

Short communication

## Identification of general features in soil fungal communities modulated by phenolic acids

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### ARTICLE INFO

#### Keywords:

Allelochemical  
Microbiome  
Assembly mechanism  
Fungal diversity

### ABSTRACT

Phenolic acids are mainly released from plant residue decomposition and play important roles in the assembly of the soil microbiome. Understanding the general features of soil microbial communities modulated by phenolic acids could provide fundamental insights into the assembly of the soil microbiome. We analyzed the effects of four phenolic acids (ferulic acid, phthalic acid, salicylic acid and tannic acid) on the soil fungal communities at two concentrations. Despite the application of different phenolic acids to the soil, we were able to identify certain general changes in the fungal communities. We found that the tested phenolic acids significantly increased the deterministic assembly process of the fungal community but decreased the fungal diversity. Moreover, the fungal community structure under each tested phenolic acid treatment was distinct at low concentrations but similar at high concentrations. Salicylic acid had the greatest impacts on the fungal community. In addition, *Fusarium* and *Aspergillus* were significantly enriched in the soil amended with all the tested phenolic acids at high concentrations. Our study revealed certain general changes in the soil fungal communities modulated by phenolic acids, which deepened our understanding of the fungal assembly mechanism and provided robust insights for identifying candidate phenolic acid-degrading microbes.

### 1. Introduction

Phenolic acids are mainly released from plant residue decomposition and not only affect plant growth but also the soil microbiome (Liu et al., 2017; Xie et al., 2018). In particular, the enrichment of phenolic acids in soil increased the abundances of fungal soil-borne pathogens (Tian et al., 2015). To relieve continuous cropping obstacle, certain microbes, such as *Paraburkholderia madseniana* (Wilhelm et al., 2021) and *Pseudomonas nitroreducens* (Zhang et al., 2010), that can efficiently degrade phenolic acids have been applied in soil. Identification of the general features of soil microbial communities modulated by phenolic acids could not only help decipher the community assembly processes but also provide the fundamental insights for identifying candidate phenolic acid-degrading

microbes.

Some studies have reported that phenolic acids can significantly change the soil microbial community composition (Liu et al., 2017; Tian et al., 2015; Zhou et al., 2018). For example, the relative abundance of *Bionectria* and *Trichoderma* was significantly increased, whereas the relative abundance of *Metarhizium* was reduced in the soil amended with benzoic acid (Liu et al., 2017). However, previous studies have focused on the effects of individual phenolic acids or concentrations on the soil microbiome (Liu et al., 2017; Zhou et al., 2018). Whether we could identify the general changes in the soil microbiome modulated by phenolic acids is still poorly known.

Generally, fungi have a higher level of enzymes secretion that can effectively degrade phenolic acids compared to bacteria (Su et al.,

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<https://doi.org/10.1016/j.apsoil.2023.104909>

Received 19 January 2023; Received in revised form 27 March 2023; Accepted 3 April 2023

Available online 11 April 2023

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2012). Furthermore, a previous study indicated that the degradation amount of phenolic acids was positively related to the abundance of the fungal community (Wang et al., 2016). Therefore, we aimed to investigate the effects of phenolic acids on the fungal community and to identify efficient phenolic acid-degrading microbes. We systematically investigated the effects of four phenolic acids at two concentrations on soil fungal communities. We found that the application of different phenolic acids in the soil resulted in changes in the fungal communities. In addition, the dominant fungi, *Fusarium* and *Aspergillus*, were significantly enriched under all tested phenolic acids, providing valuable insights for identifying potential phenolic acid-degrading microbes.

## 2. Materials and methods

### 2.1. Determination of the fungal taxa affected by the four phenolic acids in the soil

The experimental soil information was listed in Supplementary Material. The chemical properties of the tested soil were shown in Table S1. Four different phenolic acids (ferulic acid, phthalic acid, salicylic acid and tannic acid) at two concentrations were added to the soil in 100-ml bottles. The phenolic acids and concentrations were chosen based on previous studies (Table S2). In our study, the low and high concentrations of phenolic acids were  $0.06 \text{ mg g}^{-1}$  of soil and  $0.3 \text{ mg g}^{-1}$  of soil, respectively. The detailed processes of application of phenolic acids in soil were shown in the Supplementary Material. Soil that was not amended with phenolic acids was used as the control. The soil moisture was adjusted to 45 % of the soil capacity. All treatments were replicated 5 times. All bottles were incubated at  $28 \text{ }^{\circ}\text{C}$ . Three samples were randomly taken on the seventh day and then stored at  $-80 \text{ }^{\circ}\text{C}$ .

### 2.2. DNA extraction and sequencing

Total genomic DNA was isolated from the tested soils. For each sample, 0.25 g of soil was used to extract DNA using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer's protocol. The microbial community composition was characterized via MiSeq amplicon sequencing. The PCR primers and processing of the sequencing data were shown in the Supplementary Material.

### 2.3. Statistical analyses

The fungal community diversity indices Chao richness and Shannon diversity were calculated from the rarefied fungal OTU table (operational taxonomic unit) using Mothur software. The following analyses of fungal community were performed in R 4.2.2. Principal coordinate analysis (PCoA), the significant changes in the relative abundances of genera (top 50), normalized stochasticity ratio index (NST index), the associations between NST index and Shannon diversity were analyzed by vegan, DESeq, NST, and R basic package. The detailed statistical analyses were shown in the Supplementary Material.

## 3. Results

### 3.1. Phenolic acids decreased fungal diversity

We found that the application of ferulic acid and salicylic acid significantly decreased the fungal Shannon diversity (Fig. 1). The application of phthalic acid and tannic acid also slightly decreased the fungal Shannon diversity. In addition to tannic acid, the Shannon diversity was higher in the soil amended the tested phenolic acids at low concentration than that at high concentration. Moreover, the greatest decrease (approximately 1.23-fold compared with the control) in fungal Shannon diversity was found in the soil amended with salicylic acid at high concentration. Similar results were also found for the Chao index

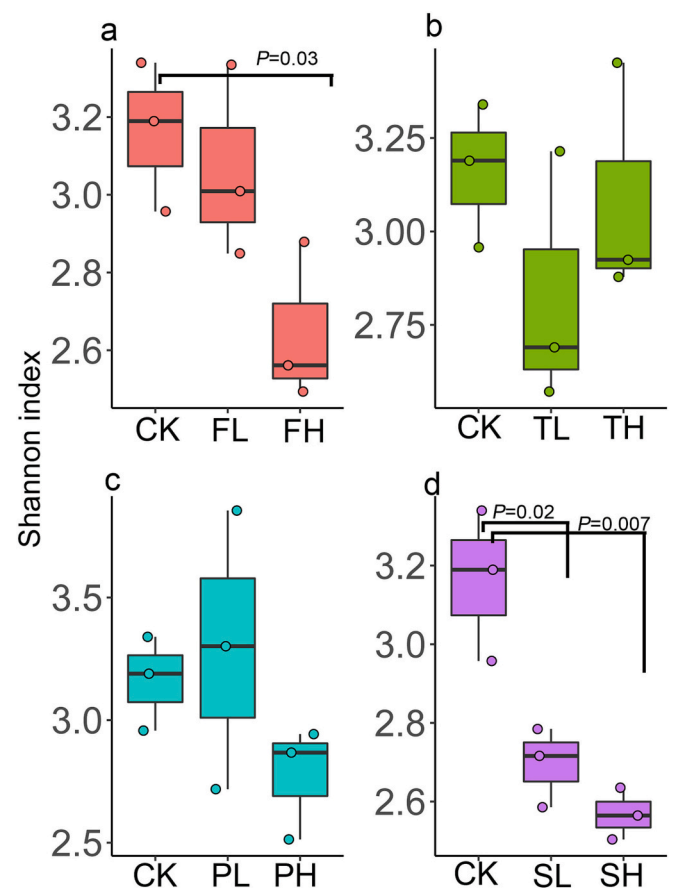


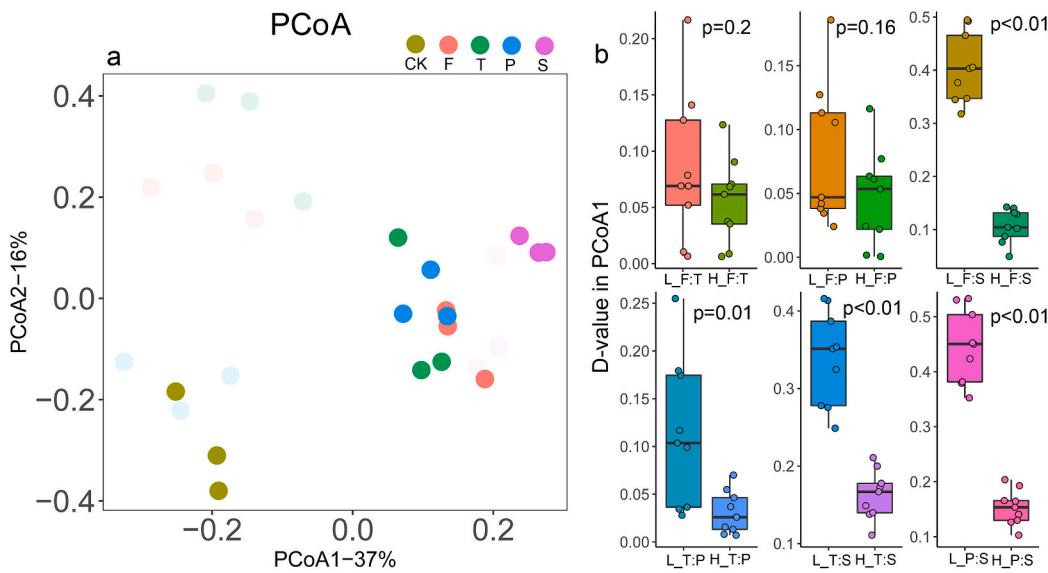
Fig. 1. Fungal Shannon diversity in the soil amended with phenolic acids. a, b, c and d correspond to ferulic acid, tannic acid, phthalic acid and salicylic acid, respectively. CK, F, T, P and S refer to control, ferulic acid, tannic acid, phthalic acid and salicylic acid, respectively. L and H refer to low and high concentrations, respectively.

(Fig. S1).

### 3.2. Phenolic acids changed the fungal community structure and increased the deterministic assembly process

We next determined the general changes in the fungal community structure in the soil amended with phenolic acids (Fig. 2). The results showed that all tested phenolic acids significantly changed the fungal community structure at low or high concentrations ( $P < 0.01$ ) (Fig. S2). In addition, salicylic acid had the greatest impacts on the fungal community structure. Moreover, the fungal community structure of the different phenolic acids differed at low concentrations. However, the fungal community structures of all tested phenolic acids were similar at high concentrations (Fig. 2a). To support the result, we compared the different values of PCoA1 in each compared phenolic acid treatment between the low and high concentrations. In addition to the different values of PCoA1 between ferulic acid and phthalic acid, the different values of PCoA1 between each compared phenolic acid at low concentration was higher than that at high concentration (Fig. 2b). Similar results were also found for the different values of PCoA2 (Fig. S3).

We determined the normalized stochasticity ratio (NST) index of the fungal community (Fig. S4). All the tested phenolic acids significantly increased the deterministic assembly process of the fungal community. Moreover, the greatest decrease (approximately 7.8-fold compared with the control) in the NST index was found in the soil amended with salicylic acid at high concentration. Furthermore, the different values of the NST index between the control and treatments were positively related to



**Fig. 2.** Changes in fungal community structure in the soil amended with phenolic acids. (a) Principal coordinate analysis of the fungal community in the soil amended with different phenolic acids. CK, F, T, P and S refer to control, ferulic acid, tannic acid, phthalic acid and salicylic acid, respectively. Dark and light points refer to low and high concentration. (b) The different values of PCoA1 between each compared phenolic acid at low and high concentration. L and H refer to low and high concentrations, respectively.

the different values of the Shannon diversity index between the control and treatments, suggesting that the decreased fungal diversity was caused by the deterministic assembly of the fungal community.

### 3.3. Phenolic acids enriched *Fusarium* and *Aspergillus*

Due to the changes in fungal community structure, we further determined the fungal compositions in the soil amended with phenolic acids (Tables S3 and S4). Supporting the above results, the numbers of significantly changed fungi were highest in the salicylic acid treatments. At the low concentration level, the relative abundances of *Fusarium* and *Aspergillus* were only significantly increased in the salicylic acid treatments (Table S3). In addition, *Fusarium* and *Aspergillus* were significantly enriched in all tested phenolic acid treatments at high concentrations (Table 1).

## 4. Discussion

Previous studies have shown the impacts of phenolic acids on the soil microbiome. However, most studies have focused on individual phenolic acids or concentrations (Bao et al., 2022; Chen et al., 2018; Liu et al., 2017). Here, we applied four phenolic acids at two concentrations to soil to compare their effects on the soil fungal community. We found that salicylic acid had the greatest impacts on the fungal community

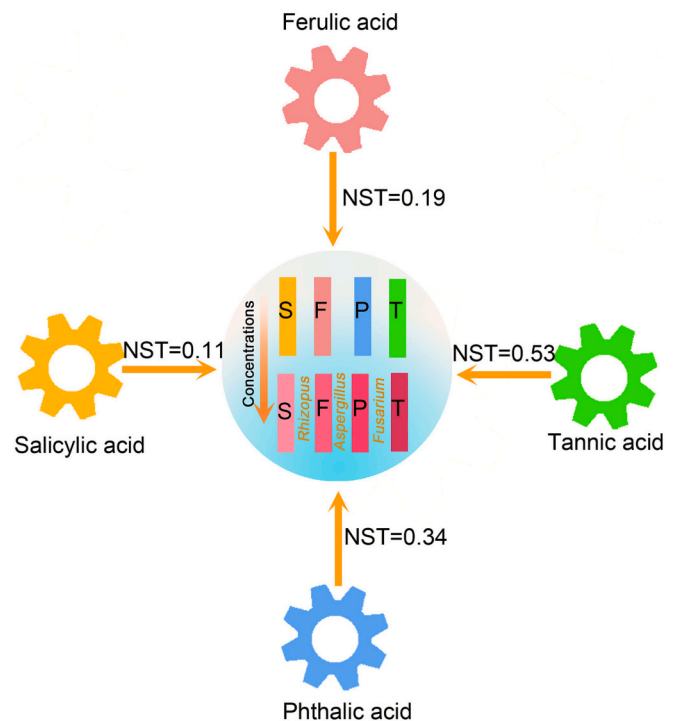
**Table 1**  
Significantly changed top 50 genera modulated by the tested phenolic acids in soil at high concentration.

Genus	CK	Ferulic	Tannic	Phthalic	Salicylic
<i>Bisifusarium</i>	0 ± 0	0.16 ± 0.14	0.11 ± 0.06	0.16 ± 0.21	0.09 ± 0.1
<i>Rhizopus</i>	0 ± 0	1.46 ± 2.33	0.18 ± 0.28	0.22 ± 0.2	0.22 ± 0.11
<i>Aspergillus</i>	0.7 ± 0.57	6.26 ± 0.51	4.41 ± 1.25	3.95 ± 1.01	9.64 ± 0.32
<i>Fusarium</i>	3.78 ± 3.87	7.44 ± 1.9	11.86 ± 2.17	17.66 ± 3	12.69 ± 1.97
<i>Rhodotorula</i>	0.02 ± 0.02	0.08 ± 0.07	0.3 ± 0.1	0.72 ± 0.24	0.11 ± 0.03
<i>Chrysosporium</i>	0.5 ± 0.23	0.04 ± 0.01	0.1 ± 0.01	0.11 ± 0.02	0.01 ± 0.01

Note: The dominant genera (relative abundances >5 %) are marked with bold type. The genera that were only significantly enriched in all the tested phenolic acid were shown.

compared to other tested phenolic acids (Figs. 2 and 3). In addition, the potential pathogen *Fusarium* was only greatly enriched under salicylic acid at low concentrations (Table S3). To support our results, a previous study showed that the combined eight phenolic acids and primary root metabolites were added in soil, salicylic acid was the only phenolic acid that stimulated a higher fungal biomass in the arable soil layer of microcosms (Clocchiatti et al., 2021). The combined information suggested that salicylic acid should be considered the primary allelochemical to be removed in soil.

We found that the fungal community structure in each phenolic acid



**Fig. 3.** Proposed model for the soil fungal communities modulated by phenolic acids. The rectangles marked with different colours represent the soil fungal communities modulated by distinct phenolic acids. The fungi between the rectangles refer to the genera that significantly enriched in all tested phenolic acids. NST refers to normalized stochasticity ratio.

treatment was distinct at the low concentrations but similar at the high concentrations (Fig. 2a). It is likely that low concentration of phenolic acids may enrich the microbes that preferentially use the corresponding phenolic acids. For example, exogenous application of cinnamic acid resulted in a different fungal community structure from that of gallic acid (Li et al., 2018). However, high concentrations may have negative effects on most microbes, but greatly enrich the microbes that have high tolerance to such concentrations. To support this, the fungal diversity at high concentrations was significantly lower than that at low concentrations (Fig. 1), suggesting that the toxic effects of high concentrations led to the disappearance of certain microbes. Moreover, the NST values at high concentrations were significantly lower than those at low concentrations. Taken together, this information suggests that the deterministic assembly processes were stronger at high concentrations, resulting in lower fungal diversity (Ning et al., 2019). Additionally, the reduced fungal diversity created more niches, which favored the enrichment of microbes with high tolerance to high concentrations, such as the plant pathogen *Fusarium*. A previous study showed that *p*-hydroxybenzoic acid, vanillic acid and ferulic acid could significantly stimulate the growth in vitro of *Fusarium graminearum*, *Fusarium langsethiae*, and *Fusarium poae* at a concentration of 100  $\mu\text{g g}^{-1}$  (Schöneberg et al., 2018). The greatly enriched *Fusarium* may contribute to the similar community structures of different phenolic acids. Overall, our results may provide reasonable information on the enrichment of *Fusarium* in soil with a high content of phenolic acids.

The application of phenolic acid-degrading microbes could relieve continuous cropping obstacle, such as *Xanthomonas* and *Rhizobium* (Zhou et al., 2020) and *Bacillus* (Duan et al., 2022) resulted in the low phenolic acid contents that contributed to soil borne disease suppression. We found that *Aspergillus* and *Fusarium* were significantly enriched in the soil amended with high concentrations of all tested phenolic acids (Table 1), indicating their potential to efficiently degrade phenolic acids (Hegde et al., 2006). *Aspergillus* and *Fusarium*, which are dominant fungi in the rhizosphere, may evolve to perform the function of degrading plant residues that contain high concentrations of phenolic acids. Although certain species of *Aspergillus* and *Fusarium* are plant pathogens, such as *Aspergillus flavus* and *Fusarium oxysporum*, some species are beneficial microbes. For example, *Aspergillus* sp. NPF7, which cannot produce aflatoxin, stimulated the growth of wheat (Pandya et al., 2018), and nonpathogenic rhizosphere strains of *Fusarium culmorum* protected rye from *Fusarium* wilt (Jarozuk-Ścisel et al., 2008). Overall, our findings provide promising insights for future improvements in the degradation of phenolic acids at high concentrations by such certain plant-beneficial microbes.

## 5. Conclusions

We obtained general results related to the effects of phenolic acids on the soil microbiome. Our results form a basis to better understand the fungal assembly mechanism in phenolic acid-rich soil and provide robust insights for identifying candidate phenolic acid-degrading microbes.

## Declaration of competing interest

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

## Data availability

Data will be made available on request.

## Acknowledgments

This work was supported by the National Natural Science Foundation of China (41571242 and 42207359) and the China Postdoctoral Science Foundation (2021M693448).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apsoil.2023.104909>.

## References

- Bao, L., Liu, Y., Ding, Y., Shang, J., Wei, Y., Tan, Y., Zi, F., 2022. Interactions between phenolic acids and microorganisms in rhizospheric soil from continuous cropping of panax notoginseng. *Front. Microbiol.* 13, 791603 <https://doi.org/10.3389/fmicb.2022.791603>.
- Chen, S., Yu, H., Zhou, X., Wu, F., 2018. Cucumber (*Cucumis sativus* L.) seedling rhizosphere trichoderma and fusarium spp. Communities altered by vanillic acid. *Front. Microbiol.* 9, 2195. <https://doi.org/10.3389/fmicb.2018.02195>.
- Clocchiatti, A., Hannula, S.E., van den Berg, M., Hundscheid, M.P.J., de Boer, W., 2021. Evaluation of phenolic root exudates as stimulants of saprotrophic fungi in the rhizosphere. *Front. Microbiol.* 12, 644046 <https://doi.org/10.3389/fmicb.2021.644046>.
- Duan, Y., Zhao, L., Jiang, W., Chen, R., Zhang, R., Chen, X., Yin, C., Mao, Z., 2022. The phlorizin-degrading bacillus licheniformis XNRB-3 mediates soil microorganisms to alleviate apple replant disease. *Front. Microbiol.* 13, 839484 <https://doi.org/10.3389/fmicb.2022.839484>.
- Hegde, S., Kavitha, S., Varadaraj, M.C., Muralikrishna, G., 2006. Degradation of cereal bran polysaccharide-phenolic acid complexes by *Aspergillus niger* CFR 1105. *Food Chem.* 96, 14–19. <https://doi.org/10.1016/j.foodchem.2005.01.050>.
- Jarozuk-Ścisel, J., Kurek, E., Winiarczyk, K., Batur, A., Łukanowski, A., 2008. Colonization of root tissues and protection against fusarium wilt of rye (*Secale cereale*) by nonpathogenic rhizosphere strains of fusarium culmorum. *Biol. Control* 45, 297–307.
- Li, Z., Fu, J., Zhou, R., Wang, D., 2018. Effects of phenolic acids from ginseng rhizosphere on soil fungi structure, richness and diversity in consecutive monoculturing of ginseng. *Saudi J. Biol. Sci.* 25, 1788–1794. <https://doi.org/10.1016/j.sjbs.2018.07.007>.
- Liu, J., Li, X., Jia, Z., Zhang, T., Wang, X., 2017. Effect of benzoic acid on soil microbial communities associated with soilborne peanut diseases. *Appl. Soil Ecol.* 110, 34–42. <https://doi.org/10.1016/j.apsoil.2016.11.001>.
- Ning, D., Deng, Y., Tiedje, J.M., Zhou, J., 2019. A general framework for quantitatively assessing ecological stochasticity. *Proc. Natl. Acad. Sci.* 116, 16892–16898. <https://doi.org/10.1073/pnas.1904623116>.
- Pandya, N.D., Desai, P.V., Jadhav, H.P., Sayyed, R.Z., 2018. Plant growth promoting potential of aspergillus sp. NPF7, isolated from wheat rhizosphere in South Gujarat India. *Environ. Sustain* 1, 245–252.
- Schöneberg, T., Kibler, K., Sulyok, M., Musa, T., Bucheli, T.D., Mascher, F., Bertossa, M., Voegelé, R.T., Vogelgsang, S., 2018. Can plant phenolic compounds reduce *Fusarium* growth and mycotoxin production in cereals? *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 35, 2455–2470. <https://doi.org/10.1080/19440049.2018.1538570>.
- Su, X., Schmitz, G., Zhang, M., Mackie, R.I., Cann, I.K., 2012. Heterologous gene expression in filamentous fungi. *Adv. Appl. Microbiol.* 81, 1–61.
- Tian, G., Bi, Y., Sun, Z., Zhang, L., 2015. Phenolic acids in the plow layer soil of strawberry fields and their effects on the occurrence of strawberry anthracnose. *Eur. J. Plant Pathol.* 143, 581–594. <https://doi.org/10.1007/s10658-015-0711-y>.
- Wang, Y., Li, C., Wang, Q., Wang, H., Duan, B., Zhang, G., 2016. Environmental behaviors of phenolic acids dominated their rhizodeposition in boreal poplar plantation forest soils. *J. Soils Sediments* 16, 1858–1870.
- Wilhelm, R.C., DeRito, C.M., Shapleigh, J.P., Madsen, E.L., Buckley, D.H., 2021. Phenolic acid-degrading paraburkholderia prime decomposition in forest soil. *ISME Commun* 1, 4. <https://doi.org/10.1038/s43705-021-00009-z>.
- Xie, D., Zhang, G., Xia, X., Lang, Y., Zhang, S., 2018. The effects of phenolic acids on the photosynthetic characteristics and growth of *Populus euramericana* cv. 'Neva' seedlings. *Photosynthetica* 56, 981–988. <https://doi.org/10.1007/s11099-018-0818-4>.
- Zhang, Z.-Y., Pan, L.-P., Li, H.-H., 2010. Isolation, identification and characterization of soil microbes which degrade phenolic allelochemicals. *J. Appl. Microbiol.* 108, 1839–1849. <https://doi.org/10.1111/j.1365-2672.2009.04589.x>.
- Zhou, X., Zhang, J., Pan, D., Ge, X., Jin, X., Chen, S., Wu, F., 2018. *p*-coumaric can alter the composition of cucumber rhizosphere microbial communities and induce negative plant-microbial interactions. *Biol. Fertil. Soils* 54, 363–372. <https://doi.org/10.1007/s00374-018-1265-x>.
- Zhou, C., Ma, Z., Lu, X., Zhu, L., Wang, J., 2020. Phenolic acid-degrading consortia increase fusarium wilt disease resistance of chrysanthemum. *Agronomy* 10, 385. <https://doi.org/10.3390/agronomy10030385>.