

The effects of two biofertilizers on the physiology of replanted *Zanthoxylum bungeanum* and soil phenolic acids

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Abstract

To solve the issues of low survival rate and poor vigor of *Zanthoxylum bungeanum* seedlings caused by long-term continuous cropping, a study was conducted using bio-organic fertilizer and microbial bacterial fertilizer as repair agents. The aim was to investigate their effects on the physiological metabolism of *Zanthoxylum bungeanum* seedlings and soil phenolic acids, with a focus on understanding the growth-promoting mechanism of replanted *Zanthoxylum bungeanum*. The results revealed that fertilization significantly increased the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), nitrate reductase (NR), as well as the levels of soluble sugar (SS) and soluble protein (SP) in *Zanthoxylum bungeanum* seedlings. Conversely, the content of malondialdehyde (MDA) and four soil phenolic acids (gallic acid (FA), ferulic acid (GA), caffeic acid (CA), and p-hydroxybenzoic acid (PHBA)) were significantly reduced. The mixed application of microbial fertilizer and bio-organic fertilizer showed better growth-promoting effects compared to single application. Specifically, when the volume ratio of microbial fertilizer and bio-organic fertilizer was 2:1, the activity of defense enzymes was most significantly promoted. Under this treatment, the activities of SOD, CAT, POD, and NR in seedlings were 1.8, 3, 3.8, and 5.3 times higher than the control (no fertilization treatment), respectively. The levels of SS and SP were 2.4 and 2.5 times higher than the control, respectively. The MDA content was 27% of the control, and the total content of the four phenolic acids was 60% of the control. Principal component analysis results showed that the scores of fertilization treatments were higher than the control, with the order being T6 > T7 > T2 > T5 > T4 > T3 > T1 > T0. Therefore, the combined application of microbial fertilizer and bio-organic fertilizer effectively promotes the physiological metabolism of seedlings, reduces the content of soil phenolic acids, and has a positive effect on alleviating the obstacles to continuous cropping of *Zanthoxylum bungeanum*.

Introduction

Zanthoxylum bungeanum, a small deciduous tree or shrub belonging to the genus *Zanthoxylum* in the family Rutaceae, exhibits early yield, wide use, high value, and strong adaptability (Luo et al. 2022; Liang et al. 2023; Lu et al. 2020). It is commonly utilized in food processing and is known for its anti-inflammatory, analgesic, anti-cancer, and other medicinal properties. However, improper management and continuous cropping in certain areas have led to the accumulation of allelopathic and autotoxic substances, resulting in a decline in soil quality and an imbalance in the microbial flora. Consequently, the yield and quality of *Zanthoxylum bungeanum* have decreased, and the survival rate of seedlings after replanting is low. This issue of continuous cropping has significantly hindered the robust growth of the *Zanthoxylum bungeanum* industry. Therefore, finding effective solutions to alleviate obstacles related to continuous land cropping has become an urgent industrial concern.

In recent years, there has been widespread use of microbial remediation agents for soil remediation. Bio-organic fertilizer, which is rich in organic and inorganic nutrients, consists of beneficial microbial flora and organic fertilizer. It contains a significant amount of organic matter components. The organic fertilizer can effectively interact with microorganisms, leading to mutual benefits (Wu et al. 2016).

Microbial fertilizer, on the other hand, is abundant in *Pseudomonas*, rhizobia, indole acetic acid, and other beneficial microorganisms. These microorganisms can enhance the release and absorption of insoluble elements like P and K in the soil (Liu et al. 2024). Therefore, combining these two types of fertilizers can better harness the synergistic effects of organic matter and beneficial microorganisms, ultimately addressing the issue of continuous cropping. Due to the numerous advantages of biofertilizers, extensive research has been conducted in this field. For instance, bioorganic fertilizers have been successfully used in various crops such as peas and oats (Jannoura et al. 2014), *Panax notoginseng* (Shi et al. 2022), grapes (Liu et al. 2024), apples (Gu et al. 2024), and walnuts (Du et al. 2022) due to their high efficiency, fast results, and cost-effectiveness. Microbial fertilizers, which contain a plethora of beneficial microorganisms, are primarily employed for biological control of plant and soil diseases. They have proven effective in mitigating postharvest diseases of fruits and vegetables (Huang et al. 2021) as well as disease problems in cotton (Liu et al. 2023), rice (Zhang et al. 2021), and apple (Duan et al. 2022). Consequently, it is evident that the appropriate application of organic and bacterial fertilizers can alleviate the issue of continuous cropping to varying degrees.

Currently, research on *Zanthoxylum bungeanum* primarily focuses on analyzing its chemical components and exploring its medical value (Wang et al. 2021; Zeng et al. 2018). Other areas of research include genetic characteristics and habitat analysis (Xiang et al. 2016), drought tolerance (Li et al. 2020; Su et al. 2023), biodiversity of endophytic fungi (Li et al. 2016), allelopathy (Ma et al. 2019; Cao et al. 2022), comparison of physiology and transcriptomics of different varieties (Tian et al. 2021), and soil physical and chemical properties (Liu et al. 2021). In the initial phase, we utilized bio-organic fertilizer and microbial bacterial fertilizer to treat soil that had been continuously cropped. By examining the growth and photosynthetic characteristics of replanted *Zanthoxylum bungeanum*, I investigated the promoting effect and photosynthetic mechanism of a mixture of biofertilizers on the growth of continuously cropped *Zanthoxylum bungeanum* (Zhang et al. 2021). However, the physiological and biochemical characteristics of replanted *Zanthoxylum bungeanum* and the impact of soil allelochemicals are still not fully understood. Hence, this study employs the principal component analysis method to assess the effects of different ratios of bio-organic fertilizers and microbial fertilizers on the physiology and biochemistry of replanted *Zanthoxylum bungeanum* seedlings, as well as soil phenolic acids. The aim is to uncover the physiological effects of applying a mixture of biofertilizers to replanted *Zanthoxylum bungeanum*, and to provide a theoretical foundation for understanding the characteristics and mitigation mechanism of soil allelopathic substances.

Materials and Methods

Study Area

The experiment was conducted at the Economic Forestry Teaching and Research Practice Base of the Forestry College of Gansu Agricultural University, which is geographically located at 36°03' north latitude and 103°40' east longitude. The region has a typical temperate continental climate, characterized by an

average annual temperature of 10.3°C, an average annual precipitation of 327 mm, and an average annual sunshine time of 2446 h.

Experimental Materials

Test plants consisted of well-growing and healthy annual 'Dahongpao' *Zanthoxylum bungeanum* seedlings obtained from Qin'an County, Tianshui City.

The soil used for testing was collected from a *Zanthoxylum bungeanum* garden that has been under continuous cultivation for 25 years in Qin'an County, Tianshui City, China.

The basic fertility indicators of the soil are presented in Table 1.

Table 1 Basic physical and chemical properties of continuous cropping soils							
pH	Organic matter	Total nitrogen	Total phosphorus	Total potassium	Available nitrogen	Available phosphorus	Available potassium
	g·kg ⁻¹	g·kg ⁻¹	g·kg ⁻¹	g·kg ⁻¹	mg·kg ⁻¹	mg·kg ⁻¹	mg·kg ⁻¹
8.29	16.21	0.79	0.61	7.85	45.00	4.36	75.68

The bioorganic fertilizer used in the test was produced by Shandong Ruipu Biotechnology Co., Ltd. It contains the following active ingredients: organic matter (≥ 30%), N + P₂O₅ + K₂O (≥ 6%), alginic acid (≥ 15%), crude protein (≥ 9%), chelated trace elements, and active biological bacteria (≥ 200 million/g). On the other hand, the microbial fertilizer tested was produced by Gansu Dahang Agricultural Science and Technology Development Co., Ltd. It mainly consists of *Bacillus subtilis*, *Bacillus licheniformis*, rhizobia, humic acid, trace elements, indoledine Potassium acid, and sodium naphthoacetate. The effective viable bacterial count in this fertilizer is ≥ 200 million/g, and the N + P₂O₅ + K₂O content is ≥ 8%.

Experimental Design

In June 2019, a 25-year continuous cropping soil was placed in plastic flower pots with an inner diameter of 32 cm and a height of 25 cm. Each pot was filled with 15 kg of soil. Uniformly growing annual *Zanthoxylum bungeanum* seedlings were selected and transplanted into the pots, with one plant per pot. The control group did not receive any fertilizer. For the other treatments, a 50 g·L⁻¹ bio-organic fertilizer was used as the base fertilizer. In the early stage of cultivation, measures such as sun protection, anti-freeze, and rainproofing were implemented. To improve the survival rate of seedlings, bio-organic fertilizer was applied as top dressing to the soil after 30 days of balancing. Before application, solid bio-organic fertilizer was ground into powder, weighed as required, and mixed with water to form a liquid. The fertilizer was applied by spraying, with an application frequency of once every 30 days. The concentrations of top dressing were 50 g·L⁻¹, 80 g·L⁻¹, and 100 g·L⁻¹ respectively, and the application amount was 500 mL per plant. This continued until October (leaf fall period) when the seedlings were cut off and moved indoors for winter safety.

After the seedlings survived the winter, top dressing was carried out in early June 2020. Spraying was done in the early morning under calm weather conditions. The test included 8 treatments: T0 (no fertilization control group), T1 (10 g·L⁻¹ microbial bacterial fertilizer), T2 (100 g·L⁻¹ bio-organic fertilizer), T3 ($V_{\text{microbial fertilizer}}:V_{\text{bio-organic fertilizer}}=1:2$), T4 ($V_{\text{microbial fertilizer}}:V_{\text{bio-organic fertilizer}}=1:4$), T5 ($V_{\text{microbial fertilizer}}:V_{\text{bio-organic fertilizer}}=1:1$), T6 ($V_{\text{microbial fertilizer}}:V_{\text{bio-organic fertilizer}}=2:1$), T7 ($V_{\text{microbial fertilizer}}:V_{\text{bio-organic fertilizer}}=4:1$); with 6 repetitions for each treatment. The mixed solution for T1 and T2 treatments was prepared according to the volume ratio, and the amount of fertilizer applied per plant was 500 mL each time. After fertilization, regular soil plowing and weeding were conducted as part of normal management.

Experimental Methods

Measurement of Physiological and Biochemical Indicators

Select several healthy leaves from different directions of the plant, including the southeast, northwest, and northeast. Ensure that the collected leaves are clean and promptly store them in an ultra-low temperature refrigerator at -80°C for future use. The determination of relevant physiological and biochemical indicators follows the methods described by Gao et al. (Gao et al. 2006) and Li et al. (Li et al. 2005). The MDA levels were measured using the thiobarbituric acid method, SOD activity was assessed using the nitroblue tetrazolium (NBT) photoreduction method, POD activity was determined using the guaiacol reduction method, SS content was measured using the anthrone colorimetric method, and SP levels were determined using the Coomassie brilliant blue (G-250) method. CAT and NR activities were determined using spectrophotometry.

Determination of Soil Phenolic Acid

To collect soil samples, the 'soil shaking method' was employed. First, the top layer of soil was removed using a shovel, followed by careful extraction of the entire plant. The plant stem was tapped to ensure complete removal of rhizosphere soil. The collected soil was then placed in a ziplock bag and transported to the laboratory for further processing. The soil was sieved through a 20-mesh sieve to remove fibrous roots, stones, and other impurities, and stored at 4°C for later use. Soil phenolic acids were determined using a quaternary gradient ultra-high performance liquid chromatograph (ACQUITY Arc, Waters Company, USA), following the methods described by Tian et al. (Tian et al. 2015) and Cheng et al. (Cheng et al. 2022). The chromatographic separation column used was Symmetry C. 18 (4.6 nm×250 nm, Column, 5 μm), with a detection wavelength of 280 nm. The injection volume was 10 μL, the column temperature was maintained at 25°C, and the flow rate was set at 1 mL·min⁻¹.

Data Analysis

Statistical analysis and significance testing were performed using SPSS 22.0. Multiple comparisons and significance analysis were conducted using the LSD minimum new complex range method and Duncan method. Graphs were created using Origin 2021 Pro. All data are presented as mean ± SE.

Results

The Effects of Two Biofertilizers on The Activity of Defense System Enzymes of Replanted *Zanthoxylum bungeanum*

Significant differences were observed in the MDA content and CAT activity of *Zanthoxylum bungeanum* leaves between the fertilization treatment and the control group T0 ($p > 0.05$)(Fig. 1A, D), and no significant differences were observed in the CAT content between T1, T2, T3, T4, T5, and T7 ($p > 0.05$). Compared to the control T0, the SOD and POD activities showed the most significant increase in the T6 treatment ($p > 0.05$)(Fig. 1B, C). There were no significant differences in the POD activity between T7 and other treatments ($p > 0.05$). Additionally, there were no significant differences in the SOD activity between T0 and other treatments except T6 ($p > 0.05$), and no significant differences were observed in the POD activity between T1, T2, T3, and T4 ($p > 0.05$).

The Effects of Two Biofertilizers on Osmoregulatory Substances in Replanted *Zanthoxylum bungeanum*

Compared to the control T0, the SS and SP contents showed a significant increase in the T6 treatment ($p < 0.05$), with an increase of 137.9% and 145.1% respectively (Table 2). Among these treatments, except for T1, all other treatments of SS showed significant differences from T0 ($p < 0.05$). The difference between the T6 treatment and other treatments was also significant ($p < 0.05$), while the differences between T2, T3, T4, and T5 were not significant ($p > 0.05$). Significant differences were observed in SP content between T6, T7, and other treatments ($p < 0.05$), but there were no significant differences between T6 and T7, as well as between T0 and T1, T2, T3, and T4 ($p > 0.05$).

Table 2
The effects of two biofertilizers on the osmoregulatory substances in replanted *Zanthoxylum bungeanum*

Treatment	Soluble sugar $\text{mg}\cdot\text{g}^{-1}$	Soluble protein $\text{mg}\cdot\text{g}^{-1}$
T0	$10.31 \pm 1.07\text{d}$	$1.57 \pm 0.12\text{c}$
T1	$13.09 \pm 1.53\text{cd}$	$2.00 \pm 0.06\text{c}$
T2	$14.54 \pm 1.11\text{bc}$	$2.44 \pm 0.43\text{bc}$
T3	$15.61 \pm 0.67\text{bc}$	$1.95 \pm 0.22\text{c}$
T4	$15.05 \pm 0.9\text{bc}$	$1.86 \pm 0.44\text{c}$
T5	$16.16 \pm 0.34\text{bc}$	$2.43 \pm 0.25\text{bc}$
T6	$24.52 \pm 1.45\text{a}$	$3.84 \pm 0.48\text{a}$
T7	$18.02 \pm 1.21\text{b}$	$3.57 \pm 0.58\text{ab}$
Note: Different lowercase letters indicate significant differences between treatments.		

The Effects of Two Biofertilizers on Nitrate Reductase Activity of Replanted *Zanthoxylum bungeanum*

The NR activity of T1 and T3 treatments is not significantly different from the control ($p > 0.05$)(Fig. 2), while the other treatments exhibit significantly higher NR activity than T0 ($p < 0.05$). Notably, the T6 treatment shows the most significant improvement in NR activity, with a remarkable increase of 428.7% compared to the control. Additionally, there is no significant difference between the T6 and T7 treatments ($p > 0.05$), but both treatments show significantly higher NR activity than T1 and T2 treatments. These findings indicate that when the volume ratio of microbial fertilizer and bio-organic fertilizer is 2:1, *Zanthoxylum bungeanum* seedlings exhibit a strong N circulation ability and improved N metabolism.

The Effects of Two Biofertilizers on the Soil Phenolic Acid Content of Replanted *Zanthoxylum bungeanum*

After fertilization treatment, the total content of four types of phenolic acids decreased significantly. Specifically, the contents of gallic acid, caffeic acid, and p-hydroxybenzoic acid were all reduced to some extent. However, the content of ferulic acid showed an increasing trend, although the difference between T0 and T1 was not significant ($p > 0.05$), and there was no significant difference between T2, T3, T4, T5, T6, and T7 ($p > 0.05$) (Fig. 3). Among the phenolic acids, gallic acid had the highest content in T0 ($1.21 \mu\text{g}\cdot\text{mL}^{-1}$) and the lowest content in T7 ($0.20 \mu\text{g}\cdot\text{mL}^{-1}$). On the other hand, ferulic acid had the highest content in T7 ($2.94 \mu\text{g}\cdot\text{mL}^{-1}$) and the lowest content in T0 ($0.93 \mu\text{g}\cdot\text{mL}^{-1}$). The contents of caffeic acid and p-hydroxybenzoic acid were both highest in T0, with values of $3.40 \mu\text{g}\cdot\text{mL}^{-1}$ and $2.65 \mu\text{g}\cdot\text{mL}^{-1}$, respectively. The lowest content of caffeic acid was observed in T6 treatment ($0.64 \mu\text{g}\cdot\text{mL}^{-1}$), while the lowest content of p-hydroxybenzoic acid was found in T1 treatment ($0.64 \mu\text{g}\cdot\text{mL}^{-1}$).

In addition to ferulic acid, the use of bioorganic fertilizer and microbial bacterial fertilizer has been found to decrease the levels of gallic acid, caffeic acid, and p-hydroxybenzoic acid. The total content of these four phenolic acids also decreases with the combined application of the two fertilizers. Furthermore, the mixed application of bioorganic and microbial bacterial fertilizers has a greater effect in reducing the accumulation of allelopathic substances compared to using a single fertilizer. This indicates that the combined use of these fertilizers can effectively reduce the accumulation of allelopathic substances. Correlation between biochemical indices of replanted *Zanthoxylum bungeanum* and soil phenolic acids

Based on the correlation analysis results presented in Fig. 4, it was found that MDA has a significant negative correlation with NR and SS, a highly significant negative correlation with SOD and CAT, and a significant negative correlation with FA. Additionally, SOD showed a highly significant positive correlation with CAT, NR and SS, and a significant positive correlation with POD, SP, a significant negative correlation with FA. POD exhibited a highly significant negative correlation with NR, SS, and SP. NR and SS, SP were found to be significantly positively correlated, and a significant negative correlation with FA. Moreover, CAT was significantly positively correlated with NR, SS, and a significant positive correlation with SP,

while showing a significant negative correlation with FA and CA. Finally, FA and GA were found to be significantly negatively correlated.

The study found that MDA is negatively correlated with SOD, POD, CAT, NR, SS, and SP in *Zanthoxylum bungeanum* seedlings. The content of MDA directly affects the expression of defense enzyme activity and the content of osmotic adjustment substances. Additionally, the FA content in the soil greatly influences the activities of SOD, CAT, and nitrate reductase in the seedlings. Higher FA content significantly inhibits the activities of these enzymes. Furthermore, an increase in CA content in the soil significantly inhibits the POD activity in the plants.

Principal Component Analysis

The eleven related indicators were analyzed using principal component analysis. Two principal components with eigenvalues greater than 1 were extracted, with eigenvalues of 7.663 and 1.439 respectively. The cumulative variance contribution rate of these two principal components was 82.74% (Table 3), indicating that they capture a significant portion of the indicator information.

Table 3
Plot of principal component loadings

Extracted ingredients	Eiges value	Proportion of variance/%	Cumulative variance/%
PC1	7.663	69.660	69.660
PC2	1.439	13.080	82.740

The relevant linear equation is obtained by calculating the coefficients based on the scores of the two principal components:

$$Y1 = 0.348X1 + 0.347X2 + 0.344X3 + 0.332X4 + 0.315X5 - 0.315X6 + 0.307X7 - 0.294X8 - 0.264X9 + 0.255X10 - 0.123X11$$

$$Y2 = -0.117X1 + 0.029X2 - 0.121X3 + 0.117X4 + 0.336X5 + 0.368X6 + 0.323X7 + 0.058X8 - 0.127X9 + 0.084X10 + 0.760X11$$

Combining the variance contribution of the two principal components, the linear equation of the total score was obtained:

$$Y = 0.6966*Y1 + 0.1308*Y2$$

Table 4
Comprehensive evaluation of two biofertilizers on the
restoration effect of replantation barriers of *Zanthoxylum
bungeanum*

Treatment	PC1	PC2	Composite score	sequence
T0	-1.41	-0.22	-1.01	8
T1	-0.13	-1.54	-0.29	7
T2	-0.13	0.38	-0.04	3
T3	-0.29	-0.31	-0.24	6
T4	-0.01	-0.97	-0.14	5
T5	-0.16	0.08	-0.10	4
T6	2.07	1.60	1.65	1
T7	0.06	0.98	0.17	2

The calculation results are shown in Table 4. From this, it can be concluded that the comprehensive restoration effects between different fertilizer ratios are in the following order: T6 > T7 > T2 > T5 > T4 > T3 > T1 > T0.

Discussion

Effect of Two Biofertilizers on Physiological Characteristics of Replanted *Zanthoxylum bungeanum*

Antioxidant enzyme activity is an important indicator of cells' ability to scavenge reactive oxygen species in plants. The content and activity of these enzymes in plants can partially reflect the health of plant growth (Roach et al. 2023). In this study, the use of microbial bacterial fertilizer and bio-organic fertilizer increased the activities of SOD, POD, and CAT in replanted *Zanthoxylum bungeanum*, as well as the content of soluble sugar and soluble protein in seedlings. This suggests that a reasonable mixed application has a positive effect, with the T6 ($V_{\text{microbial fertilizer}}: V_{\text{bio-organic fertilizer}} = 2:1$) being particularly effective in reducing membrane lipid peroxidation, promoting seedling metabolism, and enhancing plant vitality. These findings are consistent with those of Valle et al. (Valle et al. 2022) in rice and Khajeeyan et al. (Khajeeyan et al. 2019) in aloe, indicating that plants resist external adverse conditions through the synergy of defense enzymes.

The content of MDA directly reflects the degree of membrane lipid peroxidation (Akbari et al. 2023). The test results show that plants treated with T6 efficiently absorb and decompose fertilizers, with various enzymes in the body exhibiting high activity and strong metabolic ability, thus preventing the accumulation of peroxide products and enhancing plant stress resistance. Nitrate reductase is a key indicator of plants' nitrogen absorption capacity. In this study, nitrate reductase activity significantly

increased after the application of two biofertilizers, with the most significant increase observed under the T6 treatment. This finding is consistent with the research results of Cordeiro et al. (Cordeiro et al. 2022) in potatoes, suggesting that the use of bioorganic fertilizer and microbial fertilizer can enhance nitrogen metabolism and nutrient absorption in *Zanthoxylum bungeanum* seedlings and effectively mitigate the challenges associated with continuous cropping.

Effect of Two Biofertilizers on Soil Phenolic Acids in Replanted *Zanthoxylum bungeanum*

Leaves are the primary site where plants synthesize allelopathic substances. These substances can enter the soil through direct volatilization or as fallen leaves, and then release chemical compounds through leaching and decomposition, which can affect the growth and development of other plants (Yoshikawa et al. 2018). In this study, four organic acids commonly found in continuously cropped soil, namely gallic acid, ferulic acid, caffeic acid, and p-hydroxybenzoic acid, were quantitatively analyzed and measured using ultra-high performance liquid chromatography. The objective was to determine whether the application of biological fertilizers affects the content of major allelopathic substances in the soil. The experimental results indicate that the application of microbial bacterial fertilizer and bio-organic fertilizer leads to a decrease in the total content of the four phenolic acids. This finding is consistent with previous studies by Wang et al. (Wang et al. 2014) on apple seedlings and Zanelli et al. (Zanelli et al. 2022) on turf grass. Moreover, the treatment with the repair agent also resulted in varying degrees of reduction in the contents of gallic acid, caffeic acid, and p-hydroxybenzoic acid compared to the control. The results for gallic acid and p-hydroxybenzoic acid align with the findings of Kitazawa et al. (Kitazawa et al. 2005) in strawberries. However, the change in caffeic acid content differs from the experimental results, which may be attributed to variations in the allelopathic intensity of the research subjects and the crops themselves. In this study, the content of ferulic acid significantly increased after biofertilizer treatment compared to the control T0, with the highest content observed in T7 ($V_{\text{microbial fertilizer}} : V_{\text{bioorganic fertilizer}} = 4:1$). This finding aligns with the results of Tian et al. (Tian et al. 2015), but contradicts the research findings of Wu et al. (Wu et al. 2005) in cucumber. These discrepancies may be attributed to variations in fertilizer functionality or plant species. Additionally, it is possible that this specific ratio is most suitable for promoting the release of ferulic acid from the rhizosphere of *Zanthoxylum bungeanum*.

Meanwhile, The average proportion of the four phenolic acids in all treatments was as follows: p-hydroxybenzoic acid > caffeic acid > ferulic acid > gallic acid. Parahydroxybenzoic acid accounted for 40% among the four phenolic acids, while gallic acid accounted for 10%. This suggests that the overall content of gallic acid in the soil is low, while parahydroxybenzoic acid has the highest content. These results align with the findings of Zhang et al. (Zhang et al. 2015) in rice soil. In the T0 treatment (continuous cropping soil for 25 years), p-hydroxybenzoic acid and caffeic acid were the main phenolic acids, constituting approximately 75% of the four phenolic acids. However, after the application of the remediation agent, p-hydroxybenzoic acid and ferulic acid became the dominant phenolic acids, accounting for about 80% of the total. This indicates that ferulic acid and caffeic acid are the most responsive to changes after the ingestion of microbial bacterial fertilizer and bio-organic fertilizer.

Similar results were obtained by Pedersen et al. (Pedersen et al. 2018) Gallic acid and parahydroxybenzoic acid, on the other hand, remain relatively stable in terms of content.

Therefore, the use of microbial fertilizers and bio-organic fertilizers can significantly reduce the levels of phenolic acids in continuous cropping soil. Among the four phenolic acids, ferulic acid and caffeic acid are more susceptible to environmental conditions due to their larger base, while gallic acid and paraben exhibit greater stability in soil. Notably, the T4 ($V_{\text{microbial fertilizer}}: V_{\text{bio-organic fertilizer}} = 1:4$) showed the lowest content of the four phenolic acids, indicating that the reduction of phenolic acids in soil is primarily attributed to the allelopathic effect of a high proportion of bio-organic fertilizer on the rhizosphere of *Zanthoxylum bungeanum*, which exerts a certain inhibitory effect.

Conclusions

The application of bioorganic fertilizer and microbial bacterial fertilizer significantly increases the activities of enzymes such as SOD, POD, CAT, and NR in 'Dahongpao' *Zanthoxylum bungeanum* seedlings. It also increases the content of soluble sugar and soluble protein, while reducing the content of MDA and four soil phenolic acids. Based on the principal component analysis results, the T6 ($V_{\text{microbial fertilizer}}: V_{\text{bioorganic fertilizer}} = 2:1$) shows the highest comprehensive score and the most effective promotion of physiological metabolism in replanted *Zanthoxylum bungeanum* seedlings. The total amount of four phenolic acids (gallic acid, ferulic acid, caffeic acid, and p-hydroxybenzoic acid) significantly decreases. Therefore, it is recommended to use a compound ratio of microbial fertilizer and bio-organic fertilizer in a volume ratio of 2:1 to better address the issue of continuous cropping of *Zanthoxylum bungeanum*.

Declarations

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Author Contributions J.H. and S.Z. conceived and designed the research; S.Z and D.D .conducted the experiments; W.C. and J.H supervised the project administration; S.Z. wrote the manuscript; W.C. and B.W. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Figures

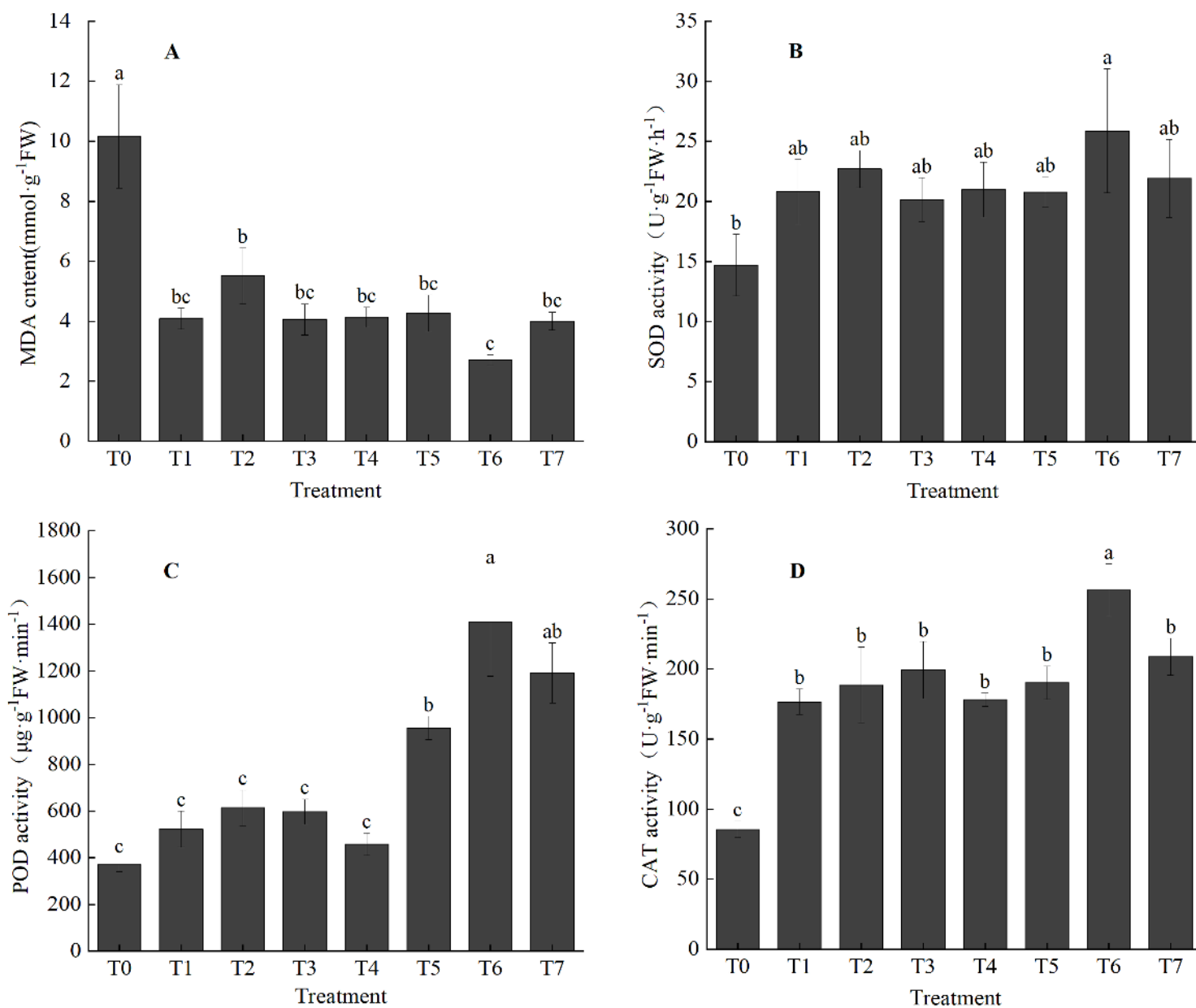


Figure 1

Effect of Two Biofertilizers on Defense Enzyme of Replanted *Zanthoxylum bungeanum*

Note: Different lowercase letters indicate significant differences between treatments.

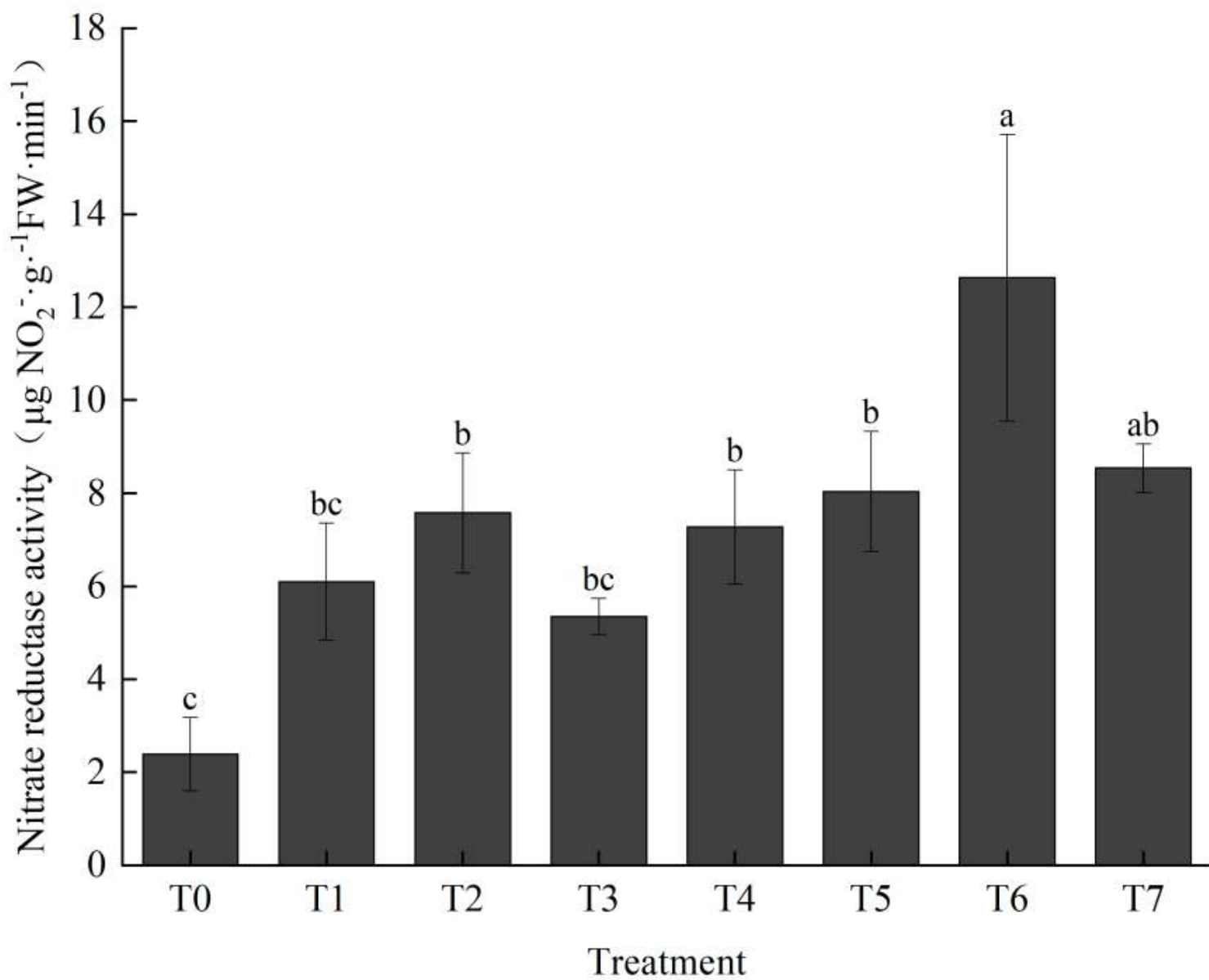


Figure 2

Effect of two biofertilizers on nitrate reductase enzyme of replanted *Zanthoxylum bungeanum*

Note: Different lowercase letters indicate significant differences between treatments.

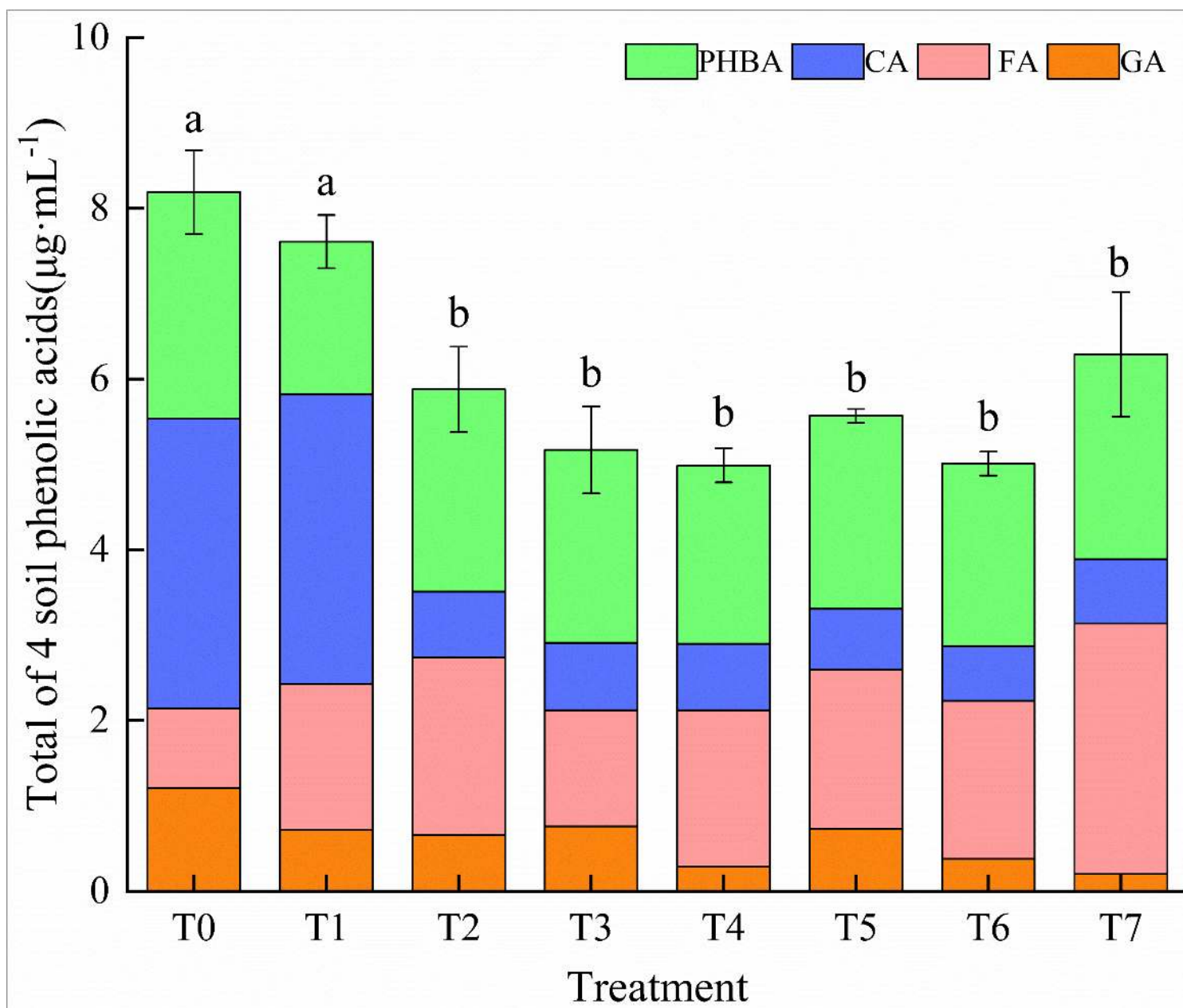


Figure 3

Effect of two biofertilizers on soil phenolic of replanted *Zanthoxylum bungeanum*

Note: Different lowercase letters indicate significant differences between treatments.

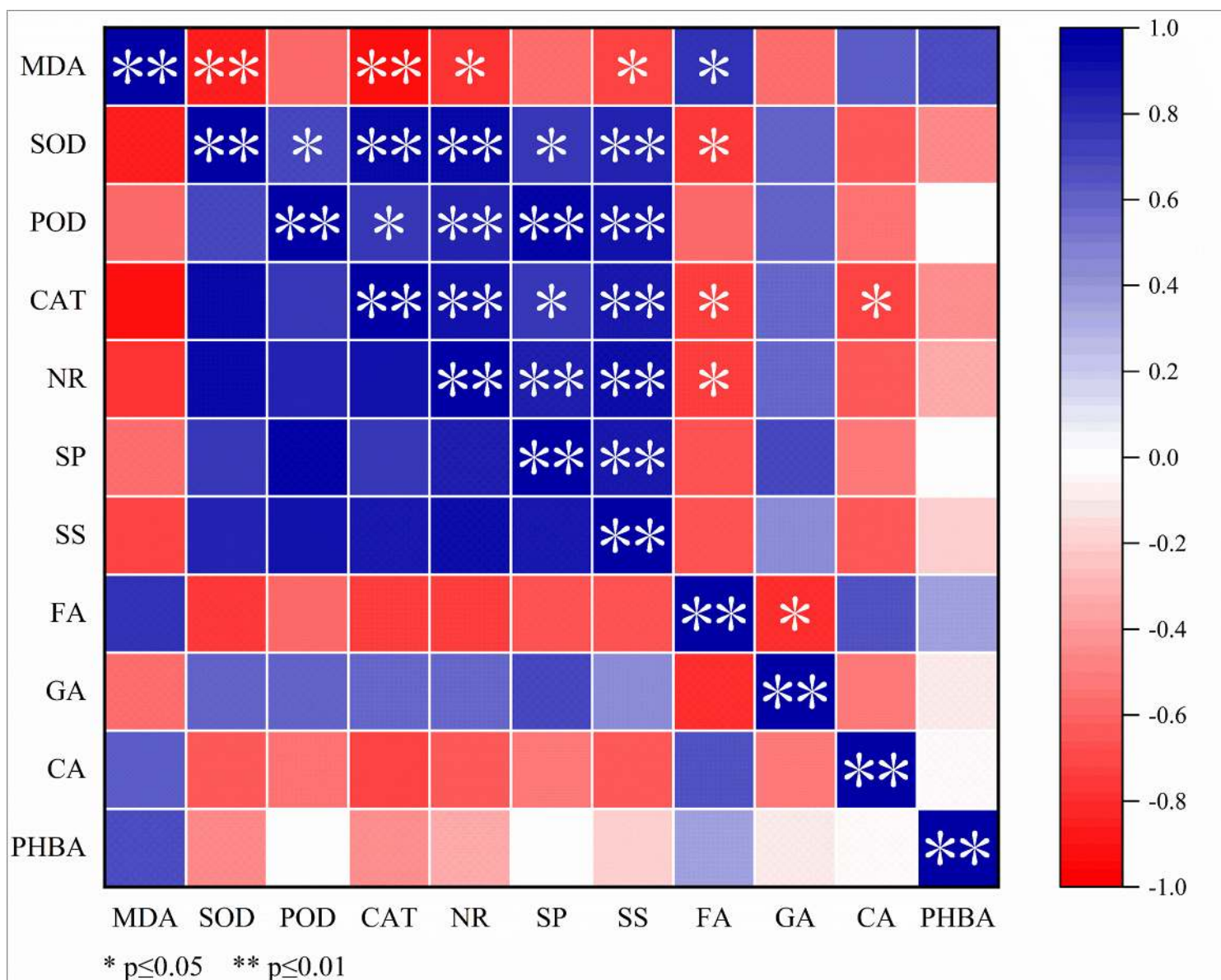


Figure 4

Correlation between biochemical indices of replanted *Zanthoxylum bungeanum* and soil phenolic acids