

Factors influencing soil enzyme activity in China's forest ecosystems

Haifeng Zheng  · Yang Liu · Jian Zhang · Yamei Chen · Lin Yang · Hongjie Li · Lifeng Wang

Received: 21 April 2017 / Accepted: 25 October 2017
© Springer Science+Business Media B.V. 2017

Abstract Enzyme activity (EA) mediates soil organic matter (SOM) degradation, transformation, and mineralization, thereby maintaining the biogeochemical cycles and energy flow of ecosystems. To determine the main factors explaining EA variations in China's forest ecosystems, we created a database of soil EAs and relevant variables using data from the literature and analysed relationships between EAs and both climatic and edaphic variables. Catalase, phenol oxidase, acid (alkaline) phosphatase, and protease activities differed significantly among different types of forests. Catalase and urease activities were generally higher in primosols, cambisols, and argosols than in ferrosols. EA largely decreased with soil depth and increased with SOM. Phenol oxidase and urease activities were negatively correlated with mean annual temperature (MAT); in contrast, catalase, invertase, and protease activities first decreased (< 2.5 °C), increased (2.5–17.5 °C), and

then decreased (> 17.5 °C) with increasing MAT. Although protease activity was slightly positively correlated with mean annual precipitation (MAP), catalase, phenol oxidase, and urease activities were all negatively related to MAP. Catalase, invertase, acid (alkaline) phosphatase, urease, and protease activities first increased (< 2000 m.a.s.l.) and then decreased (2000–4100 m.a.s.l.) with increasing elevation. Principal component analysis revealed most EAs to be correlated with climate conditions and soil pH. These findings suggest that climatic and edaphic variables directly and indirectly correlate with forest type and greatly impact soil EA.

Keywords Enzyme activity · Soil organic matter · Soil profile · Forest type · Climatic factors

Communicated by Sumanta Bagchi.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11258-017-0775-1>) contains supplementary material, which is available to authorized users.

H. Zheng · Y. Liu (✉) · J. Zhang · Y. Chen · L. Yang · H. Li · L. Wang
Key Laboratory of Ecological Forestry Engineering,
Institute of Ecology & Forest, Sichuan Agricultural
University, Chengdu 611130, China
e-mail: sicauliuyang@163.com

Introduction

Approximately one-third of the Earth's surface is covered by forests and woodlands, regions that play important roles in global carbon sequestration and nutrient cycling (Perry et al. 2008). Compared to other terrestrial plant communities, forests are often highly heterogeneous environments. Forest ecosystems in China are diverse and include various types of coniferous, hardwood, deciduous, and evergreen forests as well as shrublands and secondary forests. The type of forest affects the physical–chemical

characteristics of soil through litter and root exudates produced by the dominant tree species, in turn regulating the soil microbial community structure and activity. Microbial community metabolism is the principal driver of the biogeochemical cycles in detrital food webs (Sinsabaugh and Follstad Shah 2012), and microbes regulate the production and release of enzymes in response to environmental resource availability signals, including the products of enzymatic reactions (Sinsabaugh et al. 2014).

Soil enzymes mediate the degradation, transformation, and mineralization of soil organic matter (SOM), thereby maintaining the biogeochemical cycle and energy flow of an ecosystem (Burns and Dick 2002; Sinsabaugh 2010). Enzymes are crucial for the success of microorganisms that rely on polymeric substrate degradation, and C, N, and P allocation for enzyme production must be prioritized to prevent starvation. Enzyme activity (EA) catalyses the rate-limiting steps of the degradation of organic matter, and many studies have examined relationships among plant litter decomposition, the microbial community, and EA (Adamczyk et al. 2009, 2014; Brockett et al. 2012; Hill et al. 2014). Such evaluation of large-scale EA patterns has provided insight into the biochemistry of decomposition and nutrient cycling in aquatic systems and terrestrial soils (Sinsabaugh et al. 2008; Williams et al. 2012; Hill et al. 2014). In China, extensive research has been performed on soil EA in forest ecosystems; however, no comprehensive analysis of the magnitude and distribution of soil EA in China's forest ecosystems has been conducted to date. By identifying how EAs differ among forest types, we can better assess how plant–microbe interactions relate to ecosystem processes.

Overall, soil EAs and their relationships with ecological factors have attracted much attention (Suseela et al. 2012; McDaniel et al. 2013; Ladwig et al. 2015). EAs in forest ecosystems are determined by numerous factors, including edaphic conditions [i.e. soil order, soil depth, soil pH, and soil organic matter (SOM)], climatic factors (i.e. moisture and temperature), and geographic factors (i.e. forest type, longitude, latitude, and elevation), which often interact and regulate carbon allocation in soil (Burns et al. 2013). Moreover, edaphic conditions are key factors that determine microbial species compositions or functions (Drenovsky et al. 2004; Brockett et al. 2012), and climatic and geographic factors are

expected to influence microbial communities by determining both temperature and moisture conditions, which influence the soil microbial community structure as well as EAs (Hackl et al. 2005; Lanzen et al. 2016). In general, enzyme reactions are sensitive to temperature (Davidson and Janssens 2006), and climatic and geographic factors can affect EA by altering microbial biomass and through abiotic control of enzyme turnover and stabilization (Suseela et al. 2012; Steinweg et al. 2013). Although several studies have described relationships between soil EA and soil pH, results have been inconsistent, with positive, negative, or no correlation reported (Eivazi and Tabatabai 1990; Acosta-Martínez and Tabatabai 2000; Błońska 2010; Guan et al. 2013). Additionally, much debate remains with regard to how climatic and geographic factors affect soil EAs on large spatial scales (Allison et al. 2010; Cusack et al. 2010; Kardol et al. 2010; Puissant et al. 2013). Therefore, in the present study, we performed a meta-analysis and structural review of the eight most widely measured soil EAs in China's forest ecosystems, as reported in publications from the past 20 years. The aims of this study were (i) to identify how EAs differ between forest types (coniferous vs. hardwood, deciduous vs. evergreen forest, pure vs. mixed forest, and shrubland vs. forest), (ii) to reveal relationships between soil EAs and both climatic (mean annual temperature (MAT), mean annual precipitation (MAP), and elevation) and edaphic (soil order, SOM, soil pH, and soil depth) variables, and (iii) to explore the main factors influencing soil EAs in China's forest ecosystems.

Methods

Data description

In this study, data on soil EAs and relevant variables were collected from studies of China's forest ecosystems published from 1997 to 2016. Literature searches were mostly performed using Google Scholar and China National Knowledge Infrastructure (CNKI) online journal databases. The keywords used included but were not limited to *soil enzyme*, *enzyme activity*, *biochemical characteristics*, and *forest* and the names of specific locations such as *China*. Our searches resulted in > 10,000 hits, from which we extracted 73 studies that met the following eligibility criteria

Table 1 Soil enzymes assayed for potential activity

Enzyme	EC	Abbreviation	EU	Main function
Catalase	1.11.1.6	CAT	$\mu\text{l (KMnO}_4\text{)/g h}$	Catalyses the decomposition of hydrogen peroxide
Phenol oxidase	1.10.3.2	POX	$\mu\text{g (pyrogallol)/g 2 h}$	Plays important roles in transforming SOM
Invertase	3.2.1.26	INV	$\mu\text{g (glucose)/g 24 h}$	Hydrolyses sucrose to produce glucose and fructose
Acid (alkaline) phosphatase	3.1.3.1	AP	$\mu\text{g (phenol)/g 2 h}$	Participates in the hydrolysis of phosphate monoesters
Urease	3.5.1.5	URE	$\mu\text{g (NH}_3\text{-N)/g}\cdot\text{24 h}$	Closely related to nitrogen transformation
Protease	3.4.2.21	PRO	$\mu\text{g (NH}_3\text{-N)/g}\cdot\text{24 h}$	

EC enzyme commission classification, EU enzyme unit

(Supplementary material 1). (1) The data were from a field experiment. (2) The soil EA assay and EA unit (EU) were the same (Table 1; EA was measured using previously described methods (Guan 1986)). (3) The data were presented as specific values (including charts). (4) The data included the activity of at least one soil enzyme and one related variable. A total of 561 valid data points were collected from 73 studies. The data originated from 57 experimental sites mainly distributed in northeastern, central, eastern, southern, and southwestern China, together representing the primary forest types and climatic zones of the country (Fig. 1 and Supplementary material 1).

To analyse relationships between soil EAs and each of the influencing factors, data were collected to assess EAs in eight types of soil. These data included two types of oxidases (catalase (CAT) and polyphenol oxidase (POX)) and four types of hydrolases (invertase (INV), urease (URE), acid (alkaline) phosphatase (AP), and protease (PRO)) (Supplementary material 1). Data for the following parameters of the experimental sites were also compiled: (1) soil properties, including soil order, soil depth, pH, and SOM concentration; (2) a geographic factor (elevation); (3) climatic factors, including MAT and MAP; and (4) leaf characteristics, including leaf type (deciduous or evergreen) and morphology (coniferous or hardwood) (Table 2 and Supplementary material 1). EAs were analysed in four main soil orders (argosols, cambisols, ferrosols, and primosols). Argosols have a clay-enriched subsoil and a relatively high native fertility, and most are formed under forest vegetation with a thick soil solum. Cambisols are largely distributed in low mountains and hilly areas, where soil weathering and forming durations are quite short due to the impact

of erosion. Ferrosols are highly acidic, with a low cation exchange capacity and P availability, and are characterized by an argic horizon within the profile. Primosols are characterized by a thin soil solum due to a weak degree of weathering and an alpine ecological condition (Gong et al. 2007).

Sensitivity analysis and publication bias

We conducted sensitivity analysis to determine the robustness of the results to changes in the decisions and assumptions made in the meta-analysis (the data structure can adopt a variety of forms). Additionally, we performed descriptive statistical analysis to understand the distribution and variability of the sample observations using a curve fitted by a Gaussian function (Koricheva et al. 2014; You et al. 2017). Subsequently, we randomly sampled five datasets from the total EA sample (Fig. S1) and used *t* tests to reanalyse variations in the five groups. The results obtained from the five groups were qualitatively the same as those obtained from the total sample (Fig. S1), and this finding suggests that reducing the number of samples will not change the results of this study. Publication bias testing is also a form of sensitivity analysis (Koricheva et al. 2014) and occurs when the probability of publication depends on the statistical significance, magnitude, or direction of the effect. Publication bias can influence any synthesis or review of the literature, including narrative reviews (Koricheva et al. 2014). Accordingly, we assessed publication bias by observing whether the data followed a normal distribution (Hu et al. 2016; You et al. 2017), as large amounts of data following a normal distribution can indicate that the data are close

Fig. 1 Map of the study site locations. The map was generated with ArcGIS® version 10.2 (ESRI, Redlands, CA, USA, <http://desktop.arcgis.com/en/arcmap>). *ECF* evergreen coniferous forest, *EBF* evergreen broad-leaved forest, *DCF* Deciduous coniferous forest, *DBF* deciduous broad-leaved forest, *MF* mixed forest, *S* shrublands

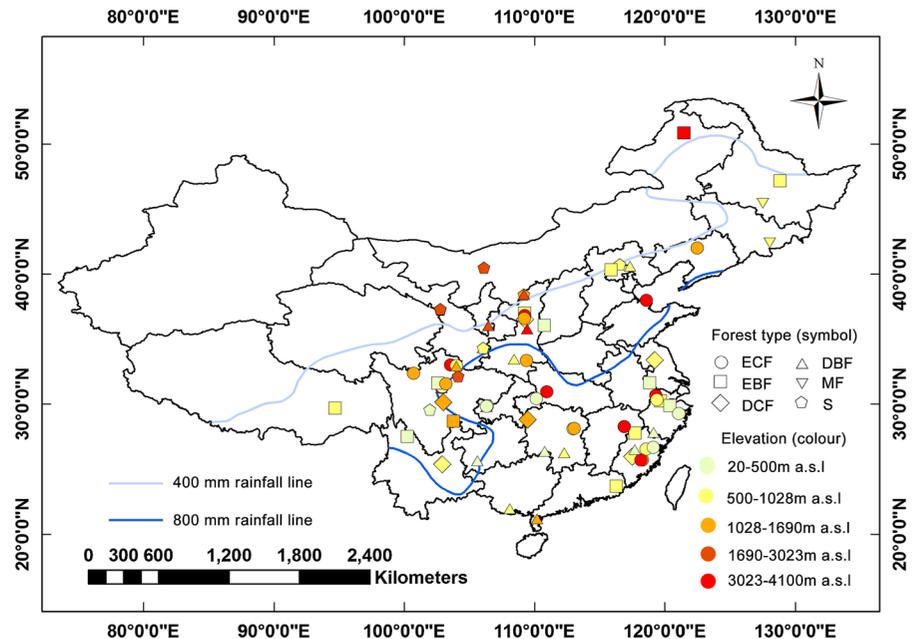


Table 2 Predictor variables included in the analyses

Factor	Levels
Forest type	Evergreen coniferous forest, evergreen broad-leaved forest, deciduous coniferous forest, deciduous broad-leaved forest, mixed forest, shrublands
Soil order	Argosols, cambisols, ferrosols, primosols
Soil organic matter	Continuous (0.79–950.67 g/kg)
Soil pH	Continuous (3.7–9.3)
Soil depth	Continuous (1–50 cm)
Mean annual temperature	Continuous (– 5.0 to 23.0 °C)
Mean annual precipitation	Continuous (145–2900 mm)
Elevation	Continuous (10–4100 m)

to reality and have good representativeness. The occurrence of publication bias can also be reduced by using a large amount of data (Lebauer and Treseder 2008). The results of normal distribution tests and the large amount of data indicated a lack of publication bias in the present study (Fig. S2).

Statistical analysis

The data were ln-transformed prior to analysis to meet the requirement for a normal distribution. Univariate

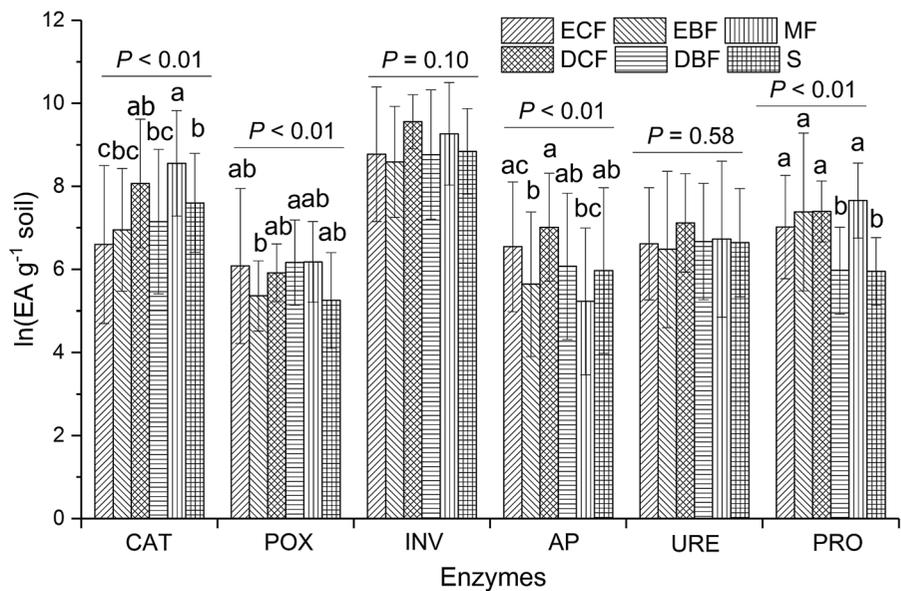
linear and non-linear (quadratic and cubic) regression analyses were performed to relate soil EAs to variations in SOM concentration, soil pH, soil depth, MAT, MAP, and elevation. Dunnett's T3 test was used when one-way analysis of variance (ANOVA) showed that the treatment effects (forest type and soil order) on soil EAs were significant. Principal component analysis (PCA) was employed to reduce the six variables (SOM, pH, soil depth, MAT, MAP, and elevation) to two factors (F1 and F2). All data-processing steps were performed using OriginPro 8.5 (OriginLab Corporation, Northampton, MA, USA), Excel 2013 (Microsoft, Redmond, WA, USA), and SPSS Statistics for Windows version 20.0 (IBM Corp., Armonk, NY, USA).

Results

Relationships between EAs and forest types

Significant differences in CAT (ANOVA, $F = 8.93$, $df = 5$, $P < 0.01$), POX (ANOVA, $F = 5.17$, $df = 5$, $P < 0.01$), AP (ANOVA, $F = 5.18$, $df = 5$, $P < 0.01$), and PRO (ANOVA, $F = 9.74$, $df = 5$, $P < 0.01$) activities were observed among the examined forest types (Fig. 2). The highest EAs were generally observed in mixed forests. CAT activity was

Fig. 2 Values of $\ln(\text{EA g}^{-1} \text{ soil})$ for different forest types. The bars indicate standard errors. Different letters indicate significant differences between different forest types, as derived using Dunnett's T3 test. *ECF* evergreen coniferous forest, *EBF* evergreen broad-leaved forest, *DCF* deciduous coniferous forest, *DBF* deciduous broad-leaved forest, *MF* mixed forest, *S* shrublands. The enzyme abbreviations are provided in Table 1



higher in mixed forest than in evergreen coniferous forest (Fig. 2 and Table S1, Dunnett's T3 test, $P < 0.05$, 29.6%), and POX activity was higher in deciduous broad-leaved forest than in evergreen broad-leaved forest (Fig. 2 and Table S1, Dunnett's T3 test, $P < 0.05$, 14.9%). Compared to evergreen broad-leaved forest, deciduous coniferous forest exhibited higher AP activity (Fig. 2 and Table S1, Dunnett's T3 test, $P < 0.05$, 24.3%). PRO activity was lower in deciduous broad-leaved forest and shrublands than in the other forest types (Fig. 2 and Table S1, Dunnett's T3 test, $P < 0.05$, 23.5%).

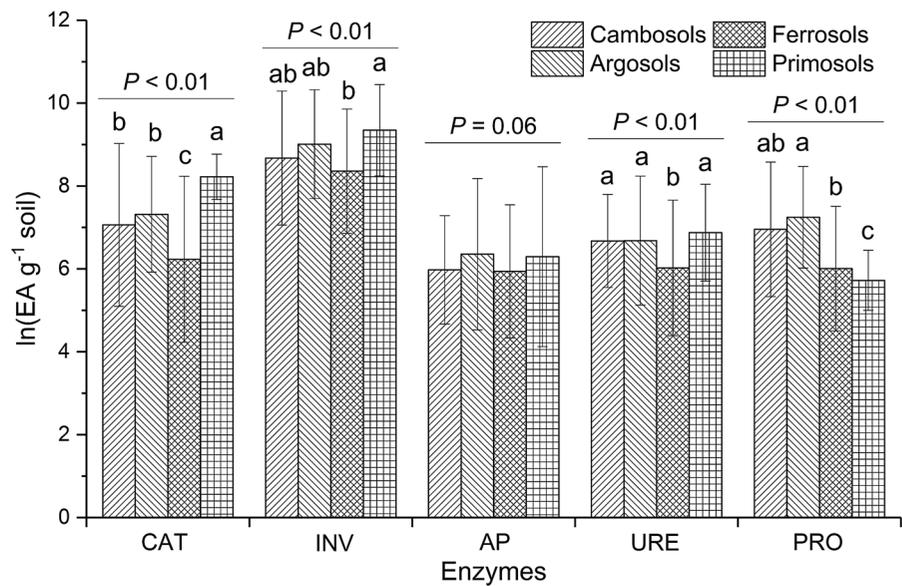
Relationships between EAs and edaphic factors

We found significant differences among soil orders with regard to the activities of CAT (ANOVA, $F = 15.05$, $df = 3$, $P < 0.01$), INV (ANOVA, $F = 4.25$, $df = 3$, $P < 0.01$), URE (ANOVA, $F = 5.53$, $df = 3$, $P < 0.01$), and PRO (ANOVA, $F = 9.84$, $df = 3$, $P < 0.01$) (Fig. 3). In general, CAT activity was higher in primosols, cambisols, and argosols than in ferrosols (Dunnett's T3 test, all $P < 0.05$, effect size: 17.5, 13.32, and 31.9%, respectively), as was URE activity (Dunnett's T3 test, all $P < 0.05$, effect size: 11.0, 10.8, and 14.3%, respectively) (Fig. 3 and Table S1). CAT activity was higher in primosols than in argosols (Fig. 3 and Table S1, Dunnett's T3 test, $P < 0.05$, 12.3%), whereas PRO activity was higher in argosols

than in primosols (Fig. 3 and Table S1, Dunnett's T3 test, $P < 0.05$, 26.8%). Conversely, AP activity did not differ significantly across the different soil orders examined in this study (Fig. 3 and Table S1, ANOVA, $F = 1.74$, $df = 3$, $P > 0.05$).

Regarding the spatial distribution of EAs in the soil profile, soil EAs generally decreased with increasing soil depth (Fig. 4, linear regression, CAT versus soil depth: $n = 398$, $R^2_{\text{adj}} = 0.04$, $F = 14.88$, $P < 0.001$; INV versus soil depth: $n = 179$, $R^2_{\text{adj}} = 0.10$, $F = 34.46$, $P < 0.001$; URE versus soil depth: $n = 398$, $R^2_{\text{adj}} = 0.04$, $F = 19.82$, $P < 0.001$; cubic regression, POX versus soil depth: $n = 398$, $R^2_{\text{adj}} = 0.14$, $F = 9.52$, $P < 0.001$; AP versus soil depth: $n = 325$, $R^2_{\text{adj}} = 0.15$, $F = 39.33$, $P < 0.001$), with the exception of PRO activity, which did not vary significantly with soil depth. PRO activity was negatively correlated with soil pH (Fig. 4, linear regression, $n = 133$, $R^2_{\text{adj}} = 0.24$, $F = 42.24$, $P < 0.001$); in contrast, CAT and POX activities were positively correlated with soil pH (Fig. 4, linear regression, CAT versus soil pH: $n = 285$, $R^2_{\text{adj}} = 0.09$, $F = 26.54$, $P < 0.001$; POX versus soil pH: $n = 171$, $R^2_{\text{adj}} = 0.02$, $F = 4.66$, $P < 0.05$). AP activity first increased and then decreased with rising soil pH (Fig. 4, quadratic regression, $n = 283$, $R^2_{\text{adj}} = 0.04$, $F = 6.95$, $P < 0.01$), whereas URE activity showed the opposite response (Fig. 4, quadratic regression,

Fig. 3 Values of $\ln(\text{EA g}^{-1} \text{ soil})$ for different soil orders. The bars indicate standard errors. Different letters indicate significant differences between different soil orders derived from Dunnett's T3 test. POX activity was not included due to insufficient data. The enzyme abbreviations are provided in Table 1



$n = 346$, $R_{\text{adj}}^2 = 0.09$, $F = 16.83$, $P < 0.001$). CAT, POX, URE, and PRO activities were all positively correlated with SOM (Fig. 4, linear regression, CAT versus SOM: $n = 232$, $R_{\text{adj}}^2 = 0.03$, $F = 8.70$, $P < 0.001$; POX versus SOM: $n = 124$, $R_{\text{adj}}^2 = 0.12$, $F = 18.29$, $P < 0.001$; URE versus SOM: $n = 272$, $R_{\text{adj}}^2 = 0.09$, $F = 26.44$, $P < 0.001$; PRO versus SOM: $n = 108$, $R_{\text{adj}}^2 = 0.09$, $F = 12.04$, $P < 0.01$).

Relationships between EAs and MAT, MAP, and elevation

POX and URE activities were negatively correlated with MAT (Fig. 5, linear regression, POX versus MAT: $n = 162$, $R_{\text{adj}}^2 = 0.09$, $F = 16.08$, $P < 0.001$; URE versus MAT: $n = 456$, $R_{\text{adj}}^2 = 0.07$, $F = 34.58$, $P < 0.001$). However, CAT, INV, and PRO activities first decreased, increased, and then decreased again with rising MAT (Fig. 5, cubic regression, CAT versus MAT: $n = 369$, $R_{\text{adj}}^2 = 0.13$, $F = 19.83$, $P < 0.001$; INV versus MAT: $n = 296$, $R_{\text{adj}}^2 = 0.08$, $F = 9.72$, $P < 0.001$; PRO versus MAT: $n = 159$, $R_{\text{adj}}^2 = 0.27$, $F = 20.68$, $P < 0.001$). Although PRO activity was slightly positively correlated with MAP (Fig. 5, linear regression, $n = 152$, $R_{\text{adj}}^2 = 0.03$, $F = 5.79$, $P < 0.05$), CAT, POX, and URE activities were all negatively related to MAP (Fig. 5, linear

regression, CAT versus MAP: $n = 378$, $R_{\text{adj}}^2 = 0.01$, $F = 5.41$, $P < 0.05$; POX versus MAP: $n = 179$, $R_{\text{adj}}^2 = 0.19$, $F = 42.28$, $P < 0.001$; URE versus MAP: $n = 446$, $R_{\text{adj}}^2 = 0.03$, $F = 13.46$, $P < 0.05$). CAT, INV, AP, URE, and PRO activities were all first enhanced and then reduced with increasing elevation (Fig. 5, quadratic regression, CAT versus elevation: $n = 398$, $R_{\text{adj}}^2 = 0.25$, $F = 66.47$, $P < 0.001$; INV versus elevation: $n = 322$, $R_{\text{adj}}^2 = 0.05$, $F = 8.63$, $P < 0.001$; AP versus elevation: $n = 371$, $R_{\text{adj}}^2 = 0.06$, $F = 13.49$, $P < 0.001$; URE versus elevation: $n = 465$, $R_{\text{adj}}^2 = 0.15$, $F = 42.63$, $P < 0.001$; PRO versus elevation: $n = 169$, $R_{\text{adj}}^2 = 0.03$, $F = 3.82$, $P < 0.05$).

Principal component analysis

PCA of the data reduced the six evaluated environmental variables (elevation, MAT, MAP, soil depth, SOM, and soil pH) to two principal components (F1 and F2), for which the cumulative contribution to the observed variance reached 67.4% (Table 3). The first principal component (F1) generally reflected climate conditions, including MAT, MAP, elevation, and soil pH (variance contribution percentage of 45.2%); the second principal component (F2) reflected soil conditions, including soil depth and SOM (variance contribution percentage of 22.4%) (Table 3).

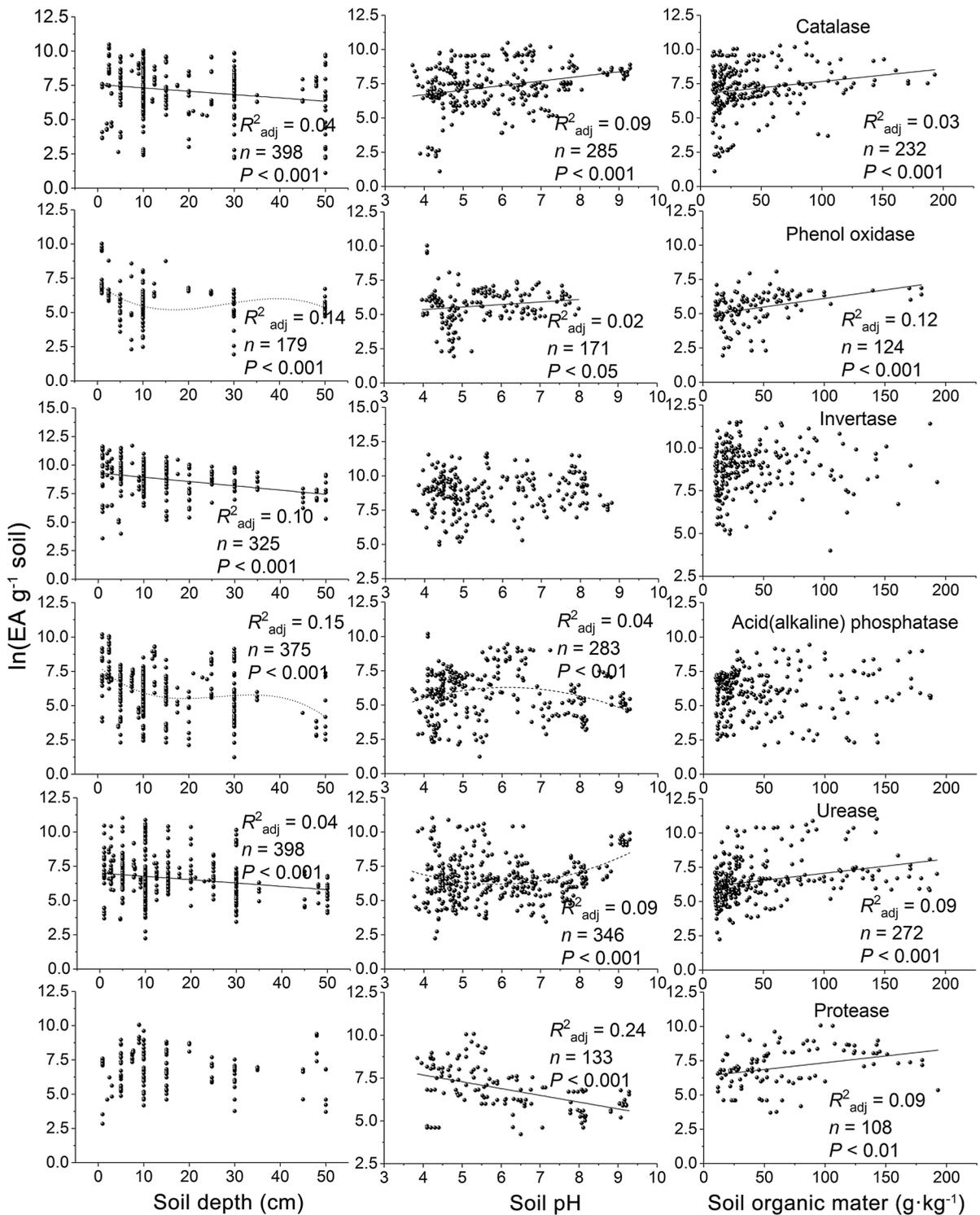


Fig. 4 Relationships of soil enzyme activities with soil depth, soil pH, and soil organic matter. The lines indicate enzymes with statistically significant relationships ($P < 0.05$, Confidence

interval 95%). The solid lines, dashed lines, and dotted lines represent linear, quadratic, and cubic regression fits, respectively

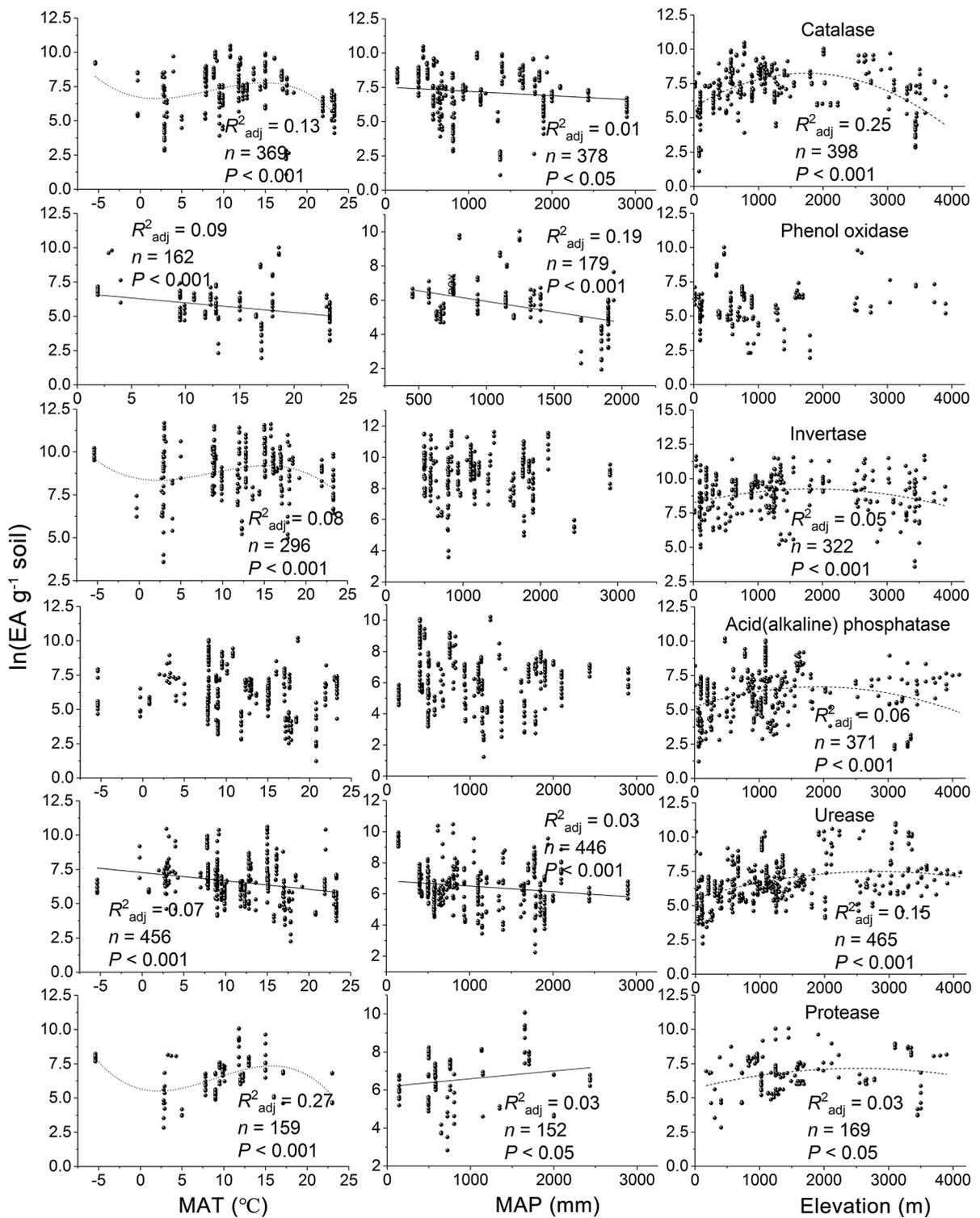


Fig. 5 Relationships of soil enzyme activities with MAT, MAP, and elevation. The lines indicate enzymes with statistically significant relationships ($P < 0.05$, Confidence interval

95%). The solid lines, dashed lines and dotted lines represent linear, quadratic and cubic regression fits, respectively

Table 3 Rotating component matrix and characteristic root of PCA of 6 environmental variables

	MAT	MAP	Elevation	Soil pH	Soil depth	SOM	CR	VCA %	CVCA %
F1	– 0.88	– 0.80	0.72	0.63	– 0.18	0.58	2.71	45.2	45.2
F2	0.04	0.41	0.37	– 0.58	– 0.54	0.65	1.34	22.4	67.5

Bold values indicate the variable with the larger loading on F1/F2. Factor analysis resulted in two common factors with a general variance of 67.4%. Each factor had a strong loading on the corresponding items

CR characteristic root, VCA variance contribution rate, CVCA cumulative variance contribution rate

Table 4 Pearson correlations of $\ln(\text{EA g}^{-1} \text{ soil})$ and F1/F2

EA	CAT		POX		INV		AP		URE		PRO	
	R	N	R	N	R	N	R	N	R	N	R	N
F1	0.36**	170	0.40**	107	0.25**	157	0.30**	177	0.25**	224	– 0.08	81
F2	0.26**	170	– 0.28**	107	– 0.10	157	0.25**	177	0.20**	224	0.16	81

R Pearson correlation coefficient. EA enzyme activity. The enzyme abbreviations are provided in Table 1

*And **indicate significance at the 0.05 and 0.01 levels, respectively

Correlation analyses of F1/F2 and EAs revealed higher correlation coefficients for CAT, POX, INV, AP, and URE activities with F1 than with F2. These results indicate that MAT, MAP, elevation, and soil pH had a greater combined effect on CAT, POX, INV, AP, and URE activities than did soil depth and SOM (Table 4).

Discussion

Forest type

Forest type is an important factor that influences variations in soil EAs as well as in C, N, and P cycling and stocks (Butterbach-Bahl and Gundersen 2011; Zhang et al. 2016). Our results showed 29.6% higher catalase activity in mixed forest than in pure forest (Fig. 2), a result that was consistent with the findings of several previous studies showing that mixed forests have higher soil fertility and EA than pure forests (Cun-Wang et al. 2007; Hou and Ma 2007; Dong 2014). Compared with pure forests, the soil of mixed forests exhibits higher total N, available N, total P, available K, and organic matter contents. Moreover, mixed forests also have a 95.9% higher total abundance of soil microorganisms and 104.5% higher bacterial numbers than pure forests (Fu et al. 2009; Yu et al. 2015).

We also found that phenol oxidase activity was 14.9% higher in deciduous broad-leaved forests than in evergreen broad-leaved forests (Fig. 2). Compared with evergreens, deciduous trees have shorter leaf lifespans, lower leaf masses per area, and higher lignin and cellulose contents (Ishida et al. 2006). Therefore, microorganisms in deciduous forests allocate more resources to ligninolytic enzyme production and improve soil fertility to meet the demands of plant and microbe growth (Sardans and Peñuelas 2013). These factors may contribute to the high phenol oxidase activity observed in deciduous broad-leaved forests.

Compared with coniferous forests, evergreen forests exhibit higher total P and available P concentrations (Burton et al. 2007; Huang et al. 2011), which can increase AP activities. This difference might explain the 24.3% higher AP activity observed in deciduous coniferous forest than in evergreen forest in this study (Fig. 2). We also found that protease activity was 23.5% lower in shrublands than in forest (Fig. 2), a difference that is likely due to the higher abundance of N-containing substrates (simple amino acids, chitin, chlorophyll, and nucleic acids) in forests compared with shrublands (Rineau et al. 2015). Differences in EAs among different forest types ranged from 14.9 to 29.6%, and these significant differences indicate that forest type has a close association with soil EA.

Edaphic conditions

We found that catalase and urease activities were overall higher in argosols, cambisols, and primosols than in ferrosols (Fig. 3). Such variations in EA with soil order are associated to some degree with the geology of the parent material, the SOM concentration, and the soil pH (Acosta-Martínez et al. 2007). For example, the availability of soil nutrients is consistently lower in ferrosols than in other soil orders due to the thick soil solum and the large amount of litter input. Argosols also showed 26.8% higher protease activity than primosols (Fig. 3), most likely because of the more advanced weathering stage of argosols, resulting in finer textures and a higher SOM content. In addition to geology and soil texture, differences in organic C content also contribute to variations in EA across different soil orders (Acosta-Martínez et al. 2007; Cruz-Rodríguez 2009).

Our results revealed a decreasing trend in EA with increasing soil depth (Fig. 4), consistent with previous studies (Hong et al. 2013; Chen et al. 2014; Stone et al. 2014). SOM strongly affects soil fertility and the physical, chemical, and biological properties of soil. Furthermore, increased litter production and rapid SOM decomposition correspond to an increase in microbial biomass and activity (Vaheri and Timpl 2006). In the present study, catalase, phenol oxidase, urease, and protease activities were positively correlated with SOM (Fig. 4), which was consistent with previous studies demonstrating that EA increases with rising levels of organic residues (Graham and Haynes 2005; Cui et al. 2011; Jin et al. 2011). Therefore, the distribution of SOM and microbial activity might explain changes in EA in the soil profile. For example, Guan et al. (2013) found that the activities of five non-ligninolytic enzymes (α -1,4-glucosidase, β -1,4-glucosidase, N-acetyl- β -glucosaminidase, β -D-cellobiosidase, and β -xylosidase) decreased with increasing soil depth, though the activities of two ligninolytic enzymes (peroxidase and phenol oxidase) did not follow this trend. In accordance with these findings, our results showed very little variation in phenol oxidase activity with soil depth (Fig. 4), which might reflect the higher lignin concentrations in deeper soil layers (Thomas et al. 2013). Soil pH affects SOM decomposition, mineral dissolution, colloid aggregation and dispersion, and microbial activity intensity and directly affects the biochemical reaction rates of

soil enzymes (Guan 1986; Tabatabai 1994). Enzymes act on a narrow range of substrates, and their optimal pH is largely defined by their reaction mechanisms (Sinsabaugh 2010). Soil pH is often assumed to affect soil EAs by altering the ionic form of enzyme active sites, thereby impacting substrate affinity (Shuler and Kargı 1992). In the present study, protease activity decreased significantly as soil pH increased (Fig. 4), consistent with the results of a previous study (Chen et al. 2014). We also found that catalase and phenol oxidase activities were higher with increasing soil pH (Fig. 4), which was in agreement with other reports (Sinsabaugh et al. 2008; Błońska 2010; Yang et al. 2013).

Geographic and climatic factors

Our analysis showed that both MAT and MAP affected the distribution patterns of soil EAs, though the temperature sensitivities of the different soil enzymes did vary. Regression analysis indicated that EA generally decreased with increasing MAT (Fig. 5). A specific range of high temperatures accelerates soil biochemical processes related to C and N cycling and suppresses soil biochemical processes related to P. Within a wider range of high MATs, biochemical processes associated with C might also be suppressed (Xiong et al. 2004), and higher MAT might affect EAs in a similar manner. Nonetheless, we also observed higher activities of certain enzymes (catalase, invertase, and protease) with increasing MAT within the range of 2.5–17.5 °C (Fig. 5). Because most soil enzymes are bioactive proteins, these increases may have been caused by higher catalytic activity with increasing environmental temperature of certain magnitudes. We found that catalase, phenol oxidase, and urease activities decreased with increasing MAP (Fig. 5), yet most controlled experiments have revealed that precipitation has little effect on soil EAs (McDaniel et al. 2013; Steinweg et al. 2013; Khalili et al. 2016). We believe that heavy rain may decrease EAs due to the leaching of soluble nutrients (Mackowiak 2014), whereas a small amount of precipitation may stimulate hydrolytic activity (Nadeau 2006; Ladwig et al. 2015).

Temperature and associated climatic factors change in a predictable manner with increasing elevation; because they are correlated with these abiotic factors, ecosystem properties and processes can also be

influenced by elevation (Vincent et al. 2014). The present study revealed that the activities of all enzymes, except for phenol oxidase, first increased (< 2000 m.a.s.l.) and then decreased (2000–4100 m.a.s.l.) with increasing elevation (Fig. 5). N availability decreases with increasing elevation, even though the microbial biomass and concentration of phospholipid-derived fatty acids (PLFAs) from fungi and bacteria rise with increasing elevation (Nottingham et al. 2015; Xu et al. 2015; He et al. 2016). Such patterns are due to the greater metabolic effort of microbes at higher elevations than at lower elevations to obtain N during litter lignin degradation or humification, which likely results in higher urease and protease activities with increasing elevation (< 2000 m.a.s.l.) (Fig. 5). However, as elevation increases, the likelihood of extreme weather and the duration and depth of snow cover also increase, and these changes likely reduce the microbial litter decomposition rate and enhance nutrient limitations for microbes (Margalef et al. 2017). Due to these biophysical constraints, microorganisms may be starved of nutrients and therefore allocate fewer resources to enzyme production. This possibility is in line with our finding that EAs decrease with increasing elevation within the range of 2000–4100 m.a.s.l. (Fig. 5). In the present study, R-square for some regressions was relatively low, albeit statistically significant. This might be because the variations in some soil EAs can be better explained by multiple factors or by other factors not analysed in this study.

Main factors influencing soil EAs

The combined effects of certain environmental factors may have a large impact on soil EAs. PCA demonstrated climate conditions and soil pH to be correlated with the activity of most of the enzymes assessed, i.e. catalase, phenol oxidase, invertase acid (alkaline) phosphatase, and urease (Table 4). Climate can greatly determine soil EA due to the sensitivity of soil biological processes to factors such as temperature and precipitation, and areas with harsh climatic conditions exhibit lower litter input, lower decomposability, and reduced numbers of microbes and EAs (Sardans et al. 2006). Previous studies of various types of biomes have reported increased plant biomass and microbial activity at higher temperatures or decreased

EA with warming trends, which is consistent with our results. Precipitation largely determines the water availability of soil, and our analyses showed that the activities of some enzymes (catalase, phenol oxidase, and urease) decreased with increasing MAP, consistent with previous studies reporting that water-saturated and flooded areas (swamps and flooded forests) have lower EAs than dryer areas (Rivas et al. 2007). However, a lack of water can also result in extremely low levels of microbial biomass and EA (Sardans et al. 2006). Soil pH has strong effects on the structure and diversity of soil bacterial communities, and a suitable pH can benefit microbial growth. Indeed, a number of studies have identified soil pH as a primary variable affecting soil microbial function and/or structure (Hackl et al. 2005; Högberg et al. 2007; Schneider et al. 2012). Our results suggest that climate conditions and soil pH are the primary factors affecting soil EA.

Conclusions

Our findings demonstrate that catalase, phenol oxidase, acid (alkaline) phosphatase, and protease activities differ significantly among different types of forests. Catalase and urease activities were generally higher in primosols, cambisols, and argosols than in ferrosols, and most EAs decreased with soil depth and increased with SOM. Phenol oxidase and urease activities were negatively correlated with mean annual temperature (MAT); in contrast, catalase, invertase, and protease activities first decreased (< 2.5 °C), increased (2.5–17.5 °C), and then decreased (> 17.5 °C) with increasing MAT. Although protease activity was slightly positively correlated with MAP, activities of catalase, phenol oxidase, and urease were all negatively related to MAP. Catalase, invertase, acid (alkaline) phosphatase, urease, and protease activities first increased (< 2000 m.a.s.l.) and then decreased (2000–4100 m.a.s.l.) with increasing elevation. PCA revealed most EAs to be affected by climate conditions and soil pH. These findings suggest that climatic and edaphic variables directly and indirectly correlate with forest type and greatly impact soil EA.

This study is the first to comprehensively analyse soil EA patterns in China's forest ecosystems. Although this study identified some soil geochemical

conditions that are empirically associated with soil EA, the complexity and importance of this issue merit a further combination of experiments using global natural soils. These findings may contribute to our understanding of how plant–microbe interactions are associated with ecosystem processes and the mechanism of the coupling of C, N, and P cycles.

Acknowledgements This study was financially supported by the National Natural Science Foundation of China (31570605). We thank the two anonymous reviewers for their very constructive feedback, which has contributed to a greatly improved manuscript. We also want to thank the guest editorial team for their very valuable feedback and guidance.

References

- Acosta-Martínez V, Tabatabai MA (2000) Enzyme activities in a limed agricultural soil. *Biol Fertil Soils* 31:85–91. <https://doi.org/10.1007/s003740050628>
- Acosta-Martínez V, Cruz L, Sotomayor-Ramírez D, Pérez-Alegría L (2007) Enzyme activities as affected by soil properties and land use in a tropical watershed. *Appl Soil Ecol* 35:35–45. <https://doi.org/10.1016/j.apsoil.2006.05.012>
- Adamczyk B, Kitunen V, Smolander A (2009) Polyphenol oxidase, tannase and proteolytic activity in relation to tannin concentration in the soil organic horizon under silver birch and Norway spruce. *Soil Biol Biochem* 41:2085–2093. <https://doi.org/10.1016/j.soilbio.2009.07.018>
- Adamczyk B, Kilpeläinen P, Kitunen V, Smolander A (2014) Potential activities of enzymes involved in N, C, P and S cycling in boreal forest soil under different tree species. *Pedobiologia* 57:97–102. <https://doi.org/10.1016/j.pedobi.2013.12.003>
- Allison SD, McGuire KL, Treseder KK (2010) Resistance of microbial and soil properties to warming treatment seven years after boreal fire. *Soil Biol Biochem* 42:1872–1878. <https://doi.org/10.1016/j.soilbio.2010.07.011>
- Błońska E (2010) Enzyme activity in forest peat soils. *Folia Forestalia Polonica Seria A Forestry* 52:20–25
- Brockett BFT, Prescott CE, Grayston SJ (2012) Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. *Soil Biol Biochem* 44:9–20
- Burns RG, Dick RP (2002) *Enzymes in the environment: activity, ecology, and applications*. CRC Press, Boca Raton
- Burns RG, Deforest JL, Marxsen J, Sinsabaugh RL, Stromberger ME, Wallenstein MD, Weintraub MN, Zoppini A (2013) Soil enzymes in a changing environment: current knowledge and future directions. *Soil Biol Biochem* 58:216–234
- Burton J, Chen C, Xu Z, Ghadiri H (2007) Soluble organic nitrogen pools in adjacent native and plantation forests of subtropical Australia. *Soil Biol Biochem* 39:2723–2734
- Butterbach-Bahl K, Gundersen P (2011) Nitrogen processes in terrestrial ecosystems. In: Sutton MA, Howard CM, Erisman JW, Billen G, Bleeker A, Grennfelt P, van Grisven H, Grizzetti B (eds) *The European nitrogen assessment: sources, effects and policy perspectives*. Cambridge University Press, Cambridge, pp 99–125
- Chen ZF, Liu JF, Wu ZY (2014) Soil physico-chemical properties and enzyme activities at different elevation gradient forest type of Daiyun Mountain. *J Henan Inst Sci Technol (Nat Sci Ed)* 42:10–14
- Cruz-Rodríguez L (2009) *Soil organic carbon and nitrogen distribution in a tropical watershed*. University of Puerto Rico, Masters of Science
- Cui XX, Wang JJ, Luo HN, Yu YC, Liang GF, Zhou CF, Wang Z, Zhang JC (2011) Variation of soil enzyme activities under different vegetation restoration stages in Karst gorge district. *J Nanjing For Univ (Nat Sci Ed)* 2011:103–107
- Cun-Wang YE, Zhai QR, Guo ZJ, Song XD, Zhao HG, Yan-Hui YE (2007) Soil nutrient, microorganism and enzyme activity of Hippophae rhamnoides and Platycladus orientalis mixed forests. *J Northwest For Univ* 22:1
- Cusack DF, Torn MS, McDowell WH, Silver WL (2010) The response of heterotrophic activity and carbon cycling to nitrogen additions and warming in two tropical soils. *Glob Change Biol* 16:2555–2572
- Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* 440:165–173
- Dong LL (2014) Characteristics of soil enzyme in artificially-established forest of Zhifanggou valley in north of Shaanxi. *Pratacultural Sci* 31:22–29
- Drenovsky RE, Vo D, Graham KJ, Scow KM (2004) Soil water content and organic carbon availability are major determinants of soil microbial community composition. *Microbial Ecol* 48:424–430
- Eivazi F, Tabatabai MA (1990) Factors affecting glucosidase and galactosidase activities in soils. *Soil Biol Biochem* 22:891–897
- Fu G, Liu Z, Cui F (2009) Features of soil enzyme activities and the number of microorganisms in plantations and their relationships with soil nutrients in the Qinling Mountains, China. *Front For China* 4:344–350
- Gong ZT, Zhang GL, Chen CZ (2007) *Pedogenesis and soil taxonomy*. Science Press, Beijing
- Graham MH, Haynes RJ (2005) Organic matter accumulation and fertilizer-induced acidification interact to affect soil microbial and enzyme activity on a long-term sugarcane management experiment. *Biol Fertil Soils* 41:249–256
- Guan SY (1986) *Soil enzymes and its methodology*. Agricultural Press, Beijing
- Guan ZJ, Luo Q, Chen X, Feng XW, Tang ZX, Wei W, Zheng YR (2013) Saline soil enzyme activities of four plant communities in Sangong River basin of Xinjiang, China. *J Arid Land* 6:164–173. <https://doi.org/10.1007/s40333-013-0223-6>
- Hackl E, Pfeffer M, Donat C, Bachmann G, Zechmeister-Boltenstern S (2005) Composition of the microbial communities in the mineral soil under different types of natural forest. *Soil Biol Biochem* 37:661–671. <https://doi.org/10.1016/j.soilbio.2004.08.023>

- He X, Hou E, Yang L, Wen D (2016) Altitudinal patterns and controls of plant and soil nutrient concentrations and stoichiometry in subtropical China. *Sci Rep* 6:24261
- Hill BH, Elonen CM, Jicha TM, Kolka RK, Lehto LL, Sebestyen SD, Seifert-Monson LR (2014) Ecoenzymatic stoichiometry and microbial processing of organic matter in northern bogs and fens reveals a common P-limitation between peatland types. *Biogeochemistry* 120:203–224
- Högberg MN, Högberg P, Myrold DD (2007) Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? *Oecologia* 150:590–601
- Hong CQ, Wu JB, Wang XD (2013) Effects of global climate change on the C, N, and P stoichiometry of terrestrial plants. *Chin J Appl Ecol* 24:2658–2665
- Hou BD, Ma FY (2007) Effect of the mixed stands of black locust with other tree species on soil chemical property and soil enzyme activity. *J Shandong Agric Univ (Nat Sci)* 38:53–57
- Hu Z, Li S, Guo Q, Niu S, He N, Li L, Yu G (2016) A synthesis of the effect of grazing exclusion on carbon dynamics in grasslands in China. *Glob Change Biol* 22:1385
- Huang W, Liu J, Zhou G, Zhang D, Deng Q (2011) Effects of precipitation on soil acid phosphatase activity in three successional forests in southern China. *Biogeosciences* 8:1901–1910. <https://doi.org/10.5194/bg-8-1901-2011>
- Ishida A, Diloksumpun S, Ladpala P, Staporn D, Panuthai S, Gamo M, Yazaki K, Ishizuka M, Puangchit L (2006) Contrasting seasonal leaf habits of canopy trees between tropical dry-deciduous and evergreen forest in Thailand. *Tree Physiol* 26:643–656
- Jin YH, Wang JS, Li LG, Ruan HH, Xu G, Han LY (2011) Soil enzyme activities in typical vegetation zones along an altitude gradient in Wuyi Mountains. *Chin J Ecol* 9:1955–1961
- Kardol P, Cregger MA, Company CE, Classen AT (2010) Soil ecosystem functioning under climate change: plant species and community effects. *Ecology* 91:767–781
- Khalili B, Ogunseitan OA, Goulden ML, Allison SD (2016) Interactive effects of precipitation manipulation and nitrogen addition on soil properties in California grassland and shrubland. *Appl Soil Ecol* 107:144–153
- Koricheva J, Gurevitch J, Gómez-Aparicio L (2014) Uses and misuses of meta-analysis in plant ecology. *J Ecol* 102:828–844
- Ladwig LM, Sinsabaugh RL, Collins SL, Thomey ML (2015) Soil enzyme responses to varying rainfall regimes in Chihuahuan Desert soils. *Ecosphere* 6:1–10. <https://doi.org/10.1890/es14-00258.1>
- Lanzen A, Epelde L, Blanco F, Martin I, Artetxe U, Garbisu C (2016) Multi-targeted metagenetic analysis of the influence of climate and environmental parameters on soil microbial communities along an elevational gradient. *Sci Rep* 6:28257. <https://doi.org/10.1038/srep28257>
- Lebauer DS, Treseder KK (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* 89:371–379
- Mackowiak C (2014) Heavy rain causing nitrogen leaching in Florida. Southeast Farm Press. <https://southeastfarmpress.com/management/heavy-rain-causing-nitrogen-leaching-florida>
- Margalef O, Sardans J, Fernándezmartínez M, Molownyhoras R, Janssens IA, Ciais P, Goll D, Richter A, Obersteiner M, Asensio D (2017) Global patterns of phosphatase activity in natural soils. *Sci Rep* 7:1337
- McDaniel MD, Kaye JP, Kaye MW (2013) Increased temperature and precipitation had limited effects on soil extracellular enzyme activities in a post-harvest forest. *Soil Biol Biochem* 56:90–98. <https://doi.org/10.1016/j.soilbio.2012.02.026>
- Nadeau JA (2006) The effects of short intense precipitation events on activities of enzymes involved in mineralization and potential bioavailability of organic carbon, nitrogen, and phosphorous in the Mojave Desert. Dissertation, University of Michigan, Ann Arbor
- Nottingham AT, Turner BL, Whitaker J, Ostle NJ, McNamara NP, Bardgett RD, Salinas N, Meir P (2015) Soil microbial nutrient constraints along a tropical forest elevation gradient: a belowground test of a biogeochemical paradigm. *Biogeosciences* 12:6071–6083. <https://doi.org/10.5194/bg-12-6071-2015>
- Perry DA, Oren R, Hart SC (2008) Forest ecosystems. JHU Press, Baltimore
- Puissant J, Cécillon L, Mills RTE, Gavazov K, Robroek BJM, Spiegelberger T, Buttler A, Brun J-J (2013) Climate effect on soil enzyme activities and dissolved organic carbon in mountain calcareous soils: a soil-transplant experiment European geosciences union general assembly conference. EGU, Vienna, p 10463
- Rineau F, Stas J, Nguyen NH, Kuyper TW, Carleer R, Vangronsveld J, Colpaert JV, Kennedy PG (2015) Soil organic nitrogen availability predicts ectomycorrhizal fungal protein degradation ability. *Appl Environ Microbiol* 82:1–23
- Rivas Y, Godoy R, Valenzuela E, Leiva J, Oyarzún C, Alvear M (2007) Actividad biológica del suelo en dos bosques de *Nothofagus* del centro sur de Chile. *Gayana Botánica* 64:81–92
- Sardans J, Peñuelas J (2013) Plant-soil interactions in Mediterranean forest and shrublands: impacts of climatic change. *Plant Soil* 365:1–33. <https://doi.org/10.1007/s11104-013-1591-6>
- Sardans J, Peñuelas J, Estiarte M (2006) Warming and drought alter soil phosphatase activity and soil P availability in a Mediterranean shrubland. *Plant Soil* 289:227–238
- Schneider T, Keiblinger KM, Schmid E, Sterflinger-Gleixner K, Ellersdorfer G, Roschitzki B, Richter A, Eberl L, Zechmeister-Boltenstern S, Riedel K (2012) Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. *ISME J* 6:1749–1762. <https://doi.org/10.1038/ismej.2012.11>
- Shuler ML, Kargi F (1992) Bioprocess engineering: basic concepts. Prentice Hall, Englewood Cliffs
- Sinsabaugh RL (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biol Biochem* 42:391–404. <https://doi.org/10.1016/j.soilbio.2009.10.014>
- Sinsabaugh RL, Follstad Shah JJ (2012) Ecoenzymatic stoichiometry and ecological theory. *Annu Rev Ecol Evol Syst* 43:313–343
- Sinsabaugh RL, Lauber CL, Weintraub MN (2008) Stoichiometry of soil enzyme activity at global scale. *Ecol Lett* 11:1252–1264. <https://doi.org/10.1111/j.1461-0248.2008.01245.x>

- Sinsabaugh RL, Belnap J, Findlay SG, Shah JFF, Hill BH, Kuehn KA, Kuske CR, Litvak ME, Martinez NG, Moorhead DL, Warnock DD (2014) Extracellular enzyme kinetics scale with resource availability. *Biogeochemistry* 121:287–304. <https://doi.org/10.1007/s10533-014-0030-y>
- Steinweg JM, Dukes JS, Paul EA, Wallenstein MD (2013) Microbial responses to multi-factor climate change: effects on soil enzymes. *Front Microbiol* 4:146. <https://doi.org/10.3389/fmicb.2013.00146>
- Stone MM, DeForest JL, Plante AF (2014) Changes in extracellular enzyme activity and microbial community structure with soil depth at the Luquillo Critical Zone Observatory. *Soil Biol Biochem* 75:237–247. <https://doi.org/10.1016/j.soilbio.2014.04.017>
- Suseela V, Conant RT, Wallenstein MD, Dukes JS (2012) Effects of soil moisture on the temperature sensitivity of heterotrophic respiration vary seasonally in an old-field climate change experiment. *Glob Change Biol* 18:336–348. <https://doi.org/10.1111/j.1365-2486.2011.02516.x>
- Tabatabai MA (1994) Soil enzymes. In: Weaver RW, Angle S, Bottomley P, Bezdicek D, Smith S, Tabatabai A, Wollum A (eds) *Methods of soil analysis, part 2 Microbiological and chemical properties*. Soil Science Society of America, Madison, pp 775–833
- Thomas FM, Molitor F, Werner W (2013) Lignin and cellulose concentrations in roots of Douglas fir and European beech of different diameter classes and soil depths. *Trees* 28:309–315
- Vaheri A, Timpl R (2006) Soil microbial communities and enzyme activities under various poultry litter application rates. *J Environ Q* 35:1309–1318
- Vincent AG, Sundqvist MK, Wardle DA, Giesler R (2014) Bioavailable soil phosphorus decreases with increasing elevation in a subarctic tundra landscape. *PLoS ONE* 9:e92942
- Williams CJ, Scott AB, Wilson HF, Xenopoulos MA (2012) Effects of land use on water column bacterial activity and enzyme stoichiometry in stream ecosystems. *Aquat Sci* 74:483–494
- Xiong HZ, Wang KY, Yang WQ (2004) Seasonal variations of soil enzyme activities in fir and birch forests in subalpine area of western Sichuan. *Chin J Appl Environ Biol* 10:416–420
- Xu G, Chen J, Berninger F, Pumpanen J, Bai J, Yu L, Duan B (2015) Labile, recalcitrant, microbial carbon and nitrogen and the microbial community composition at two *Abies faxoniana* forest elevations under elevated temperatures. *Soil Biol Biochem* 91:1–13
- Yang N, Zou DS, Yang MY, Chen J, Chen ZY, Lin ZG, Song GT (2013) Soil enzyme activities in different re-vegetation stages on sloping-land with purple soils in Hengyang of Hunan Province, China. *J Plant Nutr Fertil* 19:1516–1524
- You C, Wu F, Gan Y, Yang W, Hu Z, Xu Z, Tan B, Liu L, Ni X (2017) Grass and forbs respond differently to nitrogen addition: a meta-analysis of global grassland ecosystems. *Sci Rep* 7:1563
- Yu X, Liu X, Zhao Z, Liu J, Zhang S (2015) Effect of monospecific and mixed sea-buckthorn (*Hippophae rhamnoides*) plantations on the structure and activity of soil microbial communities. *PLoS ONE* 10:e0117505. <https://doi.org/10.1371/journal.pone.0117505>
- Zhang H, Zeng Q, An S, Dong Y, Darboux F (2016) Soil carbon fractions and enzyme activities under different vegetation types on the Loess Plateau of China. *Solid Earth Discuss* 2016:1–27. <https://doi.org/10.5194/se-2016-137>