



Soil P availability and mycorrhizal type determine root exudation in sub-tropical forests

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ABSTRACT

Root exudates determine plant's ability to acquire nutrients through influencing plant's interactions with soil microorganisms. Recent studies suggest that plant's associations with beneficial soil microorganisms explain variation in root exudation as plants opt to minimize the exudation cost through such symbiosis. Yet, we have a poor understanding of whether plants change their exudation rates through mycorrhizal symbiosis in soil environments with varying resource availability. Here, we report the effects of plant-mycorrhizal symbiosis on root exudation rates across a gradient of soil phosphorous (P) availability from a field experiment in subtropical forests. Root exudation rates were higher in plants partnering with arbuscular mycorrhizal fungi than those with ectomycorrhizal fungi, but this difference disappeared in soils with high P. Specific root surface area, specific root length and fine root vitality explained high root exudation in P-limited soils. These findings demonstrate that mycorrhizal symbiosis and root functional traits collectively determine the variation in root exudation in P-limited environments.

1. Introduction

Plants exude a wide range of organic compounds into the rhizosphere—a process known as root exudation (Shahzad et al., 2015). The quantity of carbon released as root exudates in the soil account for up to 21% of plant photosynthates (Farrar et al., 2003; Haichar et al., 2014). Root exudates are mainly composed of low molecular-weight organic compounds, such as sugars, amino acids, organic acids, and secondary metabolites (Jones et al., 2004; Bais et al., 2006). These substances provide sufficient energy resources to rhizosphere microorganisms to sustain their growth and extracellular enzyme production (Phillips et al., 2011). Stimulated microbial activities by root exudation could promote soil organic matter (SOM) decomposition and nutrient cycling, with dramatic influence on soil carbon (C) formation and

stability in terrestrial ecosystems (Kuzyakov, 2010; Guyonnet et al., 2018). Yet, despite this general knowledge on the importance of root exudation for rhizosphere processes, the main ecological drivers of root exudation are less understood, particularly so in natural settings.

Root exudation is affected by a complex array of biotic (such as plant status, root traits, and mycorrhizal types) and abiotic factors (e.g., atmosphere CO₂ concentration, air temperature and soil nutrient availability; Klein et al., 2016; Meier et al., 2020; Dror and Klein, 2021; Wang et al., 2021). As two dominant types of mycorrhizal fungi associated with trees, arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi differ significantly in forms and functions (Phillips et al., 2013; Liese et al., 2018). These two mycorrhizal fungi can determine root exudation via their effects on root traits, nutrient acquisition, and nutrient cycling (Liese et al., 2017; Meier et al., 2020; Sun et al., 2021).

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For example, AM colonization usually promotes specific root length and specific root surface area of root systems, which might lead to greater root exudation than ECM (Wen et al., 2019). To date, only a handful of studies have explored the effects of mycorrhizal types on root exudation in-situ in forest ecosystems and even those have shown mixed results. For instance, a field study with mature trees showed greater root exudation in AM trees compared to ECM trees (Sun et al., 2017), while another study reported higher root exudation in ECM trees (Yin et al., 2014), whereas there was also a study showing no significant differences in exudation rates of absorptive fine roots (the first three orders) between the trees with two mycorrhizal types (Han et al., 2020). These contradictions imply an important knowledge gap in our understanding of ecological processes in rhizosphere and their subsequent effects on biogeochemical cycles in forest soils (Finzi et al., 2015; Haichar et al., 2014).

The degree to which mycorrhizal associations affect root exudation relates to plant's nutrient economy and the changes in root functional traits (Phillips et al., 2013; Sun et al., 2021; Williams et al., 2022). Arbuscular mycorrhizal plants generally have an inorganic nutrient economy, which is their capacity (e.g., exude acid phosphatases) to acquire inorganic nutrients, especially the soil phosphorous (P) (Cornelissen et al., 2001; Talbot et al., 2008; Bilen and Turan, 2022). In nutrient-poor environments, AM plants largely depend on morphological plasticity of roots and exudation by roots for the acquisition of soil nutrients (Wen et al., 2019; Keller et al., 2021). Indeed, a study of pre-dominantly AM-colonized grassland plants showed that composition of root exudates was correlated mostly with some specific root traits, such as root diameter, root tissue density and root nitrogen content (Williams et al., 2022). ECM trees adopt an organic nutrient economy and access some forms of organic nutrients, especially for N from soils directly (Read and Perez-Moreno, 2003). ECM trees often rely on the mycelial pathway to forage soil organic nutrients in response to nutrient deficiency (Phillips et al., 2013; Zhang et al., 2019). As such, the morphology and exudates of AM roots are likely to be more responsive to inorganic nutrient deficiency than ECM associated plants (Chen et al., 2016; Keller et al., 2021). Thus, soil inorganic nutrient availability can largely regulate the effects of mycorrhizal types on root exudation. For instance, soil nitrogen (N) availability regulated the magnitude of the effects of mycorrhizal types on root exudation, especially in N-limited temperate forests (Yin et al., 2014). An important extension of these findings will be to test whether P-limitation can also affect root exudation patterns in different mycorrhizal plants. This is particularly relevant as soil P availability is a major determinant of whether mycorrhizal associations are beneficial for plants (Plassard and Dell, 2010; Johnson et al., 2015; Honvault et al., 2021).

Subtropical forests are often characterized as P-limited ecosystems, but with high vegetation productivity and C sequestration capacity (Huang et al., 2013; Yu et al., 2014), making them a major terrestrial biome for climate change mitigation (Batjes and Sombroek, 1997). Trees in subtropical ecosystems are usually associated with mycorrhizal fungi to forage the limited mineral nutrients, especially the soil available P, to meet the demand for the rapid growth of plants (Ma et al., 2018). These mycorrhizal symbioses strongly affect plant growth and belowground C allocation (Rosling et al., 2016; Cheeke et al., 2017). However, it remains unclear how soil P availability regulates the effects of mycorrhizal plant types on root exudation in subtropical forests (Akatsuki and Makita, 2020). In this study, we designed a field experiment with isolated AM and ECM plantations (located no more than 1 km) in five subtropical sites across a soil P gradient to explore the effects of AM- and ECM-associated trees and soil P availability on root exudation. We hypothesized that: i) root exudation in AM trees will be greater than those of ECM trees in subtropical forests; and ii) the degree to which mycorrhizal type affects root exudation will closely associate with the changes in soil P availability and root functional traits (e.g., specific root length and specific root surface area).

2. Materials and methods

2.1. Site description and experimental design

The experiment was conducted at five sub-tropical forest sites across a soil P gradient in subtropical areas of southern China (Fig. S1; Table S1). The regional climate is controlled by the East Asian monsoon, with high temperature and abundant precipitation (Yu et al., 2014). Mean annual temperature and precipitation at five sites ranged from 15.4 to 22.5 °C and 1375–1822 mm, respectively (Table S2). Air temperature of five sites at the sampling date ranged from 31 to 35 °C, and soil water content ranged from 33.9 to 36.7% (Table S2). Soil P availability increases with the latitude, which varies from 3.6 mg kg⁻¹ in Xishuangbanna (XSNB) site to 5.5 mg kg⁻¹ in Tiantong site, and dissolved inorganic nitrogen at five sites ranged from 39.7 to 44.9 mg kg⁻¹ (Table S1). We chose two AM and two ECM representative isolated plantations (located no more than 1 km) with similar land-use history and forest age with different species across five sites (list of species provided in Table S2). In total, ten AM and ten ECM tree species were selected in our study. These plantations have a closed canopy and little understory vegetation. The mean diameter at breast height (DBH) ranged from 22.6 to 26.4 cm, and the stem density ranged from 783 to 845 tree ha⁻¹ (Table S3). Other background information (e.g., the location elevation, climatic parameters and soil basic physicochemical properties) of the five forest sites used in this study are provided in Table S1 and Table S2.

2.2. Root exudate collection

Three 20 m × 20 m plots were selected for both root exudate measurements and soil samplings within each plantation at each field site. Root exudates were collected using an in-situ collection device during the peak of the growing season (August) of 2019 (modified from Phillips et al., 2008; Fig. S2). Field collection of root exudates always started at 9 a.m. on a sunny day to ensure the reliability and consistency in results. Five target trees (DBH: 20–30 cm) were randomly selected per plot. Fine roots of the target tree (diameter <2 mm, length 15–20 cm), which were connected with tree taproot all the time, were dug up from the topsoil of 0–15 cm, and carefully rinsed with C-free nutrient solution (0.1 mM KH₂PO₄, 0.2 mM K₂SO₄, 0.3 mM CaCl₂·2H₂O and 0.2 mM MgSO₄·7H₂O). The cleaned roots were put into a syringe and filled with sterile glass beads (diameter: ~1 mm). The syringe was covered with aluminum foil and then buried into the soil. The collection devices were filled with 50 ml of nutrient solution to maintain the root growth. After 24 h equilibration periods, the soluble C in the syringe was removed by a vacuum pump, and 50 ml nutrient solution was injected and continued the exudate collection. After a 48 h interval, the organic C solution was filtered and transferred into brown bottles with a vacuum pump. For each target tree, a device without roots was buried into the adjacent soil environment to set as a blank control.

The solution samples were immediately filtered with a 0.22-µm filter and transferred to a 4 °C refrigerator. These filtered solutions were analyzed on a total organic carbon analyzer (Multi N/C 2100; Analytic Jena, Jena, Germany). The same root samples collected from the syringe were later oven-dried at 60 °C for 48 h and then weighted. Root exudation rate was calculated as the dissolved organic carbon (DOC) content per unit time and root dry mass (Yin et al., 2013).

$$\text{Root exudation rate} = (\text{DOC}_{\text{root}} - \text{DOC}_{\text{blank}}) / (\text{Time} \times \text{Root biomass}) \quad (1)$$

Where DOC_{root} and $\text{DOC}_{\text{blank}}$ are the DOC concentrations in the solution samples collected from the root exudate collection device with roots and the blank control device (no roots), respectively.

2.3. Soil sampling and measurement

Soil samples were collected from the mineral soil at the depth of 0–15 cm. The organic layers were shallow in these plantations. In each plot, twelve soil cores (5 cm in diameter, 15 cm deep) were randomly taken from each plot, mixed thoroughly and any visible living plants materials or stones were quickly removed using a sieve (2 mm). Approximately 100 g of each soil sample was air-dried (~25 °C) to measure various soil physicochemical characteristics, and the rest was immediately stored at 4 °C in a refrigerator to collect roots (see section 2.4).

The total soil carbon and nitrogen concentrations were determined by an elemental analyzer (Vario Macro cube, Elementar, Hanau, Germany). The total soil P concentration was obtained by ICP-OES (Optima 5300 DV, PerkinElmer, Waltham, MA, USA). Soil inorganic N (DIN) in the soil sample was determined by flow injection using an AutoAnalyser III (SEAL Analytical, Germany). Air-dried soils were extracted with 0.025 M HCl and 0.03 M NH₄F, and analyzed colorimetrically to determine the soil available P concentrations using the vanadomolybdate method (Bray and Kurtz, 1945). Soil pH was measured by a glass electrode (Model PHS-2, INESA Instrument, Shanghai, China) with a 1:2.5 (w:v) soil: water solution.

2.4. Measurement of fine root morphology and vitality

The fine living roots of target species in soil cores were picked out by tweezers immediately after soil sampling. The roots were cleaned in pure water, and fine fresh roots of three cores were randomly selected to assess fine root vitality (FRV) with the triphenyl tetrazolium chloride (TTC) method to approximate dehydrogenase activity (Yin et al., 2013). The rest of fine root samples in nine cores were scanned at 400 dpi using a scanner (Epson expression 11000 XL, Seiko Epson Corporation) and analyzed by WinRhizo (Regents Instruments Inc., Québec, Canada) for root morphology (root length, root surface area, root diameter and root volume). These scanned roots were over-dried at 60 °C for 48 h to formulate the dry mass weight. The fine root biomass (FRB) was calculated by dividing root dry weight by surface area of soil cores. Specific root length (SRL) was obtained as the ratio of root length to its root dry mass; specific root surface area (SRA) was calculated as the total root surface per unit root dry weight. Root tissue density (RTD) was calculated by dividing root dry weight by root volume (Comas and Eissenstat, 2009).

2.5. Statistical analysis

Linear mixed-effects models (LMMs) were used to examine the effects of mycorrhizal types and sites on root exudation rates and root functional traits, with tree species, and forest age as two independent random intercepts (the *lme4* package in R software, Stegmann et al., 2018). We also used LMMs to evaluate the effects of mycorrhizal types on root exudation rates and root functional traits for each site and across all five forest sites. The residues were checked for normality, and log transformation was conducted when necessary to meet the model assumption (e.g., homogeneity of variance). The association of soil P availability with root exudation rates and root functional traits were examined by linear regression models. Relative contribution of root functional traits and soil properties to root exudation were explored via principal component analysis (PCA) and general linear models (GLMs). We used the PCA scores of the first and second axis (Dim1 and/or Dim 2) for linear modelling with root exudation, and then GLMs were used to quantify the best model with the lowest corrected Akaike information criterion (AICc) score to predict variation in root exudation rates (the FactoMineR package in R, Lê et al., 2008). Further, we used relative importance analysis to explore the main factors that controlled root exudation rates (the *rfPermute* R package, Jiao et al., 2018), whereas the ranking method was based on mean decrease in mean square error (%

IncMSE). Finally, structural equation model was used to analyze the direct and/or indirect relationships among the main factors in explaining the variation in root exudation rates (the *piecewiseSEM* package in R, Lefcheck, 2016). The goodness of SEM fit was evaluated using Fisher's C statistic (based on Shipley, 2009), Akaike information criterion, and the whole-model P value. The effects of mycorrhizal types on root exudation and root functional traits were calculated as the ratio of Variables_(AM-ECM) and Variables_(ECM) (Chen et al., 2016). The Variables_(AM-ECM) mean the difference between the measured variables in the AM and ECM plantations, Variables_(ECM) are the measured variables in the ECM plantations. All statistical analyses were conducted in R (R core team, version 4.1.1). Figures were drawn with origin 2018, and tables were drawn with Excel 2016.

3. Results

3.1. Root exudation rate

Across all five forest sites, the root exudation rate in AM plantations was 113.3 $\mu\text{g C g}^{-1} \text{h}^{-1}$ on average, which was 33.2% higher than that in ECM plantations (85.1 $\mu\text{g C g}^{-1} \text{h}^{-1}$; Fig. 1a; Table S4; $F_{1,14} = 28.1$, $P < 0.01$). Root exudation rates in both AM and ECM plantations decreased with soil P availability (AM: $R^2 = 0.81$, $P < 0.01$; ECM: $R^2 = 0.23$, $P < 0.01$; Fig. 1b), with the highest values at the Xishuangbanna (XSBN) site where the soil P was lowest (AM: 138.8 $\mu\text{g C g}^{-1} \text{h}^{-1}$, ECM: 90.5 $\mu\text{g C g}^{-1} \text{h}^{-1}$), whereas the lowest root exudation rates were found at the Tiantong site, which had the highest soil P availability (AM: 85.5 $\mu\text{g C g}^{-1} \text{h}^{-1}$, ECM: 81.2 $\mu\text{g C g}^{-1} \text{h}^{-1}$). Difference in root exudation rates between two mycorrhizal types further decreased in sites with higher soil P availability (Fig. S3). At the lowest P site in our study, the root exudation rate in AM plantations was 53.4% greater than that in ECM plantations (Fig. 1a; Table S5). By contrast, no obvious difference in root exudation rates between the two mycorrhizal types was found at the highest P site in our experiment (Fig. 1a; Table S5).

3.2. Root morphological and physiological traits and soil properties

Specific root length (SRL), specific root surface area (SRA), fine root biomass (FRB), and fine root vitality (FRV) in AM plantations were 8.0 m g^{-1} , 0.03 $\text{m}^2 \text{g}^{-1}$, 354.3 g m^{-2} , 11.9 $\mu\text{g g}^{-1} \text{h}^{-1}$ on average, which were 30.1%, 40.9%, 12.5% and 19.7% higher than those in ECM plantations, respectively (Fig. 2; Table S4). Root diameter (RD) and root tissue density (RTD) in AM plantations were 1.0 mm and 0.42 g cm^{-3} on average, which were 14.4% and 21.9% lower than those in ECM ones (Fig. 2; Table S4). The SRL, SRA, FRB and FRV in both AM and ECM plantations decreased with soil P availability gradient (Fig. 3). The greatest values of these four variables were observed at the XSBN site having low soil P, with 9.4 m g^{-1} , 0.04 $\text{m}^2 \text{g}^{-1}$, 392.0 g m^{-2} , 14.1 $\mu\text{g g}^{-1} \text{h}^{-1}$ in AM plantations; and 6.6 m g^{-1} , 0.03 $\text{m}^2 \text{g}^{-1}$, 338.7 g m^{-2} , 10.8 $\mu\text{g g}^{-1} \text{h}^{-1}$ in ECM ones, respectively (Fig. 2). The lowest values were found at the Tiantong site with high P availability (Fig. 2). The RD and RTD in AM and ECM plantations increased with soil P availability gradient (Fig. 3c; Fig. 3d). The lowest values of these two variables were found at the XSBN site with low P availability, with 0.9 mm and 0.27 g cm^{-3} in AM plantations; and 1.1 mm and 0.46 g cm^{-3} in ECM ones (Fig. 2c; Fig. 2d). The highest values of two variables were observed at the Tiantong site with high P (Fig. 2c; Fig. 2d). Effects of mycorrhizal types on root functional traits also decreased with soil P availability gradient (Fig. S3). The greatest values of root traits (SRL, SRA, FRB and FRV) were found at the XSBN site. The SRL, SRA, FRB and FRV in AM plantations at this site were 43.2%, 67%, 15.7%, 30.8% greater than those in ECM plantations (Fig. 2; Table S5). RD and RTD in AM plantations at this site were 21.9% and 40.4% lower than ECM ones (Fig. 2; Table S5). By contrast, no obvious differences in root functional traits between the two mycorrhizal types were found at the Tiantong site with high P availability (Fig. 2; Table S5).

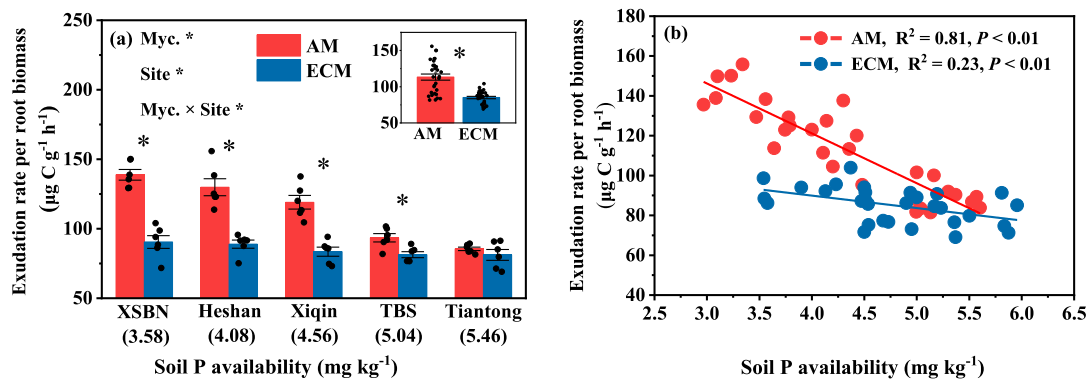


Fig. 1. Root exudation rates per root biomass (a) and its correlations with soil P availability in two mycorrhizal-type plantations (b, Mean \pm SE, $n = 3$). Bars represent means (with standard error bars shown) for AM and ECM plantations at each site. The sample locations contain Xishuangbanna (XSBN), Heshan, Xiqin, Tongboshan (TBS), and Tiantong. Insets show effects of mycorrhizal types on root exudation across all sites. The significant differences between the AM and ECM plantations are noted by asterisks ($P < 0.05$).

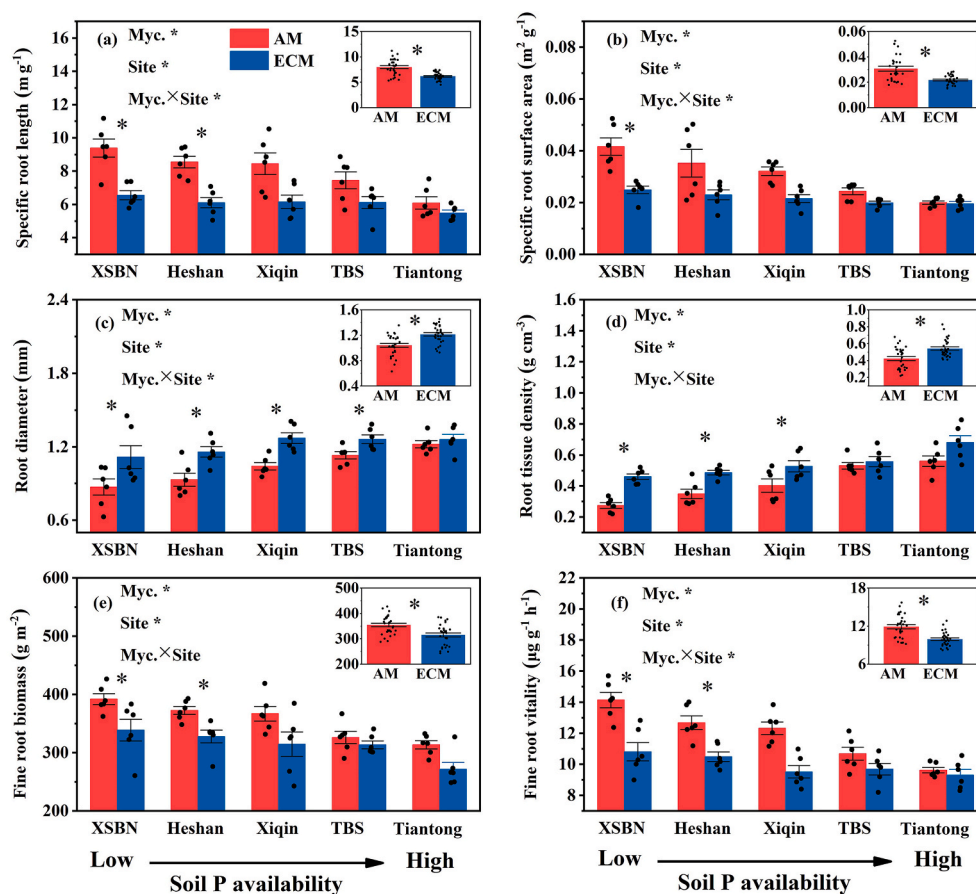


Fig. 2. Effects of mycorrhizal types on specific root length (a), specific root surface area (b), root diameter (c), root tissue density (d), fine root biomass (e), fine root vitality (f) across a soil P availability gradient (Mean \pm SE, $n = 3$). These roots were collected from soil cores. Bars represent means values of root functional traits in AM and ECM plantations at each site. Insets show effects of mycorrhizal types on root functional traits across all sites. The significant differences between the AM and ECM plantations are noted by asterisks ($P < 0.05$).

Soil P availability in AM plantations was 4.3 mg kg^{-1} on average, which was 9.8% lower than that in ECM plantations (4.8 mg kg^{-1} ; Table S3). However, no significant differences were found in soil organic carbon (SOC), total nitrogen (TN), total phosphorous (TP), the ratio of soil carbon and nitrogen (C/N), ratio of nitrogen and phosphorus (N/P), dissolved inorganic nitrogen (DIN), soil water content (SWC), and pH between AM and ECM plantations across five sites (Table S3).

3.3. Contribution of mycorrhizal type, root functional traits, and soil properties to root exudation

Principal component analysis (PCA) with root functional traits and

soil properties showed that the first component (Dim 1) and its combination with the second principal component (Dim 2) had significant effects on root exudation (Table S6). Specific responses of root functional traits and soil properties are provided in Supplementary Tables S1–S3 and Figs. 2–3. The best model on root exudation was the one including Dim 1 (AICc = 485.5; Table S6), explaining 80% and 19% of the variations in root exudation of AM and ECM plantations, respectively (Fig. 4). Variables related to soil P supply and root functional traits (e.g., soil P availability, fine root vitality, specific root length, specific root surface area, fine root biomass, root tissue density, and root diameter) had the largest loading weight in Dim 1 (Fig. 4a). Relative importance analysis showed that mycorrhizal type, soil P

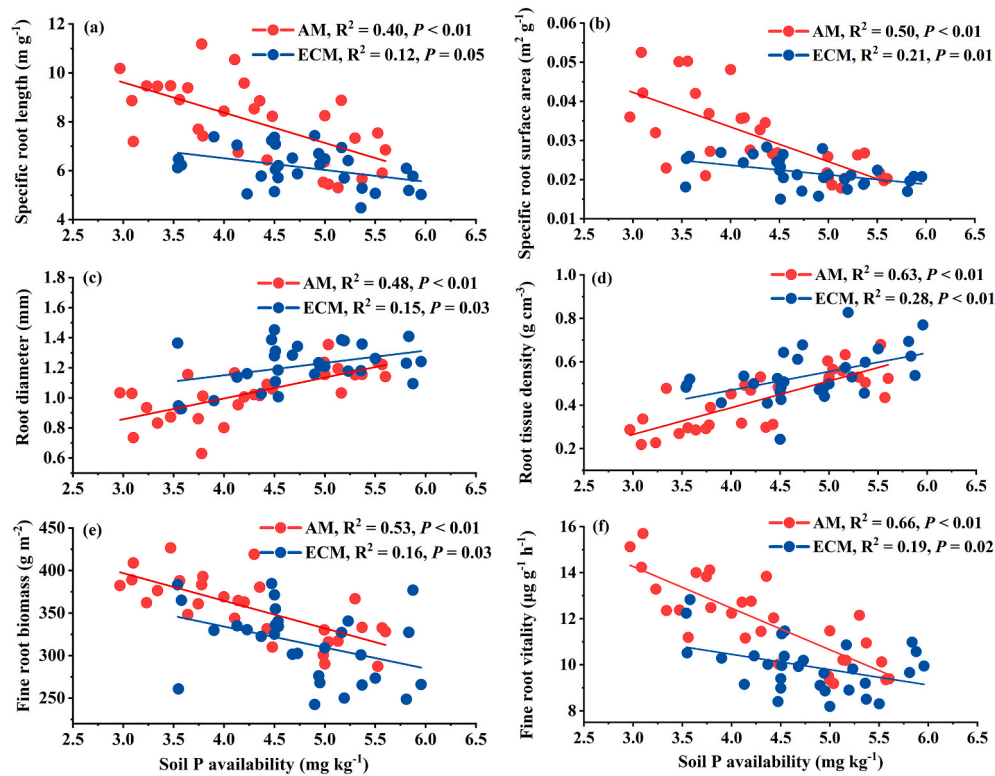


Fig. 3. The relationships of soil P availability with specific root length (a), specific root surface area (b), root diameter (c), root tissue density (d), fine root biomass (e), fine root vitality (f) in AM and ECM plantations.

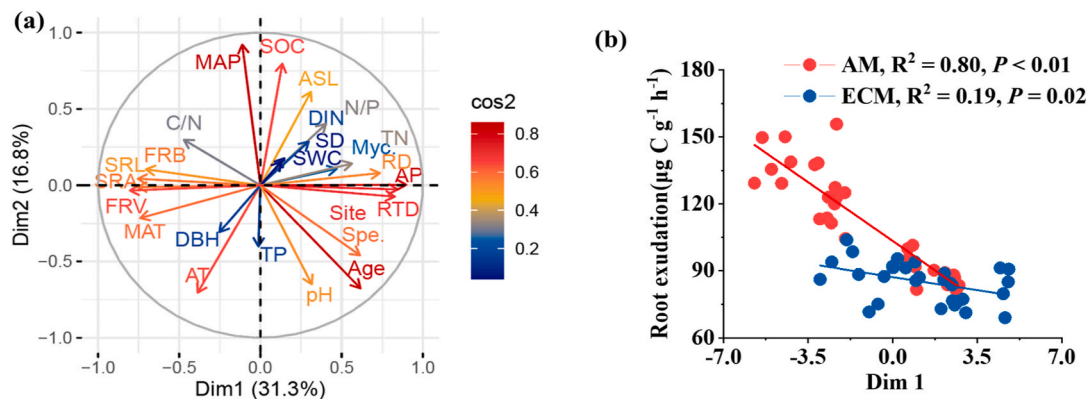


Fig. 4. Principal component analysis of root functional traits, site conditions, and soil properties of two mycorrhizal-type plantations (a) and best general linear model for root exudation of two mycorrhizal-type plantations (b). The best model (AICc = 485.5) on root exudation was that one only included Dim 1. Soil parameter includes soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), dissolved inorganic nitrogen (DIN), soil available P (AP), soil water content (SWC); site condition includes above sea level (ASL), mean annual temperature (MAT), mean annual precipitation (MAP), air temperature (AT), soil water content (SWC); plant traits includes forest age (Age), diameter at breast height (1.3 m, DBH), mycorrhizal types (Myc.), tree species (Spe.), stem density (SD); Root traits includes specific root length (SRL), specific root surface area (SRA), root diameter (RD), fine root biomass (FRB), root tissue density (RTD), and fine root vitality (FRV). The same abbreviations are used for all figures and tables.

availability, and root functional traits explained more of the variation in root exudation rates than any other abiotic and biotic factors measured in our study (e.g., air temperature and soil water content; Fig. 5a). Moreover, structural equation model (SEM) showed that the combination of these indirect and direct effects accounted for 73% of the variance in root exudation rates (Fig. 5b). It was directly modulated by the SRL, SRA, RD, FRV, and indirectly regulated by mycorrhizal type and soil P availability. Among these factors, soil P availability was the most important driver of root exudation rates (Fig. 5; Fig. S4). Soil P availability and mycorrhizal type could significantly influence root exudation rates through their effects on root functional traits (Fig. 5; Table S7).

4. Discussion

Understanding the effects of mycorrhizal types on root exudation and the underlying mechanism is crucial for predicting the feedback between the belowground C cycle and global climate change (Averill and Hawkes, 2016). Our study, for the first time to the best of our knowledge, has explored the potential role of soil P availability in regulating the effects of plant mycorrhizal types on root exudation rates at a broad geographical scale (Fig. 6). Based on the broad-scale field collection experiments, our results revealed that AM trees had higher root exudation rates than ECM trees, especially in P-limited soil

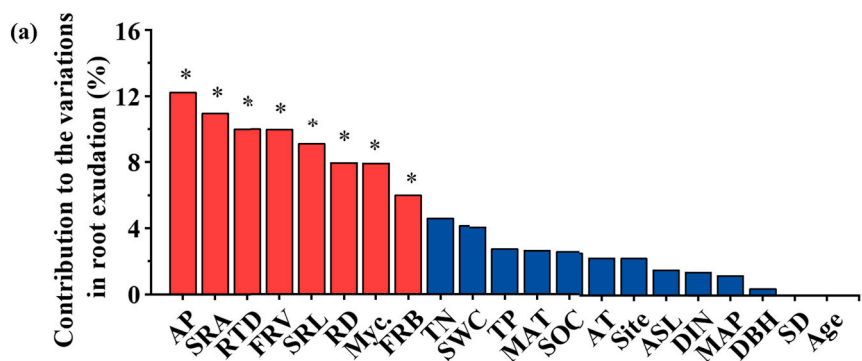
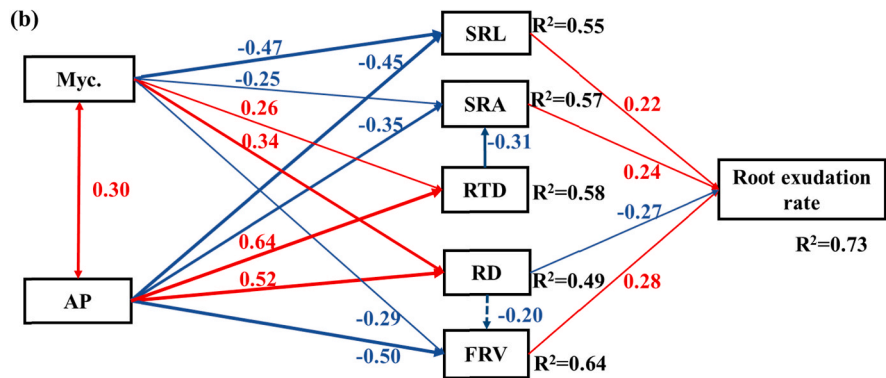


Fig. 5. Relative contribution of soil properties and root functional traits to the variation in root exudation (a) and structural equation model (b). The red line indicated a positive correlation between the two variables. A blue line indicated a negative correlation between the two variables. The solid line is a significant correlation, and the dashed line is an insignificant relationship. The thickness of the line represents the size of the correlation. The Myc. means mycorrhizal type. Significance levels are noted by asterisks ($P < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



Fisher's C = 32.997, $P = 0.062$, AIC = 88.997

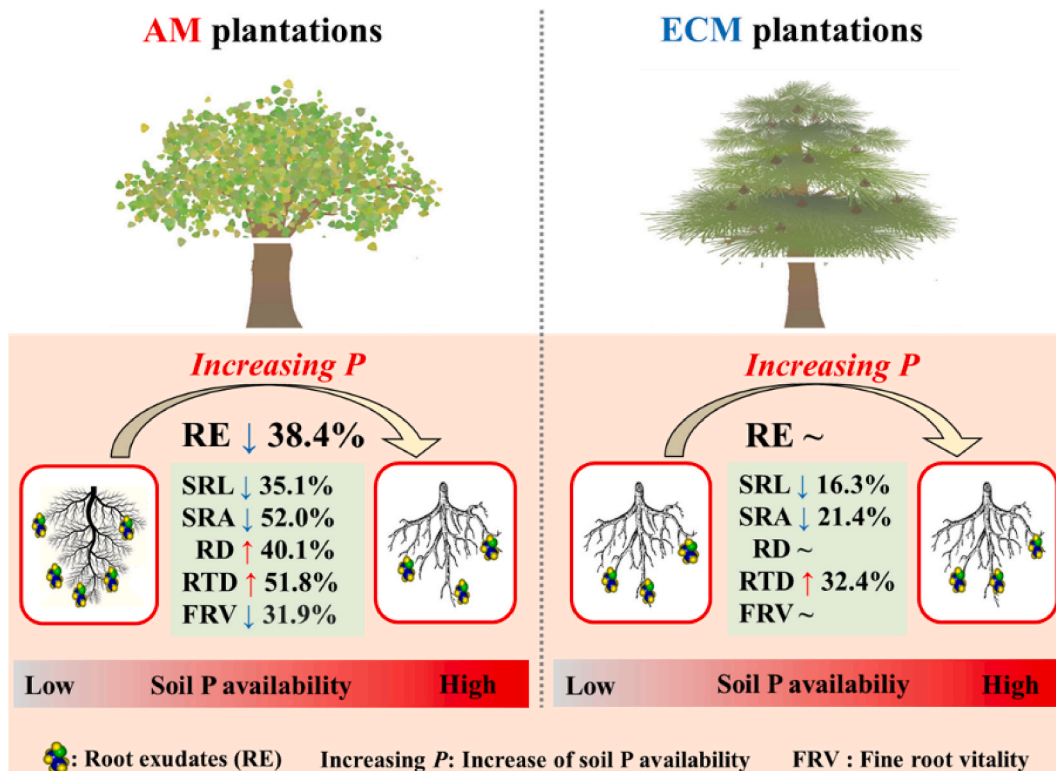


Fig. 6. A simplified conceptual framework for the root exudation of two mycorrhizal-type plantations across soil P availability gradient. The rectangle changes from gray to red indicate the increase of soil P availability. Small arrows represent the increase (red ones) and decrease (blue ones) of the variables across soil P availability gradient. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

environments (Fig. 1a). These findings highlight that soil P availability is a crucial driver of the effects of mycorrhizal types on root exudation in forest ecosystems (Phillips et al., 2013).

Mycorrhizal associations have the capacity to influence the C and P economies of their host trees (Lin et al., 2017). Our results showed that AM plantations in general had lower soil P availability than ECM ones (Table S3), which might be attributable to greater P demand and absorption capacity of AM trees compared to that of ECM trees (Talbot et al., 2008; Phillips et al., 2013). In turn, decreased soil P availability is likely to enhance root exudation in AM trees (Wen et al., 2019). According to the resource optimization theory, the C costs of root exudates should be greater in P-limited soils (Agren, 2003). The exuded carboxylates can be used as the substrate for microorganisms to stimulate the production of phosphatases and phytases that catalyze the decomposition of organic P to phosphate (Maistry et al., 2016; Maseko and Dakora, 2019). Meanwhile, P-limited plants can also exude acid phosphatases (and sometimes phytases) directly (Spohn and Kuzakov, 2013; Han et al., 2022). Previous studies have confirmed that the phosphatase activity of AM soils to be much higher than that of ECM ones in subtropical forests (Liu et al., 2018; Fang et al., 2020).

The variation in root exudation was also closely associated with root functional traits across species and soil P gradients in our study (Fig. S5; Akatsuki and Makita, 2020). AM roots had greater specific root length (SRL), specific root surface area (SRA) and fine root vitality (FRV), and lower root diameter (RD) than ECM ones (Fig. 2), which probably resulted in enhanced root exudation by AM trees compared to that by ECM trees (Sun et al., 2021). Other studies have pointed out that the relative abundance of young roots with very small diameters are most likely to regulate root exudation rates (Paterson and Sim, 1999; Darwent et al., 2003). Moreover, we suspect that the changes in root functional traits among the five forest sites are related to soil nutrient availability (Fig. 3; Xia et al., 2020). Soil nutrient deficiency generally affects the vitality and growth of the fine root system. It was demonstrated that P deficiency induced variable responses of roots, with smaller RD, larger SRL, and greater root biomass allocation (Kramer-Walter and Laughlin, 2017; Li et al., 2019), but longer root life span (Van Der Krift and Berendse, 2002). Consistently, we found a positive correlation between soil P availability and SRL, SRA or FRV, whereas a negative correlation between soil P availability and RD (Fig. 3). The response of root functional traits to soil P deficiency was greater in AM compared to that in ECM plantations (Fig. 3). These correlations are consistent with findings from previous stand-level studies investigating the effects of external P addition (Jansa et al., 2011; Xia et al., 2020). The distinct responses of root functional traits to soil P gradients between two mycorrhizal types likely resulted in the changes in mycorrhizal effects on root exudation across soil P gradients (Wen et al., 2019).

The relationships between root exudation and root functional traits also differed among two mycorrhizal types (Akatsuki and Makita, 2020; Fig. S5). This result might be related to the mycorrhizal nutrient strategy. AM mainly depends on the root pathway for nutrient utilization, whereas ECM relies on the mycelial pathway (Phillips et al., 2013). As such, root exudation and morphological plasticity of AM plants would be cohesive to cope with nutrient deficiencies than those of ECM ones (Wen et al., 2019; Keller et al., 2021). Previous studies have also reported that the relationship between root exudation and root functional traits was strongly driven by AM roots and not by ECM roots (Honvault et al., 2021). The relationship between root exudation and fine root traits should accordingly be considered to model forest belowground C process in future studies, which will help us better understand the underlying mechanisms of belowground C allocation between two mycorrhizal-type plants.

We also examined the effects of other potential factors on root exudation, such as plant growth status and soil N availability. Differences in the root exudation rates between AM and ECM trees could be driven by the aboveground C, as a result of either physiological acclimation or genotypic adaptation to their contrasting natural

environments (Yin et al., 2014). In this study, AM plantations had greater diameter at breast height (DBH) and aboveground biomass than ECM ones (Table S3). However, we did not find a significant correlation of root exudation with tree DBH and aboveground biomass (Fig. S6). Surprisingly, we found no relationship between root exudation rates and soil N availability (Fig. 5a; Fig. S6), which contradict previous studies (Yoneyama et al., 2012; Meier et al., 2020). This might be attributable to high N deposition in this region, which increased soil N availability (Mo et al., 2006). Previous studies showed that subtropical forests were more restricted by P rather than N availability (Huang et al., 2013; Yu et al., 2014). Thus, it seems reasonable to conclude that effects of mycorrhizal types on root exudation were primarily mediated by root functional traits and soil P availability in our study system.

Overall, our study found that the effects of mycorrhizal types on root exudation were dependent on soil P availability, which may provide key insights to explain C–P coupling in subtropical forests. We conclude with three key messages, our study limitation and a way forward based on our main findings. First, as AM root exudation was greater than that by ECM trees in subtropical forests, the role of mycorrhizal type on root exudation cannot be ignored. However, current manipulative experiments usually do not differentiate the effects of the mycorrhizal effects on belowground C input and the associated soil nutrient transformation (Meier et al., 2020; Ding et al., 2021), which creates a great challenge to accurately predict future feedbacks between the climate and the soil C dynamics (Peng et al., 2015).

Second, we found that soil P availability and mycorrhizal type could significantly influence root exudation rates through their effects on root functional traits (e.g., SRL and SRA). However, other essential root functional traits need to be implemented to better predict root exudation and associated inorganic nutrient economy in subtropical forests. For instance, mycorrhizal fungi attributes (e.g., colonization rate, fungal taxa, and mycelium network) have been shown to influence the nutrient use strategy (Han et al., 2020), and their impact on root exudation need to be further studied. Besides, root chemical traits (e.g., N and P concentration) can reflect the intensity of plant root metabolism (Tang et al., 2019), and these chemical traits have been found to be tightly related to root exudation rates (Meier et al., 2020; Sun et al., 2021).

Finally, root exudation is increasingly recognized as an important driver of ecosystem processes (Yin et al., 2013; Dijkstra et al., 2021). However, current Earth system models often exclusively use the root architecture and root length to predict root exudation (Finzi et al., 2015), which may trigger large uncertainty because both abiotic (e.g., air temperature and soil nutrient availability) and biotic factors (e.g., mycorrhizal types and microbial activity) greatly regulate the quantity and quality of root exudates as shown by our study. Our results further show that soil P availability plays a vital role in regulating root exudation in terrestrial biomes like subtropical forests. Hence, incorporating the soil nutrient regulated mycorrhizal effects on root exudation rates will improve our understanding of soil carbon dynamics in a changing world.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2022.108722>.

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