RESEARCH



Differential effects of exogenous VOCs on the growth and stress responses of *Cunninghamia lanceolata* seedlings under low phosphorus



Yachao Li^{1,2}, Hui Zhang^{1,2,3}, Jing Liu^{1,2}, Ming Li^{1,2}, Taimoor Hassan Farooq⁴, Xiangqing Ma^{1,2}, Xiaoli Yan^{1,2*} and Pengfei Wu^{1,2*}

Abstract

This study investigates how exogenous volatile organic compounds (VOCs) influence the root responses of Cunninghamia lanceolata under nutrient limitations. Specifically, we assessed the differential effects of syringic acid and 1-butene on root morphology, phosphorus acquisition, and stress resilience. Clonal Chinese fir seedlings were utilized to test the effects of treatment with these two exogenous VOCs under both phosphorus supply (1.0 mM KH_2PO_4) and no phosphorus supply (0 mM KH_2PO_4) treatments in a controlled pot experiment. Compared to syringic acid, 1-butene significantly enhanced root morphological traits, including root length, specific surface area, and root volume. These morphological changes enhanced the root's ability to acquire phosphorus. Moreover, the addition of 1-butene increased the underground phosphorus use efficiency (PUE) by 25.6% compared to the addition of syringic acid. Furthermore, the addition of 1-butene stimulated higher activity of antioxidant enzymes such as superoxide dismutase (SOD) and peroxidase (POD). Proteomic analysis revealed that 1-butene induced significant changes in root protein expression, particularly in proteins associated with stress responses, phenylpropanoid biosynthesis, and phosphate transport. Compared to syringic acid, 1-butene promoted the differential expression of phosphate transporter proteins, indicating its beneficial effects on the root systems of Chinese firs under low-phosphorus stress. These findings underscore the potential of 1-butene in promoting root efficiency and phosphorus acquisition in forest species, providing insights for enhancing plant adaptation to nutrient limitations.

Keywords Volatile organic compounds, 1-butene, Syringic acid, Low P environment, *Cunninghamia lanceolata*, Stress resistance

*Correspondence: Xiaoli Yan ccyanxiaoli@163.com Pengfei Wu fjwupengfei@126.com; fjwupengfei@fafu.edu.cn ¹College of Forestry, Fujian Agriculture and Forestry University, Fuzhou 350002, China



© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

 ²Chinese Fir Engineering Technology Research Center of the State Forestry and Grassland Administration, Fujian Agriculture and Forestry University, Fuzhou 350002, China
³Fujian Mawei Minjiang River Estuary Wetland Provincial Nature Reserve Management Office, Fuzhou 350002, China
⁴Bangor College China, a Joint Unit of Bangor University and Central, South University of Forestry and Technology, Changsha 410004, China

Introduction

Phosphorus (P) is an essential nutrient for all life forms, playing a critical role in biochemical processes such as nucleic acid synthesis (e.g., DNA, RNA), energy transfer (e.g., ATP), and the structural integrity of membranes (e.g., phospholipids) [1]. Despite its importance, the availability of P in soils is often limited. Over 30% of global agricultural and forestry production is constrained by insufficient soil P levels [7, 14]. Low availability is attributed to the high chemical fixation rates, slow diffusion rates, and predominance of plant-unavailable organic P forms over inorganic forms [44]. While fertilization is commonly employed to mitigate these limitations, it fails to address the long-term sustainable P scarcity and poses potential environmental risks [3]. Plants exhibit various adaptive strategies to cope with low P availability [20, 24]. Investigating these adaptations is fundamental for understanding how plants mitigate P stress and maintain growth and survival in nutrient-limited conditions.

Volatile organic compounds (VOCs) are important secondary metabolites that are typified by low emission levels and potent effects [8]. They play critical roles in various stress responses. For example, in the Scots pine (*Pinus sylvestris*), emission of aliphatic aldehydes and monoterpene compounds is altered in response to oxidative changes [33]. The Chinese fir (*Cunninghamia lanceolata*) can respond to low P stress by regulating the emission of VOCs such as syringic acid [24]. Tea plants (*Camellia sinensis*) can release nerolidol to alleviate cold stress [22].

VOCs not only affect the emitting plant but can also influence neighboring plants, thereby enhancing their stress tolerance [9, 16, 40]. For example, in response to virus attacks, tobacco plants (Nicotiana benthamiana) releases VOCs like (E)-2-octenal, 6-methyl-5-hepten-2-one, and geranylacetone after infection with Tobacco mosaic virus, enhancing the resistance of neighboring plants to the virus [19]. Sweet orange (Citrus sinensis) can absorb VOCs released by neighboring guava (Psidium guajava) and enhance its own resistance to herbivores [25]. Tea plants (Camellia sinensis) release VOCs such as methyl salicylate (MeSA), benzyl alcohol, and phenethyl alcohol in response to drought stress, enhancing the drought tolerance of neighboring tea plants [21]. Such interplant signaling mechanisms, particularly under stress conditions, are significant for enhancing overall plant resilience in nutrient-limited environments.

Chinese fir is the most commercially important coniferous species that is widely distributed in southern China [48]. However, there primary cultivation region contains an abundance of red clay, which lacks sufficient available P content. Successive pure-stand management practices have exacerbated P depletion, hindering sustainable growth [26]. In response to low P conditions, Chinese fir roots exhibit adaptive mechanisms, including enhanced VOCs emissions and morphological adjustments [5, 24]. The increasing adoption of high-density planting in Chinese fir plantations has led to overcrowded root zones and intensified nutrient competition [29]. Despite these challenges, the role of root-emitted VOCs in mediating growth responses and interplant interactions remains poorly understood. Furthermore, the difficulty in assessing root VOC emissions compared to aboveground emissions presents a significant knowledge gap.

The root VOCs syringic acid and 1-butene both exhibit significant changes in emission levels in response to low P stress [24, 41]. However, their specific roles and mechanisms of action remain unclear. This study seeks to address key questions: Do these VOCs exhibit distinct roles in plant responses to low P stress? How do these differences influence the growth and stress responses of Chinese fir?

To address these questions, this study conducted a pot experiment to investigate the effects of syringic acid and 1-butene on Chinese fir seedlings under different P conditions. The morphological, physiological, and proteomic responses of the seedlings were assessed to uncover the mechanisms behind their adaptation to low P stress. This research offers new insights into how VOCs influence plant responses to nutrient limitations, with a focus on their potential to improve the resilience and productivity of forest ecosystems under P-deficient conditions. By enhancing our understanding of VOCs-mediated stress responses, this study contributes valuable knowledge for developing sustainable management practices in Chinese fir plantations, particularly in nutrient-limited environments.

Materials and methods

Plant materials and growth conditions

The plant materials in this study consisted of robust seedlings of the clonal M25 of the Chinese fir, bred by the Chinese fir Engineering Technology Research Center of the State Forestry and Grassland Administration. The clonal M25 of Chinese fir is capable of maintaining adequate growth and development under low P conditions [45]. In this study, one-year-old Chinese fir seedlings were selected, with an average height of 25.78 ± 1.88 cm and a diameter at root collar of 5.23 ± 0.21 mm. The seedlings were initially placed in nutrient-rich substrate prior to treatment. The seedlings were transplanted into sterilized culture sand, which had been thoroughly cleaned and sterilized. The content of available P in culture sand was $0.034 \pm 0.007 \text{ mg} \cdot \text{kg}^{-1}$ [24]. An indoor simulated sand culture experiment was carried out in a greenhouse. The greenhouse environment was maintained under controlled environmental conditions with a day/night temperature of 28° C/ 25° C, a 16 h day/8 h night cycle with a

photon flux density of 21 mol·quanta·m⁻²·d⁻¹, and relative humidity of 42.7% during the day and 67.7% at night.

Experimental design

The experimental treatments were conducted using Chinese fir seedlings subjected to two P levels (1.0 mM KH₂PO₄ or 0 mM KH₂PO₄) and supplied with two VOCs (syringic acid or 1-butene), for a total of four treatments: P supply with syringic acid (P_{1sv}: 1.0 mM KH₂PO₄, 200 mg·L⁻¹ syringic acid), P supply with 1-butene (P_{1bu}: 1.0 mM KH₂PO₄, 10 mL·L⁻¹ 1-butene), no P supply with syringic acid (P_{0sv}: 0 mM KH₂PO₄, 200 mg·L⁻¹ syringic acid), and no P supply with 1-butene (P_{0bu} : 0 mM KH_2PO_4 , 10 mL·L⁻¹ 1-butene). According to the Hoagland nutrient solution and the available P content in southern Chinese forest soils, a 1.0 mM KH₂PO₄ solution was used as the P supply treatment [34]. Low P stress of 7 days was found to stimulate the production of VOCs in Chinese fir roots [24]. Therefore, a treatment period of 7 days was selected to better understand the role of root VOCs in response to low P stress. 1-butene was released through a tube buried in the pot, while syringic acid was added to the pot with the nutrient solution and applied exogenously every 3 days. To better investigate the effects of VOCs on Chinese fir seedlings under low-P conditions, we applied different P supply treatments for one month before the addition of exogenous VOCs. Each treatment was replicated five times.

To meet the remaining elemental needs of the seedlings during the experimental treatment period, other elements were supplemented using modified Hoagland nutrient solution (Table S1) [43]. Seedlings were irrigated with 200 mL of 1/4 modified Hoagland nutrient solution every 3 days. Exogenous syringic acid (McLean, Shanghai, China) is a solid that is immediately applied to the pots after being dissolved in the nutrient solution. Exogenous 1-butene (Huaxia Chemicals, Chengdu, China) is a gas and is delivered via the air tube in the pot. After each application, the pot is immediately sealed to prevent the volatilization of syringic acid and 1-butene. To prevent the accumulation of CO₂ and other substances produced by the roots, the sealing device is opened every 3 days for ventilation, and the next round of nutrient solution and exogenous 1-butene is applied. This process is repeated until the sealing time reaches 7 days, after which ventilation occurs, and no further exogenous substances are applied. After the application of exogenous substances has ended, the seedlings are harvested.

After the treatment period, the seedlings were dug out whole, rinsed with water, and then wiped with absorbent paper to remove surface water, taking care to avoid damaging the root system in the process. Cleaned and collected intact roots were quickly examined for root morphology. A portion of the Chinese fir root samples designated for physiological and proteomic analyses were stored in a -80 °C freezer, while the remaining underground and aboveground parts of the Chinese fir plants were subjected to drying.

Measurement of root morphology and physiology indexes

After harvesting, the treated Chinese fir seedlings were promptly scanned using a STD1600 scanner (Seiko Epson Corporation, Nagano-ken, Japan) to visualize their root systems. Subsequently, the root lengths, surface areas, volumes, and average diameters of each root system were analyzed using WinRHizo software version 4.0B (Regent, Canada).

After harvesting the treated Chinese fir seedlings, the superoxide dismutase (SOD) activities, peroxidase (POD) activities, malondialdehyde (MDA) contents, and acid phosphatase (Apase) activities of fresh root samples were determined. The root sample (0.2 g) in liquid nitrogen and subsequently standardizing them with phosphate buffer (pH 7.8, 0.5 M) to measurement root SOD and POD activities [47]. MDA content was determined by colorimetric using thiobarbituric acid and 0.2 g root sample of needles per replicate. The absorption values at 532 nm and 600 nm were recorded with distilled water as the control. Apase activity was estimated by calculating the extent of degradation of nitrobenzene disodium phosphate to nitrophenol per unit time [31].

Determination of biomass and root: shoot ratio

Treated Chinese fir seedlings were then dried. They were subjected to a temperature of 108 °C for 30 min and then dried at 80 °C until they reached a constant weight. The aboveground biomass was the dry weight of the aboveground parts of the treated seedlings, while the underground biomass was the dry weight of the underground parts of the tested seedlings. The total biomass was the sum of the aboveground biomass and the underground biomass. The root: shoot ratio was calculated as the underground biomass divided by the aboveground biomass.

Determination of P content and P use traits

After drying and weighing, samples were crushed and analyzed for P content. Masses of 0.2 g of crushed samples were sieved and then heated in conical flasks containing 30 mL digestion solution (nitric acid: perchloric acid = 5:1) until the samples were fully dissolved. The P content in the digestion solution was measured using Inductively Coupled Plasma Optical Emission Spectrometer Optima 8000 (Perkin Elmer Instrument Co., LTD., Shanghai, China), with the following formulas utilized to calculate P accumulation and P use efficiency (PUE): P accumulation = Dry weight * P content; PUE = Dry weight / (P accumulation) [28].

Proteomic detection in root system

Randomly select three biological samples for proteomics analysis. A mass of 0.2 g of fresh root samples were used for each replicate. Protein extraction and enzymatic digestion were conducted using Zandalinas' described method [46]. Peptides were dissolved in mobile phase A (0.1% formic acid and 2% acetonitrile in water) and separated using a NanoElute ultra-high-performance liquid chromatography (UHPLC) system (Bruker, United States). Mobile phase B consisted of 0.1% formic acid in 100% acetonitrile. The gradient elution program was set as follows: 6–24% B from 0 to 70 min, 24–32% B from 70 to 84 min, 32–80% B from 84 to 87 min, and 80% B from 87 to 90 min, with a constant flow rate of 450 nL/ min [28]. The mass spectrometry analysis conditions for peptide fragments were referenced from Li et al. [28].

Statistical analysis of data

Independent sample T-tests (p < 0.05) were used to determine the significance of differences between plants treated with different P supply levels and exogenous VOCs. SPSS 19.0 (IBM, New York, United States) was used to perform statistical analyses, with GraphPad Prism 9 (GraphPad Software, Santiago, United States) used for presenting histograms (mean ± standard error). The heatmaps were generated using Hiplot Pro (https://hiplot.com.cn/).

Fisher's exact test was used to identify differentially expressed proteins (DEPs). Proteins with a log2 fold change ≥ 0.584963 and p < 0.05 were considered DEPs. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were then performed on the set of DEPs. GO terms or

KEGG pathways with p < 0.05 were deemed significantly enriched. The subcellular localizations of proteins were predicted using WoLF PSORT software (v3.0). EggNOG was used to identify the KOG of DEPs. The Pfam database was employed to analyze the enrichment of functional domains of DEPs (p < 0.05).

In order to further analyze and compare the different effects of two exogenous VOCs on Chinese fir under low P conditions, this study compared the proteomic data of P_{0sy} and P_{0bu} . Proteomic data from Chinese fir that were not treated with either of the VOCs were also compared to proteomic data from plants treated with the two exogenous VOCs, both under P supply (P₁:1 mM KH₂PO₄ without VOCs) and no P supply (P₀: 0 mM KH₂PO₄ without VOCs) conditions. The P₀ and P₁ databases were derived from Li et al. [28].

Results

Effects of exogenous VOCs on the growth of Chinese Fir

Chinese fir seedlings were cultivated with one of two VOCs in different P treatments (Fig. 1). Under no P supply treatment with the two VOCs produced different growth responses in the Chinese fir seedlings (Fig. 2a-d). The aboveground biomass and total biomass with 1-butene treatment were 28.6% and 21.6% lower, respectively, under no P supply treatment than under supply P treatment (p < 0.05, Fig. 2a, c). Compared to syringic acid treatment, treatment with 1-butene resulted in a significant increase in underground biomass and root: shoot ratio under no P supply treatment (Fig. 2b, d). Thus, the application of 1-butene may promote the growth of underground parts of Chinese fir under low P conditions, especially as compared to treatment with syringic acid.



Fig. 1 Schematic diagram of the potted simulation experiment setup



Fig. 2 Analysis of biomass and root: shoot ratio of Chinese fir. Effects of exogenous VOCs on the aboveground biomass (a), underground biomass (b), total biomass (c), and root: shoot ratio (d) of Chinese fir seedlings. P_{1sy} and P_{1bu} indicate treatments with syringic acid and 1-butene, respectively, under supply P treatment, while P_{OSV} and P_{ObV} indicate treatments with syringic acid and 1-butene, respectively, under no P supply treatment. * indicates a significant difference (p < 0.05) between the two treatment groups

Effects of exogenous VOCs on the morphology and resistances of Chinese Fir roots

Treatment with 1-butene significantly increased root lengths by 28.1%, root surface areas by 27.4%, and root volumes by 49.7% as compared to treatment with syringic acid under no P supply treatment (p < 0.05, Fig. 3ac). However, there was no significant difference in root average diameters between treatments of the two VOCs (Fig. 3d). Root length and root surface area are significant factors that influence the P absorption capacity of Chinese fir. Treatment with 1-butene increased the root lengths and root surface areas of the Chinese fir seedlings relative to treatment with syringic acid. This suggests that 1-butene may play a role in acquiring P under low P conditions in Chinese fir, especially as compared to syringic acid.

SOD and POD are important antioxidant enzymes. MDA is the final product of free radical oxidation and is also an important antioxidant parameter in plants. Treatment with 1-butene under no P supply treatment resulted in significant increases in both SOD (12.7%) and POD (99.5%) activities as compared to supply P treatment, though MDA content was not significantly affected (Fig. 3e-g). Compared to supply P treatment, treatment with syringic acid resulted in a significant increase of MDA content (158.3%) under no P supply treatment,



Fig. 3 Analysis of root morphology and physiology of Chinese fir. The effects of treatment with exogenous VOCs on root length (**a**), root surface area (**b**), root volume (**c**), and root average diameter (**d**), along with the impacts on SOD activity (**e**), POD activity (**f**), MDA content (**g**), and Apase activity (**h**) under different treatments. P_{1sy} and P_{1bu} indicate treatments with syringic acid and 1-butene, respectively, under supply P treatment, while P_{0sy} and P_{0bu} indicate treatments with syringic acid and 1-butene, respectively, under supply P treatment, while P_{0sy} and P_{0bu} indicate treatments with syringic acid and 1-butene, respectively, under supply treatment. * indicates a significant difference (p < 0.05) between the two treatment groups

while POD and SOD activities were not significantly affected (Fig. 3e-g). The addition of exogenous VOCs did not significantly affect Apase activity (Fig. 3h). Thus, treatment with 1-butene resulted in significant enhancements in stress tolerance in Chinese fir under low P conditions as compared to treatment with syringic acid.

Effects of exogenous VOCs on P use traits in Chinese Fir

Under supply P treatment, treatment with 1-butene resulted in a significant decrease of aboveground P content (23.5%) as compared to treatment with syringic acid, though no significant differences were observed under no P supply treatment (Fig. 4a). Under no P supply treatment, syringic acid treatment resulted in a significant increase of underground P content (21.9%) as compared to treatment with 1-butene, though no significant differences were observed under supply P treatment (Fig. 4b). No significant differences in total P content and aboveground P accumulation were observed between any of the treatment conditions (Fig. 4c, d). Compared to supply P treatment, syringic acid treatment resulted in decreased P accumulation in the underground parts, and 1-butene treatment resulted in decreased total P accumulation under no P supply treatment (Fig. 4e, f). Treatment with 1-butene had no significant effect on aboveground PUE between different P treatment, though it did result in increased underground PUE in no P supply treatment. It exerted no significant effect upon the total PUE (Fig. 4gi). Consequently, while the addition of 1-butene may not facilitate an immediate augmentation in the P content in Chinese fir, it may ameliorate P deficiency stress through the enhancement of underground PUE, thereby sustaining the adequate growth and development of the root system under low P conditions.

Identification, annotation, and enrichment of deps

Proteomic analysis was conducted on Chinese fir roots treated with different exogenous VOCs. The relative standard deviation of protein quantification values among replicate samples within each group ranged from 0.1 to 0.2 (Fig. 5a). A total of 8,066 proteins were detected in this proteomic analysis, among which 6,235 were quantifiable (Fig. 5b). A comparison of P_{0sy} and P_{1sy} identified 56 DEPs, with 22 being down-regulated and 34 up-regulated. Likewise, comparison of P_{0bu} and P_{1bu} identified 201 DEPs, with 115 being down-regulated and 86 up-regulated, and comparison of P_{0sy} and P_{0bu} identified 257 DEPs, with 112 being down-regulated and 145 up-regulated.

To further investigate the effects of VOC treatments under no P supply treatment, we investigated the functions of DEPs identified between P_{0sv} and P_{0bv} . Of the 201



Fig. 4 Analysis of P use traits of Chinese fir. The effects of treatment with exogenous VOCs on Chinese fir aboveground P content (a), underground P content (b), total P content (c), aboveground P accumulation (d), underground P accumulation (e), total P accumulation (f), aboveground PUE (g), underground PUE (h), and total PUE (i). P_{1sy} and P_{1bu} indicate treatments with syringic acid and 1-butene, respectively, under supply P treatment, while P_{0sy} and P_{Obj} indicate treatments with syringic acid and 1-butene, respectively, under no P supply treatment. * indicates a significant difference ($\rho < 0.05$) between the two treatment groups

DEPs identified in this comparison, 108 could be classified using GO terms. In the Biological Process category, the most frequently observed GO terms among the DEPs included "cellular metabolic process", "organic substance metabolic process", and "primary metabolic process"; in the Cellular Component category, the most commonly observed were "intracellular", "organelle", and "cytoplasm"; and in the Molecular Function category, the most commonly observed were "organic cyclic compound binding", "heterocyclic compound binding", and "transferase activity" (Fig. 6a).

A total of 132 DEPs were classified using COG function classifications, with the most frequently observed classifications including "[O] Posttranslational modification, protein turnover, chaperones", "[G] Carbohydrate transport and metabolism", and "[C] Energy production and conversion" (Fig. 6b). Subcellular localization analysis revealed that the DEPs were most likely to localize to



Fig. 5 Quality analysis of proteins and identification of DEPs. The relative standard deviation of protein quantification values under different treatments (**a**). The numbers of DEPs identified between different treatments (**b**). UP: DEPs were up-regulated; DOWN: DEPs were down-regulated. P_{1sy} and P_{1bu} indicate treatments with syringic acid and 1-butene, respectively, under supply P treatment, while P_{0sy} and P_{0bu} indicate treatments with syringic acid and 1-butene, respectively, under supply P treatment, while P_{0sy} and P_{0bu} indicate treatments with syringic acid and 1-butene, respectively.

the chloroplast, the cytoplasm, or the nucleus (Fig. 6c). A total of 132 DEPs were classified using KEGG functional enrichment analysis, with "map03013 RNA transport" and "map00195 Photosynthesis" the most frequently observed classifications (Fig. 6d). Analysis of protein domains revealed that the most significantly enriched domains among the DEPs included "PF00150 Cellulase (glycosyl hydrolase family 5)", "PF04969 CS domain", and "PF07714 Protein tyrosine kinase" (Fig. 6e).

The expression of phosphate transport proteins

Phosphate transport proteins (PHTs) are crucial for the absorption and transportation of phosphate, particularly PHT1-4, whose expression is sensitive and responsive to no P supply treatment. Under no P supply treatment, the addition of 1-butene significantly reduced the expression of PHT1-4 by 34.7% compared to syringic acid (Fig. 7). These results indicate that the addition of 1-butene under low P conditions contributes to the alleviation of P stress in Chinese fir.

Discussion

Effects of exogenous VOCs on P use and distributions in Chinese Fir

Serving crucial sensory roles, plant root systems respond to environmental cues including nutrient availability, leading to modifications in root activities that demonstrate significant plasticity [15]. Root VOCs drive plants to adjust their growth dynamics, rapidly responding to their surroundings [42]. The release of VOCs in Chinese fir roots can be increased under low P conditions [24]. Root systems are crucial for P absorption, with changes in root morphology markedly affecting P uptake (Liu [27]). The addition of 1-butene led to significant increases in root lengths, root surface areas, and root volumes as compared to addition of syringic acid under low P conditions (Fig. 3a-c). No significant changes in morphological characteristics, such as root length, were observed under different P supply treatments without the addition of exogenous substances [28]. P uptake has been found to increase as the area of root-soil contact increases [49]. Hence, these findings suggest that 1-butene enhances root morphological traits, which may improve P uptake under low P conditions, highlighting its potential role in optimizing root function for nutrient acquisition.

Contrary to our expectations, the results from the P content analysis in Chinese fir diverged from the results of our morphological analyses. Compared to supply P conditions, the addition of 1-butene resulted in a significant decrease in underground P content under low P conditions, while underground PUE was enhanced (Fig. 4b, h). There is no significant difference in the underground PUE and underground P content of Chinese fir roots were observed between different P supply treatments without the addition of exogenous substances [28]. Although the increased root length and surface area may enhance the P absorption capacity of Chinese fir seed-lings, the low P content in the environment may still fail to meet the adequate growth requirements of Chinese fir.



Fig. 6 Identification and annotation enrichment analysis of DEPs. GO functional classifications (a), KOG classifications (b), predicted subcellular localizations (c), KEGG functional enrichments (d), and predicted protein domains (e) of DEPs identified between P_{0sy} and P_{0bu}. P_{0sy} and P_{0bu} indicate treatments with syringic acid and 1-butene, respectively, under no P supply treatment

biomass or yield from the P it has acquired [17]. One way to increase PUE in plants is to enhance P mobilization within the plant [10]. In low P environments, plants enhance their PUE by converting endosomal organic P into inorganic P to sustain standard growth and development [38]. This result seems inconsistent with the conclusion that 1-butene enhances P acquisition in Chinese fir by increasing root surface area. It suggests that in the short term under low P conditions, 1-butene actively aids Chinese fir in coping with P scarcity. On the one hand, 1-butene modulates root morphology to enhance the plant's ability to acquire P. On the other, it regulates internal P cycling to maintain adequate growth and development under low P conditions.

The acquisition and redistribution of P within the plant is primarily facilitated by the PHT1 protein family [32]. PHT1-4, an essential member of the PHT1 family, plays a key role in P absorption under low P conditions [11]. This study showed that compared to exogenous syringic acid, the addition of 1-butene significantly reduced the expression of PHT1-4 under low P conditions (Fig. 7). In a previous study, the expression of PHT1-4 was upregulated under low P stress, while it was expressed at lower levels under high P condition [35]. In comparison to syringic acid, the addition of 1-butene resulted in significant downregulation of PHT1-4 expression, suggesting that 1-butene supplementation aids in alleviating low P stress in Chinese fir.

Effects of exogenous VOCs on resistance to low P conditions in Chinese Fir

The application of exogenous VOCs not only regulates P uptake and PUE to alleviate low P stress but also maintains the adequate growth and development of the plant by regulating stress tolerance. Antioxidant resistance serves as a key indicator of plant adversity during adequate development [23]. Prominent antioxidant enzymes in plants include POD, SOD, and others, which



Fig. 7 Protein expression of PHT1-4 under different treatments. P_{1sy} and P_{1bu} indicate treatments with syringic acid and 1-butene, respectively, under supply P treatment, while P_{0sy} and P_{0bu} indicate treatments with syringic acid and 1-butene, respectively, under no P supply treatment

perform significant defensive roles in response to abiotic and biotic stresses [6, 37]. MDA is a product of lipid peroxidation and indicates oxidative membrane damage [2, 12]. Compared to the P supply treatment, we found that the addition of 1-butene significantly increased the activities of both enzymes under low P conditions while not significantly changing MDA content (Fig. 3e-g). The addition of syringic acid did not result in increased SOD and POD activities under low P conditions, but it did result in higher MDA content (Fig. 3e-g). The difference in effects also may stem from the distinct chemical properties of the two VOCs. 1-butene, an alkene, may have a significant impact on the activity of antioxidant enzymes, such as SOD [39]. In contrast, syringic acid, a phenolic acid, promotes lipid peroxidation in plants, resulting in the accumulation of substances such as H₂O₂, O₂, and MDA within the plant [18]. Compare to syringic acid, the addition of 1-butene promoted the enhancement of antioxidant capacity in the roots of Chinese fir under low P stress.

We conducted proteomic data analysis to assess differential effects of treatment with various exogenous VOCs under low P conditions on phenylpropanoid biosynthesis. Following the addition of 1-butene, DEPs were significantly enriched in phenylpropanoid biosynthesis pathway, and the DEPs were also significantly upregulated (Fig. S1). In contrast, DEPs after the addition of syringic acid were no significantly enriched for phenylpropanoid biosynthesis pathways (Fig. S2a, b). Phenylpropanoid biosynthesis secondary metabolites in plants, playing crucial roles in antioxidation, stress mitigation, and signal regulation [13]. Under low P conditions, the addition of 1-butene significantly promotes the biosynthesis and accumulation of Phenylpropanoid biosynthesis in Chinese fir seedlings. This suggests a potential link between the metabolic changes induced by 1-butene and the improved stress recovery capacity. In contrast, the addition of syringic acid did not exhibit similar effects. The promotion of Phenylpropanoid biosynthesis by 1-butene is achieved by significantly upregulating the activity of key enzymes in the Phenylpropanoid biosynthesis pathway, thereby enhancing antioxidant capacity and potentially improving stress resilience.

The upregulation of DEPs such as Phenylalanine ammonia-lyase 1, 4-coumarate-CoA ligase 7, and Peroxidase 3 upon 1-butene addition treatment (Fig. S1). These enzymes collaboratively enhance the metabolic flux toward phenylpropanoid biosynthesis and lignin production, which are crucial for antioxidative defense and structural reinforcement under low P conditions [4, 13, 50]. Furthermore, the phenylpropanoid metabolites may influence root-rhizosphere interactions, enhancing nutrient cycling and adaptive capacity in nutrient-poor environments. In contrast, the addition of 1-butene significantly enhances phenylpropanoid biosynthesis and associated stress-resilience mechanisms in Chinese fir seedlings under low P conditions, where as syringic acid did not induce similar metabolic changes, highlighting potential role of 1-butene in improving plant adaptation to nutrient limitations.

We found that the DEPs between P_{0sy} and P_{0bu} were significantly enriched in the RNA transport (Fig. 6d). RNA transport refers to the molecular exchange between the nucleus and cytoplasm, which is mediated by nuclear pore complexes embedded in the nuclear membrane [36]. Under the 1-butene addition, the expression of DEPs associated with RNA transport in the roots of Chinese fir was significantly upregulated compared to the syringic acid addition (Table S2). During plant stress responses, the synthesis and transport of stress-related factors rely on nucleocytoplasmic transport, frequently activating that enhance stress resilience [30]. These results indicate that the upregulation of RNA transport proteins by 1-butene may contribute to enhancing stress adaptation in Chinese fir under low P conditions.

Conclusion

This study demonstrated that 1-butene and syringic acid distinctly regulate root growth and physiological responses in Chinese fir seedlings under low P stress. Among these, 1-butene is associated with enhanced P uptake capacity, PUE, and allocation within the plant, as well as improved antioxidant capacity, compared to syringic acid. This increased antioxidant capacity is likely attributed to the upregulation of key antioxidant enzymes and secondary metabolites, which helps mitigate oxidative stress under low P conditions. These results highlight the potential of specific VOCs in optimizing root function and improving stress resilience in nutrient-limited plant. However, the long-term effects of these VOCs on plant growth and nutrient dynamics were not investigated, and future studies should address the sustainability of these responses over extended periods.

Abbreviations

Р	Phosphorus
VOCs	Volatile organic compounds
P _{1sv}	Phosphorus supply with syringic acid
P _{1bu}	Phosphorus supply with 1-butene
P _{0sy}	No phosphorus supply with syringic acid
Pobu	No phosphorus supply with 1-butene
SOD	Superoxide dismutase
POD	Peroxidase
MDA	Malondialdehyde
Apase	Acid phosphatase
PUE	Phosphorus use efficiency
DPEs	Differentially expressed proteins
GO	Gene ontology
KEGG	Kyoto encyclopedia of genes and genomes
P ₁	Phosphorus supply without VOCs
Po	No phosphorus supply without VOCs
PHTs	Phosphate transport proteins

Supplementary Information

The online version contains supplementary material available at https://doi.or g/10.1186/s12870-025-06319-x.

Supplementary Material 1

Acknowledgements

Not applicable.

Author contributions

P.W. X.Y. Y.L. and H.Z. designed the experiment and wrote the manuscript. Y.L. H.Z. and J.L. performed data collection, analysis, and interpretation. X.M. and M.L. offered suggestions to the manuscript. T.F. revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by National Natural Science Foundation of China (grant no. 31870614) and the Key Program of Natural Science of Fujian Province, China (grant no. 2024J02010).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 23 December 2024 / Accepted: 28 February 2025 Published online: 07 March 2025

References

- Alexova R, Millar AH. Proteomics of phosphate use and deprivation in plants. Proteomics. 2013;13(3–4):609–23. https://doi.org/10.1002/pmic.201200266.
- 2 Ali E, Hussain N, Shamsi IH, Jabeen Z, Siddiqui MH, Jiang LX. Role of jasmonic acid in improving tolerance of rapeseed (*Brassica napus*) to cd toxicity. J Zhejiang University-Science B. 2018;19(2):130–46. https://doi.org/10.1631/jzu s.B1700191.
- 3 Bindraban PS, Dimkpa CO, Pandey R. Exploring phosphorus fertilizers and fertilization strategies for improved human and environmental health. Biol Fert Soils. 2020;56(3):299–317. https://doi.org/10.1007/s00374-019-01430-2.
- 4 Chen XH, Su WL, Zhang H, Zhan YG, Zeng FS. Fraxinus mandshurica 4-coumarate-CoA ligase 2 enhances drought and osmotic stress tolerance of tobacco by increasing coniferyl alcohol content. Plant Physiol Biochem. 2020;155:697–708. https://doi.org/10.1016/j.plaphy.2020.08.031.
- 5 Chen WT, Zhou MY, Zhao MZ, Chen RH, Tigabu M, Wu PF, Li M, Ma XQ. Transcriptome analysis provides insights into the root response of Chinese Fir to phosphorus deficiency. BMC Plant Biol. 2021;21(1):525. https://doi.org/10.118 6/s12870-021-03245-6.
- 6 Cocozza C, Bartolini P, Brunetti C, Miozzi L, Pignattelli S, Podda A, Scippa GS, Trupiano D, Rotunno S, Brilli F, Maserti BE. Modulation of class III peroxidase pathways and phenylpropanoids in *Arundo donax* under salt and phosphorus stress. Plant Physiol Biochem. 2022;183:151–9. https://doi.org/10.1016/j.plaph y.2022.05.002.
- 7 Cunha HFV, Andersen KM, Lugli LF, Santana FD, Aleixo IF, Moraes AM, Garcia S, Di Ponzio R, Mendoza EO, Brum B, Rosa JS, Cordeiro AL, Portela BTT, Ribeiro G, Coelho SD, de Souza ST, Silva LS, Antonieto F, Pires M, Salomao AC, Miron AC, de Assis RL, Domingues TF, Aragao LEOC, Meir P, Camargo JL, Manzi AO, Nagy L, Mercado LM, Hartley IP, Quesada CA. Direct evidence for phosphorus limitation on Amazon forest productivity. Nature. 2022;608(7923):558–62. htt ps://doi.org/10.1038/s41586-022-05085-2.
- 8 Dani KGS, Loreto F. Plant volatiles as regulators of hormone homeostasis. New Phytol. 2023;234(3):804–12. https://doi.org/10.1111/nph.18035.
- 9 Dicke M, Agrawal AA, Bruin J. Plants talk, but are they deaf? Trends Plant Sci. 2003;8(9):403–5. https://doi.org/10.1016/S1360-1385(03)00183-3.
- 10 Dissanayaka DMSB, Plaxton WC, Lambers H, Siebers M, Marambe B, Wasaki J. Molecular mechanisms underpinning phosphorus-use efficiency in rice. Plant Cell Environ. 2018;41(7):1483–96. https://doi.org/10.1111/pce.13191.
- 11 Dindas J, DeFalco TA, Yu G, Zhang L, David P, Bjornson M, Thibaud MC, Custódio V, Castrillo G, Nussaume L, Macho AP, Zipfel C. Direct Inhibition of phosphate transport by immune signaling in *Arabidopsis*. Curr Biol. 2022;32(2):488–95. https://doi.org/10.1016/j.cub.2021.11.063.
- 12 Ding HN, Ma DY, Huang X, Hou JF, Wang CY, Xie YX, Wang YH, Qin HX, Guo TC. Exogenous hydrogen sulfide alleviates salt stress by improving antioxidant defenses and the salt overly sensitive pathway in wheat seedlings. Acta Physiol Plant. 2019;41(7):123. https://doi.org/10.1007/s11738-019-2918-6.
- 13 Dong NQ, Lin HX. Contribution of phenylpropanoid metabolism to plant development and plant–environment interactions. J Integr Plant Biol. 2021;63(1):180–209. https://doi.org/10.1111/jipb.13054.
- 14 Fort F, Cruz P, Catrice O, Delbrut A, Luzarreta M, Stroia C, Jouany C. Root functional trait syndromes and plasticity drive the ability of grassland (*Fabaceae*) to tolerate water and phosphorus shortage. Environ Exp Bot. 2015;110:62–72. https://doi.org/10.1016/j.envexpbot.2014.09.007.
- 15 Garlick K, Drew RE, Rajaniemi TK. Root responses to neighbors depend on neighbor identity and resource distribution. Plant Soil. 2021;467(1–2):227–37. https://doi.org/10.1007/s11104-021-05083-9.
- 16 Gfeller V, Huber M, Förster C, Huang W, Köllner TG, Erb M. Root volatiles in plant-plant interactions I: high root sesquiterpene release is associated with increased germination and growth of plant neighbours. Plant Cell Environ. 2019;42(6):1950–63. https://doi.org/10.1111/pce.13532.
- 17 Han Y, White PJ, Cheng LY. Mechanisms for improving phosphorus utilization efficiency in plants. Ann Bot. 2022;129(3):247–58. https://doi.org/10.1093/aob /mcab145.
- 18 He ZG, Wang YF, Yan Y, Qin SW, He H, Mao RJ, Liang ZS. Dynamic analysis of physiological indices and transcriptome profiling revealing the mechanisms

of the allelopathic effects of phenolic acids on *Pinellia ternate*. Front Plant Sci. 2022;13:1039507. https://doi.org/10.3389/fpls.2022.1039507.

- 19 Hong Y, Zhen QX, Cheng LT, Liu PP, Xu GY, Zhang H, Cao PJ, Zhou HA. Identification and characterization of TMV-induced volatile signals in *Nicotiana benthamiana*: evidence for JA/ET defense pathway priming in congeneric neighbors via airborne (E)-2-octenal. Funct Integr Genomic. 2023;23(3):272. h ttps://doi.org/10.1007/s10142-023-01203-z.
- 20 Jia XC, Liu P, Lynch JP. Greater lateral root branching density in maize improves phosphorus acquisition from low phosphorus soil. J Exp Bot. 2018;691(20):4961–70. https://doi.org/10.1093/jxb/ery252.
- 21 Jin JY, Zhao MY, Gao T, Jing TT, Zhang N, Wang JM, Zhang XC, Huang J, Schwab W, Song CK. Amplification of early drought responses caused by volatile cues emitted from neighboring tea plants. Hort Res. 2021;8(1):243. htt ps://doi.org/10.1038/s41438-021-00704-x.
- 22 Jin JY, Zhao MY, Jing TT, Zhang MT, Lu MQ, Yu GM, Wang JM, Guo DY, Pan YT, Hoffmann TD, Schwab W, Song CK. Volatile compound-mediated plant-plant interactions under stress with the tea plant as a model. Hort Res. 2023;10(9):uhad143. https://doi.org/10.1093/hr/uhad143.
- 23 Kapoor D, Singh S, Kumar V, Romero R, Prasad R, Singh J. Antioxidant enzymes regulation in plants in reference to reactive oxygen species (ROS) and reactive nitrogen species (RNS). Plant Gene. 2019;19:100182. https://doi.o rg/10.1016/j.plgene.2019.100182.
- 24 Lai HY, Wu K, Wang NM, Wu WJ, Zou XH, Ma XQ, Wu PF. Relationship between volatile organic compounds released and growth of *Cunninghamia lanceolata* roots under low-phosphorus conditions. lforest. 2018;11:713–20. https:// doi.org/10.3832/ifor2797-011.
- 25 Ling SQ, Rizvi SAH, Xiong T, Liu JL, Gu YP, Wang SW, Zeng XN. Volatile signals from guava plants prime defense signaling and increase jasmonatedependent herbivore resistance in neighboring Citrus plants. Front Plant Sci. 2022;13:833562. https://doi.org/10.3389/fpls.2022.833562.
- 26 Liu B, Liu QQ, Daryanto S, Guo S, Huang ZJ, Wang ZN, Wang LX, Ma XQ. Responses of Chinese Fir and Schima Superba seedlings to light gradients: implications for the restoration of mixed broadleaf-conifer forests from Chinese Fir monocultures. For Ecol Manag. 2018;419:51–7. https://doi.org/10. 1016/j.foreco.2018.03.033.
- 27 Liu D, Integr J. Plant Biol. 2021;63(6):1065–90. https://doi.org/10.1111/jipb.130 90.
- 28 Li YC, Zhang H, Tian YL, Farooq TH, Ma XQ, Wu PF. L-Arginine enhances stress resilience against P deficiency of Chinese Fir in root system: physiological and proteomics analysis. Environ Exp Bot. 2024;225:105864. https://doi.org/10.101 6/j.envexpbot.2024.105864.
- 29 Lin DC, Lu JA, Li Q, Zou XH, Li M, Wu PF. The effect of underground space crowding on the endogenous organic acids in the roots of Chinese Fir seedlings. J Fujian Agric Forestry Univ (Natural Sci Edition). 2021;50(1):54–60.
- 30 Lüdke D, Yan QQ, Rohmann PFW, Wiermer M. NLR we there yet? Nucleocytoplasmic coordination of NLR-mediated immunity. New Phytol. 2022;236(1):24–42. https://doi.org/10.1111/nph.18359.
- 31 Mclachlan KD. Acid phosphatase activity of intact roots and phosphorus nutrition in plants (I): assay conditions and phosphatase activity. Aust J Agric Res. 1980;31:429–40. https://doi.org/10.1071/AR9800429.
- 32 Roch GV, Maharajan T, Ceasar SA, Ignacimuthu S. The role of Pht1 family transporters in the acquisition and redistribution of phosphorus in plants. Crit Rev Plant Sci. 2019;38(3):171–98. https://doi.org/10.1080/07352689.2019.164 5402.
- 33 Roffael E, Schneider T, Dix B. Effect of oxidising and reducing agents on the release of volatile organic compounds (VOCs) from strands made of Scots pine (*Pinus sylvestris* L). Wood Sci Technol. 2015;49(5):957–67. https://doi.org/ 10.1007/s00226-015-0744-6.
- 34 Sheng WT, Fan SH. Study on long-term productivity maintenance mechanism of Chinese Fir plantation. Beijing: Science; 2005. p. 251.
- 35 Shin H, Shin HS, Dewbre GR, Harrison MJ. Phosphate transport in Arabidopsis: Pht1;1 and Pht1;4 play a major role in phosphate acquisition from both

low- and high-phosphate environments. Plant J. 2004;39(4):629–42. https://doi.org/10.1111/j.1365-313X.2004.02161.

- 36 Tamura K, Hara-Nishimura I. Functional insights of nucleocytoplasmic transport in plants. Front Plant Sci. 2014;5:118. https://doi.org/10.3389/fpls.2014.00 118.
- 37 Tewari RK, Yadav N, Gupta R, Kumar P. Oxidative stress under macronutrient deficiency in plants. J Soil Sci Plant Nut. 2022;21(1):832–59. https://doi.org/10. 1007/s42729-020-00405-9.
- 38 Tóth B, Moloi MJ, Szöke L, Labuschagne M. Low nitrogen and phosphorus effects on wheat Fe, Zn, phytic acid and phenotypic traits. S Afr J Sci. 2021;117(3–4):28–34. https://doi.org/10.17159/sajs.2021/8414.
- 39 van Doorn MM, Merl-Pham J, Ghirardo A, Fink S, Polle A, Schnitzler JP, Rosenkranz M. Root isoprene formation alters lateral root development. Plant Cell Environ. 2020;43(9):2207–23. https://doi.org/10.1111/pce.13814.
- 40 Vlot AC, Rosenkranz M. Volatile compounds-the Language of all kingdoms? J Exp Bot. 2022;73(2):445–8. https://doi.org/10.1093/jxb/erab528.
- 41 Wang HB, He HB, Ye CY, Lu JC, Chen RS, Liu CH, Guo XK, Lin W. Molecular physiological mechanism of increased weed suppression ability of allelopathic rice mediated by low phosphorus stress. Allelopathy J. 2010;25(1):239– 48. https://doi.org/10.2134/agronj2009.0183.
- 42 Wang NQ, Kong CH, Wang P, Meiners SJ. Root exudate signals in plant-plant interactions. Plant Cell Environ. 2020;44(4):1044–58. https://doi.org/10.1111/p ce.13892.
- 43 Wu PF, Ma XQ, Tigabu M, Wang C, Liu AQ, Oden PC. Root morphological plasticity and biomass production of two Chinese Fir clones with high phosphorus efficiency under low phosphorus stress. Can J Res. 2011;41(2):228–34. https://doi.org/10.1139/X10-198.
- 44 Wu P, Shou HX, Xu GH, Lian XM. Improvement of phosphorus efficiency in rice on the basis of Understanding phosphate signaling and homeostasis. Curr Opin Plant Biol. 2013;16(2):205–12. https://doi.org/10.1016/j.pbi.2013.03. 002.
- 45 Wu WJ, Wang P, Chen NL, Ma XQ, Lin WJ, Wu PF. Phosphorus seeking ability of roots of different Chinese Fir families under low phosphorus stress. J Fujian Agric Forestry Univ (Natural Sci Edition). 2019;48(2):174–81.
- 46 Zandalinas SI, Song LH, Sengupta S, McInturf SA, Grant DG, Marjault HB, Castro-Guerrero NA, Burks D, Azad RK, Mendoza-Cozatl DG, Nechushtai R, Mittler R. Expression of a dominant-negative AtNEET-H89C protein disrupts iron-sulfur metabolism and iron homeostasis in *Arabidopsis*. Plant J. 2020;101(5):1152–69. https://doi.org/10.1111/tpj.14581.
- 47 Zhang XZ. Determination of superoxide dismutase and peroxidase activities. In: Zhang XZ (Editor-in-Chief), Su ZS (ed.). Research methodology of crop physiology. Beijing: Agriculture. 1992;pp. 208–11.
- 48 Zhao Y, Deng XW, Xiang WH, Chen L, Ouyang S. Predicting potential suitable habitats of Chinese Fir under current and future Climatic scenarios based on maxent model. Ecol Inf. 2021;64:101393. https://doi.org/10.1016/j.ecoinf.2021 .101393.
- 49 Zhou CF, Jiang WY, Li Y, Hou XL, Liu AQ, Cai LP. Morphological plasticity and phosphorus uptake mechanisms of hybrid Eucalyptus roots under spatially heterogeneous phosphorus stress. J Res. 2017;28(4):713–24. https://doi.org/1 0.1007/s11676-016-0335-x.
- 50 Zhu BF, Liu Y, Pei XQ, Wu ZL. Characterization of phenylalanine ammonia lyases from lettuce (*Lactuca sativa* L.) as robust biocatalysts for the production of d- and l-amino acids. J Agric Food Chem. 2023;71(6):2935–42. https:// doi.org/10.1021/acs.jafc.2c07890.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.