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Research Article

Metabolite Profiling of Urban and Forest Tree Rhizosphere Soil using Analytical Instruments

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Abstract

The rhizosphere is the area of the soil affected by plant roots and plays an important role in plant health, nutrient cycling, and soil structure. This study investigated differences in rhizosphere soil metabolites between trees in urban and forest environments using LC/MS-Q-TOF and GC/MS. Urban environments can cause soil pollution and ecosystem disturbance due to the accumulation of heavy metals and petrochemicals, while forest environments are rich in organic matter and fallen leaves, maintaining a nutrient-rich environment in which various microorganisms coexist. Rhizosphere soil samples were collected from Dankook University (city) and Taejo Mountain (forest) in Cheonan, South Korea, extracted using methanol and chloroform using the Soxhlet extraction method, and then analyzed by LC/MS-Q-TOF and GC/MS. N-Nitrosopyrrolidine, a possible carcinogen from automobile exhaust and industrial activities, has been found in the rhizosphere soil of urban trees. Forest rhizosphere samples showed high concentrations of momilactone A, which is important for plant defense mechanisms. 140 significant metabolites were identified through MPP analysis. Furthermore, GC/MS analysis confirmed that siloxanes and oleamides were predominantly detected in urban and forest samples, respectively. Overall, this study highlights the impact of environmental conditions on rhizosphere soil chemistry and microbial ecosystems and provides insights for urban greening and forest conservation strategies. Comprehensive profiling of rhizosphere metabolites will improve our understanding of plant-soil interactions and contribute to maintaining soil health and plant diversity in diverse ecosystems.

Abbreviations

LC/MS/Q-TOF: Liquid Chromatography Coupled with High-Resolution Mass Spectrometry, Quadrupole Time of Flight; GC/MS: Gas Chromatography-Mass Spectrometry.

Introduction

The rhizosphere is the area of the soil affected by plant roots and is a highly dynamic and complex environment that plays an important role in plant health, nutrient cycling, and soil structure [1,2]. The chemical composition of rhizosphere soils is shaped by a variety of factors, including root exudates, microbial activity, and environmental conditions, and urban and forest environments provide contrasting environments that can significantly vary the chemical composition of rhizosphere soils [3,4]. Urban environments are mainly affected by high population density and industrial activities, making them prone to the accumulation of heavy metals, petrochemicals, and other pollutants [5,6]. This can lead to soil pollution, ecosystem disturbance, and the destruction of natural habitats. Additionally, the urban heat island effect increases soil temperatures, which may affect microbial activity and the chemical composition of root exudates [7]. Finally, soils in urban areas are often composed of artificial fill and debris, which can change soil structure and permeability and impede the movement of water and nutrients [8]. On the other

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hand, the forest environment maintains a relatively natural state and has little human interference. Forest soil is rich in nutrients due to the fallen leaf layer and high organic matter content, and various plants and microorganisms coexist [9,10]. This environment contributes to natural nutrient cycling and maintenance of soil structure. In addition, forest soils generally have high water permeability and water retention capacity, enabling smooth movement of water and nutrients, rich biodiversity, and active interactions between plants and microorganisms [11].

Comparing the metabolite profiles of urban and forest rhizosphere soils revealed significant differences. In urban environments, carcinogenic substances such as N-Nitrosopyrrolidine were predominantly detected, reflecting the impact of vehicle exhaust and industrial activities on environmental pollution. In contrast, forest environments showed the presence of bioactive compounds like Momilactone A, which plays a crucial role in plant defense mechanisms. Recent advancements in analytical techniques such as Liquid Chromatography Coupled with High-Resolution Mass Spectrometry (LC/MS-Q-TOF, Quadrupole Time-of-Flight: Q-TOF) and Gas Chromatography-Mass Spectrometry (GC/MS) have revolutionized the field of soil metabolomics, enabling detailed profiling of complex mixtures of organic compounds [12,13]. LC/MS-Q-TOF is particularly effective in analyzing polar and high molecular weight compounds and is an equipment that can perform qualitative/quantitative analysis of basic substances of biomaterial drugs and raw material production samples. In addition, it is widely used in various fields such as biotechnology, environment, food, and petrochemistry [14]. GC/MS is suitable for analyzing volatile nonpolar compounds and is a powerful tool for separating and qualitatively/quantitatively analyzing the components of complex mixtures [15]. It, along with LC/MS-Q-TOF, is widely used in a variety of fields, including pharmaceuticals, biotechnology, environmental research, food and beverage, and clinical diagnostics [16]. Comparing metabolites in urban and forest rhizospheres based on these techniques can provide insight into plant-soil interactions by comprehensively characterizing chemical interactions.

In this study, we sought to investigate differences in the chemical composition of rhizosphere soils in urban and forest environments through LC/MS and GC/MS analysis. To extract various metabolites from the soil, we used the Soxhlet extraction method, which can efficiently extract various metabolites including microorganisms from soil samples [17]. The polar solvent methanol was used for extracting organic acids, sugars, and other hydrophilic compounds for LC/MS-Q-TOF analysis, while the nonpolar solvent chloroform excelled at extracting lipophilic, volatile substances, making it ideal for profiling volatile and semi-volatile organic compounds using GC/MS [18-20]. Ultimately, through this study, we hope to contribute to elucidating the impact of environmental conditions on soil chemistry and microbial ecosystems through rhizosphere soil metabolite profiling in urban and forest environments, and how these differences can be used to develop urban greening and forest conservation strategies. In addition to providing useful information, it will provide a comprehensive framework for investigating the chemical diversity of rhizosphere soils and improve understanding of the interactions that maintain diverse plant and soil health.

Materials and methods

Methods for collecting tree rhizosphere soil samples in urban and forest areas

On June 17, 2024, rhizosphere soil samples were collected from two distinct locations in Cheonan, South Korea. Urban samples were obtained from Dankook University (Dongnam-gu, Cheonan), while forest samples were collected from Taejo mauntain (Dongnam-gu, Cheonan). The samples were collected under typical summer conditions, with an average temperature of 30 °C and no precipitation. *Juniperus chinensis* (Chinese juniper), *Quercus dentata*, and *Pinus densiflora* were selected as trees for obtaining rhizosphere soil samples. To collect rhizosphere soil samples, the soil was dug up to the root exposure point using a sterilized shovel treated with 35% hydrogen peroxide and 70% ethanol. The sealed samples were placed in an icebox filled with dry ice and transported to the laboratory. Immediately thereafter, foreign substances except soil were removed using a sterilized sieve. Samples from which foreign substances were removed were placed in 50mL conical tubes and stored in a refrigerator at 4 degrees Celsius until extraction.

Sample preparation of rhizosphere soil for LC/MS-Q-TOF and GC/MS analysis

10 g of soil samples contained in a conical tube were taken, distributed in a cellulose extraction thimble (Whatman, Maidstone, UK), and then mounted in a Soxhlet extraction apparatus (Pyrex, New York, USA). 100 mL LC/MS grade methanol (Sigma-Aldrich, Missouri, USA) for extraction of substances from samples for LC/MS-Q-TOF analysis, and 100 mL GC/MS grade chloroform for extraction of substances from samples for GC/MS analysis. (Sigma-Aldrich, Missouri, USA) was dispensed into a round bottom flask. Each sample was extracted at 70 °C for 24 hours and stored in a -20 °C freezer until analysis. All samples were filtered using a 0.45uL nylon syringe filter (HYUNDAI MICRO, Seoul, Korea) in 5mL quantities. Additionally, samples for LC/MS-Q-TOF were diluted 1:50 in methanol. Afterwards, all samples were dispensed three times, 1 mL each, into 2 mL sample vials.

Non-targeted screening of soil samples using LC/MS-Q-TOF

Non-targeted screening was conducted to identify differences in polar metabolites in the rhizosphere of urban and forest trees. To perform non-targeted screening, 6456 LC/MS-Q-TOF (Agilent, California, USA) and RRHD Eclipse Plus C18 column (1.8 µm, 2.1 × 150 mm, Agilent, California, USA) were used. Analysis was conducted through the MassHunter Workstation Data Acquisition (Agilent, California, USA) program. The conditions of the mobile phases used in the analysis were as follows; Mobile phase A: 97.9% LC/MS grade water (Daejung, Siheung, Korea), 2% LC/MS grade

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acetonitrile (Duksan, Ansan, Korea), and 0.1% LC/MS grade formic acid (Sigma-Aldrich, Missouri, USA), mobile phase B: 99.9% acetonitrile, and 0.1% formic acid. Binary pump flow was performed at 0.3mL/min. The initial mobile phase ratio for 0-0.5 min was A: 95%, B: 5%, and at 0.5-30 min, A was set from 95% to 5%, and B was set from 5% to 95%. At 30-35 min, A was set from 5% to 2%, and B was set at 95% to 98% and maintained at 35-40 min. At 40-40.1 min, A was set at 2% to 95% and B was set at 98% to 5%.40.1-45 min and was maintained continuously. Binary pump pressure limits were set to a minimum of 0.00 bar and a maximum of 1,300.00 bar, and the column temperature was set to 40 °C. The Q-TOF ion source used was dual AJS ESI (Agilent Jet Stream Electrospray Ionization) and was conducted in positive ion polarity mode. The gas temperature was 210 °C and the drying gas flow rate was 6 L min^{-1}. The nebulizer pressure was 35 psi, the sheath gas temperature was 380 °C, and the sheath gas flow was 11 L min^{-1}. The capillary voltage was set to 4000V and the nozzle voltage was set to 1500V. The mass scan range was 50-1100 m/z and the acquisition rate was set at 1 spectra/sec. Acquisition time was set to 1000 ms/spectrum.

GC/MS Non-targeted screening conditions and NIST library searching

To compare the differences in non-polar metabolites between urban and forest tree rhizosphere, 7010C GC/MS-QQQ (Agilent, California, USA) and HP-5ms Ultra Inert column (30 m, 0.25 mm, 0.25 µm, Agilent, California, USA) were used. Analysis was conducted through the 7010C/Enhanced MassHunter (Agilent, California, USA) program. The analysis conditions are as follows. The sample was injected in a volume of 2 uL and He (99.999%) was used as the mobile phase. Inlet temperature was set at 240 °C, pressure at 11.962 psi, and septum purge flow at 3 mL min^{-1}. Pulsed splitless inlet mode was used, the injection pulse pressure was 25 psi, and the injection was performed for 2 min. Purge flow to the split vent was performed at 100 mL min^{-1} for 1 minute. Column flow was set at 1.5 mL min^{-1} and pressure was set at 11.962 psi. The initial column temperature was 40 °C and held for 1 min, then proceeded at 15 °C min^{-1} until 250 °C and then at 30 °C min^{-1} until 300 °C. It was held at 300 °C for 5 minutes. Analysis was performed at 250 °C at 70ev (electron energy) using EI (electron ionization) as the ion source. The solvent delay was set to 4 min and the mass scan range was set to 40-500 m/z. Rhizosphere Samples were each analyzed in three replicates. Peak height and peak area were obtained using Masshunter acquisition software version 10.0, and candidate substances were identified using Masshunter unknown analysis and NIST library. The match factor for unknown analysis was set to 0.85.

Results

Comparison of metabolite candidates in urban and forest groups through LC/MS-Q-TOF non-targeted screening

Candidate substances in urban and forest tree rhizosphere soils were investigated using Agilent MassHunter Qualitative Analysis 10.00 software. Analysis conditions for the detected peaks are separately indicated in Supplementary Table S1. As a result of identifying substances with target/suspect screening scores (Tgt) of 75 and 90 points or higher in urban areas and forests, the number of substances specified in Table 1 was identified. Information on substances with a score of 90 or higher is provided in Supplementary Tables S2 and S3. The results of re-checking the same detected substances in three repeated samples by setting the target/suspect screening score (Tgt) to 90 or higher are included in Supplementary Table S4. The number of identically detected substances in replicate samples of C. juniper, *Q. dentata*, and *P. densiflora* from urban areas was 186, 151, and 147, respectively. 144, 164, and 166 were detected in the forest, respectively (Figure 1). The results of confirming the differences in metabolites between the same trees in the city and forest are shown in Figure 2. C. juniper shared 95 substances regardless of city and forest, but 91 different substances were found in the city and 41 in the forest. *Q. dentata* shared 75 different substances regardless of the city and forest, and 76 different substances were detected in the city and 89 in the forest. Finally, *P. densiflora* shared 84 substances in the city and forest, and 63 different substances were identified in the city and 82 in the forest.

As a result of checking whether the substances detected only in urban areas and those detected only in forest areas are shared by each tree (Figures 2d,f), it was found that three substances (3-Pentadecenal, N-Nitrosopyrrolidine, Dicyclohexyl disulfide) were shared in urban areas, and five substances (10-Eicosene, 1-Phenylbiguanide, Isoleucyl-Isoleucine, Metalaxyl, Momilactone A) were shared in forest areas.

According to the repetitive sample inspection results, N-Nitrosopyrrolidine, a type of nitrosamine compound, was found exclusively in the rhizosphere soil of trees in urban environments. More than 200 nitrosamines are known to be carcinogenic, and N-Nitrosopyrrolidine is also known to cause various cancers, including liver cancer [21-23]. The accumulation of N-Nitrosopyrrolidine, a nitrosamine, in urban areas may be a result of environmental pollution resulting from urban vehicle exhaust and industrial activities. Because these substances can pose serious risks to human and animal health, efforts are needed to manage urban environments and reduce pollutants.

Table 1: Agilent MassHunter Qualitative Analysis metabolites screening results.

Area	Target/suspect screening score (Tgt)	C. juniper_1	C. juniper_2	C. juniper_3	*Q. dentata*_1	*Q. dentata*_2	*Q. dentata*_3	*P. densiflora*_1	*P. densiflora*_2	*P. densiflora*_3
Urban	75 ≥	1228	1185	1197	1189	1179	1214	1186	1212	1185
	90 ≥	504	485	517	478	465	497	496	514	477
Forest	75 ≥	1288	1241	1216	2027	2009	1810	1552	1616	1617
	90 ≥	524	486	488	708	700	652	587	616	571

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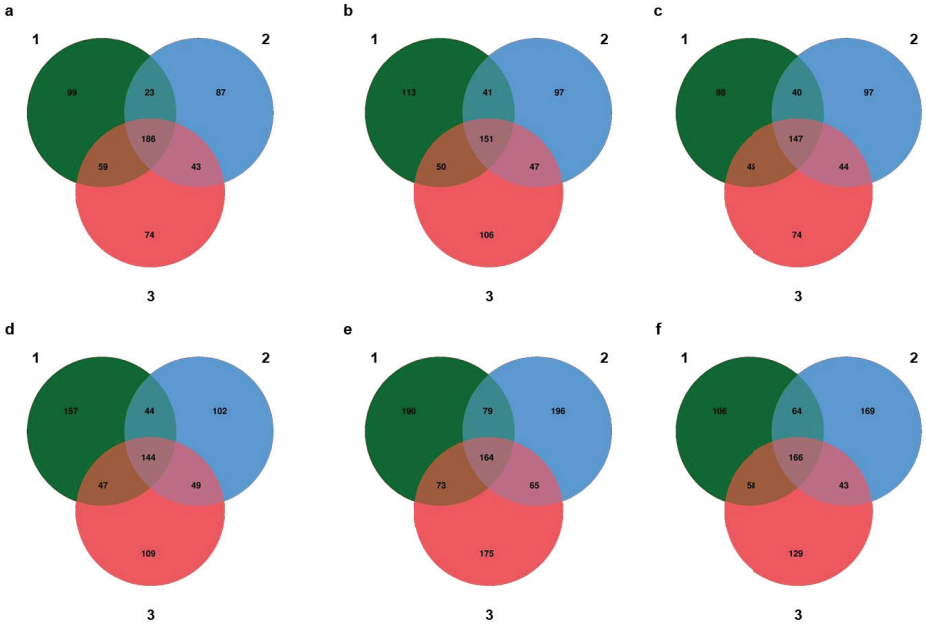

Figure 1: Venn diagrams comparing the number of identically detected metabolites in replicate samples of C. juniper, *Q. dentata*, and *P. densiflora* from urban areas and forests. The Venn diagram shows the overlap and unique metabolites detected in replicate samples. (a): C. juniper in urban (total 186), (b) *Q. dentata* in urban (total 151), (c) *P. densiflora* in urban (total 147), (d): C. juniper in forest (total 144), (e) *Q. dentata* in forest (total 164), and (f) *P. densiflora* in forest (total 166).

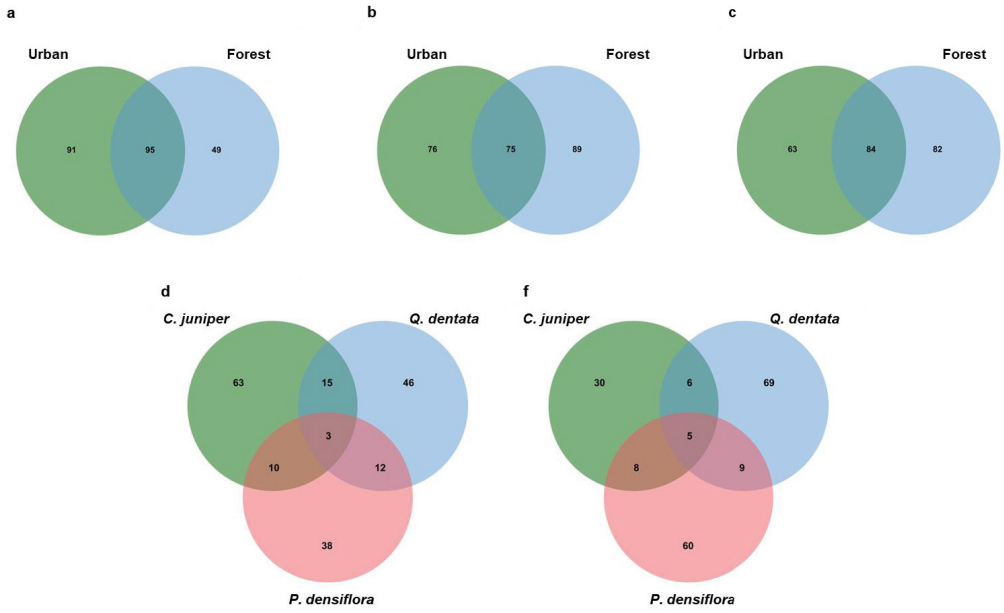

Figure 2: Comparison of detected metabolites in C. juniper, *Q. dentata*, and *P. densiflora* between urban and forest environments. (a), (b), and (c) are Venn diagrams of metabolites found in each species in urban and forest environments. (a): C. juniper, (b): *Q. dentata*, and (c): *P. densiflora*. These Venn diagrams highlight the differences and commonalities in the metabolites detected in each species when comparing urban and forest rhizosphere environments. This Venn diagram (d,e): Shows metabolites shared between C. juniper, *Q. dentata*, and *P. densiflora* in an urban and forest area. (d,e) analyzes metabolites detected in cities and forests to determine how many are shared between the three species. In particular, among all three species, three metabolites (3-Pentadecenal, N-Nitrosopyrrolidine, Dicyclohexyl disulfide) are shared in urban areas, and five metabolites are shared in forest areas (10-Eicosene, 1-Phenylbiguanide, Isoleucyl-Isoleucine, Metalaxyl, Momilactone A).

On the other hand, Momilactone A, which was identified only in forests, is known to have strong physiological activity and plays an important role in the defense mechanism of plants. Substances found only in forest rhizosphere soils suggest that environmental differences between urban centers and forests affect rhizosphere soils [24]. Natural ecological processes and interactions lead to the accumulation and production of these substances in forests, which can contribute to the survival, adaptation, and competitiveness of plants.

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Differences in relative proportions of metabolites in urban and forest rhizosphere samples through MPP analysis

The raw data obtained from LC/MS-Q-TOF analysis (.d format) was converted into a new data format (.pfa format) using the Agilent Profinder 10.0 program. This conversion was done to perform Mass Profiler Professional (MPP) analysis, which is used to identify substances showing statistically significant differences. Afterward, MPP (Agilent, version 15.1) analysis was performed, and the statistical analysis and settings are shown in Supplementary Table S5. 140 substances were identified, and the relative amounts were displayed as a heatmap (Figure 3a, R version 4.3.1). Of these, 20 substances were identified by name rather than molecular weight, as shown in Figure 3b. Each substance showed different behavior in urban areas and forests, and detailed information on the substances is provided in Supplementary Table S6. Out of the 20 substances, six, including Spirasine I, Methyl 5-(1-Propynyl)-2-thiophenepropanoate, APC, 3-Vinylbacteriochlorophyllide a, 3'-Sialyllactosamine, and 2,3-Dinor-6-keto-prostaglandin F1 a, were predicted to be relatively more abundant in urban areas compared to forest areas. On the other hand, in forest areas, higher levels of Rifabutin, PE(14:1(9Z)/P-18:1(11Z)), Palmitic Acid, Hydroxyhemin, Epifisetinidol-(4beta-8)-epicatechin-(6-4beta)-epifisetinidol, Dihydrospheroidene/Methoxyneurosporene, DG(14:0/18:1(11Z)/0:0), DG(14:0/15:0/0:0), Cyanidin-3-arabinoside, beta-Estradiol 17-acetate, Aromatized deshydroxy-C-1027 chromophore, 4-Hydroxy-3-(16-methylheptadecyl)-2H-pyran-2-one Esi+29.147, 4-Hydroxy-3-(16-methylheptadecyl)-2H-pyran-2-one, and higher proportions of 10-Eicosene are predicted.

Differences in metabolite composition revealed by GC/MS untargeted screening through NIST library search

As a result of library searching, a total of 31 metabolites were identified. Metabolites were classified into siloxanes, alkanes, fatty acids and derivatives, amides and nitriles, aromatic compounds, terpenes and terpene derivatives, and other organic compounds according to their structural and chemical properties (Table 2). There were 9 substances commonly found in both urban and forest tree rhizosphere soils, and among the 9 substances, oleamide showed the highest proportion in all samples (Figure 4, Table 2). Oleamide is a bioactive lipid molecule found in various plant root exudates and is known to stimulate nitrogen metabolism in rhizospheric bacteria, promote nitrogen removal through denitrification, and have an antibacterial effect [25-29]. In our results, when comparing cities and forests, forests showed higher rates, suggesting that forest trees may secrete more oleamides due to more fertile soil, suitable climatic conditions, and diverse microbial interactions. The fatty acid n-Hexadecanoic acid was present in both urban and forest areas, and hexadecanoic acid, methyl ester, and methyl stearate were detected only in *Q. dentata* in the urban area. Additionally, fatty acid accounted for a higher composition compared to most other metabolites.

Siloxanes such as octamethylcyclotetrasiloxane (D5), hexamethylcyclotrisiloxane (D3), and decamethylcyclopentasiloxane (D4) were detected through GC/MS analysis. Siloxane is an artificial compound that combines silicon and oxygen and is mainly used in a variety of products such as household goods, building materials, electronic products, and textiles [30,31]. Siloxanes are rarely found in nature and are mainly discharged into the environment through artificial activities and are detected in high concentrations in waste landfills, sewage treatment plants, and indoor dust. The

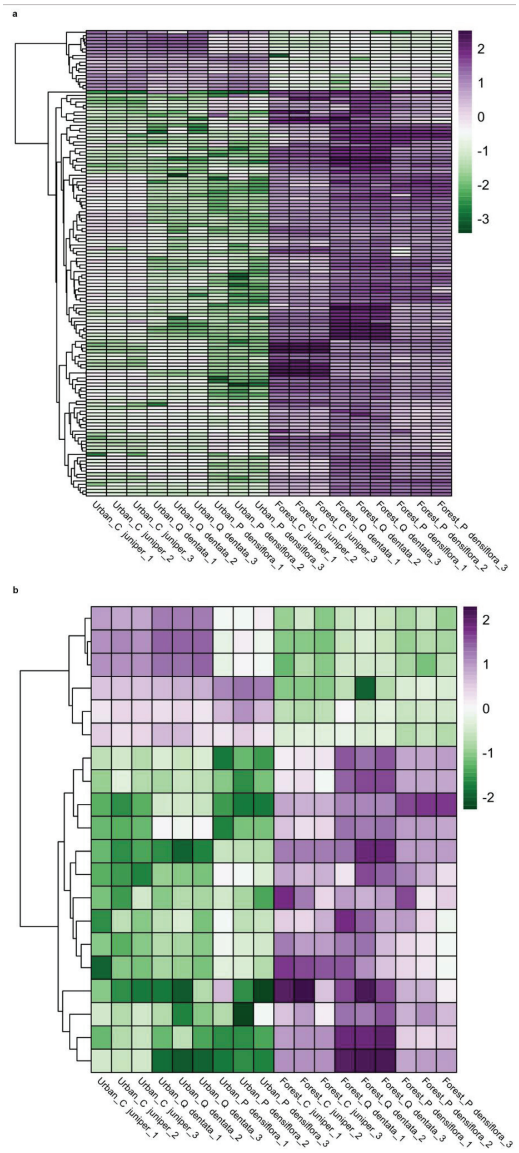

Figure 3: Heatmap of MPP analysis of C. juniper, *Q. dentata*, and *P. densiflora* in urban and forest environments. (a): Raw data obtained through LC/MS/Q-TOF analysis was transformed for MPP (Mass Profiler Professional) analysis and 140 substances showing statistically significant differences were identified. This heatmap shows the relative amounts of metabolites identified for each tree species in urban and forest environments. Heatmaps were generated using R version 4.3.1. The x-axis of the heatmap represents the composition of the samples, with each sample being C. juniper, *Q. dentata*, and *P. densiflora* collected from an urban or forest environment. The y-axis represents the identified metabolites, with their relative amounts indicated in color. The colors are on a scale from -3 to 2, with green meaning low relative quantity and purple meaning high relative quantity. Clustering was used to group samples with similar metabolic profiles. (b): Of the 140 substances identified in 3a, 20 were identified by name rather than molecular weight. This heatmap shows the relative amounts of 20 metabolites identified by name. The x- and y-axes represent each sample and metabolite as in 3a, and the colors also represent relative amounts.

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Table 2: Metabolites classified according to structural and chemical properties, and metabolite composition.

Group	Metabolite	Urban			Forest		
		C. juniper	Q. dentata	P.densiflora	C. juniper	Q. dentata	P.densiflora
Siloxanes	Octamethylcyclotetrasiloxane	0.60 ± 0.03	0.63 ± 0.03	0.66 ± 0.02	0.51 ± 0.07	0.48 ± 0.06	0.54 ± 0.06
	Hexamethylcyclotrisiloxane	0.23 ± 0.05		0.28 ± 0.01			
	Decamethylcyclopentasiloxane			0.21 ± 0.02			
Alkanes	Dodecane	2.07 ± 0.08	1.56 ± 0.05	1.52 ± 0.04	1.12 ± 0.01	2.05 ± 0.10	1.66 ± 0.10
	Tetradecane	2.28 ± 0.03	1.89 ± 0.10	1.84 ± 0.02	1.26 ± 0.05	2.05 ± 0.03	1.83 ± 0.11
	Hexadecane	2.40 ± 0.04	0.89 ± 0.04	0.88 ± 0.01		0.89 ± 0.06	0.84 ± 0.01
	Farnesane				0.53 ± 0.01		
Fatty acids and derivatives	n-Hexadecanoic acid	2.94 ± 0.23	4.53 ± 0.40	4.26 ± 0.03	3.80 ± 0.16	3.14 ± 0.30	7.13 ± 0.13
	Hexadecanoic acid, methyl ester		1.99 ± 0.11				
	Methyl stearate		0.91 ± 0.02				
Amides and Nitriles	Palmitoleamide	5.76 ± 0.46	4.94 ± 0.30	6.04 ± 0.19	5.82 ± 0.34	4.18 ± 1.18	2.08 ± 1.20
	Hexadecanamide	5.55 ± 0.16	4.28 ± 0.34	5.75 ± 0.09	5.51 ± 0.06	4.00 ± 0.50	3.88 ± 0.15
	Oleamide	73.02 ± 0.46	72.00 ± 1.07	73.96 ± 0.27	77.18 ± 0.43	79.82 ± 2.17	74.47 ± 1.14
	9-Octadecenenitrile, (Z)	1.02 ± 0.01		0.84 ± 0.02			
Aromatic compounds	Benzaldehyde, 4-propyl-	1.77 ± 0.13	1.97 ± 0.03	1.49 ± 0.04	1.22 ± 0.08	1.33 ± 0.10	1.52 ± 0.04
	Ethylbenzene					0.35 ± 0.03	0.34 ± 0.03
	(2S,3R)-3-Phenyl-2-butanol					0.22 ± 0.01	
	Phenanthrene		0.22 ± 0.07				
	Pyrene		2.15 ± 0.14				
Terpenes and Terpene derivatives	α-Phellandrene						0.25 ± 0.04
	β-Phellandrene						1.10 ± 0.05
	γ-Terpinene						0.45 ± 0.03
	Longifolene						0.91 ± 0.03
Other organic compounds	2,4-Di-tert-butylphenol	1.22 ± 0.08	1.59 ± 0.05	1.86 ± 0.01	1.81 ± 0.07	1.17 ± 0.12	2.15 ± 0.07
	Triethyl phosphate			0.30 ± 0.00	0.32 ± 0.00		0.30 ± 0.01
	Phthalic acid, di(2-propylpentyl) ester	1.15 ± 0.13					
	Ethyl 5,5-dimethyl-4-oxo-2-(trifluoromethyl) hexanoate			0.10 ± 0.07			
	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione				0.61 ± 0.02		
	3-Thujene						0.56 ± 0.07
	S-Tetrachloroethane		0.44 ± 0.02				
	Glycerin				0.32 ± 0.02	0.34 ± 0.15	

siloxanes detected in this analysis appear to have spread into the air or precipitated in water and soil, as reported in previous studies. D4 can affect reproductive health when inhaled and orally exposed, and D5 is known to be potentially carcinogenic. In particular, D4, unlike D3 and D5, has been detected not only in urban areas but also in forests [32-34]. Additional investigation is needed to determine what effect D4, which appears to be relatively easy to settle in the environment, will have.

α-Phellandrene, β-Phellandrene, γ-Terpinene, and longifolene, which belong to terpenes and terpene derivatives, are compounds that can be found in *Pinus* species but were not found in urban *P. densiflora* [35,36]. These causes may include air pollution, soil conditions, climatic conditions, and ecological interactions. Since the number of repetitions of the trees analyzed in this study is small, it is necessary to conduct experiments with an increased number of repetitions in the future to verify this.

Discussion

This study focused on identifying the effects of environmental conditions on plant-soil interactions by analyzing differences in the chemical composition and metabolite profiles of rhizosphere soils in urban and forest environments. In addition, it was intended to provide a scientific basis for establishing urban greening and forest conservation strategies. In urban environments, heavy metals, petrochemicals, and other pollutants accumulate, causing soil pollution and ecosystem disturbance. In this study, N-nitrosopyrrolidine, a nitrosamine compound, was exclusively detected in urban rhizosphere soil. This is believed to be a result of environmental pollution caused by urban vehicle emissions and industrial activities. N-nitrosopyrrolidine is a known carcinogen, and its accumulation can pose a serious risk to human and animal health.

The rhizosphere, the narrow region of soil influenced by root secretions and associated soil microorganisms, plays a critical role in plant health [37]. It acts as a dynamic interface where plants, soil, and microorganisms interact intensively, affecting nutrient uptake, disease resistance, and overall plant growth. The chemical composition of rhizosphere soils is shaped by a variety of factors, including plant species, soil type, environmental conditions, and microbial communities [38]. Root exudates, for instance, release organic acids, sugars, and amino acids into the soil, which can alter the soil's pH, nutrient availability, and microbial activity. In general, trees play an essential role in ecosystems. For starters, trees absorb carbon dioxide and release oxygen through photosynthesis,

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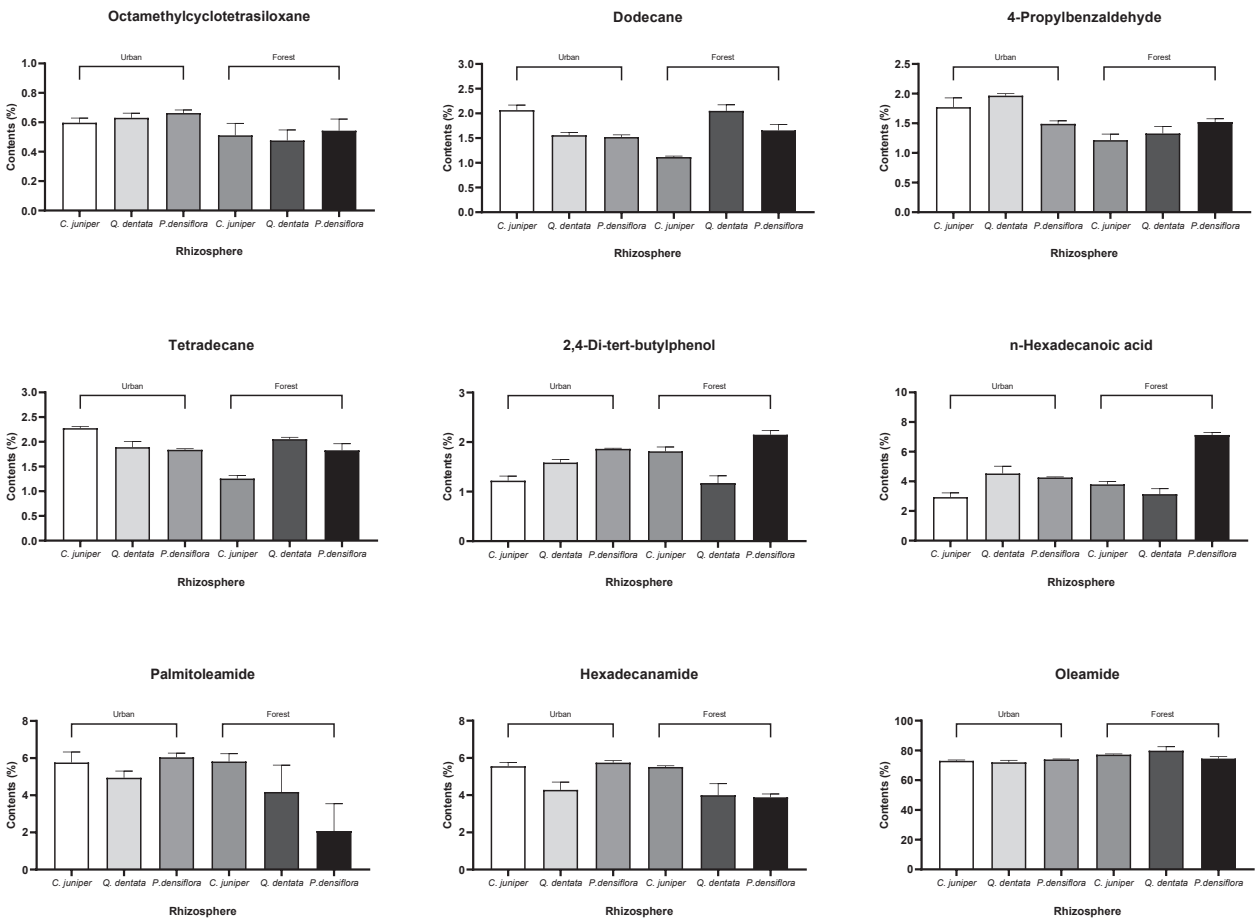

Figure 4: Comparison of the contents of nine major metabolites identified in urban and forest environments. As a result of the library search, a total of 31 metabolites were identified, which were classified according to their structural and chemical properties into siloxanes, alkanes, fatty acids and derivatives, amides and nitriles, aromatics, terpenes and terpene derivatives, and other organic compounds (Table 2). Among these, the relative contents of nine metabolites commonly found in the soil around the roots of trees in cities and forests are shown in bar graphs.

reducing the concentration of carbon dioxide in the atmosphere and providing oxygen. Their roots also hold the soil firmly in place, preventing erosion and improving water quality, while their leaves and branches intercept rainfall and regulate the runoff of surface water, preventing flooding. Furthermore, they maintain biodiversity by providing habitat and food for various species. Finally, they create economic and cultural value by providing a variety of resources for humans, including timber, medicine, and fruit. These roles make trees essential to maintaining the balance of the global environment. However, the accumulation of pollutants that comes with human development disrupts this balance, making it imperative to address this issue and conduct in-depth research.

On the other hand, forest environments have less human interference, contributing to natural nutrient circulation and maintaining soil structure. Momilactone A, discovered in forest rhizosphere soil, is known to have strong physiological activity and play an important role in plants' defense mechanisms. These substances accumulate and are produced in forest environments through natural ecological processes and interactions. Therefore, this can contribute to plant survival, adaptation, and competitiveness.

LC/MS-Q-TOF and GC/MS analyses revealed significant differences in metabolite profiles between urban and forest environments. In the forest environment, high concentrations of various bioactive compounds such as rifabutin and palmitic acid were found. This suggests that the abundance of organic matter and microbial activity in forest environments may promote the production of these compounds. In particular, siloxanes detected through GC/MS analysis are compounds mainly released into the environment through anthropogenic activities and were detected at high concentrations in urban environments. This also shows that siloxane contamination in urban areas can be serious. Siloxanes are known to pose reproductive health and carcinogenic risks, which is one of the reasons why the need to manage urban environments is becoming increasingly important. Beyond their ecological roles, rhizosphere soils have important applications in agriculture, livestock, and land restoration. In agriculture, healthy rhizosphere conditions can enhance crop yields and reduce the need for chemical fertilizers and pesticides. In livestock farming, maintaining robust pastureland with healthy soil-rhizosphere interactions can improve forage quality and animal health. In restored areas, understanding and manipulating the rhizosphere can accelerate the recovery

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of degraded lands, promoting the re-establishment of native plant species and ecosystem functions.

This study contributed to a comprehensive understanding of the impact of environmental conditions on the rhizosphere soil ecosystem by comparatively analyzing the chemical diversity and metabolite profiles of rhizosphere soils in urban and forest environments. These results provide an important scientific basis for establishing urban greening and forest conservation strategies and will advance our understanding of the interactions that maintain plant and soil health in diverse ecosystems. However, this study was based on a limited number of samples and results limited to a specific region, so future research is needed in various regions and with more samples. Additionally, standardization of experimental conditions and in-depth study of the functional roles of metabolites are needed.

Conclusion

The chemical composition and metabolite profiles of rhizosphere soils in urban and forest environments vary greatly depending on environmental conditions, which has important implications for plant-soil interactions. The results obtained through this study will contribute to understanding the impact of environmental conditions on the root zone ecosystem and to establishing urban greening and forest conservation strategies based on this. It is also of great significance that high-efficiency analytical equipment LC/MS-Q-TOF and GC/MS were utilized to analyze various metabolites present in the soil, thus providing a method that different researchers can efficiently analyze in the future. In the future, if more in-depth research is conducted on changes in the chemical composition and metabolites of rhizosphere soil through various environmental conditions and more samples, it will be possible to present standards that can effectively understand the ecological situation of various urban environments around the world. Additionally, this research underscores the pivotal role of the rhizosphere in plant health, shaped by various biotic and abiotic factors. Future studies should also explore practical applications of rhizosphere knowledge in agriculture, livestock management, and ecosystem restoration, thereby broadening the impact of this field of study.

Data availability statement

The data that support the findings of this study are available within the article.

Author contributions

Data Curation: Y.P, Writing – Original Draft Preparation: Y.P and J.C, Writing – Review & Editing: B.Y, Conceptualization: Y.P, and B.Y, Validation: B.Y, Investigation: J.C, Methodology: J.C, Visualization: Y.P. All authors have read and agreed to the published version of the manuscript.

(Supplementary-Table)

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