

Root exudation as a major competitive fine-root functional trait of 18 coexisting species in a subtropical forest

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Summary

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- Root exudation stimulates microbial decomposition and enhances nutrient availability to plants. It remains difficult to measure and predict this carbon flux in natural conditions, especially for mature woody plants. Based on a known conceptual framework of root functional traits coordination, we proposed that root functional traits may predict root exudation.
- We measured root exudation and other seven root morphological/chemical/physiological traits for 18 coexisting woody species in a deciduous-evergreen mixed forest in subtropical China.
- Root exudation, respiration, diameter and nitrogen (N) concentration all exhibited significant phylogenetic signals. We found that root exudation positively correlated with competitive traits (root respiration, N concentration) and negatively with a conservative trait (root tissue density). Furthermore, these relationships were independent of phylogenetic signals. A principal component analysis showed that root exudation and morphological traits loaded on two perpendicular axes.
- Root exudation is a competitive trait in a multidimensional fine-root functional coordination. The metabolic dimension on which root exudation loaded was relatively independent of the morphological dimension, indicating that increasing nutrient availability by root exudation might be a complementary strategy for plant nutrient acquisition. The positive relationship between root exudation and root respiration and N concentration is a promising approach for the future prediction of root exudation.

Introduction

Fine roots are essential systems through which plants acquire nutrients and water, and their functions could determine plant performance and survival (Comas, 2017). Fine roots not only take up readily usable soil nutrients but also affect nutrient availability through root carbon (C) deposition (Phillips *et al.*, 2011; Cheng *et al.*, 2014; Finzi *et al.*, 2015). Among different types of root C deposition such as root exudates, root litter and mycorrhizal hyphae, exudates alone contribute *c.* 58–96% of total root C deposition to rhizosphere soils (Shahzad *et al.*, 2015). Root exudates mainly contain organic acids, sugars and amino acids, all of which can increase the availability of nitrogen (N), phosphorus (P), calcium (Ca), and magnesium (Mg) through microbial decomposition of soil organic matter or abiotic processes (Dakora & Phillips, 2002; Phillips *et al.*, 2011; Aoki *et al.*, 2012; Keiluweit *et al.*, 2015; Ohta & Hiura, 2016). Root exudates affect soil dissolved organic carbon and stimulate microbial respiration (Sun *et al.*, 2017b; Erktan *et al.*, 2018). Moreover, signal and allelopathy compounds in root exudates shape the rhizosphere microbial community (Bais *et al.*, 2006; Badri & Vivanco,

2009). Together, root exudates make further contributions to belowground C cycling (de Vries *et al.*, 2019). At the individual plant level, the magnitude of root-induced N availability varies from 6% to 100% (Frank & Groffman, 2009). At the ecosystem level, model simulations have suggested that soil C release and N mineralisation induced by root exudates can contribute up to one-third of the total C and N cycling (Finzi *et al.*, 2015). Despite the fact that root exudates have a great effect on soil C and nutrient cycles, only a few models have estimated this flux and its impact (Cheng *et al.*, 2014; Perveen *et al.*, 2014; Finzi *et al.*, 2015). One main reason for this lack is that direct measurement on root-exudate fluxes is difficult and limited (Oburger & Jones, 2018; Pausch & Kuzyakov, 2018).

Pioneer studies targeting woody species have revealed that root-exudate fluxes vary among mycorrhizal types and life forms, possibly due to species-specific N-acquisition strategies and photosynthesis capacities (Brzostek *et al.*, 2012; Yin *et al.*, 2014; Sun *et al.*, 2017a; Wang *et al.*, 2019). However, a convenient tool to predict the variation of root-exudate fluxes among species is still lacking. Aboveground leaf morphological, chemical and metabolic traits correlate with each other to form an economic

spectrum that can predict plant performance and adaptation to environmental factors (Donovan *et al.*, 2011). Competing leaves have high specific leaf area and tissue N concentration, and therefore have a high photosynthetic capacity and respiration rate (Wright *et al.*, 2004; Reich *et al.*, 2008). Analogous to the leaf economic spectrum aboveground, coordination of belowground fine-root functional traits has been proposed based on the function of nutrient acquisition (Fig. 1a). Competing fine roots forage soil nutrients using a relatively efficient morphological structure involving highly specific root length, high branching intensity, low root tissue density and low diameter (Valverde-Barrantes *et al.*, 2015, 2017; Weemstra *et al.*, 2016; Laliberté, 2017). A metabolic root trait, namely respiration, positively correlated with N concentration (Reich *et al.*, 2008) and specific root length (Makita *et al.*, 2009; Makita *et al.*, 2012) locally and globally among different species. Recent analyses have shown that root functional traits vary among species in relation to their phylogenetic clades, life forms and symbiotic mycorrhizal types (Comas *et al.*, 2014; Kong *et al.*, 2014; Valverde-Barrantes *et al.*, 2015, 2017; Ma *et al.*, 2018; Bergmann *et al.*, 2020). For example, the branching ratio of fine roots for ectomycorrhizal trees is consistently higher than that of arbuscular mycorrhizal trees in subtropical forests (Kong *et al.*, 2014); and evolutionarily ancient species tend to have coarse roots (Ma *et al.*, 2018). Considering that root exudates can stimulate the availability of various soil nutrients, which is essential for plant nutrient acquisition, it is promising that root-exudate flux also is connected to fine-root functional trait coordination (Bardgett, 2018; Moreau *et al.*, 2019; Fig. 1a,b).

Is root exudation a competitive or a conservative root trait (Fig. 1b)? Among six *Poacea* species, root exudation was negatively correlated with root dry matter content and positively with specific root length, indicating that root exudation is a competitive trait (Guyonnet *et al.*, 2018; Fig. 1b; H1). For woody species, there is only limited intraspecific evidence that root exudation might be a competitive trait. For example, positive correlations were previously found between root exudation and two competitive traits, root respiration and tissue N concentration, in dominant *Quercus* species from two different biomes (Sun *et al.*,

2017b; Ataka *et al.*, 2020). Along a gradient of soil nutrient availability, root exudation of European beech (*Fagus sylvatica*) also exhibited a positive correlation with a competitive trait, specific root length (Meier *et al.*, 2020). Accordingly, aspen (*Populus tremuloides*) roots of high nonstructural C concentration also exuded more C to the surrounding soils (Karst *et al.*, 2017). Alternatively, root exudation and respiration consumed *c.* 21% and 30%, respectively, of total photosynthates allocated belowground (Lambers *et al.*, 2003; Haichar *et al.*, 2014), such that C allocation to root exudates of crops was often sacrificed for root respiration at an individual plant level under external disturbances (Dilkes *et al.*, 2004; Hill *et al.*, 2006). Among 16 crop species, species with competitive morphological traits (i.e. high branching intensity) invested less C to carboxylate in root exudates or their mycorrhizal symbionts (Wen *et al.*, 2019). This indicated an alternative strategy for nonwoody species by which to acquire nutrients, by foraging readily usable soil nutrients with an efficient morphological structure rather than increasing nutrient availability with root exudates. It remains an open question for woody plants whether root exudation correlates negatively with competitive traits among species (Fig. 1b; H2).

Most studies on root trait coordination put forward hypotheses based on a single-dimensional spectrum of acquiring resources efficiently or not (Roumet *et al.*, 2016; Weemstra *et al.*, 2016) that were in parallel with a single-dimensional 'fast-slow growing' leaf economic spectrum and whole-plant strategy (Westoby & Wright, 2006; Reich, 2014; Weemstra *et al.*, 2016). However, field studies have revealed both single-dimensional (Prieto *et al.*, 2015; Roumet *et al.*, 2016; Li *et al.*, 2017; Wen *et al.*, 2019) and multidimensional root trait coordination (Valverde-Barrantes *et al.*, 2015; Kramer-Walter *et al.*, 2016; Liese *et al.*, 2017; Erktan *et al.*, 2018; Ding *et al.*, 2020). One reason is that symbiotic mycorrhizal fungi provide an alternative strategy for plant nutrient acquisition rather than a competitive morphological structure (McCormack & Iversen, 2019; Bergmann *et al.*, 2020). Therefore, functional groups of different whole-plant strategies, for example different mycorrhizal types, should be considered in root coordination studies (Laliberté, 2017). To date, pioneer studies including root exudation in root trait coordination have focussed

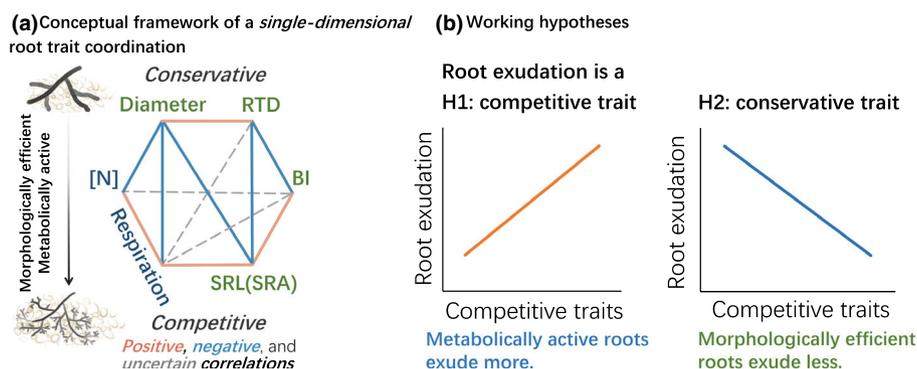


Fig. 1 Diagram of (a) a conceptual framework of a proposed coordination of fine-root functional traits converted from Kong *et al.* (2014), Roumet *et al.* (2016), and Weemstra *et al.* (2016). Diagram of (b) working hypotheses: the direction of the correlation between root exudation and competitive root traits based on a single-dimensional root traits coordination. BI, branching intensity; [N]: nitrogen concentration; RTD, root tissue density; SRA, specific root area; SRL, specific root length.

on nonwoody species of nonmycorrhizal or arbuscular mycorrhizal types (e.g. Ryan *et al.*, 2012; Guyonnet *et al.*, 2018; Wen *et al.*, 2019). Therefore, this study aimed to reveal correlations between root exudation and other root traits for woody species with a broad range of whole-plant strategies, including different mycorrhizal types, life forms and successional stages.

In a deciduous-evergreen mixed forest of high biodiversity in subtropical China, we measured root exudation fluxes, as well as seven other root functional traits (e.g. root diameter, specific root length and area, tissue N concentration, root tissue density, root respiration, and branching intensity) for 18 coexisting tree species. The target species were chosen from different clades, life forms and mycorrhizal types (Table 1). These had different nutrient-acquisition capacities and exhibited different root morphological, chemical traits and root-exudate fluxes. We put forward two alternative hypotheses to predict the relationship between root-exudate flux rate and other root functional traits based on a single-dimensional root trait coordination (Fig. 1b): (1) the flux rate of root exudates would relate *positively* to competitive root functional trait coordination (i.e. high N concentration, respiration rate, specific root length and branching intensity, as well as low root tissue density and diameter) among these species; and (2) the flux rate of root exudates would relate *negatively* to competitive root functional trait coordination among these species because the competitive morphological structure and other physiological activity are efficient enough for plants to acquire nutrients. By exploring the relationship between root exudation and other root functional traits, we can

better predict soil carbon and nutrient cycling induced by root exudation.

Materials and methods

Site description

The study was performed in the southern slope of the Shennongjia Mountains. The Shennongjia region is a biodiversity hotspot in central China and also a habitat for glacial relic species (Ge *et al.*, 1997). At an elevation between 1200 m and 1800 m a.s.l., the forest is a mixture of deciduous and evergreen species (Ge *et al.*, 1997). The mean annual temperature is 10.6°C, and the mean annual precipitation is 1330 mm (Ge *et al.*, 2012; Chi, 2015). Field work was performed near the long-term plot No. SNFZQ64 of the National Ecosystem Research Network of China (31°32'40"N, 110°49'20"E, 1360 m) in July 2018.

Target species and individual trees

We sampled 18 coexisting species (Table 1) which comprised: (1) the most abundant families formerly reported in the nearby permanent plot of the Chinese ecology network at a similar elevation (Ge *et al.*, 2012); (2) as many families and genera from different clades as possible; and (3) different mycorrhizal types, life forms and successional stages. In total, 18 species from 14 genera and 10 families were sampled (see Supporting Information Fig. S2

Table 1 Phylogenetic information, mycorrhizal type, life form and successional stages of 18 target woody species and diameter at breast height (DBH) and replicate numbers of each individual target tree.

Species	Abbreviation	Family	Mycorrhizal type ¹	Life form	Successional stage	DBH (cm)			n ²
						Min	Max	Mean (SE)	
<i>Acer davidii</i>	AcD	Aceraceae	AM ³	Deciduous	Pioneer	5.9	20.6	13.4 (3.5)	5
<i>Acer palmatum</i>	AcP	Aceraceae	AM ³	Deciduous	Late	2.5	6.2	17.3 (2.4)	4
<i>Acer mono</i>	AcM	Aceraceae	AM ³	Deciduous	Early	13.1	21.8	4.5 (4.6)	4
<i>Castanea mollissima</i>	CaM	Fagaceae	ECM	Deciduous	Early	9.7	45.5	30.6(8.9)	4
<i>Cerasus clarofolia</i>	CeC	Rosaceae	AM ³	Deciduous	Early	14.2	44.8	30.4 (8.5)	4
<i>Cornus controversa</i>	CoC	Cornaceae	AM	Deciduous	Early	14.6	21.2	17.7 (1.5)	5
<i>Cornus ulotricha</i>	CoU	Cornaceae	AM	Deciduous	Early	10.5	27.6	17.2 (3.5)	5
<i>Cyclobalanopsis oxyodon</i>	CyO	Fagaceae	ECM	Evergreen	Late	4.4	39.0	23.9(11.6)	5
<i>Diospyros lotus</i>	DiL	Ebenaceae	AM	Deciduous	Pioneer	15.2	37.8	23.2 (5.8)	4
<i>Euptelea pleiosperma</i>	EuP	Eupteleaceae	AM ³	Deciduous	Early	6.2	65.4	23.7 (12.1)	5
<i>Lindera fragrans</i>	LiF	Lauraceae	AM	Evergreen	Late	2.1	5.8	4.3 (0.8)	5
<i>Lindera obtusiloba</i>	LiO	Lauraceae	AM	Deciduous	Early	3.4	25.7	10.9 (4.4)	5
<i>Litsea ichangensis</i>	Lil	Lauraceae	AM	Deciduous	Early	7.2	34.7	17.3 (5.6)	5
<i>Quercus serrata</i>	QuS	Fagaceae	ECM	Deciduous	Early	8.8	32.4	23.9 (6.4)	4
<i>Rhododendron hypoglaucaum</i>	RhO	Ericaceae	ERM	Evergreen	Late	5.5	37.8	14.1 (6.8)	5
<i>Sorbus folgneri</i>	SoF	Rosaceae	AM ³	Deciduous	Early	3.6	7.0	4.1 (1.2)	4
<i>Viburnum sympodiale</i>	ViS	Caprifoliaceae	AM	Deciduous	Late	2.1	7.0	10.3 (2.6)	5
<i>Yulania sprengeri</i>	YuL	Magnoliaceae	AM	Deciduous	Early	7.0	39.7	18.1 (6.6)	5

¹AM, arbuscular mycorrhizal type; ECM, ectomycorrhizal type; ERM, ericoid mycorrhizal type. For most families that formed certain kind of obligatory mycorrhizal association, the mycorrhizal type was recognised from a checklist of 3617 vascular species (Wang & Qiu, 2006).

²The lower letter 'n' denotes the individual trees sampled for each species.

³The species have no record in the former checklist or belong to a family that can form both arbuscular mycorrhizal and ectomycorrhizal type fungi (Teste *et al.*, 2019) and were further checked for the presence or absence of arbuscular mycorrhizal colonisation (see Fig. S1 for microscope pictures).

for the phylogenetic tree and approach for drawing it). Target species included different life forms (evergreen and deciduous species), mycorrhizal types (arbuscular, ecto and ericoid mycorrhizal types). Both upper and lower canopy-layer species were sampled.

We sampled our target species along a 3-km transect. The elevation ranged from 1290 m to 1380 m. Field work was done on 10 d consecutively (9–18 July 2018) with no precipitation. We set up root exudation collection syringes and harvested them the following day for respiration measurements. Every 2 d consecutively 15–19 root branches were sampled (to be described later). Individuals from different species were sampled randomly (Table S1) during this period to minimise the possible effects induced by daily variations of meteorological conditions. Root traits that were sensitive to meteorological conditions, namely fine-root respiration and exudation, exhibited no difference among sampling days (Fig. S3).

Individual trees were also randomly distributed spatially along this transect. For most species, five individuals were sampled as replicates. For some species only four individuals were successfully sampled to avoid those too close to other individuals (especially an individual from a kin species) and those at extreme locations where root exudation could not be collected.

The definition of fine roots

In the last 2 decades, the definition of fine roots by root diameter (e.g. < 2 mm) has been replaced by that of root order (e.g. first three orders) from a functional perspective (Pregitzer *et al.*, 2002; Iversen, 2014). This is because root diameter varies largely among species (Ma *et al.*, 2018), while root-order definition provides a more precise cutoff between absorptive and transport roots (Pregitzer *et al.*, 2002; Xia *et al.*, 2010; Iversen, 2014; McCormack *et al.*, 2015). The change from the diameter definition to the order definition also favours interspecies comparison such that fine roots of similar function are compared (Sun *et al.*, 2017a; Shao *et al.*, 2019). Both first order and first three orders cut-offs have been commonly used in recent studies (Freschet *et al.*, 2017b). For certain plant and ecosystem functions such as survey on root exudation, the first three orders is a reasonable and representative cutoff of fine roots (Freschet *et al.*, 2017b). Current technology cannot deal with most lateral roots of a single order (i.e. measurements on only first order or second order), so that root exudation collection requires an intact root branch (Phillips *et al.*, 2008; Oburger & Jones, 2018). Thus, we adopted the cut-offs of first three orders as a unit for measuring root exudation and other root traits.

Measurement of root exudation

We followed the soil-hydroponic hybrid protocol of Phillips *et al.* (2008) to sample root exudates *in situ* (Oburger & Jones, 2018). This methodology has been widely used over the last decade and is suitable for sampling root exudates on mature woody species under field conditions in which only intact root segments, but not a whole root system, can be sampled

(Oburger & Jones, 2018). This soil-hydroponic hybrid protocol allows targeted fine roots grown under natural conditions and only cultured in a hydroponic solution for a relatively short period, so that the measured exudation is representative for the root status under natural conditions (Phillips *et al.*, 2008). We first dug around a target tree trunk and prepared two or three intact root branches as candidates. For root exudation, we chose one well branched mature root by its colour and elasticity collectively. Briefly, the first three order roots were excavated from top soil (within 5 cm depth) carefully so that the branches remained connected to the mother tree. The roots were then washed gently with de-ionised water from a spray bottle. Afterwards, the roots were put in a syringe filled with acid-washed glass beads and 25 ml of C-free nutrient solution (0.1 mM KH_2PO_4 , 0.2 mM K_2SO_4 , 0.2 mM MgSO_4 , 0.3 mM CaCl_2 ; see more details in Phillips *et al.*, 2008). The whole syringe and roots were then covered with wet paper towel and aluminium foil to prevent roots from drying out and to protect from sunlight. After 24 h, the solutions were recovered from syringes. The syringes were flushed with 10 ml nutrient solutions twice to achieve a full recovery of root exudates. The recovered solutions were filtered using a 0.22 μm Minisart syringe filters (Sartorius, Göttingen, Germany) immediately and thereafter stored and transported to the laboratory in a cooler box containing ice packs. The roots were then harvested to measure root respiration. Harvested roots were washed and blotted with a paper towel before root respiration measurement. Total carbon in the collected solution was measured using a TOC analyser in the NPOC mode (multi N/C 3100; Analytikjena, Jena, Germany). Potassium biphthalate was used as the standard. The flux rate of root exudates was calculated by dividing the total C by incubation time and root dry weight.

Measurement of root respiration

Respiration measurement used the root branches harvested from the root exudation collection. Measurements started within 15 min after the root was cut-down from the mother tree. Measurements were performed with a static chamber connected to a Vaisala CO_2 probe (GMP343; Vaisala, Vantaa, Finland; Makita *et al.*, 2009; Sun *et al.*, 2017b). Briefly, the fine roots were put in the chamber for 15 min and the data-logger recorded the CO_2 concentration every second. To remove any disturbance from chamber opening and closure, only data after 5 min of chamber closure were used. The temperature of the chamber was also recorded. During the whole experiment, the temperature of the chamber was in the range of 22.3–27.7°C. Accordingly, temperature-dependent Q_{10} ranged from 1.97 to 2.19 following the equation of Tjoelker *et al.* (2001). Because the chamber temperature dynamics was relatively narrow and the range of temperature-dependent Q_{10} was very similar to 2.0, when adjusting respiration to 25°C, a Q_{10} of 2.0 was adopted in which all data were adjusted to respiration at 25°C. The adjusted respiration rates exhibited a close correlation with the original respiration rates, showing no significant difference with the 1:1 line (Fig. S4).

Root morphological analyses

Fine roots were transported to the laboratory on ice packs and stored at 4°C. Fresh roots were scanned using a Microtek i800-plus scanner (Microtek, Shanghai, China) at 300 d post inoculation and analysed using WINRHIZO PRO 2013 software (Regents Instruments Inc., Quebec City, QC, Canada) to extract mean diameter, total root length (area and volume) and tip number. Fine roots were then oven dried at 65°C and weighed. Specific root length (area) was calculated by dividing total root length (area) by root dry weight. Branching intensity was calculated by dividing root tip number by root length. Root tissue density was calculated by dividing root dry weight by volume.

Soil sampling

Rhizosphere soils were also sampled to harvest the fine roots for respiration measurements. Six soil cores were sampled (diameter = 7.5 cm) of top 5 cm soils around the trunk of each target tree within a 1 m radius. The root branches of the target species were sorted according to their morphological features (note that we avoided sampling another kin species in the vicinity to ensure that the roots were from the targeted individual). After gently shaking, soil still adhering to the fine-root branches was considered as rhizosphere soil (Phillips & Fahey, 2006). We collected rhizosphere soil from root branches gently with soft brushes. These soil samples were transported to and stored in the laboratory at 4°C until further analysis.

Chemical analyses on fine roots and soils

The water content of all soils was calculated based on the weight loss of a subsample (*c.* 5 g in fresh weight) that was oven dried for 72 h at 105°C. Another soil subsample (equivalent to 2 g in fresh weight) was extracted with 2M K₂SO₄ at a soil : solution ratio of 1 : 5. The extraction was shaken for 30 min, filtered through a filter paper, and analysed for nitrate and ammonium nitrogen using a continuous-flow analyser (Autoanalyser 3; Seal Analytical, GmbH Germany). A third soil subsample was air-dried and ground for total C and N analyses. The C and N concentrations of soils and roots were analysed using an elementary analyser (Vario EL III; Elementar, Langensfeld, Germany).

Statistical analyses

The minimum, maximum, mean, median and coefficient of variation (CV) of each root trait were calculated. We used Blomberg's *K* value to evaluate the influence of phylogenetic signals on each root trait using the R package PICANTE (Blomberg *et al.*, 2003). A large *K* value and $P < 0.05$ indicated conservatism for a trait. The interspecies variation of root exudation was tested using a nested ANOVA against the functional groups (mycorrhizal types, life forms and successional stages). The four or five individuals from the same species were nested. Correlations between root traits and soil properties were also performed using Pearson's correlations at both individual tree level and species-

mean level. Pairwise trait relationships were assessed using Pearson's correlations, while correlations excluding phylogenetic signals were also performed with phylogenetic independent contrasts (PICs) using the R package APE. For those traits correlated with root exudation at both species-mean level and PICs, the slopes of major-axis regressions of standardised trait data and standardised PICs were further compared to distinguish if excluding phylogenetic signals affected the correlations (Yang *et al.*, 2014; Kramer-Walter *et al.*, 2016). We used principal components analysis (PCA) to show an overall fine-root trait coordination. The differences of the trait syndrome among plant groups (*i.e.* mycorrhizal types, life forms and successional stages) were tested by MANOVA. All statistical analyses were performed using R v.3.5.3 (R Core Team, 2017).

Results

Interspecies trait variations

The root diameter varied from 0.18 mm (*Rhododendron hypoglaucom*) to 0.72 mm (*Lindera obtusiloba*), with a CV of 32.4% (Tables 2, S2). The variation exhibited a strong phylogenetic signal (Table 3). Species from basal clades, such as species from Magnolia and Lauraceae families, had the coarsest roots among the target species. By contrast, species from more divergent clades, such as Fagaceae, Ericaceae and Rosaceae, had the finest roots (Table S2). Similarly, root N concentration ([N]) and root tissue density (RTD) exhibited CVs of *c.* 30% and significant phylogenetic signals. Although specific root length (SRL) and specific root area (SRA) exhibited small CVs and branching intensity (BI) exhibited a much greater CV (87.3%) than other morphological traits, none of them exhibited significant phylogenetic signals among the target species (Table 3). Two carbon fluxes, namely root respiration and exudation, exhibited large CVs as well as significant phylogenetic signals (Fig. 2a; Table 3). Root exudation exhibited marginally significant differences among mycorrhizal types ($P = 0.08$, Fig. 2b) and life forms ($P = 0.07$, Fig. 2c).

Interspecies trait correlations

Root diameter correlated with almost every other morphological and chemical trait at species level and individual tree level (Fig. 3a,c). Species that had coarser fine roots tended to have a lower RTD, SRL and BI. When excluding the phylogenetic signals, root diameter no longer correlated with RTD. Surprisingly, root [N] only correlated negatively with one morphological trait, namely RTD, at both individual tree and species levels whether or not phylogenetic signals were considered (Fig. 3). When phylogenetic signals were not excluded, root [N] positively and marginally correlated with root diameter ($P = 0.07$; Fig. 3b).

Respiration positively correlated with root [N] and SRA but negatively with RTD. These correlations were robust whenever phylogenetic signals were included or excluded, and on both individual tree and species levels (Fig. 3). Similar to root respiration, root exudation also positively correlated with root [N] but

Table 2 Summary of the seven root traits of 18 coexisting species in a deciduous-evergreen forest.

Root traits	Diameter (mm)	Specific root length (m g ⁻¹)	Specific root area (cm ² g ⁻¹)	N concentration (%)	Root tissue density (g cm ⁻³)	Branching intensity (cm ⁻¹)	Fine-root respiration (mg C g ⁻¹ h ⁻¹)
Min ^a	0.18	10.7	81.5	0.86	0.11	0.7	0.11
Max ^b	0.70	77.1	814.4	3.61	0.48	17.6	6.32
Median	0.36	38.9	438.8	1.89	0.25	2.3	2.51
Mean	0.41	38.6	434.0	1.93	0.26	3.3	2.68
sd	0.13	18.0	140.8	0.54	0.09	2.8	1.30
CV%	32.4	46.6	32.5	28.1	33.4	87.3	48.4

CV%, coefficient of variation; sd, standard deviation.

^aMin, the minimum value of the trait across the 18 species.

^bMax, the maximum value of the trait across the 18 species.

Table 3 Blomberg's *K* values for eight root traits of 18 coexisting species in a deciduous-evergreen forest.

Fine-root traits (abbreviations)	Blomberg's <i>K</i>	<i>P</i>
Diameter	1.450	0.001
Specific root length (SRL)	0.609	0.20
Specific root area (SRA)	0.481	0.37
Nitrogen concentration ([N])	1.099	0.005
Root tissue density (RTD)	0.773	0.08
Branching intensity (BI)	0.495	0.50
Respiration	0.821	0.04
Exudation	0.947	0.04

The significance level was set at $P < 0.05$. Bold value denotes that the trait exhibited a significant phylogenetic signal.

negatively with RTD, at individual tree level (Fig. 3c) and at species level with phylogenetic signals excluded or not (Fig. 3a,c). Root exudation also positively correlated with SRA but only at species level when phylogenetic signals were excluded (Fig. 3b) or at individual tree level (Fig. 3c). Therefore, root exudation also positively correlated with root respiration (Fig. 3). Furthermore, a standardised major-axis analysis comparing the central standardised PICs and species-level data showed that the relationship between root exudation and root respiration, root [N] and RTD remained unchanged whether or not phylogenetic signals were considered (Fig. 4).

Additionally, root exudation and SRA correlated positively with soil nitrate concentration, while root diameter negatively correlated with soil ammonium concentration consistently at the individual tree level and species-mean level (Table S3a,b). Root [N] correlated positively with both soil nitrate concentration and total soil nitrogen at two levels (Table S3a,b). Root respiration positively correlated with soil nitrate concentration only at the species-mean level (Table S3b).

Two-dimensional root functional trait coordination

Based on the PCA results, the eight root functional traits were distributed on two perpendicular axes (Fig. 5a). Flux rate of root respiration and exudation and root [N] were mainly loaded on the PCA axis 1. Root diameter was almost fully loaded on the PCA axis 2, while SRL and BI were loaded on the same axis but

in the opposite direction. RTD and SRA were loaded equally on both axes 1 and 2. Together, two dimensions explained 64.3% of the total variation of all eight root traits. Considering the eight traits together, the trait syndromes of mycorrhizal types (Fig. 5b, $P < 0.001$) and successional stages (Fig. S5a, $P = 0.049$) were significantly different, but the category of life forms exhibited no difference (Fig. S5b; $P = 0.21$). The arbuscular mycorrhizal species were mostly distributed on the lower half of PCA axis 1, while the ecto and ericoid mycorrhizal species were both distributed on the upper half of the PCA axis 1 (Fig. 5b).

Discussion

Root exudation is a competitive fine-root trait: its correlations with other fine-root morphological and metabolic traits

In the deciduous-evergreen mixed forest at the Shennongjia site, root exudation was found to be a competitive trait among 18 coexisting woody species because there were positive correlations between fine-root exudation and competitive traits (i.e. fine-root respiration, root [N]), and negative correlation with a conservative trait (i.e. RTD) whenever phylogenetic signals were considered or not (Figs 3,4). The positive correlations with another competitive trait SRA were only found on PICs and at the individual tree level (Fig. 3a,c). Our findings are consistent with correlations between root exudation with root respiration and tissue N within three *Quercus* species in temperate forests (Sun *et al.*, 2017b; Ataka *et al.*, 2020), or with SRL and SRA along gradients of soil N and P availability (Aoki *et al.*, 2012; Meier *et al.*, 2020). Both fine-root respiration and tissue N are indicators of root metabolic activity (Ryan *et al.*, 1996; Reich, 2014), while RTD implies the construction cost of roots (Eissenstat & Yanai, 1997). Root exudation is released from root surface and induces a microbial response in the rhizosphere very close to the root surface. Therefore, the correlation between fine-root exudation and SRA reflected the C load strategy of target trees, indicating higher C load from fine roots of higher SRA. The positive correlations between fine-root exudation with respiration, tissue N and SRA suggested that competitive roots with high N concentration and large C respiration also released more C into the exudate. Accordingly, the negative correlation between exudation and RTD

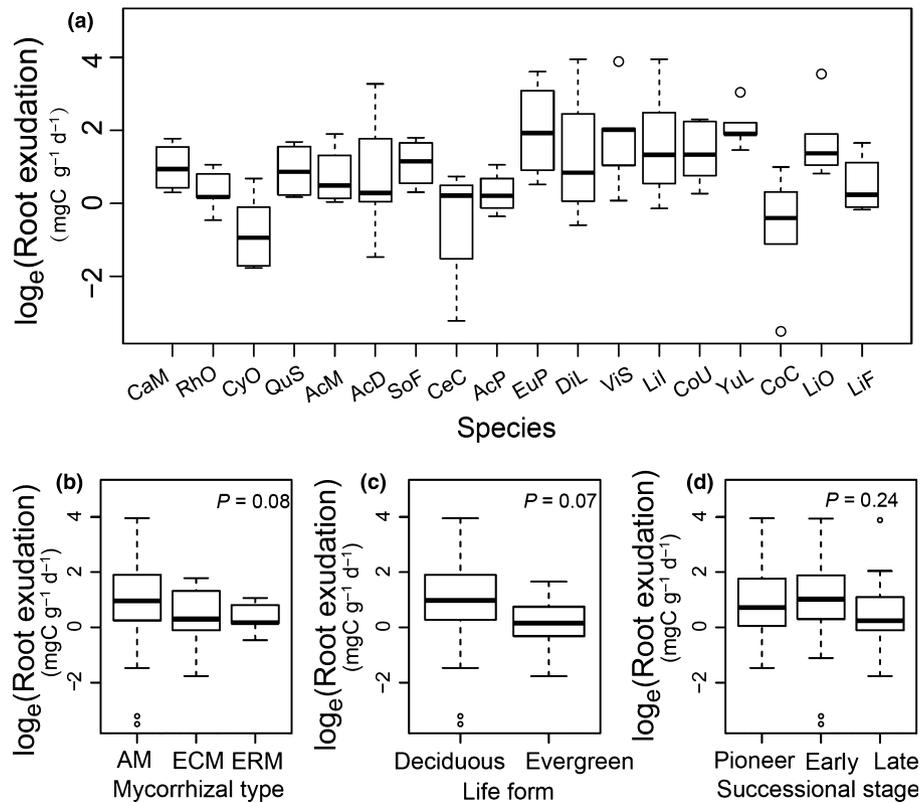


Fig. 2 Boxplot of root exudation variation among: (a) all target species, (b) different mycorrhizal types, (c) different life forms (deciduous vs evergreen) and (d) different successional stages. All data were natural log-transformed. In (a), the species aligned in the order of their root diameter. The central box in each boxplot shows the median and the interquartile range. The whiskers extend to $1.5 \times$ the interquartile range or to the minimum and maximum value. Open circles denote outliers. The abbreviations of species are consistent with Table 1. The finest-root species were on the left end and the coarsest-root species were on the right end. In (b–d), the difference among functional types was tested by a nested ANOVA with the individuals from a same species nested. AM, arbuscular mycorrhizal type, ECM, ectomycorrhizal type, ERM, ericoid mycorrhizal type.

showed that conservative roots of high construction cost tended to have low C fluxes of respiration and exudation. Therefore, fine-root exudation is likely to be a competitive trait among the coexisting woody species in our experimental forest. Notably, we represented the root trait correlations at both individual tree and species-mean levels. Root exudation and tissue N concentration exhibited a positive correlation with soil N availability at individual levels (Table S3a), indicating that the correlation among root traits at individual levels may reflect the plasticity of root traits to soil properties (Freschet *et al.*, 2017a). Furthermore, several root traits covaried in response to soil nitrogen status and the correlations were robust at species-mean level (Table S3b), showing a syndrome of trait coordination based on a whole-plant strategy (Freschet *et al.*, 2017a; Freschet *et al.*, 2018).

Fine-root functional trait coordination was multidimensional

Partly inconsistent with our single-dimensional predictions (H1; Fig. 1b), exudation rates did not consistently correlate with those morphologically and metabolically competitive traits. However, the morphological traits measured followed the classic competitive-conservative coordination (Figs 1a,3). Diameter was a leading functional trait as many researches had proposed and verified

(Kong *et al.*, 2014; Weemstra *et al.*, 2016; Ma *et al.*, 2018). It correlated with almost every other morphological trait, representing a commonly known morphologically competitive-conservative coordination (Valverde-Barrantes *et al.*, 2015, 2017; Weemstra *et al.*, 2016; Laliberté, 2017; Liese *et al.*, 2017). The PCA results of all eight traits showed that root trait coordination in the Shennongjia forest seemed to be multidimensional, with two perpendicular main axes representing root metabolic activity and morphology, respectively (Fig. 5a,b). Apparently, one reason for this multidimensional coordination was that root [N] only correlated with RTD but not with any other morphological traits (Fig. 3), which is similar to the PCA results on local and global scales (Erktan *et al.*, 2018; Ma *et al.*, 2018; Wang *et al.*, 2018; McCormack & Iversen, 2019; Bergmann *et al.*, 2020). A comparison of 34 arbuscular mycorrhizal woody species showed that root [N] was independent of SRL, and challenged the idea that high metabolic activity positively correlated with morphologically competitive traits (Valverde-Barrantes *et al.*, 2015). Another comparison among 66 species of tree seedlings showed that SRL was independent of RTD (Kramer-Walter *et al.*, 2016). Moreover, root exudation contained secondary compounds that served the function of communication with soil microbes and other plants or alleviation of environmental stress such as drought (Bais *et al.*, 2006; Badri & Vivanco, 2009; de Vries *et al.*, 2019). Fine

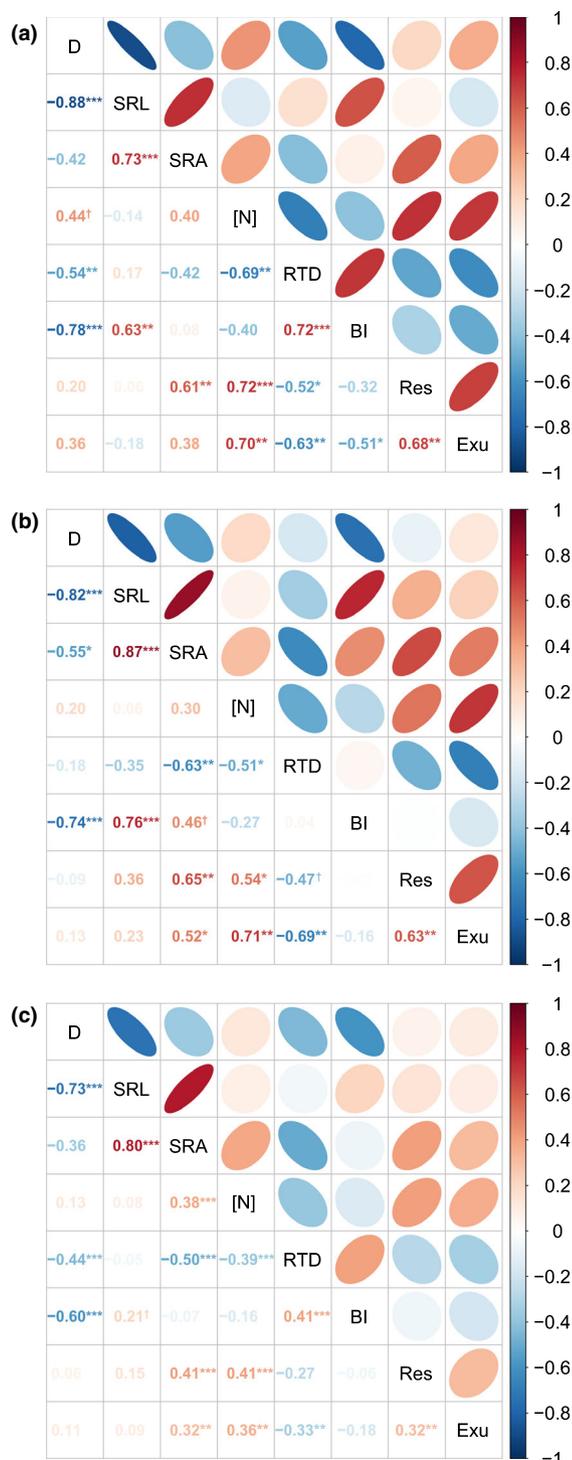


Fig. 3 Matrix of the Spearman's correlation coefficients and 95% confidential ellipse on (a) species level ($n=18$), (b) phylogenetically independent contrasts ($n=17$), and (c) individual tree level ($n=83$) among eight root traits for 18 coexisting species. A narrower confidential ellipse denotes a stronger correlation. Significance level of correlations are indicated; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; †, $P < 0.10$. BI, branching intensity; D, diameter; Exu, exudation; [N], nitrogen concentration; Res, respiration; RTD, root tissue density; SRA, specific root area; SRL, specific root length.

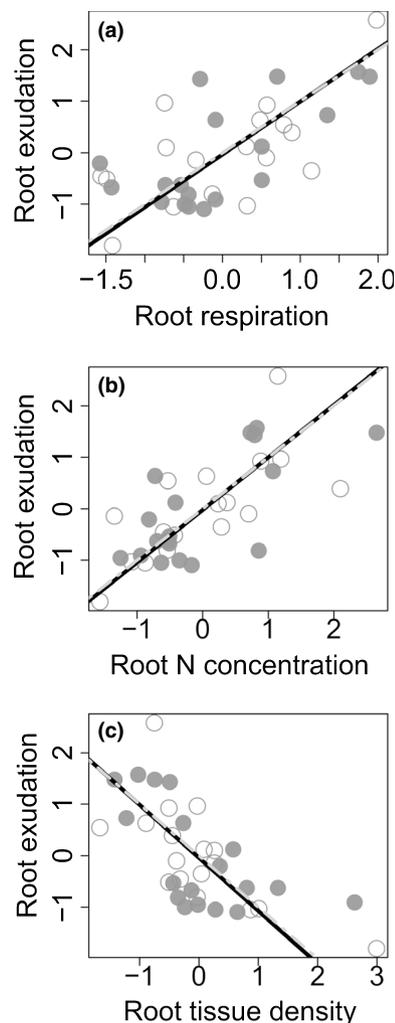


Fig. 4 Correlations of fine-root exudation with (a) fine-root respiration, (b) fine-root N concentration ([N]) and (c) fine-root tissue density (RTD). All data were standardised (unitless) to allow species level and phylogenetic independent contrast (PIC) data be displayed on the same figure. All correlations are significant (see detailed coefficients and significance level in Fig. 3a,c). The closed circles denote the species means. The open circles denote phylogenetic independent contrasts. There was no difference between slopes of species-level data and PICs for the exudation–respiration relationship ($P=0.926$), exudation–[N] relationship ($P=0.938$) or exudation–RTD relationship ($P=0.915$).

roots confronted multistresses from the complex soil matrix so that coordination of fine-root functional traits could reasonably be multidimensional (Erktan *et al.*, 2018; Kramer-Walter *et al.*, 2016; Weemstra *et al.*, 2016).

The MANOVA results of the 18 target species showed that the matrix of eight root functional traits was significantly different among mycorrhizal types (Fig. 5b). The target species of ecto and ericoid mycorrhizal types were loaded on the upper half of the PCA second axis, while the arbuscular ones were loaded on the lower half of the PCA second axis. Woody species can form different types of symbiotic associations and could be one reason that the coordination was split into two axes (McCormack &

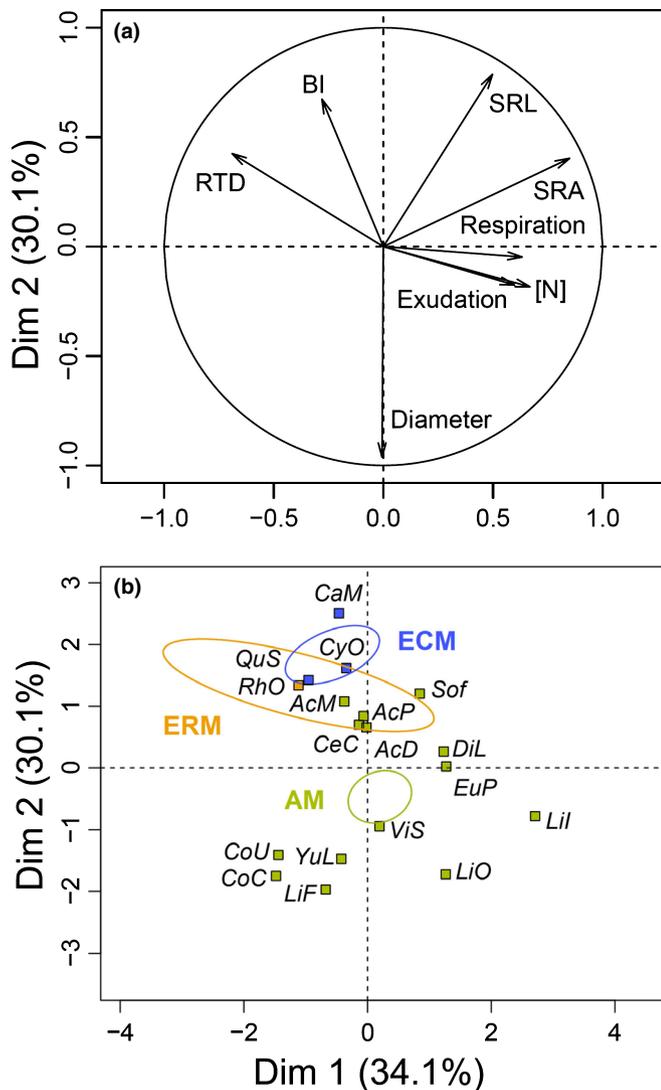


Fig. 5 Principal components analysis (PCA) of eight fine-root functional traits on an individual tree level. (a) Load of functional traits on two axes. In total, the first two dimensions explained c. 64% of the variation. (b) Species distribution on the first two dimensions. Ellipses represent the 95% confidence interval of three mycorrhizal groups on the two dimensions. The three groups (arbuscular mycorrhizal, AM; ectomycorrhizal, ECM; and ericoid mycorrhizal, ERM) exhibited significantly different functional trait coordination tested by MANOVA ($P < 0.001$). Species and the 95% confidence ellipses of a corresponding mycorrhizal type are indicated by the same colour (ECM, blue; ERM, orange; AM, green). BI, branching intensity; [N], nitrogen concentration; RTD, root tissue density; SRA, specific root area; SRL, specific root length. Abbreviations of species are consistent with those in Table 1.

Iversen, 2019; Bergmann *et al.*, 2020). Firstly, the symbiosis with mycorrhizal partners can be a complementary foraging strategy for fine roots of less competitive morphological structure to take up nutrients efficiently (Chen *et al.*, 2016; Cheng *et al.*, 2016; Li *et al.*, 2017; Bergmann *et al.*, 2020). Indeed, for arbuscular and ectomycorrhizal fungi types, host species of large diameter and low SRL tend to exhibit higher mycorrhizal colonisation at both local and global scales (Comas *et al.*, 2014; Liu *et al.*, 2015; Chen *et al.*, 2016; Li *et al.*, 2017; Ma *et al.*, 2018). Secondly, the colonisation of arbuscular and ectomycorrhizal fungi as well as

their mycorrhizal identity modifies root diameter (Sun *et al.*, 2017b), root [N] and root respiration (Hughes *et al.*, 2008; Trocha *et al.*, 2010; Fahey *et al.*, 2016). As a result, fine roots become metabolically more active but morphologically less efficient as mycorrhizal fungi colonisation increases. Nevertheless, higher colonisation of both arbuscular and ectomycorrhizal fungi was likely to be a trade-off of C allocation to root exudation (Ryan *et al.*, 2012; Meier *et al.*, 2013; Raven *et al.*, 2018; Wen *et al.*, 2019). Notably, the number of species of the three mycorrhizal types was not even (AM 14 : ECM 3 : ERM 1) in this study. Thus, whether symbiotic mycorrhiza leads to an independent metabolic dimension of a morphological dimension among woody species requires future investigation.

The root trait coordination of deciduous to evergreen species and pioneer to late successional stages were distributed on the first axis in the direction from a more competitive end to a more conservative end (Fig. S5a,b). Although root exudation flux is regulated by both abiotic and biotic factors (Bais *et al.*, 2006; Badri & Vivanco, 2009), it may also follow the leaf economics spectrum and the whole-plant ‘fast–slow growing’ strategy in parallel (Reich, 2014). Root exudation correlated with aboveground plant status and environmental conditions, such as vapour pressure deficit and solar radiation (Gougherty *et al.*, 2017; Nakayama & Tatenno, 2018). Despite the fact that the number of deciduous and evergreen species was not even (deciduous 15 : evergreen 3) in our subtropical montane forest, deciduous species consistently exhibited higher root exudation than evergreen species in forest ecosystems (Fig. 2c; Sun *et al.*, 2017a; Wang *et al.*, 2019).

Root exudation increases soil nutrient availability: a complementary strategy of plant nutrient acquisition?

We hypothesised that root exudation of woody species may correlate with other root functional traits based on a single-dimensional root trait coordination; an efficient morphological structure (high SRL/SRA, fine diameter) represents a competitive syndrome in nutrient uptake, which also has high metabolic activity (Roumet *et al.*, 2016; Weemstra *et al.*, 2016). From this perspective, plants solely take up the available nutrients released from decomposition of soil organic matter (Figs 1,6a). Later on, meta-analyses showed that cooperation with symbiotic mycorrhizal partners can be a relatively independent strategy for plants to acquire soil nutrients from forging morphologically competitive roots (McCormack & Iversen, 2019; Bergmann *et al.*, 2020). Similarly, the concept of rhizosphere priming effects challenged the paradigm because root exudates mobilise nutrients from mineral association or soil parent material (Dakora & Phillips, 2002; Aoki *et al.*, 2012; Keiluweit *et al.*, 2015; Ohta & Hiura, 2016) or accelerate microbial decomposition (Phillips *et al.*, 2011; Zhu *et al.*, 2014; Yin *et al.*, 2018), that is plants do not wait for available nutrients but also promote soil nutrient availability. Therefore, other than exploring the soil matrix for available nutrients with morphologically competitive traits (i.e. high SRL and SRA roots), fine roots can be less mobile and utilise nutrients nearby. In a new paradigm (Fig. 6c), root exudation is a complementary

strategy for plant nutrient acquisition. However, plants need to compete with rhizosphere microbes for the nutrients mobilised by root exudates, thus root exudation should not be completely independent of morphological traits (Fig. 6b,c). In the above-ground part of plants, leaf economics traits showed independence with hydraulic traits (Li *et al.*, 2015) and mechanical strength (He *et al.*, 2019), which in fact increases the possible combinations of functional traits for different species to coexist. It is interesting that anatomical traits (e.g. stele diameter to root diameter) representing root hydraulic traits exhibited inconsistent correlation with metabolically competitive and morphologically competitive root traits (Kong *et al.*, 2014). Our findings of a multidimensional root morphological and metabolic traits may also emphasise the multiple combination of strategies adapting to belowground stresses (Weemstra *et al.*, 2016).

Limitations and conclusions

Although there is a global database of fine-root functional traits (Fine Root Ecology Database, FRED, Iversen *et al.*, 2017; Global Root Traits Database, GRooT, Guerrero-Ramirez *et al.*, 2020), we do not make any prediction on root exudation rates from the correlations with root [N] and root respiration rates at this time because of limitations in the method and design of this study. First, the method of root exudation collection cannot eliminate the effects of C uptake by roots and soil microbes or temperature dependence (Hill *et al.*, 2006; Phillips *et al.*, 2008; Oburger & Jones, 2018). Therefore, the measured exudation rates did not indicate precise values. Second, although we had considered different factors such as mycorrhizal types, successional stages and phylogenetic signals in our experimental design, our findings from 18 species in a subtropical forest should be tested with more species in other ecosystems in the future.

Root exudation is a key component in soil C and nutrient cycling. Models did not include the nutrient induced by root exudation until recently (Brzostek *et al.*, 2012; Perveen *et al.*, 2014), partly because there are only limited root exudation data and we do not know how to predict this flux (Finzi *et al.*, 2015; Sun *et al.*, 2017b). In this study, we proposed a linkage between root exudation with root functional trait coordination, and verified that root exudation is a competitive trait among the 18 woody species. We found that root exudation rates correlated positively with competitive root traits (root [N], respiration, SRA) and negatively with conservative root trait (RTD) independent of phylogenetic signals. Root [N] and respiration are easier to measure than root exudation and other root functional traits. There is a global dataset which documented plenty of records of fine-root respiration, root [N] and other functional traits (Guerrero-Ramirez *et al.*, 2020; Iversen *et al.*, 2017). The correlations between root exudation with root [N], respiration, SRA and RTD indicated a promising approach to predict root exudation and thereafter the amount of nutrient mobilised by root exudation. Furthermore, we found a multidimensional root trait coordination in which root exudation loaded on a major axis perpendicularly with another major axis representing root morphology. This finding emphasised that increasing nutrient availability by root exudates is probably a complementary strategy to foraging nutrients with competitive morphological traits for plant nutrient acquisition.

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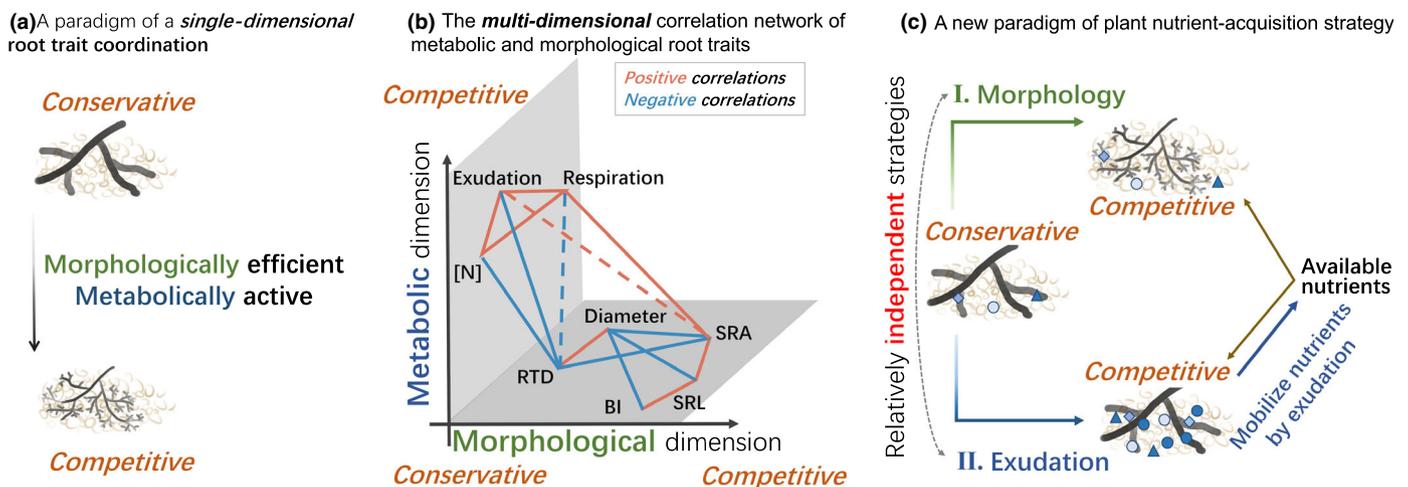


Fig. 6 (a) Single-dimensional root trait coordination that was originally hypothesised describing the correlations between root exudation with other root traits based on a single-dimensional paradigm (also see Fig. 1a,b), that is morphological and metabolic traits ally on the same axis. (b) Multidimensional root trait coordination found in this study. The two perpendicular axes represent a metabolic dimension and a morphological dimension. Solid lines denote significant correlations at three levels (individual tree level, species-mean level and phylogenetic independent contrasts). The dashed line denotes significant correlations at only two of the mentioned levels. The correlation is not shown if it is significant at less than two levels. (c) New diagram of plant nutrient-acquisition strategy. According to correlation networks in (b), increasing nutrient availability by root exudates is likely to be an independent strategy from the strategy of foraging soil nutrients with competitive morphological traits

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Author contributions

BZ, LJS and MA designed the experiments. LJS, MGH, YFH, DYG and TLX performed the field and laboratory work. LJS, MA and YPG analysed the data. LJS, MA, MGH and BZ wrote the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Microscope photographs on confirmation of mycorrhizal type (arbuscular mycorrhizal fungi).

Fig. S2 Phylogenetic tree of the target species.

Fig. S3 One-way ANOVA of fine-root exudation and respiration on different sampling dates.

Fig. S4 Correlation of original respiration rates and temperature-adjusted rates.

Fig. S5 Species distribution of the principal components analysis (PCA) of eight fine-root functional traits loaded on two axes and based on successional stages and life forms (deciduous vs evergreen).

Table S1 Sampling date of every individuals from each species.

Table S2 Species-specific means \pm SDs of the eight target root traits.

Table S3 Correlation of eight root traits with rhizosphere soil properties.

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