417

* $P < 0.05$; ** $P < 0.01$. BG, β -1,4-glucosidase; NAG, β -1,4-N-acetylglucosaminidase; AP, acid phosphatase; MAT, mean annual temperature; MAP, mean annual precipitation; DBH, diameter at breast height of 1.3 m; C, carbon; N, nitrogen; P, phosphorus; soil C:N, C:P and N:P are the mass ratios of soil carbon to nitrogen, carbon to phosphorus and nitrogen to phosphorus, respectively. The enzymatic C:N:P acquisition ratios were calculated by using the enzyme activities after the ln-transformations.

Table 3

Multiple regression analyses among soil extracellular enzyme activities, enzymatic acquisition ratios and the selected variables (n = 21).

Models	Enzyme activities			Enzymatic acquisition ratios		
	BG	NAG	AP	C:N	C:P	N:P
MAT	0.257*	0.606***	0.232*	0.417**	0.202*	0.541***
MAP	0.259*	0.566***	0.260*	0.336**	0.194*	0.491***
MAT + MAP	0.266 ns	0.610***	0.260 ns	0.422**	0.205 ns	0.542**
Soil available P (aP)	0.309**	0.316**	0.193*	0.043 ns	0.276*	0.244*
MAT + Soil aP	0.388*	0.657***	0.291*	0.428**	0.330*	0.571***
MAP + Soil aP	0.374*	0.604***	0.300*	0.350*	0.313*	0.512**
MAT + MAP + Soil aP	0.388*	0.657***	0.301 ns	0.429*	0.333 ns	0.572**
DBH	0.095 ns	0.237*	0.322**	0.171 ns	0.037 ns	0.174 ns
MAT + DBH	0.267 ns	0.634***	0.392*	0.440**	0.202 ns	0.554**
MAP + DBH	0.262 ns	0.580***	0.387*	0.354*	0.195 ns	0.495**
MAT + MAP + DBH	0.271 ns	0.634**	0.392*	0.459*	0.206 ns	0.554**
Soil aP + DBH	0.353*	0.467**	0.438**	0.189 ns	0.285*	0.354*
MAT + Soil aP + DBH	0.400*	0.687***	0.454*	0.450*	0.330 ns	0.585**
MAP + Soil aP + DBH	0.383*	0.624**	0.446*	0.365*	0.313 ns	0.519**
MAT + MAP + Soil aP + DBH	0.403 ns	0.693**	0.458*	0.462*	0.334 ns	0.590**

The values represent the determination coefficients (R^2). Overall model significances are ns (not significant, $P > 0.05$), * ($P < 0.05$), ** ($P < 0.01$) or *** ($P < 0.001$). MAT, mean annual temperature; MAP, mean annual precipitation; aP, available phosphorus; DBH, diameter at breast height of 1.3 m. All data of enzyme activity (i.e., BG, NAG, and AP) were transformed by using natural logarithm, and the enzymatic C:N:P acquisition ratios were calculated by using the enzyme activities after the ln-transformations.

study, however, soil available P content increased along the latitudinal gradient (Table S1) and the corresponding soil AP activity also increased from south to north (Fig. 2C). These results do not match the resource allocation theory that microbes allocate their resources optimally to acquire relatively limited nutrients (Allison and Vitousek, 2005; Bloom, et al., 1985; Bowles et al., 2014; Fujita et al., 2019; Sinsabaugh et al., 2009).

In this study, the soil enzymatic C:P and N:P acquisition ratios increased significantly with increasing latitude in pure pine plantations (Fig. 2E, F). Similarly, Zhou et al. (2020) found that the soil enzymatic C:P and N:P acquisition ratios increased over latitude in natural forests, while Xu et al. (2017) observed that the soil enzymatic acquisition ratios did not show any clear latitudinal trends. Our results demonstrated that microbial metabolisms tended to involve higher investments in P-acquiring enzymes than in C- and N-acquiring enzymes from south to north. Then soil EEAs appear to make a trade-off in nutrient acquisition among C, N, and P in response to variations in substrate quality and nutrient supply. Consequently, the soil enzymatic acquisition ratios along latitudinal gradients in planted forests are resource dependent rather than homeostatic. A similar conclusion was found for grassland ecosystems (Peng and Wang, 2016).

Interestingly, these three enzyme activities and their acquisition ratios along this latitudinal gradient were closer to those along the eastern latitudinal gradient reported by Zhou et al. (2020) than by Xu et al. (2017) (Fig. 5). One possible reason was the differences in the sampling times. For example, in the tropical monsoon forests of the Jianfengling Nature Reserve of southern China, the soil BG activity collected in July 2013 ($37 \pm 3.4 \text{ nmol h}^{-1} \text{ g}^{-1}$ soil; Xu et al., 2017) was higher than that collected in July 2018 ($13 \pm 3.5 \text{ nmol h}^{-1} \text{ g}^{-1}$ soil; Zhou et al., 2020). On the other hand, vegetation types and compositions significantly affected soil EEAs and enzymatic acquisition ratios (He et al., 2020; Redel et al., 2008). In this study, soil samples were collected in pure pine plantations, which contributed to these lower soil EEAs and enzymatic acquisition ratios than those in the natural forests of eastern China (Fig. 5; Xu et al., 2017; Zhou et al., 2020). Additionally, previous studies have indicated that soil microorganism properties and EEAs varied significantly with soil depth (Feng et al., 2019; Peng and Wang, 2016; Zhou et al., 2020). However, the use of soil samples, which were collected from only the 0–10 cm layer in this study, limits the understanding of the spatial patterns of soil EEAs and enzymatic acquisition ratios in the deeper soil layers and their controlling factors in subtropical pine plantations.

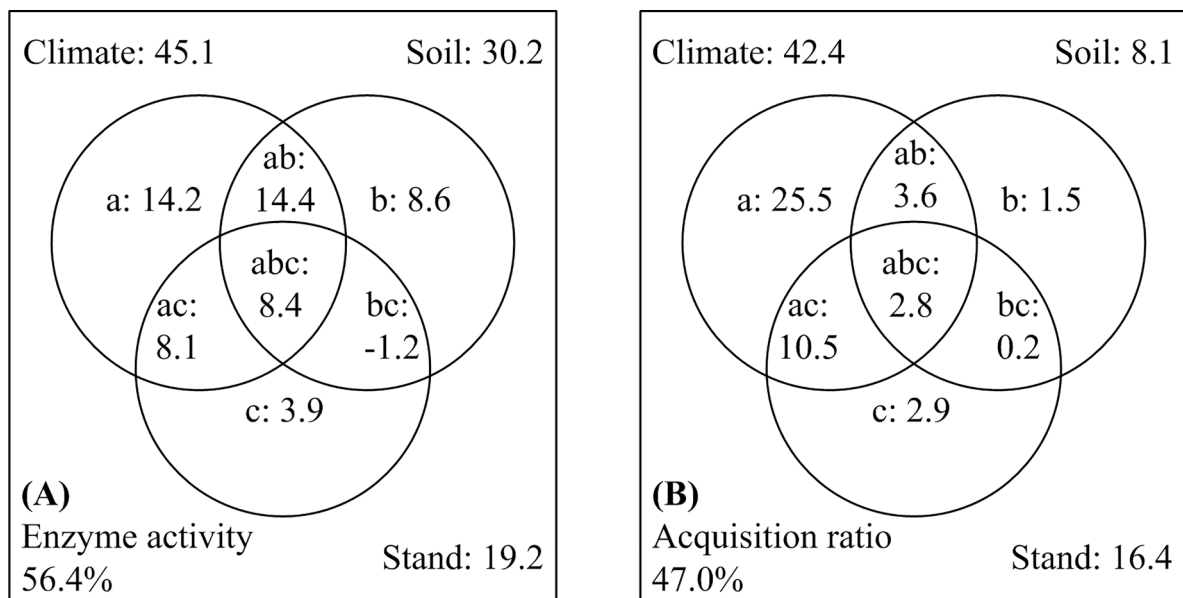


Fig. 4. Venn diagrams illustrating the relative contributions of climate (a), soil (b), stand (c) and their intersections (e.g., ab, bc, ac, and abc) to the variances in the latitudinal patterns of soil extracellular enzyme activities (A) and enzymatic acquisition ratios (B) by using redundancy analysis (RDA) ($n = 21$). Climate included mean annual temperature and mean annual precipitation, soil represented soil available P content, and stand represented diameter at breast height of 1.3 m. All data of enzyme activities (i.e., BG, NAG, and AP) were transformed by using natural logarithm, and the enzymatic C:N:P acquisition ratios were calculated by using the enzyme activities after the ln-transformations.

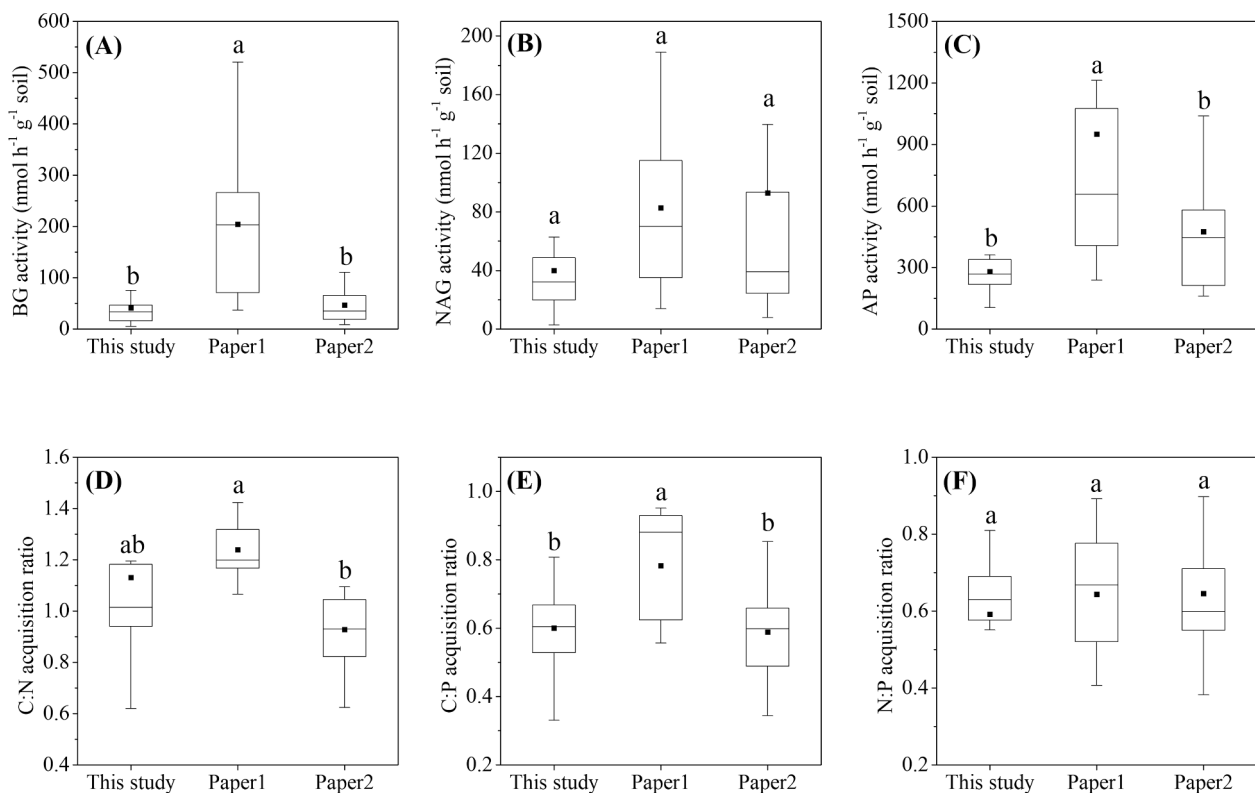


Fig. 5. Comparisons of soil extracellular enzyme activities and their acquisition ratios (0–10 cm) among this study and those in published datasets. The boxes show the median values surrounded by the 25th and 75th percentile values, and the black squares in the middle of the boxes show the average values. The outliers are not shown in the figures. Different lowercase letters indicate significant differences at a level of 0.05 ($P < 0.05$) based on Duncan's multiple range tests. The data of Paper1 and Paper2 were extracted from forest sites along latitudinal gradients in eastern China, as described by Xu et al. (2017) and Zhou et al. (2020), respectively. (A) BG, β -1,4-glucosidase; (B) NAG, β -1,4-N-acetylglucosaminidase; (C) AP, acid phosphatase; (D) enzymatic C:N acquisition ratio; (E) enzymatic C:P acquisition ratio; and (F) enzymatic N:P acquisition ratio. The enzymatic C:N:P acquisition ratios were calculated by using the enzyme activities after the ln-transformations.

4.2. Drivers of latitudinal variations in soil EEAs and their acquisition ratios

In agreement with the second hypothesis, climate (e.g., MAT and MAP) was the main driver of the latitudinal variations in the soil EEAs and their acquisition ratios in subtropical pure pine plantations (Fig. 4; Tables 3 and S4). This result supports previous findings (Kittredge et al., 2018; Stone et al., 2012; Xu et al., 2017; Zhou et al., 2020). Climatic factors affect soil EEAs and enzymatic acquisition ratios by influencing microbial nutrient demand and/or soil nutrient availability (Kivlin and Treseder, 2014; Manzoni et al., 2012). In this study, climatic factors had negative impacts on the latitudinal patterns of soil EEAs and enzymatic acquisition ratios (Table 2), which agreed with the results in Xu et al. (2017) but differed from the findings in Zhou et al., (2020). The dissimilar effects of climatic factors on soil EEAs and their acquisition ratios seem to be universal and are dependent on spatial scales or geographic regions. For example, based on regional data, Waring et al. (2014) reported positive temperature effects on BG activity and enzymatic C:P acquisition ratio in tropical soils. By using a global dataset, however, Sinsabaugh et al. (2008) showed that there were no significant correlations of enzyme activities with temperature. Two meta-analyses by Henry (2012) and Xiao et al. (2018) also showed that the activities of both hydrolytic and oxidative enzymes often did not respond to warming. In contrast, Peng and Wang (2016) observed negative temperature effects on longitudinal variations in BG and NAG activities as well as enzymatic acquisition ratios in Chinese grasslands. Moreover, Zhou et al. (2013) and Kittredge et al. (2018) suggested that the temperature effects on soil EEAs depend on seasons and soil depths. Therefore, to further understand and maintain the functionality of soil enzymes in terrestrial ecosystems under rapid climate change (Henry, 2012; Kittredge et al., 2018; Zhou et al., 2013; Xiao et al., 2018), controlled experiments are needed to precisely identify the relationships among climatic factors, EEAs and enzymatic acquisition ratios.

Our results were inconsistent with previous studies showing that edaphic variables (e.g., properties or stoichiometry) had greater influences on soil EEAs and their acquisition ratios (Cui et al., 2018; Jones et al., 2019; Liu et al., 2008; Peng and Wang, 2016; Stark et al., 2014). We found that only soil available P content was significantly and positively related to soil EEAs and their acquisition ratios in subtropical pure pine plantations (Table 2). The relationship between soil AP activity and soil P availability also varied with spatial scale and geographical region. For instance, at the local scale of New Zealand, Allison et al. (2007) observed that the efficiency of P-hydrolyzing enzymes was negatively affected by soil P content, which was confirmed by the responses of phosphatase activities to P additions (Allison and Vitousek, 2005; DeForest et al., 2012; Xiao et al., 2018). In contrast, Liu et al. (2008) and Peng and Wang (2016) found significant and positive relationships between soil AP activity and soil P content along spatial gradients in northern China. However, Waring et al. (2014) did not find any clear relationship between AP activity and soil P content based on regional data from tropical forests. The positive relationship between AP activity and available P content found in the present study (Table 2) may provide evidence for the one case described by Burns et al. (2013), who stated the positive relationship between enzyme activity and resource availability when an increase in enzyme activity in response to resource demand results in the release of excess reaction products. In other words, when the soil available P content that is produced by microorganism decomposition is not sufficient to meet the growth demand, soil AP activity would increase with increasing soil P availability. For example, phosphatase production and activity may increase with soil microbial P demand in P-limited ecosystems (DeForest et al., 2012; Jing et al., 2020). Additionally, soil AP activity was significantly influenced by soil P fractions (Chen, 2003; DeForest and Scott, 2010; Redel et al., 2008; Rojo, et al., 1990). Chen (2003) found that phosphatase activity was significantly and positively correlated with total, inorganic, organic and occluded P as well as with acid extractable, aluminum bound, and

iron bound P in a Chinese fir plantation in subtropical China. Unfortunately, we did not examine the relationships between phosphatase activity and P fractions in the present study.

The selected abiotic and biotic factors (e.g., MAT, MAP, soil available P content and DBH) explained only 33.4–69.3% of the variations in all or individual soil EEAs and their acquisition ratios in the present study (Fig. 4 and Table S4). These results suggest that other drivers, such as soil physical (e.g., texture) and chemical (e.g., P fractions) properties and biotic factors (e.g., plant nutrient and vegetation compositions) (DeForest and Scott, 2010; Cui et al., 2018; Henry, 2012; Peng and Wang, 2016; Xu et al., 2017; Zhou et al., 2020), might contribute to their latitudinal variations. Further studies, including large-scale investigations and manipulation experiments, are needed to clarify the effects of abiotic and biotic factors on soil EEAs and their acquisition ratios in planted forests across multiple scales and soil depths.

4.3. Evidence for P limitation in pure pine plantations in subtropical China

Soil enzymatic C:N:P acquisition ratios are usually used to identify microbial nutrient demands (Sinsabaugh et al., 2009). Enzymatic C:P acquisition ratio tends to be lower in highly weathered soil (Waring et al., 2014), which is generally considered to be P-limited for plant growth (Tian et al., 2010; Vitousek et al., 2010; Zhang et al., 2005). Therefore, the lower enzymatic C:P and N:P acquisition ratios suggest that soil P is the limiting element in tropical and subtropical ecosystems (Waring et al., 2014; Xu et al., 2017; Zhou et al., 2020). The enzymatic C:P and N:P acquisition ratios in our study were similar to those reported in previous studies (Fig. 5E, F). And the enzymatic C:P (BG:AP) and N:P (NAG:AP) acquisition ratios (0.13 and 0.14, respectively) in our study were also comparable to the ratios reported for tropical (0.21 and 0.13, respectively; Waring et al., 2014) and subtropical (0.22 and 0.09, respectively; Xu et al., 2017) forest ecosystems when no natural logarithmic transformations were applied. Moreover, there were lower soil total P content in the 0–10 cm soil layer (0.33 g kg^{-1}) along the latitudinal gradient (Table S1) than in the 0–10 cm soil layer (0.78 g kg^{-1}) at a national scale (Tian et al., 2010; Zhang et al., 2005). Therefore, these results indicate that this planted forest may be subject to P limitation. Instead, the latitudinal patterns of enzymatic C:N acquisition ratio (Fig. 2D) and soil N availability (Table S1) indicated that this planted forest may become co-limited by both N and P at higher latitude sites. However, because of the significantly lower soil P supply capacity (i.e., the ratio of available P to total P; Zhang et al., 2005) than soil N supply capacity (i.e., the ratio of available N to total N) that we observed (Mann-Whitney *U* test, $P < 0.001$; Fig. S1), P limitation appears to be prevalent along the entire latitudinal gradient in this pure pine plantation in subtropical China. Notably, a recent study concluded that the enzymatic acquisition ratios did not reveal the limiting element for bacterial, fungal and plant growth in subtropical soils (Rosinger et al., 2019). Therefore, P-limited pine growth should be further confirmed by the effects of P cycling and addition experiments.

5. Conclusions

We investigated soil EEAs and their acquisition ratios as well as their controlling factors in pure pine plantations along a 1100 km transect in subtropical China. Except for the enzymatic C:N acquisition ratio, three enzyme activities (i.e., BG, NAG, and AP) and two enzymatic acquisition ratios (i.e., C:P and N:P) increased significantly with increasing latitude, and these latitudinal patterns were mainly controlled by MAT, MAP, soil available P and DBH. Climatic factors (e.g., MAT and MAP) had greater effects on the latitudinal variations in soil EEAs and their acquisition ratios than soil available P content and DBH. Moreover, the lower average enzymatic C:P and N:P acquisition ratios along the latitudinal gradient imply that the pure pine plantations of subtropical China may suffer from P limitation for microbial and plant growth. Our results are

helpful for understand the factors that control soil nutrient cycling in planted forests in subtropical China.

CRedit authorship contribution statement

Zunji Jian: Investigation, Formal analysis, Writing - original draft. **Yanyan Ni:** Investigation, Formal analysis. **Lixiong Zeng:** Conceptualization, Project administration. **Lei Lei:** Conceptualization, Data curation, Writing - review & editing. **Jin Xu:** Methodology. **Wenfa Xiao:** Conceptualization, Funding acquisition. **Mai-He Li:** Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank our colleagues, Le Li, Liang Ma, and Yu Tian, for their assistance with soil sampling, and Lijun Wang for assisting soil enzyme assays. We thank the staff who provided assistance in sampling and our investigations in the planted forest sites and the National Forest Ecosystem Station of Three Gorges Reservoir in Zigui County. We thank anonymous reviewers for very helpful comments and suggestions. This work was supported by the National Key Research and Development Program of China (grant number 2016YFD0600201).

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foreco.2021.119358>.

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