Folia Forestalia Polonica, Series A – Forestry, 2024, Vol. 66 (3), 270–284

SHORT COMMUNICATION

DOI: 10.2478/ffp-2024-0020

Differences in phenolic acids in soil substrates of forest deciduous tree species

Michal Jakl[1a] ✉, *Ivan Kuneš*[2b], *Sanja Ćavar Zeljković*[3,4c], *Petr Tarkowski*[3,4d], *Jana Jaklová Dytrtová*[2e]

[1] Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Agroenvironmental Chemistry and Plant Nutrition, Kamýcká 129, 165 00 Prague–Suchdol, Czech Republic, e-mail: m.jakl@chemist.com

[2] Czech University of Life Sciences Prague, Faculty of Forestry and Wood Sciences, Department of Silviculture, Kamýcká 129, 165 00 Prague–Suchdol, Czech Republic

[3] Centre of the Region Haná for Biotechnological and Agricultural Research, Medicinal and Special Plants, Crop Research Institute, Department of Genetic Resources for Vegetables, Šlechtitelů 29, 783 71 Olomouc, Czech Republic

[4] Palacký University, Czech Advanced Technology and Research Institute, Šlechtitelů 27, 783 71 Olomouc, Czech Republic

ORCID ID: a – https://orcid.org/0000-0002-3072-1226; b – https://orcid.org/0000-0002-1875-384X; c – https://orcid.org/0000-0002-8534-3146; d – https://orcid.org/0000-0002-7581-7686; e – https://orcid.org/0000-0002-6024-8679

ABSTRACT

Currently, reforestation efforts focus primarily on deciduous species replacing coniferous monocultures. Related to this are efforts to identify their interactions in the soil. Root exudation has many functions, including plant communication with soil microorganisms and the solubilisation of nutrients. Root exudates reflect the situation in the soil, as well as refer to the plant species and health/fitness. The idea here was to reveal the typical content of low-molecular-weight phenolic acids in the soil solution that occur in the early-life stages of most typical temperate deciduous trees *Quercus robur*, *Sorbus torminalis*, *Fagus sylvatica* and *Acer pseudoplatanus*, including the fast-growing invasive *Robinia pseudoacacia*. To compare their initial strategies and ambient, seedlings were planted in the pot experiment for one season since emergence. The following phenolic acids were detected in detectable concentrations: vanillic, 4-benzoic, syringic, *p*-coumaric and salicylic. Each tree species tested showed a unique fingerprint in these acids, which can be considered species-specific, i.e., their presence differed among the species. *Robinia pseudoacacia* (unlike the other trees tested) showed the ability to maintain high levels of *p*-coumaric acid in the soil solution, indicating its potential to survive in nutrient-poor soil and achieve rapid growth. On the contrary, the levels of all phenolic acids detected in the soil solution of *Quercus robur* and *Sorbus torminalis* were very low. These fingerprints should be extended to other compounds and also to older trees.

KEY WORDS

LMWOAs, soil environment, soil solution, tree exudation, tree seedlings

Received 28 December 2023 / Accepted 6 May 2024

INTRODUCTION

Root exudation (mostly located at the root tips) belongs to one of the strategies of how the plant may affect the soil environment (Kaiser et al. 2002; Vives-Peris et al. 2020). The root tip gets in contact with the soil environment and reacts to various chemical, biological and physical stimuli. In addition to dissolved mineral substances, the soil solution also contains many soluble organic compounds (dissolved organic carbon – fraction smaller than 0.45 µm), which get here mainly *via* the plant exudation, microbial activity and such mediated decomposition of soil organic matter (Herbert and Bertsch 1995). Organic plant exudates can be divided into primary metabolites (sugars, polysaccharides, and organic and amino acids) and secondary metabolites (terpenes, phenolics, alkaloids, etc.), enzymes (Wen et al. 2007a), defence substances (Baetz and Martinoia 2014), proteins (Wen et al. 2007b), and extracellular DNA (Wen et al. 2009). The most important chemical groups of root exudates are summarised in Figure 1 (Vives-Peris et al. 2020).

Root exudation is frequently associated with the mobilisation of soil nutrients based on the exudation of low-molecular-weight organic acids (LMWOAs) into the rhizosphere or with the exudation of chemicals supporting rhizosphere symbiosis with soil microorganisms or fungi and last but not least chemicals affecting soil environment as a strategy of interspecific competition (Bergmann et al. 2020; Canarini et al. 2019; Sanon et al. 2009; Vives-Peris et al. 2020). LMWOAs are a relatively large group of compounds containing the carboxylic group (-COOH) that has a maximum molecular weight of 300 g/mol (Herbert and Bertsch 1995). Their character and therefore the behaviour in the soil (environment) are strongly affected by other functional parts of the molecule. LMWOAs can create chelates with nutrient/hazardous elements (Adeleke et al. 2017; Jaklová Dytrtová et al. 2012) and in this way can mobilise (dissolve) the elements and potentially influence their bioavailability. Consequently, also plant interactions with microorganisms play a crucial role (Canarini et al. 2019; Sasse et al. 2018). Root exudation can be understood as a process that drives organic metabolites to the root and ensures their exudation to the soil. Once the exudate is in the apoplast, its release into the soil environment is driven by diffusion (controlled by the concentration gradient). It seems that microorganisms play a role in the adjustment of the concentration gradient. Microorganisms can consume the plant exudates and thus maintain a high concentration gradient (Figure 2. Illustration of the transport of organic acids through the last plasma membrane (the apoplastic transport) and its control by the concentration gradient). In this manner, the diffusion would regulate the flux of exudates into the soil. However, this mechanism works only in the absence of any physical obstacle, which can differentiate the distance from the meristem to the differentiation zone (Somssich et al. 2016).

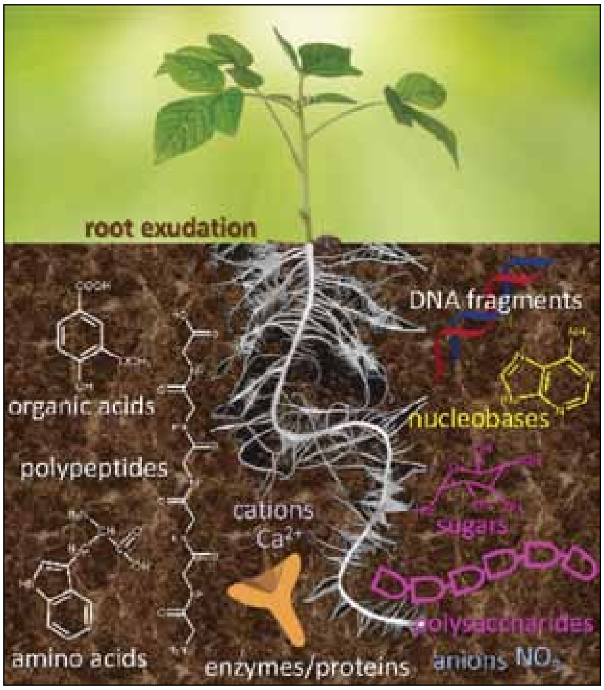

Figure 1. Overview of the most important chemical groups of root exudates (not scaled)

In particular, the smallest aliphatic molecules, whose production and importance within the soil and plant are indisputable (Jones 1998), have been mentioned above. In this study, we focus on LMWOAs, which contain a benzene ring in the molecule and at least one hydroxyl (-OH) group (Fig. 3), that is, phenolic acids.

These compounds are probably mostly produced by plant roots (as a secondary metabolite) and soil microorganisms, and they affect the regulation of root growth (Strobel 2001). These structures are expected to be effective antioxidants. Their OH groups make them reactive, and the benzene ring allows the delocalisation of accepted free electrons, and consequently, freeelectron radicals can be quenched. The LMWOAs with the aromatic ring may differ in their effects on plants and microorganisms. It appears that the plant is able to adjust the activity of symbiotic microorganisms in this way depending on the current conditions (e.g., drought) and the nutrient needs of the plant (de Vries et al. 2019; Oppenheimer-Shaanan et al. 2022).

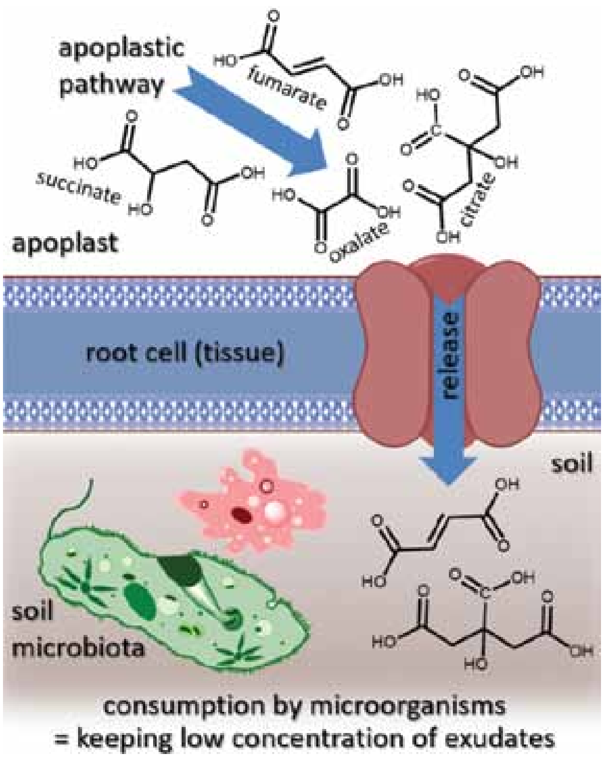

Figure 2. Illustration of the transport of organic acids through the last plasma membrane (the apoplastic transport) and its control by the concentration gradient

Namely, salicylic acid plays a decisive role in the mechanisms of tolerance against abiotic stress that controls the main plant metabolic processes (Khan et al. 2015). In some deciduous trees, it increases tolerance to salinity and hazardous elements by protecting membrane integrity (Drzewiecka et al. 2019; Rao et al. 2019). Salicylic acid also participates in growth, development, photosynthesis, transpiration, ion uptake and transport (Vlot et al. 2009); therefore, it is also classified as a phytohormone.

There is a very limited amount of information on the precise action of LMWOAs with aromatic rings and a hydroxyl group. From the chemical structure, a high reactivity and antioxidant capacity can be estimated. On the other hand, it was described (Jaklová Dytrtová et al. 2018) that the presence of metal cations (copper, iron and others) can change the antioxidant properties of some compounds to pro-oxidant. In soils, copper and iron are present in a wide range of concentrations and forms. Therefore, some interactions can be expected. It is disputable whether the high content of these LMWOAs with redox properties is advantageous for plants. In the presence of copper or iron cations, they can destroy root cell structures *via* the production of free radicals in the Fenton (Haber-Weiss) reaction.

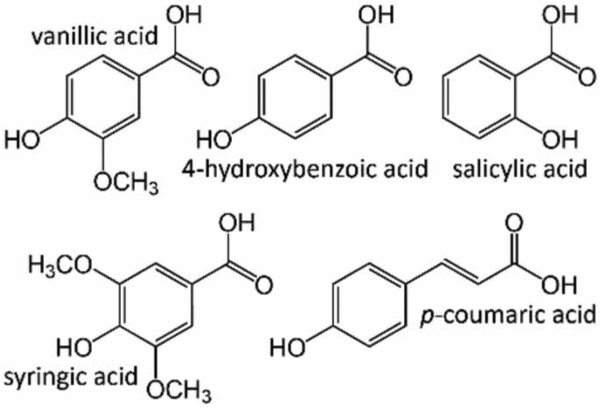

Figure 3. Chemical structures of phenolic acids detected and analysed in our study

For forest tree species, at least those that grow in Europe, the information on phenolic acids is even less than for crops. Therefore, we selected five species of deciduous forest trees to focus on this issue from a European perspective. At lower elevations of European temperate forests, English oak (*Quercus robur*) is typically the dominant tree species, whereas the wild service tree (*Sorbus torminalis*) is a representative of admixed (associated) trees in the species composition. At the mid to higher elevations of European temperate forests, European beech (*Fagus sylvatica*) is typically the dominant or co-dominant species, and sycamore maple (*Acer pseudoplatanus*) is mainly the admixed (associated) tree in the species composition. In addition to the four native European trees listed above, we also included the non-native North American species black locust (*Robinia pseudoacacia*) in our study. Back locust is considered an invasive tree species in many countries in Europe and elsewhere in the world (Richardson and Rejmánek 2011; Vítková et al. 2017), possibly (Bartha et al. 2008; Nasir et al. 2005) capable of allelopathically influencing neighbouring trees in forest stands through its metabolites.

This study aimed to monitor the contents of selected low-molecular-weight organic acids with potentially redox properties in soil solution sampled from different tree species (*Robinia pseudoacacia*, *Quercus robur*, *Sorbus torminalis*, *Fagus sylvatica* and *Acer pseudoplatanus*). We expect that the phenolic compounds found in the soil solution collected from *Robinia pseudoacatia* will differ in their composition and concentration from other cultivated species.

MATERIAL AND METHODS

Pot experiment

The trees were grown from seeds on the growth substrate composed of fertilised peat, conifer bark compost and trace elements in a greenhouse from March 10 (2021; Prague, Czech Republic). On June 21, the seedlings were transplanted in triplicates into 1 L pots and moved to the vegetation hall (temperature equal to outdoors, i.e., in temperate summer conditions, under controlled precipitation and shading). In the vegetation hall, the control (soil substrate without plants) was also installed in three replicates. The trees were watered with demineralised water to avoid increasing the number of minerals from the tap water. The scheme of the experiment is depicted in Figure 4.

Selected pedochemical parameters of the growth substrate are described in Tables 1–3. The pseudototal content of selected elements in the substrate was determined after decomposition of the dried, ground (<2 mm) and homogenised sample in Aqua regia heated in a microwave-assisted wet digestion system Ethos 1 (MLS GmbH, Germany) for 33 min at 210 °C. For the water-soluble and bioavailable fractions, the samples were extracted in 1:10 mixtures with demineralised water and appropriate CAT agent (0.01 mol/L $CaCl_2$

Table 1. Active and exchangeable pH and electrical conductance (**κ**) of the substrate (1:10 (w/v); mean ± st. dev., n = 3)

pH(H_2O)	pH(KCl)	κ (mS/cm)
5.17±0.00	4.65±0.00	0.955±0.005

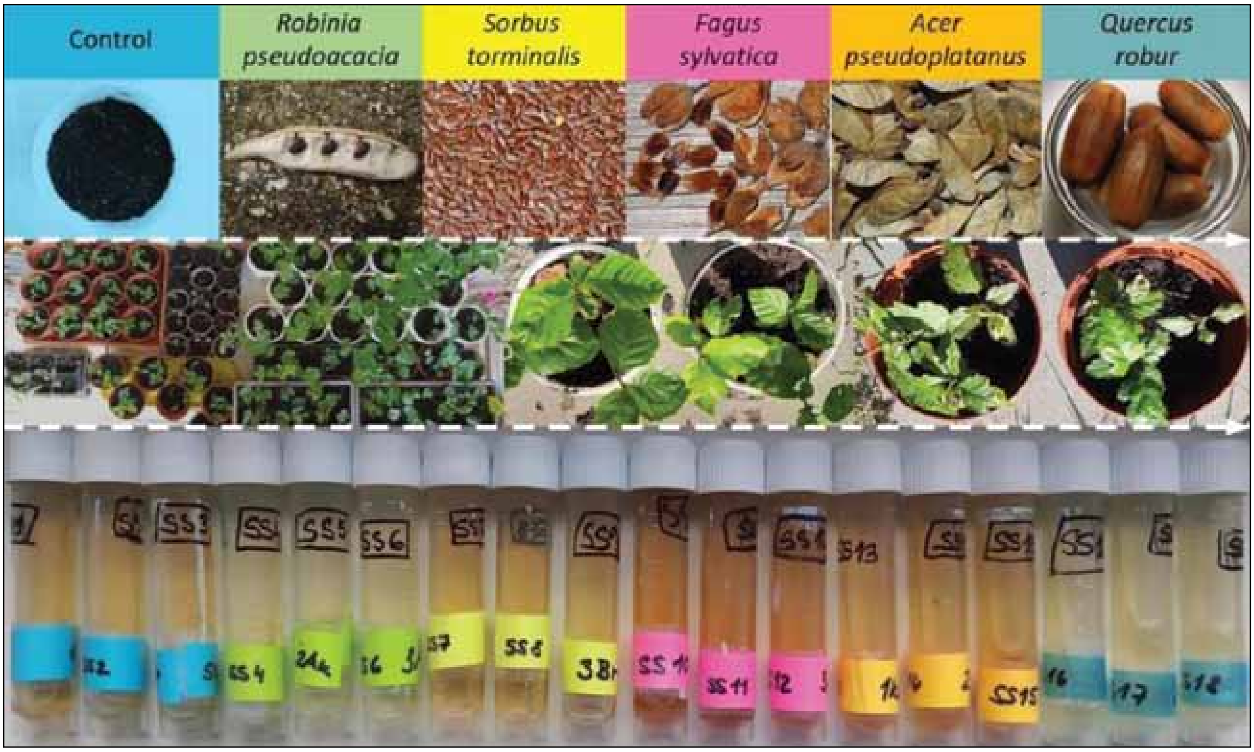

Figure 4. Basic scheme of the experiment from each seed through all seedlings in small and experimental pots (chronologically in the central row) to the soil solution

Table 2. Total, bioavailable (CAT) and water-soluble fractions of selected elements in the substrate (mg/kg dry wt.; mean ± st. dev., n = 3)

Fraction	K	Ca	Mg	P	Zn	Fe	Mn	Cu
Total	2321±48	9655±103	1748±42	872±11	74.1±2.8	2089±25	462.5±8.6	6.03±0.21
CAT	1272±4	–	717±6	585±19	82.5±3.7	508±14	407.4±6.7	2.02±0.42
H_2O	808±8	225±6	93±3	572±5	0.952±0.007	6.14±0.00	7.15±0.04	0.188±0.026

Table 3. Content of mineral nitrogen forms, total organic carbon (TOC) and humic substances (HS), the degree of polymerisation (DP) and the humification index (HI) of the dry substrate (mean ± st. dev.; n = 3)

$N\text{-}NH_4$ (mg/kg)	$N\text{-}NO_3$ (mg/kg)	TOC (% C)	HS (% C)	DP (HA : FA)	HI (HA : TOC)
1421.5±2.6	36.6±5.2	13.4±0.3	6.14±0.03	1.80±0.06	0.231

and 0.002 mol/L DTPA, according to EN 13651:2001 standard (Committee for European Standardization)), respectively, for 1 hour and filtered. All these fractions of selected elements were determined using optical emission spectrometry with inductively coupled plasma (Agilent 720, Agilent Technologies Inc., USA), and only the fractions of potassium were (for higher precision) determined using flame atomic absorption spectrometry (Varian 280FS, Varian, Australia). The content of mineral nitrogen (ammonia and nitrate form) was determined in the extract of 0.01 mol/L $CaCl_2$ using the method based on Kjeldahl on VAPODEST 50s device (Gerhardt, Germany), using Devarda's alloy for $N\text{-}NH_4$ form. The amount of oxidisable carbon was determined based on its oxidation using potassium dichromate in an acidic environment of sulphuric acid, followed by the determination of the amount of reduced Cr^{3+} spectrophotometrically (Lambda 25 UV/Vis, PerkinElmer, USA). Humic substances and their ratios were determined using the methodology mentioned in Sedlář et al. (2023).

The substrate can be characterised as moderately acidic with an adequate soil reaction to grow seedlings of forest trees. The content of selected nutrients in the substrate, namely, P, Ca and Mg, is also on an adequate to a luxurious level. The content of potentially hazardous Mn and Cu is low and that of Zn is at a well-acceptable level (Kopinga and Van den Burg 1995; Landis et al. 1989; Šrámek et al. 2004).

Sampling and analysis of phenolic acids

The soil solution was sampled from the pots with a growing seedling of *Robinia pseudoacacia* (RP), *Acer pseudoplatanus* (AP), *Quercus robur* (QR), *Fagus sylvatica* (FS), *Sorbus torminalis* (ST), and control (soil substrate only). On September 22 (at the end of the growing season before the leaves began to senesce), the soil solutions were obtained from each pot (one pot – one sample) after 24-hour saturation of the substrate with deionised water to 100% water holding capacity, sucked off using a 0.22 μm suction filter. The soil solutions were immediately frozen at –20°C and delivered for analysis. Exactly 5 mL of soil solution was lyophilised (Christ Beta 1-8 LOplus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and remained solid was dissolved in 500 μL of milli-Q water where the content of phenolic compounds was analysed.

LCMS analysis of the exudates was performed on Nexera X2 UHPLC (Shimadzu Handels GmbH) coupled with an MS-8050 (Shimadzu Handels GmbH). Chromatographic separation was performed on a UHPLC Acquity BEH C18 (50 × 2.1 mm; 1.7 μm particle size) column (Waters Corp., Milford, MA, USA) that was kept at 40 °C. The mobile phase consisted of 15 mmol/L formic acid in water, pH 3 (adjusted with NH_4OH) (component A) and acetonitrile (component B). Totally, 32 analytes were separated using a binary gradient starting at 5% B for 0.7 min, 5–15% B for the next 1.3 min, 15% B isocratic for 1 min, 15–30% B for 1.5 min, 30% B isocratic for 1.5 min, 30–70% B for next 1.5 min, 70–100% B for 0.1 min, 100% isocratic for 0.5 min, back to 5% B within 0.1 min, and equilibration for 3.8 min. The flow rate was 0.4 mL/min, and the injection volume was 2 μL.

All analytes were analysed in an electrospray negative ionisation mode (ESI^-) using a multiple reaction monitoring (MRM) mode for their identification and quantification (Jakl *et al.* 2021). Standard solutions of 32 target compounds (2,3-dihydroxybenzoic acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, 5-hydroxyferulic acid, apigenin, caffeic acid, catechin, chlorogenic acid, chrysin, ferulic acid, galangin, gallic acid, hesperidin, kaempferol, luteolin, methyl *p*-coumarate, morin, myricetin, naringenin, naringin, *p*-coumaric acid, pinocembrin, quercetin, quercitrin, rosmarinic acid, rutin, salicylic acid, salicylic acid 2-O-*β*-D-glucoside, sinapic acid, syringic acid, *trans*-cinnamic acid and vanillic acid), purchased from Sigma-Aldrich (Czech Republic), were firstly prepared in methanol at 1 mmol/L concentration. All solutions were gradually diluted in the mobile phase to working concentrations ranging from 0.01 to 50 µmol/L. Quantification was performed by isotope diluting method with *p*-coumaric acid-d_6 and salicylic acid-d_4 (Toronto Research Chemicals, Canada) as internal standards.

Statistical analysis

The results of the analyses performed in three replicates and two technical repeats were statistically processed by one-way ANOVA (Tukey method), t-test, and MANOVA using the statistical software TIBCO Statistica (v.14; Statistica.pro) and by Pearson Product-Moment Correlation using SigmaPlot (v.12.5; Systat Software Inc.). The differences were considered statistically significant at $P \leq 0.05$.

RESULTS

In detectable amounts (LOD ~ µmol/kg lyophilisate), five phenolic acids were found: 4-hydroxybenzoic acid, vanillic acid, syringic acid, *p*-coumaric acid and salicylic acid (Fig. 5). The others (listed in the experimental section) have not been found in detectable amounts.

Vanillic acid was found in the soil solution from its highest content in the control, followed by (*RP*) > (AP) = (*QR*) > (FS), (ST). The highest 4-hydroxybenzoic acid content was also found in the control soil solution, further followed by RP = AP > ST > FS = QR. The content of syringic acid was similar in the control, RP and FS followed by a very low content recorded in the QR and ST treatments. Furthermore, the salicylic acid content was highest in the control, further followed by QR = RP > AP = ST > FS treatments. *p*-Coumaric acid was highest in RP followed by AP = control > QR = FS > ST.

DISCUSSION

The low contents (mostly available or water-soluble) of nutrients lead to increased plant exudation (Tato et al. 2021). However, the growth substrate was prepared to have optimum chemistry that would prevent nutritional and physiological stresses, as documented in Tables 1–3. Therefore, in our experiment, the exudation stimulated by low nutrition was not expected. Additionally, other conditions (humidity, light and temperature) were set to be optimal. The aim was to record the typical contents of phenolic acid under each tree seedling. The number of individual phenolic acids that were detected varied from units (*p*-coumaric acid) to higher tens of pmol (vanillic acid) in 1 mg of the lyophilised soil solution. These findings reflect the no-stress situation for the one-year-old seedlings – the baseline.

In the lyophilised soil solutions, only 4-hydroxybenzoic acid, vanillic acid, syringic acid, *p*-coumaric acid and salicylic acid were found in detectable amounts. In the control, the concentrations of vanillic acid, 4-hydroxybenzoic acid and salicylic acid were significantly higher than those in the variants of the cultivated trees (Tab. SI1). This finding supports the assumption that nutrient stress-induced exudation was not expected due to sufficient nutrient balance in the soil substrate.

Vanillic acid belongs to one of the curcumin metabolites and is known for its antioxidant effect (Sharma et al. 2022). Its content was the highest among the assessed acids in all treatments, including the control. In the control, the content also reached the highest value among the compared treatments. In the soil solution taken from the seedlings, its content was 3 times (RP) to 15 times (ST) lower than that in the control. From this point of view, it could be suggested that its occurrence was mainly caused by microbial activity related to the degradation of biomass (lignin as a part of the substrate). Therefore, it is expected that vanillic acid created from biomass decomposition can be in the pots with tree seedlings converted (and spread) to a variety of C4 dicarboxylic acids, such as succinic, malic, maleic and fumaric acid, whose individual concentrations were below our detection limit (Vega-Aguilar et al. 2020).

4-Hydroxybenzoic acid is a phenolic derivative of benzoic acid with a potential antioxidant (anti-inflammatory) effect (Taofiq et al. 2017). It is slightly soluble in water and more soluble in polar organic solvents. Its

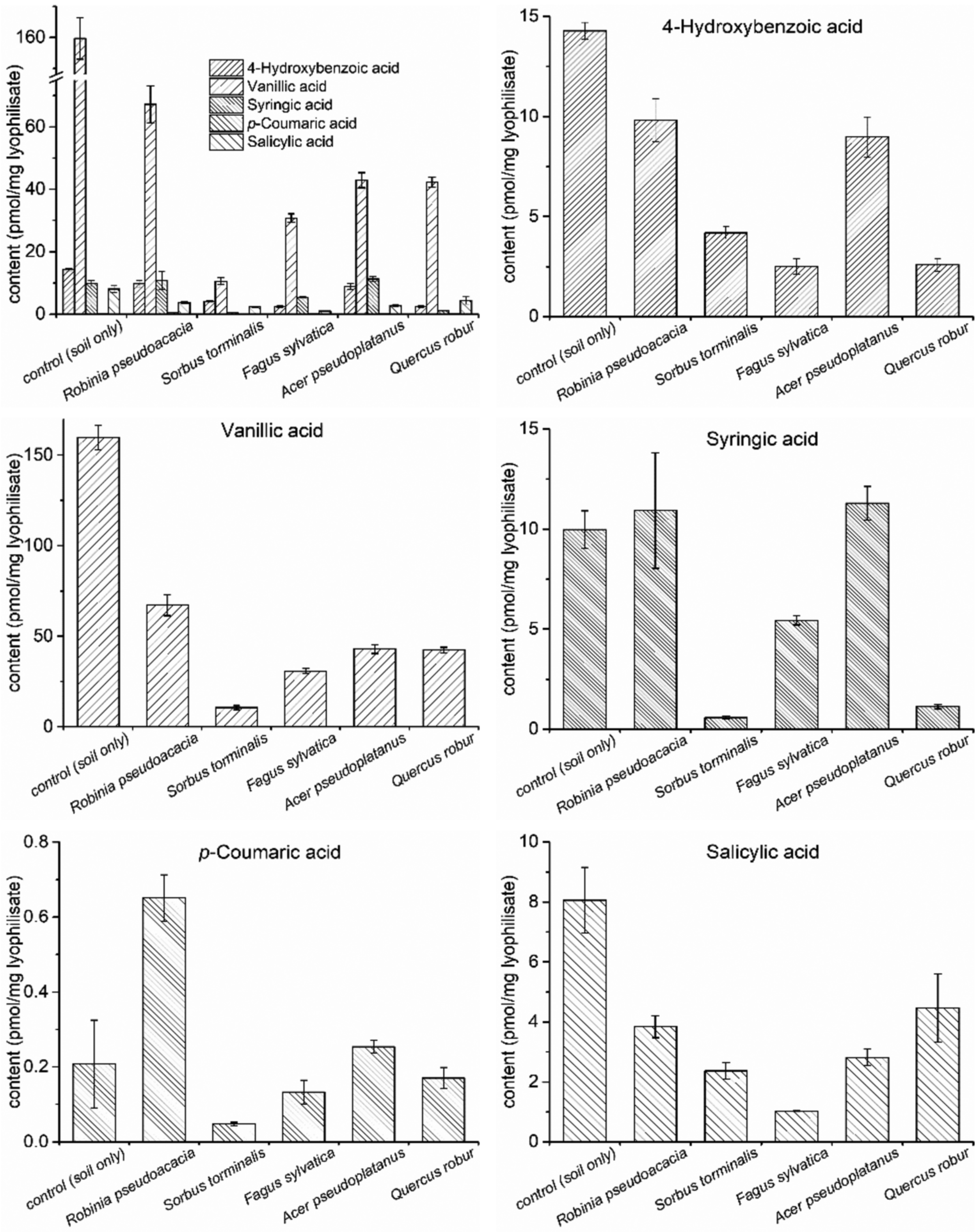

Figure 5. Content of selected phenolic acids in the soil solution of focused trees (n = 3, mean ± st. dev.)

isomer (2-hydroxybenzoic acid) is known as salicylic acid. Salicylic acid is known for its protective antifungal/anti-inflammatory effect (Bi et al. 2022). Both acids occurred in the studied soil solutions in a similar ratio. The 4-hydroxybenzoic acid content was roughly twice as high as salicylic acid, and this is the case for variants except for QR. Research results (Pizzeghello et al. 2006) have confirmed the positive effect of this acid on root growth. Its presence in the soil solution of beech (FS) seedlings was significantly lower compared to the others (Tab. SI1). Beech belongs to slow-growing trees and its roots develop more slowly. It is therefore possible to infer a lower support for their growth due to a lower production of this acid by symbiotic microorganisms.

p-Coumaric acid is known to have a strong antioxidant effect (Kannan et al. 2013). It is well known to suppress *Fusarium oxysporum* (Were et al. 2022). It also seems that this phenolic compound is produced in the case of elevated oxidative stress. *p*-Coumaric acid is also exuded concerning higher content of copper (Achary et al. 2012) or other metals. The highest content of *p*-coumaric acid was detected in the soil solution sampled from RP. Its concentration is several times higher than that in the control and other trees (3x on average). *p*-Coumaric acid is reported in the literature as a substance with allelopathic effects on plants (Blum 1996; Han Kyaw et al. 2022; Lima et al. 2013). Therefore, we suggest that *p*-coumaric acid may play a role in the influence of the soil environment and may be related to the reported ability of RP to suppress its competitors. In an older publication (Inderjit and Mallik 1997), *p*-coumaric acid was shown to reduce soil organic matter and increase microbial activity. RP is a leguminous tree known to be capable of using symbiotic microorganisms (*Rhizobium*) to fix atmospheric nitrogen and alter the soil to increase mineral nitrogen forms and thus promote its growth (Medina-Villar et al. 2016). From this, it would be possible to conclude that this phenolic substance is purposefully produced to support symbiotic bacteria and also to mediate the release of nutrients from the soil. However, the data to support these hypotheses are non-significant; therefore, further research is needed.

Syringic acid is known to have antioxidant, antimicrobial and anti-inflammatory properties (Cheemanapalli et al. 2018). It can be found as a significant part of the phenolic exudates of tree species (Bi et al. 2022). In addition, the presence of syringic acid in the substrate indicates the presence of S-lignin (Zhao et al. 2022). Furthermore, syringic acid is demethylated to 3-O-methyl gallic acid, which is transformed into 2-pyranone-4,6-dicarboxylic acid and further converted to acetyl-CoA (Kasai et al. 2007). In the end, it enters the Krebs cycle and is metabolised into CO_2 and H_2O. The highest content of syringic acid (approx. 10 pmol/mg) can be found in the control, RP and AP soil solution. Therefore, it seems that its presence might refer to lignin degradation as well as to intense exudation by RP and AP. Its presence in the FA soil solution is twice as and in ST and QR ten times lower than in the control/RP/AP. From this result, it seems that the content of syringic acid refers to the species and may be species-specific.

Statistical analysis shows (Tab. SI2) that some of these organic acids are interdependent regardless of the tree species. Strong positive correlations between the acids were found:

4-hydroxybenzoic vs. vanillic acid (0.842) and 4-hydroxybenzoic vs. syringic acid (0.783), indicating a significant relationship where both variables increase together. Even a very strong positive correlation between vanillic and salicylic acid (0.881) was observed, indicating a very close linear relationship with high dependency. This fundamental finding of the closed model system implies that to fingerprint individual trees, mainly the concentrations and ratios of 4-hydroxybenzoic vs. *p*-coumaric acid and syringic vs. *p*-coumaric acid need to be jointly assessed. When our measured values of acid concentrations from all species are statistically compared (MANOVA), they are highly significantly different among the tree species (Tab. SI3). The determined amounts of phenolic substances represent their total amount in the soil solution. It is very difficult to distinguish the origin of these exudates; it is not possible to determine which part is formed by microbial exudation, which by decomposition of organic matter, and which is the origin of seedling root exudation. Unfortunately, even the introduction of the control variant (i.e., the soil without plants) will not help to resolve what proportion of the given exudate is of plant origin. It is known (Siqueira et al. 1991) that (as in our control variant increased) 4-hydroxybenzoic acid and vanillic acid (and many others) can originate in soil from lignin degradation, and salicylic acid derivatives (and many others) can originate from the activity of soil fungi. In other cases, the abundance is the result of interactions between soil microorganisms and the plants, which influence the degradation of soil organic matter in their specific way. Increased exudation is known to increase the rate of decomposition of organic matter by enhancing its microbial access, thereby causing net carbon loss (Keiluweit et al. 2015). As a result of the transformation of soil organic matter into stable cyclic carbonaceous substances, part of the original carbonaceous substances is transformed into CO_2, and thus, the soil is depleted of carbon (Liang et al. 2023). From this point of view, a higher amount of exudates found in the soil solution should correspond to an overall lower organic carbon content.

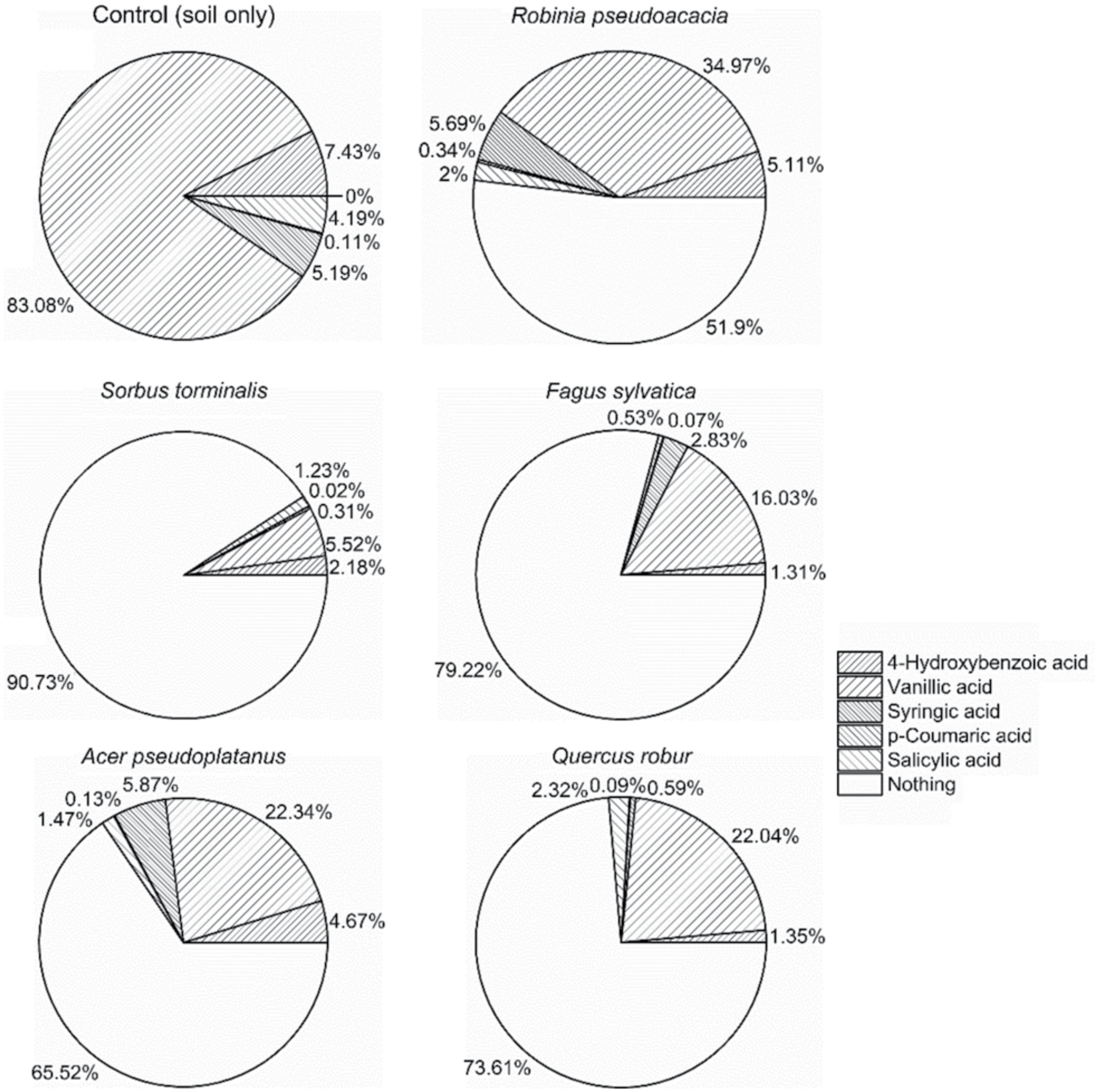

Figure 6. Relative content of phenolic acids detected in the soil solution of each tree compared to their sum in the control (100%)

As for the distribution of root exudates within the entire rhizosphere, it can be said that it is non-homogeneous. In studies dealing with a more detailed description of exudation and mechanisms in a particular system, this distribution needs to be precisely mapped and/or mathematically modelled (Luster et al. 2009). Various rhizobox designs (with the ability to separate soil at different distances from the roots) are used for these purposes, and a controlled carbon dioxide isotope atmosphere is used to determine fluxes, with the soil subsequently determined for molecules containing isotopic carbon. It appears that as the distance from the roots increases in the order of millimetres, the amount of exudates decreases rapidly and follows the laws of diffusion (Kuzyakov et al. 2003). However, in our study, we focused on the fingerprint of exudates present in the (bulk) soil solution, which can be a readily available parameter in classifying different tree species and their exudative impact. These fingerprints are based on the proportions of the individual phenolic substances found.

Based on the results (Fig. 6), it can be assumed that RP creates an environment for a higher concentration of phenolic acids in the substrate than the other species. In the soil solution from RP, vanillic and *p*-coumaric acid were present in significantly higher concentrations than from all other tested tree species. As well as 4-hydroxybenzoic acid in the soil solution from RP is present in a significantly higher concentration than that from ST, FS and QR. Syringic acid is also higher in the soil solution from RP than that from ST, FS and QR. In contrast, the presence of salicylic acid in the soil solution of RP is on a level similar to that of QR, ST and AP.

Overall, the total abundance of individual exudates depends on the specific interplay between the microorganisms and the plant; the microbial populations present in the soil are very important. If there is a high multiplication of root bacteria, there could be a significant increase in the exudation of several phenolic compounds that were not detected in our case. This phenomenon of microbial initiation has been observed, for example, in the rhizosphere of *Cupressus* after rapid bacterial inoculation (Oppenheimer-Shaanan et al. 2022). By exposing plants to abiotic stress (drought), the pattern of exudates can change fundamentally, as well as depending on the specific bacterial (pathogen) population. We did not change the representation of individual strains, and we assumed that the horticultural substrate used was homogeneous enough to ensure the same input conditions for all variants.

In this study, a targeted analysis of 32 compounds in soil solution samples was performed, but only six of them were present in detectable amounts. It was also surprising that all these phenolic substances were present in both, the control and in all variants with seedlings, as we expected the interspecific variation in the representation of individual exudates to be higher. There are several explanations for this phenomenon besides the targeted strategies of plants to control the activity of microorganisms. First, increased exudation is commonly associated with the acceleration of soil organic matter depletion (Zwetsloot et al. 2018). Once the organomineral complex has decomposed (over a longer period), the nutrient acceptability increases and energy-intensive exudation is no longer needed. Due to the fact that the substrate we used contained a high proportion of peat and stabilised humus, no significant loss of organic matter was observed in the containers over a period of 4 months. However, soil mineralisation clearly occurs more in the long term in temperate deciduous trees than that in conifers (Malý et al. 2014). Second, the already mentioned priming effect (Keiluweit et al. 2015) can promote microbial activity to such an extent that palatable low-molecular-weight organic matter is rapidly degraded *via* biotic pathways (Kumar et al. 2024). Furthermore, the loss of carbon from the soil depends very strongly on the climate (temperature and humidity conditions) which also a key role in the representation and quantity of individual exudates (de Vries et al. 2019).

Primarily, we expected that the exudates of the phenolic compounds found in the soil solution collected from *Robinia pseudoacacia* would differ both in the representation of the phenolic compounds and in their concentrations from the other soil solutions collected from the other tested tree species. Only half of the hypothesis was confirmed. Five of the same phenolic compounds were found in all soil solutions tested (starting with the control variant through all tree species). However, differences in concentrations were found between the different woody species, with the soil solution from *Robinia* differing significantly from the others, as evident from both the phenolic compound fingerprints (Fig. 6) and the statistical analysis.

CONCLUSIONS

The purpose of the study was to find out whether individual six-month-old seedlings of five different tree species (*Robinia pseudoacacia*, *Quercus robur*, *Sorbus torminalis*, *Fagus sylvatica* and *Acer pseudoplatanus*) would differ in the content of phenolic low-molecular-weight organic acids in the soil solution. Among the acids above the detection limit (vanillic, 4-benzoic, syringic, *p*-coumaric and salicylic), vanillic acid had the highest concentration of all. As for the treatment comparison, the highest content of vanillic, 4-hydroxybenzoic and salicylic acids was found in the control (soil substrate without seedlings). However, at least some portion of vanillic acid is related to the degradation of biomass (e.g., wood chips) contained in the soil substrate. The presence of these substances in the control variant therefore refers to microbial degradation processes. The highest content of *p*-coumaric acid was found in *Robinia pseudoacacia*.

Differences in the contents of phenolic acids in the tree seedling soil solutions after half a year of growth were expressed as fingerprints. Soil solutions from *Sorbus torminalis*, followed by *Fagus sylvatica* and *Quercus robur*, show their relatively low content in our list of tested trees. In the soil solution from *Acer pseudoplatanus*, more phenolic acids than those from the aforementioned trees were found. On the contrary, the soil solution sampled from *Robinia pseudoacacia* contained their highest content among the tested trees. This may correspond to the fact that it is a resistant species capable of surviving in soils poor in available nutrients. Additionally, it may contribute to the fact that *Robinia pseudoacatia* tends to reduce the species diversity of both the undergrowth and intermingled tree species.

ACKNOWLEDGEMENT

This work was supported by the project no. QK22020045 (Potential of geographically non-native species in the forestry sector of the Czech Republic) and no. RO0423 funded by the Ministry of Agriculture of the Czech Republic, the project no. CZ.02.1.01/0.0/0.0/16_019/00008 27 (Plants as a tool for sustainable global development) within the Operational Programme Research, Development and Education (OP RDE) managed by the Ministry of Education, Youth and Sports, and by the project no. MHMP 789027/2020 (Reproduction potential of black locust derived from its seed bank) funded by the Capital City of Prague.

REFERENCES

Adeleke, R., Nwangburuka, C., Oboirien, B. 2017. Origins, roles and fate of organic acids in soils: A review. *South African Journal of Botany*, 108, 393–406. DOI: 10.1016/j.sajb.2016.09.002.

Achary, V.M.M., Patnaik, A.R., Panda, B.B. 2012. Oxidative biomarkers in leaf tissue of barley seedlings in response to aluminum stress. *Ecotoxicology and Environmental Safety*, 75, 16–26. DOI: 10.1016/j.ecoenv.2011.08.015.

Baetz, U., Martinoia, E. 2014. Root exudates: the hidden part of plant defense. *Trends in Plant Science*, 19 (2), 90–98. DOI: 10.1016/j.tplants.2013.11.006.

Bartha, D., Csiszár, Á., Zsigmond, V. 2008. Black locust (*Robinia pseudoacacia* L.). In: The most important invasive plants in Hungary (eds. Z. Botta-Dukát, L. Balogh). Institute of Ecology and Botany – Hungarian Academy of Science, Vácrátrót, Hungary, 63–76. DOI: 10.13140/2.1.2088.6085.

Bergmann, J. et al. 2020. The fungal collaboration gradient dominates the root economics space in plants. *Science Advances*, 6 (27), eaba3756. DOI: 10.1126/sciadv.aba3756.

Bi, B.Y., Tong, Q., Wan, C.Y., Wang, K., Han, F.P. 2022. *Pinus sylvestris* var. *mongolica* mediates interspecific belowground chemical interactions through root exudates. *Forest Ecology and Management*, 511, 120158. DOI: 10.1016/j.foreco.2022.120158.

Blum, U. 1996. Allelopathic interactions involving phenolic acids. *Journal of Nematology*, 28 (3), 259–267.

Canarini, A., Kaiser, C., Merchant, A., Richter, A., Wanek, W. 2019. Root exudation of primary metabolites: Mechanisms and their roles in plant responses to environmental stimuli. *Frontiers in Plant Science*, 10, 157. DOI: 10.3389/fpls.2019.00157.

de Vries, F.T. et al. 2019. Changes in root-exudate-induced respiration reveal a novel mechanism through which drought affects ecosystem carbon cycling. *New Phytologist*, 224 (1), 132–145. DOI: 10.1111/nph.16001.

Drzewiecka, K. et al. 2019. Differences of *Acer platanoides* L. and *Tilia cordata* Mill. Response patterns/survival strategies during cultivation in extremely polluted mining sludge – A pot trial. *Chemosphere*, 229, 589–601. DOI: 10.1016/j.chemosphere.2019.05.051.

Han Kyaw, E., Iwasaki, A., Suenaga, K., Kato-Noguchi, H. 2022. Phytotoxic activity of *Clerodendrum indicum* (L.) Kuntze and its potential phytotoxic substance. *Emirates Journal of Food and Agriculture*, 33 (10), 884–892. DOI: 10.9755/ejfa.2021.v33.i10.2779.

Herbert, B.E., Bertsch, P.M. 1995. Characterization of dissolved and colloidal organic matter in soil solution: a review. In: Carbon Forms and Functions in Forest Soils (eds. W.W. McFee, J.M. Kelly). Soil Science Society of America, Inc. Madison, Wisconsin USA, 63–88. DOI: 10.2136/1995.carbonforms.c5.

Cheemanapalli, S., Mopuri, R., Golla, R., Anuradha, C.M., Chitta, S.K. 2018. Syringic acid (SA) – A review of its occurrence, biosynthesis, pharmacological and industrial importance. *Biomedicine and Pharmacotherapy*, 108, 547–557. DOI: 10.1016/j.biopha.2018.09.069.

Inderjit, Mallik, A.U. 1997. Effect of phenolic compounds on selected soil properties. *Forest Ecology and Management*, 92 (1/3), 11–18. DOI: 10.1016/s0378-1127(96)03957-6.

Jakl, M., Ćavar Zeljković, S., Kovač, I., Bělonožníková, K., Jaklová Dytrtová, J. 2021. Side effects of triazoles on treated crops. *Chemosphere*, 277, 130242. DOI: 10.1016/j.chemosphere.2021.130242.

Jaklová Dytrtová, J., Jakl, M., Schröder, D. 2012. Complexation of malic acid with cadmium(II) probed by electrospray ionization mass spectrometry. *Talanta*, 90C, 63–68. DOI: 10.1016/j.talanta.2011.12.076.

Jaklová Dytrtová, J., Straka, M., Bělonožníková, K., Jakl, M., Ryšlavá, H. 2018. Does resveratrol retain its antioxidative properties in wine? Redox behaviour of resveratrol in the presence of Cu(II) and tebuconazole. *Food Chemistry*, 262, 221–225. DOI: 10.1016/j.foodchem.2018.04.096.

Jones, D.L. 1998. Organic acids in the rhizosphere – a critical review. *Plant and Soil*, 205 (1), 25–44. DOI: 10.1023/a:1004356007312.

Kaiser, K., Guggenberger, G., Haumaier, L., Zech, W. 2002. The composition of dissolved organic matter in forest soil solutions: changes induced by seasons and passage through the mineral soil. *Organic Geochemistry*, 33 (3), 307–318. DOI: 10.1016/s0146-6380(01)00162-0.

Kannan, R.R.R., Arumugam, R., Thangaradjou, T., Anantharaman, P. 2013. Phytochemical constituents, antioxidant properties and *p*-coumaric acid analysis in some seagrasses. *Food Research International*, 54 (1), 1229–1236. DOI: 10.1016/j.foodres.2013.01.027.

Kasai, D., Masai, E., Katayama, Y., Fukuda, M. 2007. Degradation of 3-O-methylgallate in *Sphingomonas paucimobilis* SYK-6 by pathways involving protocatechuate 4,5-dioxygenase. *FEMS Microbiology Letters*, 274 (2), 323–328. DOI: 10.1111/j.1574-6968.2007.00855.x.

Keiluweit, M., Bougoure, J.J., Nico, P.S., Pett-Ridge, J., Weber, P.K., Kleber, M. 2015. Mineral protection of soil carbon counteracted by root exudates. *Nature Climate Change*, 5 (6), 588–595. DOI: 10.1038/nclimate2580.

Khan, M.I.R., Fatma, M., Per, T.S., Anjum, N.A., Khan, N.A. 2015. Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. *Frontiers in Plant Science*, 6, 462. DOI: 10.3389/fpls.2015.00462.

Kopinga, J., Van den Burg, J. 1995. Using soil and foliar analysis to diagnose the nutritional status of urban trees. *Journal of Arboriculture*, 21 (1), 17–24. DOI: 10.48044/jauf.1995.004.

Kumar, G.A. et al. 2024. Recent advancements in multifaceted roles of flavonoids in plant-rhizomicrobiome interactions. *Frontiers in Plant Science*, 14, 1297706. DOI: 10.3389/fpls.2023.1297706.

Kuzyakov, Y., Raskatov, A., Kaupenjohann, M. 2003. Turnover and distribution of root exudates of *Zea mays*. *Plant and Soil*, 254 (2), 317–327. DOI: 10.1023/a:1025515708093.

Landis, T.D., Tinus, R.W., McDonald, S.E., Barnett, J.P. 1989. Seedling Nutrition and Irrigation, Vol. 4. The container tree nursery manual. Agriculture Hanbook 674. U.S. Department of Agriculture, Forest Service, Washington, DC.

Liang, G.P., Stark, J., Waring, B.G. 2023. Mineral reactivity determines root effects on soil organic carbon. *Nature Communications*, 14 (1), 4962. DOI: 10.1038/s41467-023-40768-y.

Lima, R.B. et al. 2013. Enhanced lignin monomer production caused by cinnamic acid and its hydroxylated derivatives inhibits soybean root growth. *PLoS One*, 8 (12), e80542. DOI: 10.1371/journal.pone.0080542.

Luster, J., Göttlein, A., Nowack, B., Sarret, G. 2009. Sampling, defining, characterising and modeling the rhizosphere-the soil science tool box. *Plant and Soil*, 321 (1/2), 457–482. DOI: 10.1007/s11104-008-9781-3.

Malý, S., Fiala, P., Reininger, D., Obdržálková, E. 2014. The relationships among microbial parameters and the rate of organic matter mineralization in forest soils, as influenced by forest type. *Pedobiologia*, 57 (4/6), 235–244. DOI: 10.1016/j.pedobi.2014.09.003.

Medina-Villar, S., Rodriguez-Echeverria, S., Lorenzo, P., Alonso, A., Perez-Corona, E., Castro-Diez, P. 2016. Impacts of the alien trees *Ailanthus altissima* (Mill.) Swingle and *Robinia pseudoacacia* L. on soil nutrients and microbial communities. *Soil Biology and Biochemistry*, 96, 65–73. DOI: 10.1016/j.soilbio.2016.01.015.

Nasir, H., Iqbal, Z., Hiradate, S., Fujii, Y. 2005. Allelopathic potential of *Robinia pseudo-acacia* L. *Journal of Chemical Ecology*, 31 (9), 2179–2192. DOI: 10.1007/s10886-005-6084-5.

Oppenheimer-Shaanan, Y. et al. 2022. A dynamic rhizosphere interplay between tree roots and soil bacteria under drought stress. *Elife*, 11, e79679. DOI: 10.7554/eLife.79679.

Pizzeghello, D., Zanella, A., Carletti, P., Nardi, S. 2006. Chemical and biological characterization of dissolved organic matter from silver fir and beech forest soils. *Chemosphere*, 65 (2), 190–200. DOI: 10.1016/j.chemosphere.2006.03.001.

Rao, S.P., Du, C., Li, A.J., Xia, X.L., Yin, W.L., Chen, J.H. 2019. Salicylic acid alleviated salt damage of *Populus euphratica*: A physiological and transcriptomic analysis. *Forests*, 10 (5), 423. DOI: 10.3390/f10050423.

Richardson, D.M., Rejmánek, M. 2011. Trees and shrubs as invasive alien species – a global review. *Diversity and Distributions*, 17 (5), 788–809. DOI: 10.1111/j.1472-4642.2011.00782.x.

Sanon, A. et al. 2009. Rhizosphere microbiota interfers with plant-plant interactions. *Plant and Soil*, 321 (1/2), 259–278. DOI: 10.1007/s11104-009-0010-5.

Sasse, J., Martinoia, E., Northen, T. 2018. Feed your friends: Do plant exudates shape the root microbiome? *Trends in Plant Science*, 23 (1), 25–41. DOI: 10.1016/j.tplants.2017.09.003.

Sedlář, O., Balík, J., Černý, J., Kulhánek, M., Smatanová, M. 2023. Long-term application of organic fertilizers in relation to soil organic matter quality. *Agronomy-Basel*, 13 (1), 175. DOI: 10.3390/agronomy13010175.

Sharma, D., Shree, B., Kumar, S., Kumar, V., Sharma, S., Sharma, S. 2022. Stress induced production of plant secondary metabolites in vegetables: Functional approach for designing next generation super foods. *Plant Physiology and Biochemistry*, 192, 252–272. DOI: 10.1016/j.plaphy.2022.09.034.

Siqueira, J.O., Nair, M.G., Hammerschmidt, R., Safir, G.R. 1991. Significance of phenolic compounds in plant-soil-microbial systems. *Critical Reviews in Plant Sciences*, 10 (1), 63–121. DOI: 10.1080/07352689109382307.

Somssich, M., Khan, G.A., Persson, S. 2016. Cell wall heterogeneity in root development of *Arabidopsis*. *Frontiers in Plant Science*, 7, 1242. DOI: 10.3389/fpls.2016.01242.

Strobel, B.W. 2001. Influence of vegetation on low-molecular-weight carboxylic acids in soil solution – a review. *Geoderma*, 99 (3/4), 169–198. DOI: 10.1016/S0016-7061(00)00102-6.

Šrámek, V., Novotný, R., Lomský, B., Maxa, M., Neuman, L., Fadrhonsová, V. 2004. Změny obsahů prvků v porostech smrku, buku, jeřábu a břízy v průběhu roku [Report]. VÚLHM, Jíloviště – Strnady.

Taofiq, O. et al. 2017. The potential of *Ganoderma lucidum* extracts as bioactive ingredients in topical formulations, beyond its nutritional benefits. *Food and Chemical Toxicology*, 108, 139–147. DOI: 10.1016/j.fct.2017.07.051.

Tato, L. et al. 2021. Plasticity, exudation and microbiome-association of the root system of Pellitory-of-the-wall plants grown in environments impaired in iron availability. *Plant Physiology and Biochemistry*, 168, 27–42. DOI: 10.1016/j.plaphy.2021.09.040.

Vega-Aguilar, C.A., Barreiro, M.F., Rodrigues, A.E. 2020. Catalytic wet peroxide oxidation of vanillic acid as a lignin model compound towards the renewable production of dicarboxylic acids. *Chemical*

Engineering Research and Design, 159, 115–124. DOI: 10.1016/j.cherd.2070.04.021.

Vítková, M., Müllerová, J., Sádlo, J., Pergl, J., Pyšek, P. 2017. Black locust (*Robinia pseudoacacia*) beloved and despised: A story of an invasive tree in Central Europe. *Forest Ecology and Management*, 384, 287–302. DOI: 10.1016/j.foreco.2016.10.057.

Vives-Peris, V., de Ollas, C., Gomez-Cadenas, A., Perez-Clemente, R.M. 2020. Root exudates: from plant to rhizosphere and beyond. *Plant Cell Reports*, 39 (1), 3–17. DOI: 10.1007/s00299-019-02447-5.

Vlot, A.C., Dempsey, D.A., Klessig, D.F. 2009. Salicylic acid, a multifaceted hormone to combat disease. *Annual Review of Phytopathology*, 47, 177–206. DOI: 10.1146/annurev.phyto.050908.135202.

Wen, F., Curlango-Rivera, G., Hawes, M.C. 2007a. Proteins among the polysaccharides. A new perspective on root cap slime. *Plant Signaling and Behavior*, 2 (5), 410–412. DOI: 10.4161/psb.2.5.4344.

Wen, F.S., VanEtten, H.D., Tsaprailis, G., Hawes, M.C. 2007b. Extracellular proteins in pea root tip and border cell exudates. *Plant Physiology*, 143 (2), 773–783. DOI: 10.1104/pp.106.091637.

Wen, F.S., White, G.J., VanEtten, H.D., Xiong, Z.G., Hawes, M.C. 2009. Extracellular DNA is required for root tip resistance to fungal infection. *Plant Physiology*, 151 (2), 820–829. DOI: 10.1104/pp.109.142067.

Were, E., Schone, J., Viljoen, A., Rasche, F. 2022. Phenolics mediate suppression of *Fusarium oxysporum* f. sp. cubense TR4 by legume root exudates. *Rhizosphere*, 21, 100459. DOI: 10.1016/j.rhisph.2021.100459.

Zhao, L. et al. 2022. Biological degradation of lignin: A critical review on progress and perspectives. *Industrial Crops and Products*, 188, 115715. DOI: 10.1016/j.indcrop.2022.115715.

Zwetsloot, M.J., Kessler, A., Bauerle, T.L. 2018. Phenolic root exudate and tissue compounds vary widely among temperate forest tree species and have contrasting effects on soil microbial respiration. *New Phytologist*, 218 (2), 530–541. DOI: 10.1111/nph.15041.

SUPPORTING INFORMATION

Table SI.1. Tukey's HSD test for 4-hydroxybenzoic acid among tree species indicating whether the values are statistically different at the significance level of $\alpha \leq 0.05$

Group 1	Group 2	p-value	Reject	Group 1	Group 2	p-value	Reject
1	2	3	4	5	6	7	8
4-hydroxybenzoic acid				*p*-coumaric acid			
AP	FS	0.0000	true	AP	FS	0.3396	false
aP	QR	0.0000	true	AP	QR	0.6966	false
aP	RP	0.7967	false	AP	RP	0.0002	true
aP	ST	0.0001	true	AP	ST	0.0331	true
aP	control	0.0000	true	AP	control	0.9609	false
fS	QR	1.0000	false	FS	QR	0.9827	false
fS	RP	0.0000	true	FS	RP	0.0000	true
fS	ST	0.1973	false	FS	ST	0.6792	false
fS	control	0.0000	true	FS	control	0.7731	false
qR	RP	0.0000	true	QR	RP	0.0000	true
qR	ST	0.2274	false	QR	ST	0.3260	false
qR	control	0.0000	true	QR	control	0.9845	false
rP	ST	0.0000	true	RP	ST	0.0000	true
rP	control	0.0003	true	RP	control	0.0001	true
sT	control	0.0000	true	ST	control	0.1253	false
vanillic acid				Salicylic acid			
AP	FS	0.0749	false	AP	FS	0.1614	false
aP	QR	1.0000	false	AP	QR	0.2248	false
aP	RP	0.0005	true	AP	RP	0.6738	false
aP	ST	0.0000	true	AP	ST	0.9833	false

1	2	3	4	5	6	7	8
aP	control	0.0000	true	AP	control	0.0001	true
fS	QR	0.0951	false	FS	QR	0.0030	true
fS	RP	0.0000	true	FS	RP	0.0136	true
fS	ST	0.0024	true	FS	ST	0.4073	false
fS	control	0.0000	true	FS	control	0.0000	true
qR	RP	0.0004	true	QR	RP	0.9335	false
qR	ST	0.0000	true	QR	ST	0.0800	false
qR	control	0.0000	true	QR	control	0.0021	true
rP	ST	0.0000	true	RP	ST	0.3240	false
rP	control	0.0000	true	RP	control	0.0005	true
sT	control	0.0000	true	ST	control	0.0000	true
Syringic acid							
AP	FS	0.0069	true				
AP	QR	0.0001	true				
AP	RP	0.9997	false				
AP	ST	0.0000	true				
AP	control	0.9031	false				
FS	QR	0.0509	false				
FS	RP	0.0110	true				
FS	ST	0.0251	true				
FS	control	0.0384	true				
QR	RP	0.0001	true				
QR	ST	0.9979	false				
QR	control	0.0002	true				
RP	ST	0.0000	true				
RP	control	0.9727	false				
ST	control	0.0001	true				

AP – *Acer pseudoplatanus*; FS – *Fagus sylvatica*; QR – *Quercus robur*; RP – *Robinia pseudoacacia*; ST – *Sorbus torminalis.*

Table SI.2. Pearson product-moment correlation coefficients among the organic acids

	HBA	VA	SyA	pCA	SaA
HBA		0.842*	0.783*	0.421	0.702*
VA			0.539	0.238	0.881**
SyA				0.595	0.284
pCA					0.199

* indicates strong positive correlation, ** indicates very strong positive correlation;
HBA – 4-hydroxybenzoic acid, VA – vanillic acid, SyA – syringic acid, pCA – *p*-coumaric acid, SaA – salicylic acid

Table SI3. MANOVA analysis of the differences in acid contents among the tree species

Effect of Subject	Value	Num DF	Den DF	F Value	Pr>F
Wilks' lambda	0.00	25.00	31.22	45.71	0.000
Pillai's trace	4.05	25.00	60.00	10.22	0.000
Hotelling-Lawley trace	248.27	25.00	12.13	71.35	0.000
Roy's greatest root	188.40	5.00	12.00	452.17	0.000