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Effects of *Amaranthus palmeri* Invasion on Soil Extracellular Enzyme Activities and Enzymatic Stoichiometry

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Abstract

Soil extracellular enzyme activities (EEAs) and enzymatic stoichiometry (ES) play essential roles in plant invasion processes. Nevertheless, how soil EEAs and ES respond to *Amaranthus palmeri* invasion and what their driving mechanisms remain poorly understood. We compared 22 pairs of *A. palmeri*–invaded plots and native vegetation plots in North China. Soil physicochemical properties and the potential activities of C-acquiring enzyme (β-1,4-glucosidase, BG), N-acquiring enzyme (β-N-acetyl-glucosaminidase, NAG), and P-acquiring enzyme (alkaline phosphatase, AKP) were determined. Compared with native vegetation soil, invasion by *A. palmeri* significantly altered the soil total C, ammonium nitrogen (N-NH$_4$) content, and C:N ratio. Pearson's correlation and random forest analysis showed that soil EEAs and ES were mostly correlated with soil pH, total N, total C, and available phosphorus (P-PO$_4$). Redundant analysis demonstrated that soil total C was the major factor significantly explaining the variations in soil EEAs and ES of *A. palmeri*. Our findings highlight the effects of invasive species (*A. palmeri*) on soil chemical and biological properties, and enhance the understanding of soil microbial nutrient limitation; they also serve to reveal the mechanism of nutrient cycling and underground ecosystem processes mediated by *A. palmeri* invasion.

Keywords Extracellular enzyme · Enzymatic stoichiometry · Rhizosphere · Invasive plants

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1 Introduction

Biological invasion has been recognized as an important threat to biodiversity, ecology, and the economy (Richardson and Ricciardi 2013; Fang et al. 2021). Invasive exotic plants may become vectors of diseases and even promote species extinction (van der Putten et al. 2007; Vilà et al. 2010). It is expected that in the coming decades, various anthropogenic activities such as global trade and agriculture, will further intensify plant invasion (Stefanowicz et al. 2019). Plant invasion often alters and benefits from microbially mediated root-soil processes (Hawkes et al. 2005; Yin et al. 2020). Root-soil-microbe interaction systems constitute the rhizosphere microenvironment, and soil extracellular enzymes, as the key intermediate medium, are sensitive to changes in the soil environment (Nardi et al. 2000; Hawes et al. 2002). Soil extracellular enzyme activities can reflect the magnitude and direction of the soil biogeochemical cycle, which is a vital biological indicator for evaluating soil fertility, soil quality, and plant invasion (Xiao et al. 2020 Xie et al. 2021; Araujo et al. 2022). Microbes secrete different enzymes, but only a few can explain the majority of measurable activities

because they target the most abundant substrates in the environment (Moorhead et al. 2016). For example, β-1,4-glucosidase, β-1,4-N-acetyl-glucosaminidase, and acid (alkaline) phosphatase, which are linked to carbon, nitrogen, and phosphorus metabolism, have been well researched in recent years (Allison et al. 2010, 2018; Jian et al. 2021). These three enzymes are considered representative indicators of overall C, N, and P acquisition for the following two reasons: (1) the activities of other related enzymes are usually lower than those of the three enzymes and have a strong correlation with them; (2) these three enzymes are not easily denatured and have a long half-life (Moorhead et al. 2016; Jian et al. 2021).

Soil extracellular enzymes involved in C, N, and P cycling have been thought to reflect soil nutrient availability and stability in the process of plant invasion. For instance, invasion by *Falcataria moluccana* considerably improved the activity of acid phosphatase, consistent with phosphorus limitation in the invaded area (Allison et al. 2006). The soil EEAs of acid phosphatase, β-glucosidase, urease, and N-acetyl-glucosaminidase increased significantly with the chronosequence of *Acacia dealbata* invasion (Souza-Alonso 2015). *Pennisetum setaceum* altered the protease activity related to N cycling and the structure and composition of soil bacterial communities, thereby changing the function of invaded ecosystems (Rodríguez-Caballero et al. 2017). *Chenopodium ambrosioides* enhanced soil EEAs and soil microbial biomass at the fruit stage, creating favorable conditions for its reproduction (Wang et al. 2017). Invasion by *Solidago canadensis* (Canada goldenrod) influenced the extracellular enzymatic activities related to C, N, and P cycling, thereby changing the biogeochemical cycle (Hu et al. 2021).

Amaranthus palmeri, native to the Sonoran Desert in North America, has become a serious invasive plant in China. This species is commonly found in wastelands, roadsides, riverbanks, and other habitats. To date, it has been widely spread to various habitats and even economic crop areas, such as farmlands and orchards, seriously damaging the native ecosystem and causing vast economic losses (Ward et al. 2013; Zhang et al. 2022). However, few studies have focused on the impact of *A. palmeri* on the soil ecosystem, and previous studies on the EEAs of invasive plant soils were limited in scale. Here, we investigated soil extracellular enzyme activities and their dominant drivers in 22 major invaded areas of *A. palmeri*. The spatial distribution of extracellular enzymes in rhizosphere soil was analyzed, the impacts of *A. palmeri* invasion on soil extracellular enzyme activities were discussed, and the main driving factors of soil extracellular enzyme activities were explored. Our results provide important knowledge for further clarifying the change in soil nutrient availability and nutrient limitation during *A. palmeri* invasion as well as a theoretical basis for

comprehensively evaluating the effect of plant invasion on ecosystems.

2 Materials and Methods

2.1 Description of the Study Area

We established 22 research sites (38.74°–40.04°N, 116.36°–117.85°E) in areas without obvious human interference in Beijing, Tianjin, and Hebei, China (Fig. 1). The geographic information, environmental properties, habitat types, and accompanying plants of the invasive and native plots in the sampling sites are listed in Table S1 (Supplemental material). A paired plot design was used to explore the differences between invasive and native species by pairing the elevation, slope, and landscape to minimize the differences in environmental properties (McLeod et al. 2016). The study plots included (1) *A. palmeri* invasion patches, where the invasion period of *A. palmeri* exceeded 5 years and its cover exceeded 80%. (2) Native vegetation patches (control) with no occurrence of *A. palmeri*, only native species. The native patches were dominated by *Setaria viridis*, *Chenopodium album*, *Chloris virgata*, and *Digitaria sanguinalis*. The native patches were approximately 2–5 m away from the invasive patches to ensure that factors other than vegetation were relatively consistent. A total of 44 patches were established, which included 22 replicates (sites) × 2 patches (invasive vs. native), and the patch areas ranged from 5 × 5 to 25 × 25 m.
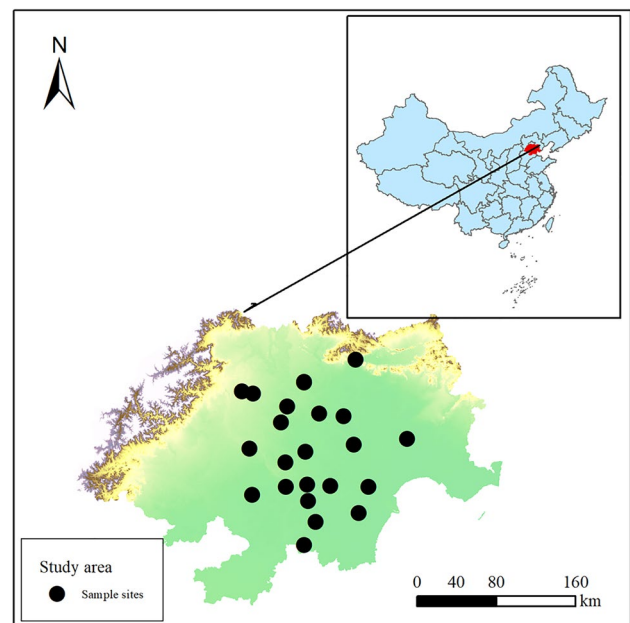

Fig. 1 Location of the study area and sample sites

2.2 Sample Collection

At the end of August 2021, three 1 m^2 plots were set up in each patch. In each plot, three soil samples were collected by pulling plants and shaking roots. Specifically, 8–10 *A. palmeri* plants were harvested in each invasive plot and all dominant plants were harvested in each native plot, and shaken vigorously until ~80% of the soil was removed. The remaining soil that was tightly adhered to the roots was brushed off and collected as the rhizosphere soil (Fan et al. 2017). The soil samples were sieved through 2-mm mesh sieves to remove stones, visible roots, and plant material. Soil samples from the three plots were mixed into one sample, and 44 soil samples were ultimately obtained. The collected soil samples were placed in an ice box and transported to the laboratory. All samples were divided into two subsamples. One subsample was stored in a 4 °C refrigerator for the determination of enzyme activity and soil ammonium/nitrate nitrogen content. The other subsample was air-dried for the determination of physical and chemical properties.

2.3 Soil Physicochemical Properties and Extracellular Enzyme Assays

The soil pH was measured by a pH meter at a water/soil ratio of 2.5:1, and the soil total C and total N content were measured by a Vario MACRO cube elemental analyzer (Elementar, Germany). The contents of nitrate nitrogen (N-NO$_3$) and ammonia nitrogen (N-NH$_4$) were extracted with 200 mL of 2 mol/L potassium chloride (KCl) solution and determined by a colorimetric method. The soil's total P content was analyzed by the H$_2$SO$_4$-HNO$_3$ fusion method. The soil available phosphorus (P-PO$_4$) was determined by molybdenum antimony colorimetry with a spectrophotometer.

The activities of *β*-1,4-glucosidase (BG, C-acquiring enzyme), *β*-N-acetyl-glucosaminidase (NAG, N-acquiring enzyme), and alkaline phosphatase (AKP, P-acquiring enzyme) were determined by test kits (Grace Biotechnology Co., Ltd, Suzhou). Briefly, for NAG, 0.0500 g of fresh soil was incubated at 37 °C for 1 h with 150 μL of fluorometric substrate (4-nitrophenyl-N-acetyl-β-D-glucosaminide) dissolved in buffer (pH 6.2), 350 μL of terminator solution was added, and the mixture was then centrifuged at 12,000 rpm for 10 min. The absorbance was measured at 405 nm using a spectrophotometer. BG was estimated similarly to NAG, except that *p*-nitrophenyl-β-D-glucopyranoside was used as the substrate. For AKP, the buffer solution (pH 10) and substrate (nitrophenol phosphate) differed from the method applied to determine the activity of NAG. The soil enzyme activity was expressed as mmol h^{-1} g^{-1} fresh soil.

2.4 Statistical Analyses

2.4.1 Calculation Formula of Soil Microbial Element Limitation

The enzymatic C:N, C:P, and N:P ratios were calculated as follows: ln(BG):ln(NAG), ln(BG):ln(AP), and ln(NAG):ln(AP), respectively. Vector length (VL) and vector angle (VA) were used to characterize the element limitation of soil microorganisms. The calculation formula was as follows (Moorhead et al. 2016):

$X = BG/(BG + AP)$
$Y = BG/(BG + NAG)$
Vector length $= SQRT(X^2 + Y^2)$
Vector angle $= DEGREES\ ((ATAN2(X; Y))$

The vector length represented the energy limitation relative to nutrient limitation, and the vector angle represented the phosphorus limitation relative to nitrogen limitation. A vector angle greater than 45° represented phosphorus limitation, and a vector angle less than 45° represented nitrogen limitation.

2.4.2 Data Analysis

The differences in physicochemical properties and extracellular enzymes in rhizosphere soil between invasive and native plants were analyzed with independent sample *t* tests in SPSS 23.0 (IBM, USA). We performed Pearson's correlation analysis between soil physicochemical properties and soil extracellular enzyme activities (EEAs) and enzymatic stoichiometry (ES) using OriginLab 2021 (OriginLab, USA). The inverse distance weighted (IDW) method was applied to examine the spatial distribution of soil EEAs and ES using ArcGIS 10.2 (Environmental Systems Research Institute, USA). Redundancy analysis (RDA) using Canoco 4.5 (Microcomputer Power, USA) was to explore the constraint relationship between selected factors and extracellular enzyme activities. The interpretation variable soil's total N was eliminated due to the variance inflation factor (VIF) exceeding 10, and the main variables affecting the variation in EEAs were obtained by the forward selection method and Monte Carlo replacement test ($P < 0.05$). Random forest (RF) analysis was used to determine the predominant drivers of the three soil extracellular enzyme activities. To estimate the importance of soil variables, we used percentage increases in the MSE (mean squared error) of variables: higher MSE% values imply more important variables (Jiao et al. 2018). The RF analysis was performed using the lm and calc.relimp functions in the "relaimpo" package using R software version 4.0.5.

3 Results

3.1 Soil Physicochemical Properties in Invasive Plots and Native Plots

The soil in the study area was alkaline, with an average value of 8.3 in the invasive plots and 8.33 in the native plots. The soil physicochemical property values and stoichiometric ratio were higher in the native plots than in the invasive plots for all but one property (soil nitrate content). Significant differences were observed between *A. palmeri*–invaded plots and native vegetation plots for soil total C ($P = 0.002$), soil N-NH$_4$ content ($P = 0.041$), and soil C:N ratio ($P = 0.026$). The differences in the remaining indicators were marginally significant or non-significant (Table 1).

3.2 Differences in Soil EEAs and Their Acquisition Ratios Between A. palmeri and Native Vegetation

The average values of C-, N-, and P-acquiring enzyme activities were 168 mmol h^{-1} g^{-1} soil, 292 mmol h^{-1} g^{-1} soil, and 364 mmol h^{-1} g^{-1} soil in the rhizosphere of *A. palmeri*, respectively. The average values of C-, N-, and P-acquiring enzyme activities were 156 mmol h^{-1} g^{-1} soil, 267 mmol h^{-1} g^{-1} soil, and 375 mmol h^{-1} g^{-1} soil in the rhizosphere of native vegetation, respectively. The C- and N-acquiring enzyme activities in the rhizosphere soil of *A. palmeri* were slightly higher than those in the rhizosphere soil of native plants, whereas the P-acquiring enzyme activity exhibited the opposite patterns. These three EEAs

did not show significant differences between *A. palmeri* and native vegetation soil (Fig. 2).

Inverse distance weight (IDW) analysis further indicated that N-acquiring enzyme activity in invasive and native plots showed similar spatial distribution patterns across the 22 sampling sites, with a higher activity in the eastern region. The C-acquiring enzyme activity in the invasive plots presented spatial distribution patterns similar to those of the N-acquiring enzyme activity. However, spatial variation in P- and C-acquiring enzyme activities was not observed across the native patches (Fig. 3).

The average values of soil enzyme C:N, C:P, and N:P ratio in the rhizosphere soil of *A. palmeri* were 1.85, 1.08, and 0.63, respectively. The average values of soil enzyme C:N, C:P, and N:P ratios in the rhizosphere soil of native vegetation were 2.05, 0.89, and 0.50, respectively (Fig. 2). Additionally, the C:N acquisition ratio showed similar spatial distribution patterns in the rhizosphere soil of *A. palmeri* and native vegetation (Fig. S2).

3.3 Pearson's Correlation and Random Forest Analysis of the Soil Chemical and Biological Properties of Invasive and Native Plants

Pearson's correlation analysis showed that N-acquiring enzyme activity in the rhizosphere soil of *A. palmeri* was significantly positively correlated with soil total N, total C, ammonia nitrogen content (N-NH$_4$), and available phosphorus (P-PO$_4$), and significantly negatively correlated with soil pH ($P < 0.05$). Soil N-acquiring enzyme activity responded to soil variables similarly in native plots. Significant correlations between C-acquiring enzyme activity and soil pH and P-PO$_4$ and between

Table 1 Soil physicochemical properties under invasive *A. palmeri* and native vegetation within 22 sampling plots

Variables	A. palmeri plots (AP)		Native plots (N)		p-value
	Means±standard deviations	Variation coefficients (%)	Means ± standard deviations	Variation coefficients (%)	AP vs N
pH	8.30±0.18	2.24	8.33±0.24	2.85	0.389
Total N (g·kg^{-1})	1.4±0.77	55.11	1.65±1.08	65.24	0.197
Total C (g·kg^{-1})	21.47±9.87	45.99	29.64±22.40	75.59	**0.002**
Total P (g·kg^{-1})	0.155±0.08	55.34	0.172±0.07	42.66	0.642
N-NH$_4$ (mg·kg^{-1})	24.76±11.02	44.51	29.18±20.21	69.26	**0.041**
N-NO$_3$ (mg·kg^{-1})	21.99±14.17	64.48	18.86±14.23	75.42	0.451
P-PO$_4$ (mg·kg^{-1})	24.22±14.02	57.91	25.41±20.46	80.50	0.114
Soil C:N ratio	16.79±5.30	31.58	19.89±14.54	73.08	**0.026**
Soil C:P ratio	167.54±92.33	55.11	184.81±149.41	80.85	0.238
Soil N:P ratio	10.43±5.81	55.74	10.18±5.49	53.97	0.913

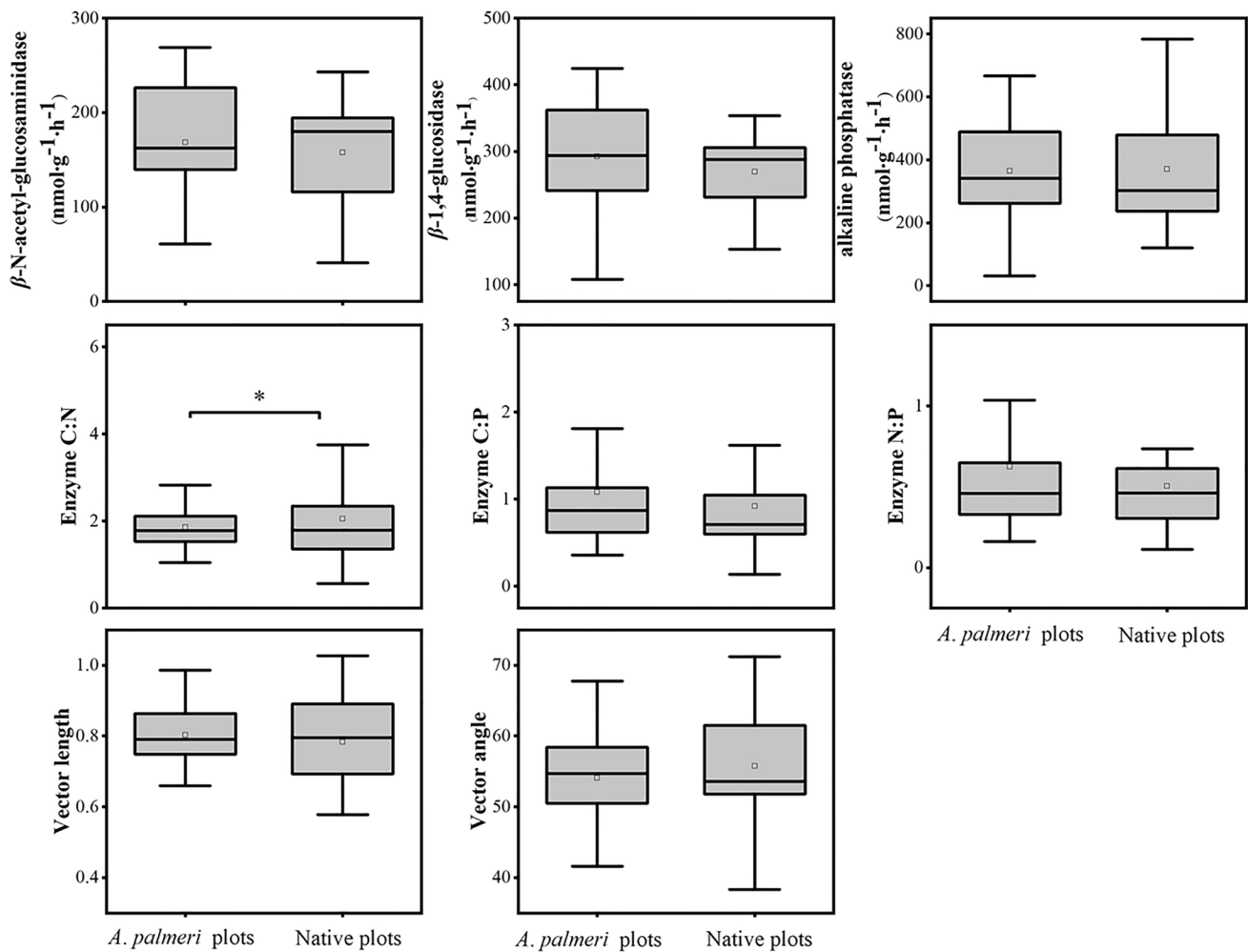[a]Bold values indicated $P < 0.05$

Fig. 2 Soil extracellular enzymatic activities, enzymatic stoichiometry, and element limitation of *A. palmeri* and native plants. The asterisk indicates significant differences between the invasive and native plots (Duncan's test, $P < 0.05$)

P-acquiring enzyme activity and soil total C and P-PO$_4$ were observed across the invaded plots. Moreover, the stoichiometric C:N imbalance was positively associated with soil pH and negatively associated with total N, total C, and N-NH$_4$ content in invaded patches ($P < 0.05$). The three kinds of soil extracellular enzymes and their stoichiometric ratios were mainly related to soil pH, total N, total C, N-NH$_4$ content, and P-PO$_4$ (Fig. 4). Random forest analysis was used to test the relative importance of the variables affecting the extracellular enzymes (Fig. 5). Soil N-NH$_4$ content and total C were the main variables driving the N-acquiring enzyme activity in invaded plots, and soil pH and total N were predominant for explaining N-acquiring enzyme (NAG) activity in native plots. C-acquiring enzyme (BG) activity in the invaded area was mainly affected by soil P-PO$_4$ and total C, while soil N-NO$_3$, pH, and P-PO$_4$ mainly drove BG activity in the native area. The prevailing driver of P-acquiring enzyme

(AKP) activity in invaded plots was soil total C, and AKP activity in native plots was mainly affected by soil pH. The selected factors can explain more than half of the variation in N-acquiring enzyme activity (*A. palmeri*, 62%; native vegetation, 53%), but most of the variation in P-acquiring enzyme activity has not been explained.

3.4 Driving Variations in Soil Extracellular Enzymes and Enzymatic Stoichiometry of Invasive and Native Plants

Redundancy analysis (RDA) can reflect the constraint ability of selected variables on soil extracellular enzyme activities (Fig. 6). The first two axes (RDA1 and RDA2) explained 50.6% of the variation between extracellular enzyme activity and selected factors in the rhizosphere soil of *A. palmeri*, and 20.8% of the variation was significantly explained by soil total C ($F = 5.25$, $P = 0.006$).
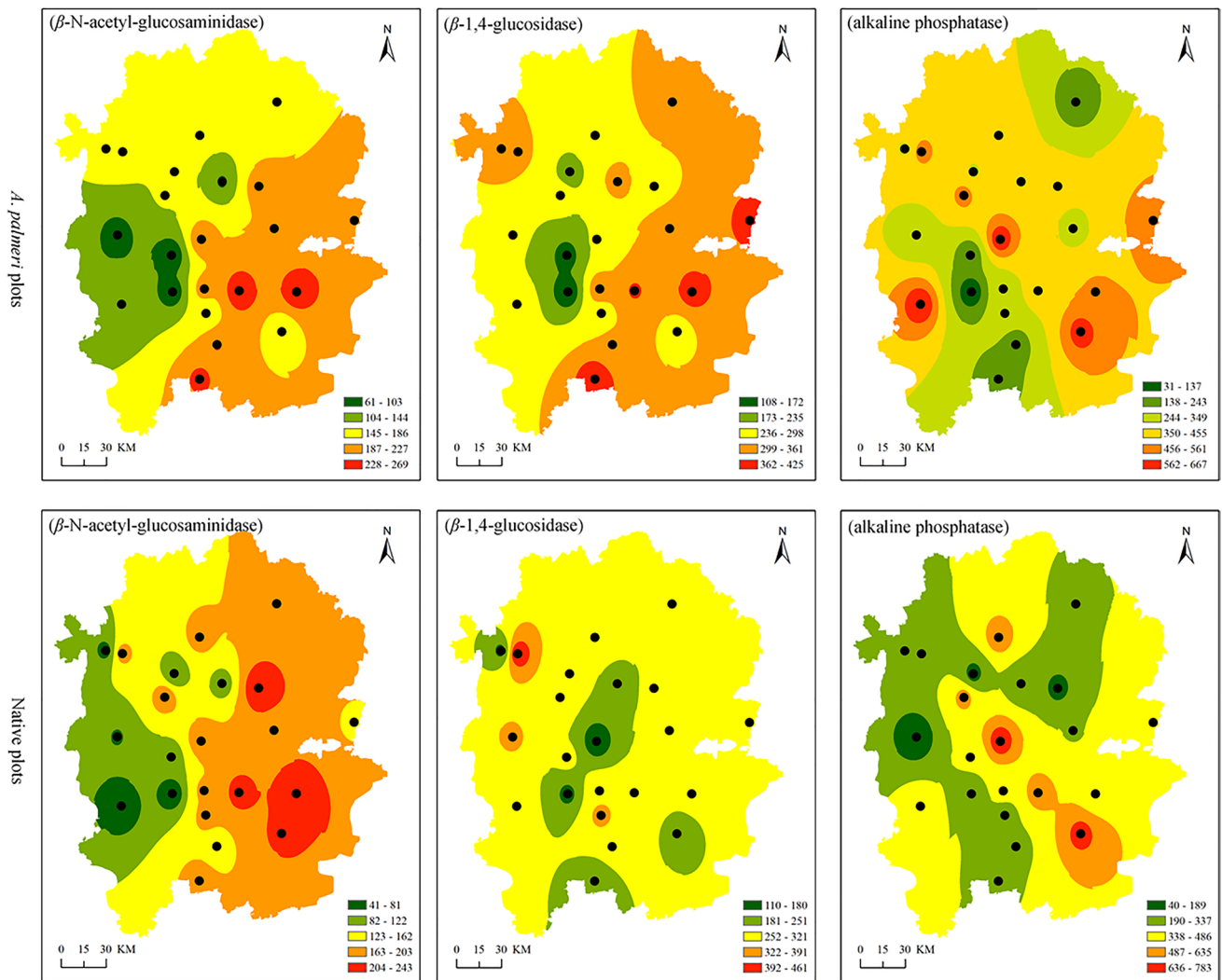
Fig. 3 The activities and spatial distributions of the soil extracellular enzyme. Black dots indicated the sampling sites

The first two axes (RDA1 and RDA2) explained 45.2% of the variation in extracellular enzyme activities in the rhizosphere soil of native plants, and 35.4% of the variation was significantly explained by soil pH ($F = 6.06$, $P = 0.002$) and P-PO$_4$ ($F = 3.66$, $P = 0.016$).

4 Discussion

4.1 Response of Soil Physicochemical Properties to A. palmeri Invasion

The interaction between invasive plants and the underground ecosystem has been proposed as one of the potential mechanisms driving their establishment (Lee et al. 2012; Mincheva et al. 2014; Zhang et al. 2017). In this study, *A. palmeri* generally worsened the nutrient status of the invaded area

(Table 1), but the change in nutrients also showed regional specificity (Fig. S1). Our results found that the soil total C content was significantly higher in native plots than in invasive plots ($P = 0.002$). This might be because most of the native sampling areas contained Poaceae species, such as *Setaria viridis*, which provided sufficient carbon sources for microorganisms and reduced the degree of microbial carbon restriction to native vegetation (Zhang et al. 2018). The soil C:N ratio is often inversely proportional to the decomposition rate of organic matter. The soil C:N ratio of native vegetation was significantly higher than that of invasive plants, indicating its lower decomposition rate of organic matter and consequent weakening or restriction of the metabolic activity of soil microorganisms (Allison and Vitousek 2005; Song et al. 2018). Invasive plants can affect the content of soil N-NH$_4$ and N-NO$_3$ through root activity (Kourtev et al. 2003; Rippel et al. 2020). The invasion by *A. palmeri*

Fig. 4 Pearson's correlation analysis of the soil variables for the extracellular enzymatic activities and stoichiometry of invaded plots (AP) and native plots (N). Abbreviation: MAP, mean annual precipitation; MAT, mean annual temperature
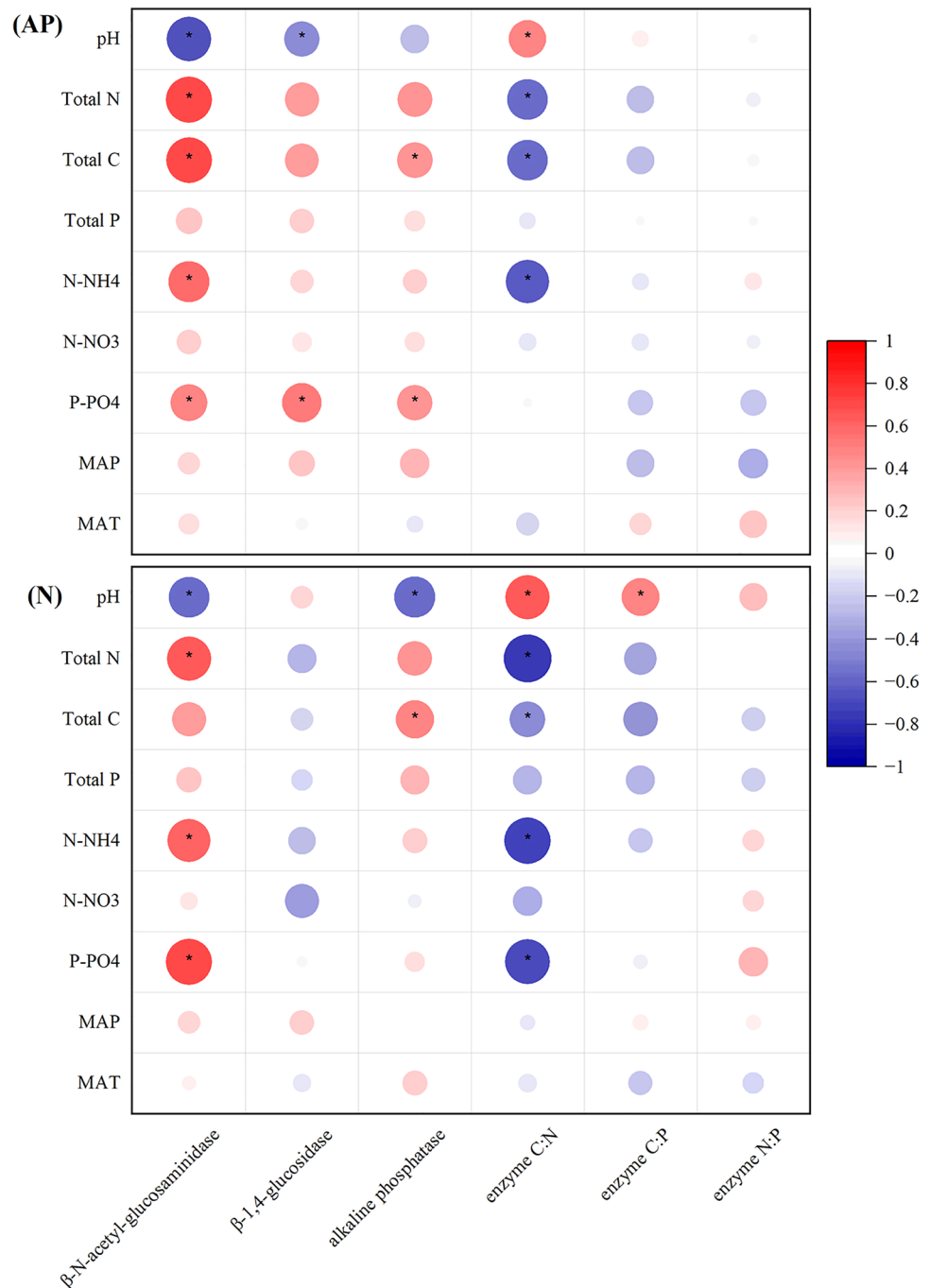

increased the N-NO$_3$ content, but significantly reduced the N-NH$_4$ content in rhizosphere soil ($P < 0.05$), indicating that *A. palmeri* may prefer to absorb and utilize N-NH$_4$. Previous studies have indicated that the soil C:N:P ratio is mainly affected by the P content. Although the contents of C and N in soil change greatly, P deficiency is often the main reason for the increase in the soil C:P and N:P ratios (Tian et al. 2010). In the present study, the soil N:P ratio was higher than the mean value of land N:P in China (5.2), indicating the lack of phosphorus in the recipient ecosystems.

4.2 Response of Soil Extracellular Enzyme Activities to A. palmeri Invasion

The persistence of invasive plants can affect soil nutrient availability via the release of allelopathic chemicals to influence soil extracellular enzyme activities, making the soil environment not conducive to the growth of surrounding vegetation, thereby suppressing and excluding plants with higher nutrition requirements (Wang et al. 2017). The soil P- and C-acquiring enzyme activities in the current research
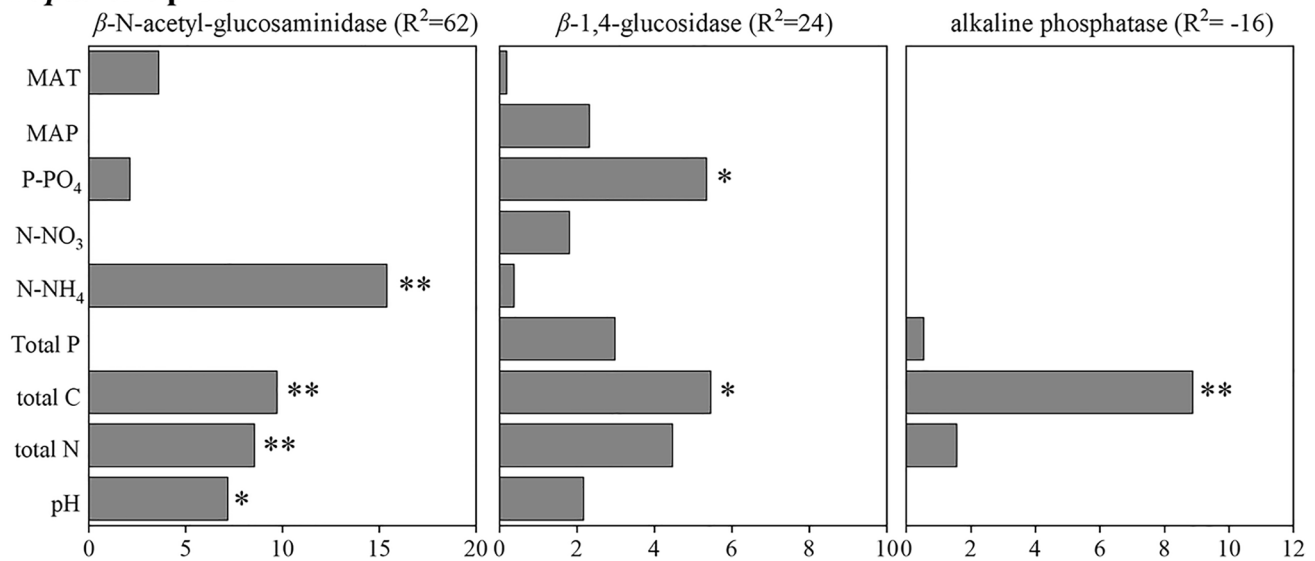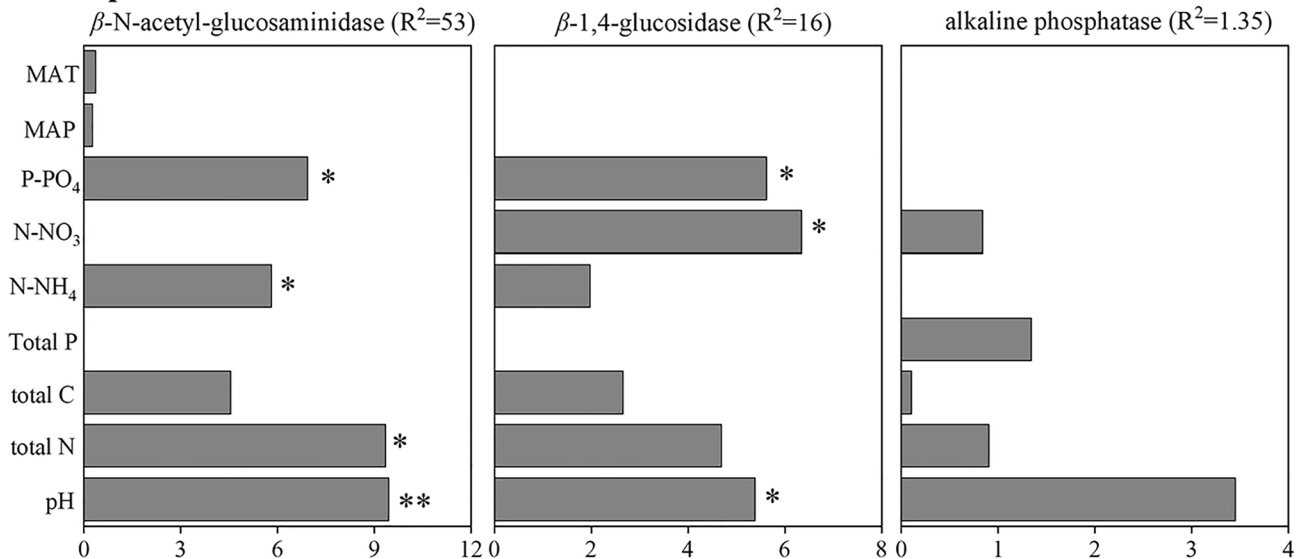
A. palmeri plots

Fig. 5 The importance of selected factors in explaining the variations of extracellular enzymatic activities of *A. palmeri* plots and native plots. Random forest was used to determine the variable importance. R^2 (pseudo) represents the total variance that could be explained by selected factors. Negative R^2 value indicates that the variance explained by the observed factors is less than that explained by their random values. **$P < 0.01$; *$P < 0.05$. Abbreviation: MAP, mean annual precipitation; MAT, mean annual temperature

were much higher than the N-acquiring enzyme activity, which suggested that microbes of *A. palmeri* allocate more resources for the production of enzymes related to P and C cycling. This result may be due to the higher carbon and nitrogen content in the rhizosphere of native plants reducing the secretion of microbial carbon and nitrogen hydrolases (Zhu et al. 2014). Compared with native vegetation, the increased C-acquiring enzyme activity (BG) consumed soil organic carbon and reduced the carbon source available for microorganisms. Moreover, more degradation products may be absorbed by plants to build biomass, underlying an intensified carbon limitation of *A. palmeri* (Lemanski et al.

2019; Zhong et al. 2020). This result was in accordance with the larger vector length of *A. palmeri* (Fig. 2). Resource allocation theory suggests that microorganisms increase the synthesis of EEAs corresponding to limited elements to meet their own nutrient requirements (Allison and Vitousek 2005; Waring et al. 2014). The enzymatic C:P and N:P ratios increased, and the enzymatic C:N ratio decreased significantly in the rhizosphere soil of *A. palmeri*, which further indicated that the carbon limitation in the invasive plots was relatively aggravated, while the P limitation was relatively alleviated. This was also confirmed by the vector angle and vector length analysis.

Fig. 6 Redundant analysis and Monte Carlo test of selected variables for the extracellular enzymatic activities and stoichiometry of invaded plots and native plots. Abbreviation: MAP, mean annual precipitation; MAT, mean annual temperature
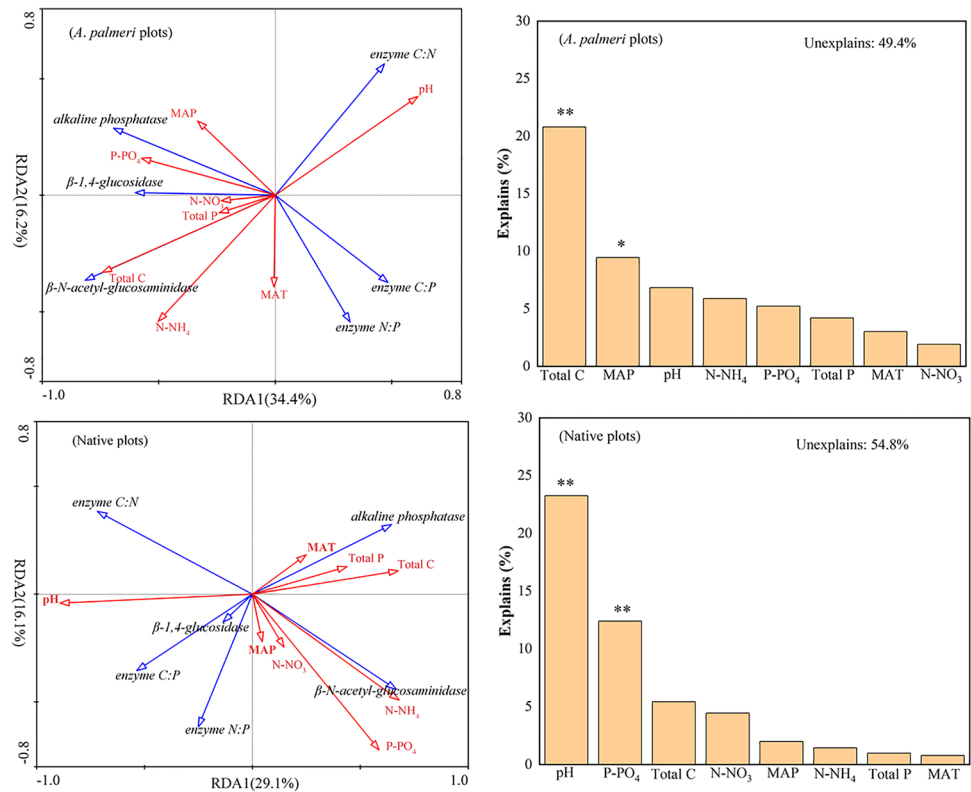
The soil N- and C-acquiring enzyme activities of the invasive plots and the N-acquiring enzyme activity of the native plots exhibited similar spatial distribution patterns, with a higher enzyme activity in the western parts of the sampling sites (Fig. 3), which was also reflected in the random forest analysis results. Only the N- and C-acquiring enzyme activities could be largely explained by the soil variables (Fig. 5). One possible reason for the lower explanation of the P-acquiring enzyme activity could be that the enzymes respond to the same factors in a site-specific manner, which may lead to neutral effects (Wang et al. 2022).

4.3 Drivers of Soil Extracellular Enzymes and Enzymatic Stoichiometry

Plant communities alter the soil nutrient content by stimulating nutrient metabolism, impacting soil enzyme activity (Pandey et al. 2014; Li et al. 2019). The N-, C-, and P-acquiring enzymes in the current study were strongly correlated with soil properties, especially with soil pH, total N, total C, N-NH$_4$, and P-PO$_4$ (Fig. 4). The strong correlations indicated that microbes could obtain relatively scarce resources by optimizing the distribution of C, N, and P during enzyme synthesis (Zhang et al. 2018; Dong et al. 2019; Gai et al. 2021). The RF analysis and RDA indicated that pH was the most important driving factor for soil extracellular enzymes and enzymatic stoichiometry in the rhizosphere soil of *A. palmeri* (Figs. 5,

6). Considerable investigations have confirmed the role of pH as a key factor influencing soil EEAs (Stone et al. 2014; Peng and Wang 2016; Wei et al. 2017).

The response of the soil N acquisition enzyme (NAG) was most strongly associated with the N-NH$_4$ content in the rhizosphere soil of *A. palmeri* (Fig. 5). This was likely associated with the difference in nutrient utilization rate between invasive plants and native plants, resulting in a significant decrease in the N-NH$_4$ content of *A. palmeri* ($P < 0.05$). Soil total C demonstrated significant or even extremely significant explanation rates for N-, C-, and P-acquiring enzymes in the rhizosphere soil of *A. palmeri* (Fig. 5). Moreover, RDA also showed that total C had the greatest explanation rate (20.8%) for C-, N-, P-acquiring enzymes and enzymatic stoichiometry (Fig. 5). Because it is a fast-growing plant, *A. palmeri* tended to allocate more resources for the growth of its aboveground biomass, and the input of available carbon sources (i.e., rhizosphere secretions) to its underground was reduced, which lead to accelerated metabolism of soil microorganisms, release of more extracellular enzymes into the rhizosphere, and provision of more nutrients and resources for the growth of invasive plants (Zhu et al. 2014; Zuo et al. 2018). The soil C:N and C:P ratios of native plants increased considerably, which caused phosphorus limitation in native vegetation (Kitayama et al. 2013; Fan et al. 2015; Zhang et al. 2018). Therefore, the explanation rate of soil P-PO4 for N- and C-acquiring enzyme activities was greater in the native vegetation rhizosphere soil (Fig. 5). The results of Monte Carlo

analysis also showed that the EEAs and ES of native plants were largely dependent upon the soil P-PO$_4$ concentrations (Fig. 6). Overall, the P limitation was alleviated, and the carbon limitation intensified in the *A. palmeri*–invaded ecosystems.

5 Conclusions

Our study found that invasion by *A. palmeri* was associated with higher soil C- and N-acquiring enzyme activity, and lower P-acquiring enzyme activity than that found in native vegetation soil. The enzymatic C:N ratio decreased significantly ($P < 0.05$), and the enzymic C:P and N:P ratios were higher in the invasive plots, indicating strengthened carbon limitation and weakened phosphorus limitation due to encroachment by *A. palmeri*. The alterations in soil extracellular enzyme activities and enzymatic stoichiometry were better explained by the total carbon in *A. palmeri* soil, whereas pH and available phosphorus were dominant drivers of soil extracellular enzyme activities and enzymatic stoichiometry in native vegetation soil. Our findings will be crucial in understanding the nutrient limitation and nutrient cycles of invasive plant soils.

Declarations

Conflict of Interest The authors declare no competing interests.

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