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Root functional traits determine the magnitude of the rhizosphere priming effect among eight tree species

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Living roots and their rhizodeposits can accelerate or decelerate the decomposition of soil organic matter which refers to the rhizosphere priming effect (RPE). However, whereas plant traits are thought to be key factors controlling the RPE, little is known about how root traits representative of plant biomass allocation, morphology, architecture, or physiology influence the magnitude of the RPE. Using a natural abundance ^{13}C tracer method allowing partitioning of native soil organic carbon (SOC) decomposition and plant rhizosphere respiration, we studied here the effects of eight C_3 tree species featuring contrasting functional traits on C_4 soil respiration over a 204-day period in a microcosm experiment. All tree species enhanced the rate of SOC decomposition, by 82% on average, but the strength of the rhizosphere priming significantly differed among species. Mean diameter of first-order roots and root exudate-derived respiration were positively correlated with the RPE, together explaining a large part of observed variation in the RPE ($R^2 = 0.72$), whereas root branching density was negatively associated with the RPE. Path analyses further suggested that mean diameter of first-order roots was the main driver of the RPE owing to its positive direct effect on the RPE and its indirect effects via root exudate-derived respiration and root branching density. Our study demonstrates that the magnitude of the RPE is regulated by complementary aspects of root morphology, architecture and physiology, implying that comprehensive approaches are needed to reveal the multiple mechanisms driving plant effects on the RPE. Overall, our results emphasize the relevance of integrating root traits in biogeochemical cycling models to improve model performance for predicting soil C dynamics.

Keywords: ^{13}C natural abundance, C_4 soil, fine roots, organic matter decomposition, root functional traits, soil organic carbon

Introduction

Soil organic matter (SOM) represents the largest reservoir of organic carbon (C) in terrestrial ecosystems, holding approximately 2500 pg C (up to 1 m depth) (Tifafi et al. 2018). Therefore, even a minor change in soil organic C (SOC) stocks would have major effects on atmospheric CO_2 concentration and would have potential feedback to climate (Davidson and Janssens 2006, Heimann and Reichstein 2008, Qiao et al. 2014). The size of SOC pools is largely determined by the balance between C inputs from plant production and C outputs from soil decomposers, whereas its stability is reflected in the microbial mineralization of SOC (Davidson and Janssens 2006, Cotrufo et al. 2015, Guenet et al. 2018). Soil CO_2 output consists of two distinct components, rhizosphere respiration by roots and associated microbes utilizing root-derived substrates, and microbial decomposition of native SOC (Zhu et al. 2014). Historically, soil temperature and water content have been considered as the primary drivers of SOC decomposition (Davidson and Janssens 2006). However, emerging evidence indicates that plant roots and rhizosphere inputs are also major drivers of decomposition processes (Fontaine et al. 2007, Schmidt et al. 2011, Finzi et al. 2015, Keiluweit et al. 2015). The presence of live roots can accelerate or decelerate SOC decomposition via the 'rhizosphere priming effect' (RPE, defined as a change in SOC decomposition by the supply of root-derived substrates). For example, in the presence of living roots, SOC decomposition can decrease by up to 79% (negative RPE), or increase by as much as 500% (positive RPE), relative to rootless soil (Cheng et al. 2014, Huo et al. 2017).

The magnitude, direction and duration of the RPE can be influenced by multiple factors, including plant species identity (Cheng et al. 2014, Henneron et al. 2019) and plant traits (e.g. photosynthesis rate, plant biomass and phenology; Yin et al. 2018, Henneron et al. 2019), and soil properties (e.g. soil type, nutrient availability, soil temperature and water content; Zhu and Cheng 2011, 2013, Dijkstra et al. 2013). Several hypotheses have been proposed to explain the underlying mechanisms of contrasting RPEs. Generally, positive RPEs could be explained by co-metabolism of SOC with root-released available substrates (i.e. root exudates) that stimulate microbial growth and extracellular enzyme production (Kuzyakov 2002, Cheng and Kuzyakov 2005). Additionally, root exudates may also destabilize mineral-associated organic SOC, thereby enhancing microbial access to previously mineral-protected compounds (Keiluweit et al. 2015). By contrast, negative RPEs might occur if microbial communities switch from degrading recalcitrant SOC to utilizing energy-rich root exudates ('preferential substrate utilization hypothesis', Cheng and Kuzyakov 2005), or if plants and microorganisms compete for nutrients ('nutrient competition hypothesis', Kuzyakov and Xu 2013, Chang et al. 2020).

Over the past decade, many studies have indicated that plant biomass and traits significantly influence the intensity of the RPE (Cheng et al. 2003, Pausch et al. 2013, Henneron et al. 2019). Previous studies have demonstrated that plant biomass was generally positively correlated with RPE, at least for herbaceous species (Dijkstra et al. 2006, Zhu and Cheng 2013, Huo et al. 2017). Furthermore, the magnitude of the RPE is strongly influenced by the quantity and chemical composition of root exudates (Cheng et al. 2014, Wang et al. 2016), which are not only regulated by plant biomass (Zhu and Cheng 2013), but also related to some root functional traits (Meier et al. 2020, Sun et al. 2021). Root traits may indeed be particularly important drivers of the magnitude and dynamics of the RPE due to their intricate relationship with the soil matrix and microorganisms (Finzi et al. 2015, Carrillo et al. 2017, Henneron et al. 2019). Root exudation rate (approximated in the present study by the CO_2 released from root exudates) is likely to be one of the strongest drivers of the RPE (Shahzad et al. 2015, Wang et al. 2016). In addition, fine roots with high metabolism (e.g. high respiration rate; Sun et al. 2021) and large surface of exchange (as represented by fine-root length; Freschet et al. 2021a) could strongly influence rhizosphere processes by changing rhizosphere properties (e.g. soil pH, nutrient status, microbial communities; Bais et al. 2006, Freschet et al. 2021b). For example, species with high root length increase the contact of roots with soil and the input of C to soil (Bardgett et al. 2014). Similarly, species with a high proportion of root tips (as represented by fine-root branching density, RBD; Freschet et al. 2021b) could show increased root exudation (mostly located at the root tips; Canarini et al. 2019). As such, these traits may all be important drivers of the RPE.

Recent studies further suggest that root exudation may be linked to root morphology, as observed at the intraspecific level between specific root length (SRL) and root exudation rate (positive relationship; Meier et al. 2020). Negative relationship of root tissue density (RTD) and root diameter with root exudation rate was also observed at the interspecific level (Han et al. 2020, Sun et al. 2021). However, the influence of such traits morphological traits on the RPE is likely to be largely indirect, through covariation of these traits with traits more directly linked to root exudation and the RPE, for example, root length and root respiration rate (see discussion in Freschet et al. 2021a). In support for this, several studies showed that root exudation may also be largely decoupled from both root morphology and mycorrhizal colonization (across 16 crop species; Wen et al. 2019), and that root morphological traits, such as SRL and diameter, were weak predictors of the RPE (Wang et al. 2016, Henneron et al. 2019).

Our understanding of root trait-RPE relationships is currently limited for several reasons. First, most studies to date on root traits-RPE relationships used a relatively small pool of species (Cheng et al. 2003, Pausch et al. 2013, Yin et al. 2020, but see Han et al. 2020). Second, although a meta-analysis can partly solve this problem by including a large pool of plant species (Huo et al. 2017), the lack of data on root morphology does not allow us to establish reliable relationships between root morphological traits and the RPE (Cheng et al. 2014, Huo et al. 2017). Moreover, studies suggesting an

absence of relationship between root morphological traits and the RPE are based on observations made on herbaceous species only (Henneron et al. 2019, Wang et al. 2016) and cannot be simply extrapolated to tree species as trait-trait and trait-function relationships may differ across plant functional types (Freschet et al. 2021a). Interestingly, while, in a study of 14 woody species, Han et al. (2020) observed that root functional traits such as root diameter strongly correlated with the rhizosphere effect on SOM decomposition their results included both the effect of rhizodeposits decomposition and the RPE. Finally, not accounting for trait differences between root orders may also influence root morphology-RPE relationships (Henneron et al. 2019) as root functions vary along root orders (Freschet and Roumet 2017). Particularly, first-order roots show high metabolic activity and respiration rate, and host a large part of root exudation processes (Canarini et al. 2019). Therefore, we differentiated here between root traits of first-order roots, which we expected to be most strongly related to root exudation processes, and root traits of absorptive roots.

The primary aim of this experiment was to examine the main drivers of root-trait effect on the RPE. To do so, the isotope-based method was used to determine RPE and identify its correlation with plant traits across woody species. Specifically, we studied the ^{13}C isotopic signature of CO_2 respired from microcosms where eight C_3 tree species from different families were planted in a C_4 soil. Owing to large species differences in plant biomass and a range of fine-root morphological, architectural and physiological traits, we expected that plant species would exert a strong influence on the magnitude of the RPE. We further expected that the stimulation of SOC decomposition should depend simultaneously on contrasting aspects of plant functioning, including biomass, morphology, architecture and physiology. More precisely, we tested the hypotheses that 1) among a range of tree species, high biomass, fine root length, root branching density, and root exudation rate would be the main drivers of the RPE; and that 2) these traits would have complementary (i.e. non-redundant) effects on the RPE because of their key role in determining the amount, location and activity of the roots.

Materials and methods

Experimental setup

The ^{13}C natural abundance approach was used to separate plant-derived CO_2–C from soil-derived CO_2–C by planting C_3 plants in C_4 soil. The soil used in this study was collected from the plow layer (0–20 cm) of a farm plot that has been grown with a C_4 maize crop for over 23 years. The soil was air-dried, thoroughly homogenized and passed through a 4 mm mesh sieve. The soil is a clay loam (43% sand, 22% silt, 35% clay) with a pH of 6.9. The C and nitrogen (N) concentrations were 17.3 g kg^{-1} and 1.5 g kg^{-1}, respectively, corresponding to a C:N ratio of 11.6. The $\delta^{13}C$ value of C_4 maize soil was −16.5‰.

Eight common and relatively abundant tree species in a subtropical forest were used in this experiment (Table 1). Tree seedlings of similar size (height and diameter were ca 37.8 and 5.4 cm) were taken from a common garden and transplanted into polyvinyl chloride (PVC) pots (diameter 16 cm, height 40 cm), equipped with a PVC lid at the bottom. A nylon bag filled with 2 kg sand was placed at the bottom of each pot for drainage, and 6.6 kg air-dried C_4 soil was packed into each pot at a bulk density of 1.27 g cm^{-3}. Pots without plants were also included as control. There were 45 pots in total, with five replicates of each species (for *Quercus acutissima*, *Carya cathayensis* and *Schima superba* only four replicates were available at the end of the experiment, owing to the death of one tree replicate). We note that a ninth species, *Metasequoia glyptostroboides*, was also planted, but was excluded from this study due to very poor development during the experiment. The plants were left to grow for 204 days (the whole growing season, from March to October 2018, with a temperature range of 20.5–31 °C, 23.8 °C on average). The pots were placed under natural conditions located at the Huitong Natural Research Station of Forest Ecosystem (26°48′N, 109°30′E) in the Hunan province, central China. Local mean annual temperature and precipitation are 16.5 °C and 1200 mm. The soil moisture in each pot was measured gravimetrically and maintained at 80% of its water holding capacity via regular watering and the use of a rain shelter to exclude rainfall during rain events.

Analysis of CO_2 fluxes

We measured the total respiration of the plant–soil system from each pot with an air-tight, opaque CO_2 chamber trapping system (Supporting information). The dark conditions inside the chamber prevented photosynthesis and, thereby, the uptake of CO_2 by the plant (Shahzad et al. 2015). The CO_2 released by the plant–soil systems was quantified by taking air samples from chambers on day 54, 90, 120 and 204 after planting. Specifically, two holes were punched on the top of PVC chamber lids and installed with bulkhead connectors. Polyurethane tubes were used for linking the bulkhead connectors with a manual valve. Pots were placed inside the PVC chamber (diameter 20 cm, height 100 cm) and the chambers were flushed by circulating air for 5 min to ensure a common CO_2 starting point, using an air compressor. An air sample was taken to measure the initial CO_2 concentration, then chambers were sealed immediately by closing the manual valve. After 48 h, the gas was collected using a portable Gas sampling pump and stored in a pre-evacuated Gas sampling bag, and pots were taken out of the chamber. Dark incubations of 48 h or less prevent the substantial decrease in root activity and soil respiration typically observed for longer incubations (Kuzyakov and Cheng 2001). The CO_2 concentration and $\delta^{13}C$ were analyzed by a high-precision isotopic CO_2 cavity ring-down spectrometer (CRDS) (Picarro G2131-i Analyzer, Picarro, Inc.). The amount of CO_2 derived from the plant–soil system respiration and its $\delta^{13}C$ were obtained by correcting for the initial atmospheric CO_2 (Wang et al. 2016).

Table 1. Plant biomass and root functional traits at the end of the experiment. Values represent means ± SE; n = 5 for all treatments, except for *Quercus acutissima*, *Carya cathayensis* and *Schima superba* treatments where n = 4. Different letters indicate significant differences among species (post hoc Tukey–Kramer honest significant difference (HSD) test, $p < 0.05$); in case of significance, 'a' represents the smaller value, while 'e' represents the larger value. ECM, ectomycorrhizal; AM, arbuscular.

Species	*Liquidambar formosana*	*Quercus acutissima*	*Carya cathayensis*	*Cunninghamia lanceolata*	*Ginkgo biloba*	*Schima superba*	*Triadica sebifera*	*Zanthoxylum bungeanum*	
Family	Altingiaceae	Fagaceae	Juglandaceae	Cupressaceae	Ginkgoaceae	Theaceae	Euphorbiaceae	Rutaceae	
Growth form	Deciduous broadleaf	Deciduous broadleaf	Deciduous broadleaf	Evergreen broadleaf	Deciduous broadleaf	Deciduous broadleaf	Deciduous broadleaf	Deciduous broadleaf	
Mycorrhizal types	ECM	ECM	ECM	AM	AM	AM	AM	AM	CV (%)
Plant biomass									
Plant total biomass (g per pot)	4.3 ± 1.0^{a}	9.6 ± 1.3^{b}	12.0 ± 1.7^{bc}	15.0 ± 1.9^{c}	7.2 ± 0.4^{ab}	3.8 ± 0.3^{a}	11.1 ± 0.7^{bc}	7.1 ± 0.4^{ab}	40.9
Aboveground biomass (g per pot)	2.8 ± 0.6^{a}	5.0 ± 1.0^{ab}	7.8 ± 0.9^{c}	8.2 ± 0.7^{c}	4.1 ± 0.3^{a}	2.6 ± 0.3^{a}	6.8 ± 0.4^{bc}	4.0 ± 0.2^{a}	40.0
Belowground biomass (g per pot)	1.5 ± 0.4^{ab}	4.5 ± 0.6^{cd}	4.2 ± 0.8^{bcd}	6.7 ± 1.2^{d}	3.2 ± 0.2^{abc}	1.2 ± 0.1^{a}	4.3 ± 0.5^{bcd}	3.1 ± 0.2^{abc}	44.1
Leaf biomass (g per pot)	0.2 ± 0.1^{ab}	0.4 ± 0.1^{cd}	1.5 ± 0.4^{bc}	4.0 ± 0.4^{d}	1.1 ± 0.1^{abc}	0.5 ± 0.1^{a}	2.6 ± 0.1^{bcd}	1.2 ± 0.1^{abc}	78.6
First-order root biomass (g per pot)	0.01 ± 0.0^{a}	0.02 ± 0.0^{a}	0.01 ± 0.0^{a}	0.28 ± 0.1^{bc}	0.10 ± 0.0^{ab}	0.01 ± 0.0^{a}	0.39 ± 0.1^{c}	0.44 ± 0.1^{c}	100.6
Absorptive root biomass (g per pot)	0.06 ± 0.0^{a}	0.21 ± 0.0^{ab}	0.09 ± 0.0^{a}	1.67 ± 0.4^{c}	0.89 ± 0.1^{abc}	1.02 ± 0.3^{bc}	1.60 ± 0.1^{c}	1.66 ± 0.2^{c}	63.7
Morphological root traits									
Mean first-order root diameter (mm)	0.37 ± 0.0^{ab}	0.50 ± 0.1^{cd}	0.35 ± 0.1^{a}	0.58 ± 0.0^{d}	0.76 ± 0.0^{e}	0.41 ± 0.0^{abc}	0.33 ± 0.0^{a}	0.49 ± 0.0^{bcd}	27.0
Mean absorptive root diameter (mm)	0.64 ± 0.08^{ab}	0.90 ± 0.08^{b}	0.57 ± 0.07^{ab}	0.88 ± 0.08^{b}	1.24 ± 0.07^{c}	1.63 ± 0.14^{d}	0.42 ± 0.01^{a}	0.73 ± 0.01^{ab}	41.4
First-order root tissue density (g cm^{-3})	0.08 ± 0.01^{a}	0.22 ± 0.06^{bc}	0.06 ± 0.01^{a}	0.13 ± 0.01^{ab}	0.07 ± 0.00^{a}	0.24 ± 0.04^{c}	0.10 ± 0.01^{a}	0.08 ± 0.01^{a}	51.5
Absorptive root tissue density (g cm^{-3})	0.14 ± 0.03^{a}	0.28 ± 0.02^{c}	0.12 ± 0.02^{a}	0.23 ± 0.02^{bc}	0.16 ± 0.01^{ab}	0.37 ± 0.02^{d}	0.14 ± 0.01^{a}	0.16 ± 0.02^{ab}	40.1
First-order specific root length (m g^{-1})	116.2 ± 11.5^{c}	33.4 ± 5.4^{ab}	211.2 ± 21.0^{d}	29.6 ± 1.8^{a}	30.0 ± 0.7^{ab}	37.0 ± 11.3^{a}	118.8 ± 6.5^{c}	77.7 ± 12.4^{bc}	66.6
Absorptive specific root length (m g^{-1})	55.55 ± 15.1^{bc}	7.99 ± 1.5^{a}	69.12 ± 14.1^{c}	10.92 ± 1.0^{a}	8.34 ± 0.6^{a}	1.49 ± 0.6^{a}	64.64 ± 5.1^{bc}	33.45 ± 2.9^{ab}	81.8
Architectural root traits									
Root branching density (cm^{-1})	3.25 ± 0.54	2.65 ± 0.70	3.32 ± 0.70	1.76 ± 0.21	2.47 ± 0.33	3.85 ± 1.03	2.97 ± 0.32	1.97 ± 0.15	23.8
Physiological root traits									
Root exudate-derived respiration (mg C kg^{-1} soil day^{-1})	0.42 ± 0.10^{ab}	0.59 ± 0.09^{b}	0.37 ± 0.02^{ab}	0.49 ± 0.05^{ab}	0.49 ± 0.03^{ab}	0.34 ± 0.02^{ab}	0.30 ± 0.06^{a}	0.34 ± 0.04^{ab}	21.9
Rhizosphere soil respiration (mg C kg^{-1} soil day^{-1})	2.30 ± 0.08	2.16 ± 0.13	2.46 ± 0.11	2.36 ± 0.13	2.28 ± 0.12	2.08 ± 0.08	2.05 ± 0.04	2.34 ± 0.09	5.9
Composite (*biomass-related*) *root traits*									
First-order root length (m)	1.12 ± 0.2^{a}	0.48 ± 0.1^{a}	2.41 ± 0.5^{a}	8.04 ± 2.1^{a}	3.00 ± 0.5^{a}	0.54 ± 0.2^{a}	45.87 ± 5.7^{c}	31.39 ± 2.2^{b}	137.2
Absorptive root length (m)	2.04 ± 0.39^{ab}	1.04 ± 0.20^{a}	4.83 ± 0.92^{bc}	15.11 ± 3.69^{d}	5.86 ± 0.78^{c}	0.90 ± 0.23^{a}	87.66 ± 7.20^{e}	50.10 ± 2.71^{e}	138.9
First-order root length density (cm cm^{-3})	0.01 ± 0.003^{a}	0.01 ± 0.001^{a}	0.03 ± 0.006^{a}	0.10 ± 0.026^{a}	0.04 ± 0.006^{a}	0.01 ± 0.002^{a}	0.57 ± 0.070^{c}	0.39 ± 0.027^{b}	137.1
Absorptive root length density (cm cm^{-3})	0.01 ± 0.002^{a}	0.01 ± 0.002^{a}	0.03 ± 0.007^{a}	0.08 ± 0.020^{a}	0.03 ± 0.004^{a}	0.00 ± 0.000^{a}	0.50 ± 0.072^{c}	0.23 ± 0.016^{b}	143.0

Harvest and measurements

The pots were destructively harvested 204 days after the planting of tree seedlings (Supporting information). Plant shoots were cut off at the base, then the pots were cut into two halves longitudinally to ensure the structural integrity of root systems. We shook the root system gently until only well-attached soil remained on the root system and carefully collected the soil still adhering to the roots (Supporting information), which was defined as rhizosphere soil (Phillips and Fahey 2006, Sun et al. 2021). Bulk soil was collected from unplanted treatment. Rhizosphere and bulk soil respiration were measured during 12 h incubation at 25 °C (Wang et al. 2016). Briefly, 10 g of soil (either fresh rhizosphere or bulk) was weighed into a 1 liter Mason jar and gas was collected 12 h after sealing using a portable gas sampling pump and stored in a pre-evacuated gas sampling bag. Previous results typically assume that the amount of root exudate is proportional to root-derived CO_2 (Ataka et al. 2020). Another proxy for the amount of root exudate can be measured as the respired CO_2 derived from rhizosphere soil within a few hours after sampling, as this respiration should be strongly related to the microbial utilization of root exudates (Fischer et al. 2010, Wang et al. 2016). Here, we tried to refine these relatively rough proxies to more accurately estimate root exudation. As such, in our study, eight C_3 tree species were planted in C_4 soil, so we were able to use the ^{13}C natural tracer approach to further partition root exudate-derived CO_2 (C_3–C) from rhizosphere soil-derived CO_2 (C_4–C). The CO_2 concentration and $\delta^{13}C$ value of respired CO_2 were also analyzed by a high-precision isotopic CO_2 CRDS, as described above.

After harvest, plant roots were gently washed with deionized water to remove residual soil particles adhering to roots. The root samples were stored in clean plastic bags and frozen at −20 °C until subsequent morphology and architecture measurements. Root orders were described and dissected according to stream ordering system, where the most distal roots are first order and where second-order roots begin at the junction of two first-order roots and so on (Pregitzer et al. 2002, McCormack et al. 2015). Roots from each order were scanned in deionized water using a clear water tray at 600 dpi on a scanner and analyzed with WinRhizo (Regent Instruments, Quebec, Canada). Root length, diameter, and root volume can be directly obtained through WinRhizo software output. However, root volume was recalculated from the sum of the volumes of all diameter classes, as the volume provided by Winrhizo is strongly biased (Freschet et al. 2021a). All plant samples were oven-dried at 65 °C for three days to constant weight and weighed.

Specific root length was calculated on first-order roots and on absorptive roots (the first three root branch order following a morphometric classification; McCormack et al. 2015) by dividing the length of these root entities by their oven-dried mass (m g^{-1}). For these same root entities, root tissue density was calculated as the dry mass of root per unit volume of fresh root (g cm^{-3}), root length density was calculated as the length of root per unit soil volume (cm cm^{-3}). Root branching density was expressed as the number of first-order laterals per centimeter of second order root (cm^{-1}) to allow for comparison with the literature (such as Eissenstat et al. 2015). Root exudate-derived respiration (C_3–C) was calculated by subtracting the CO_2 efflux of the rhizospheric soil (C_4–C) from the total CO_2 efflux, as further described below, and was denoted as mg C kg^{-1} soil day^{-1}.

Calculations

The total respiration of the plant–soil system was calculated for each pot and harvest date as follows:

$$R_{\text{total}} = \frac{C \times V \times M}{22.4 \times W \times t} / \left(\frac{273}{273 + T} \right)$$

where R is the CO_2 efflux (μg C kg^{-1} soil day^{-1}); C is the measured CO_2 concentration (ppm); V is the effective volume of a PVC chamber (21.3 L); M is the molar mass of C (12 g mol^{-1}); W is the dry weight of soil (g); t is the time of CO_2 accumulation (day); and T is the temperature (°C) of incubation.

We partitioned the total CO_2 efflux (R_{total}) (mg C kg^{-1} soil day^{-1}) of the plant–soil system incubation into SOC decomposition (R_{soil}) and plant-derived CO_2 (R_{plant}) using a two-source isotopic mixing-model:

$$R_{\text{soil}} = R_{\text{total}} \times \frac{(\delta^{13}C_{\text{plant}} - \delta^{13}C_{\text{total}})}{(\delta^{13}C_{\text{plant}} - \delta^{13}C_{\text{soil}})}$$

$$R_{\text{plant}} = R_{\text{total}} - R_{\text{soil}}$$

where $\delta^{13}C_{\text{total}}$ and $\delta^{13}C_{\text{soil}}$ are $\delta^{13}C$ values of CO_2 emitted from planted and unplanted treatment at each sampling date, respectively, and $\delta^{13}C_{\text{plant}}$ is the $\delta^{13}C$ value of root respiration. Previous studies showed that the separation of root- and SOM-derived CO_2, as a prerequisite to calculate RPE, often involves the assumption that the net isotopic fractionation during respiration processes is negligible (Schnyder and Lattanzi 2005, Dijkstra et al. 2010, Henneron et al. 2019). However, isotopic fractionation between root tissue and root-respired CO_2 has been increasingly recognized (Zhu et al. 2011, Yin et al. 2018). Therefore, we used the ^{13}C value of root respiration in this study, i.e. we considered the $\delta^{13}C$ fractionation between root-derived CO_2–C and root biomass. R_{plant} includes both the plant autotrophic respiration and the respiration of C contained in rhizodeposits by soil micro-organisms. The average $\delta^{13}C_{\text{plant}}$ ranged from −32.4‰ to −28.1‰ across species (Supporting information), with an average difference of 13.6‰ relative to $\delta^{13}C_{\text{soil}}$ (−16.5‰). The RPE was calculated by subtracting the CO_2 derived from unplanted soil from the SOM-derived CO_2 of the planted soil (mg C kg^{-1} soil day^{-1}):

$$\text{PRE} = R_{\text{soil(planted)}} - R_{\text{soil(unplanted)}}$$

The average daily R_{soil}, R_{plant} and *RPE* were calculated as the average of all four sampling dates.

Rhizospheric soil respiration ($R_{rhizospheric\ soil}$) and root exudate-derived respiration ($R_{exudates}$) were further calculated based on the incubation of rhizospheric soil sampled at the end of the experiment. We partitioned the total CO_2 efflux (R_{sum}) (mg C kg^{-1} soil day^{-1}) of the rhizospheric soil incubation using a two-source isotopic mixing-model:

$$R_{\text{rhizospheric soil}} = R_{\text{sum}} \times \frac{\left(\delta^{13}C_{\text{exudates}} - \delta^{13}C_{\text{sum}}\right)}{\left(\delta^{13}C_{\text{exudates}} - \delta^{13}C_{\text{bulk soil}}\right)}$$

$$R_{\text{exudates}} = R_{\text{sum}} - R_{\text{rhizospheric soil}}$$

where $\delta^{13}C_{sum}$ and $\delta^{13}C_{bulk\ soil}$ are $\delta^{13}C$ values of CO_2 emitted from the incubation of rhizospheric (planted treatment) and bulk soil (unplanted treatment), respectively; $\delta^{13}C_{exudates}$ is the δ^{13} value of $\delta^{13}C_{root}$ (‰). Based on a previous study on three tree species sampled five times along the year (Gougherty et al. 2018), where an average difference of 1.2‰ was observed between $\delta^{13}C_{exudates}$ and $\delta^{13}C_{root}$, we conducted a sensitivity analysis to estimate the consequence of using $\delta^{13}C_{root}$ as a proxy for $\delta^{13}C_{exudates}$ on root-exudate respiration values and relationships between root-exudate respiration and RPE, and observed a relatively limited impact (Supporting information).

Statistical analysis

To comply with normality and homogeneity assumptions of all parametric tests, root tissue density, root length and root length density of first-order roots and absorptive roots were log_{10}-transformed. Two-way ANOVA was used to assess the effects of plant species, sampling time and their interaction on the level of RPE among species. One-way ANOVA with Tukey's HSD post hoc tests were further used to compare plant above and belowground biomass and traits among different species. A correlation analysis was carried out to test collinearity among all these plant variables (Supporting information). The absence of relationship between plant biomass variables and traits indicated the likely absence of allometric effects of plant size on trait values. The relationships between all of these plant variables and the RPE was first tested using univariate linear regression analyses. Then, a cut-off of $R^2 > 0.4$ was used to retain variables with highest explanatory power on RPE in univariate regressions (namely, mean diameter of first-order roots, root branching density and root exudate-derived respiration) and conduct multivariate regressions. All possible combinations of these retained variables were used in multiple linear regression models to test their potential to explain the variance in RPE (no collinearity issue was detected among these variables, with all pairwise Pearson correlation coefficients < 0.6). The best models were selected based on corrected Akaike information criterion (AICc). Statistical analyses for all data were carried out using SPSS version 16.0 (SPSS Inc.) and the significance level was set at $p < 0.05$.

Additionally, we used path analysis (Shipley 2015) to develop and test hypotheses regarding the causal relationships between plant traits that showed (marginally-) significant univariate relationships with the RPE. Our initial hypothesized causal structure (Fig. 1a) generally reflects the idea that plant investment towards first-order roots (via high root biomass of first-order root and high root branching density) that increases the presence and distribution of first-order roots highly active in exuding compounds would stimulate soil carbon priming, as discussed earlier in this manuscript. The same initial model including traits measured on absorptive roots rather than first-order roots was also tested. These two versions of the initial model were then strongly adapted, based on results of univariate relationships between root traits and the RPE to obtain a final model that successfully accounted for the patterns of conditional dependencies in the data. Path coefficients that were not statistically different from zero were removed unless they had clear biological justifications and increased the fit of the model. We maintained two such marginally non-significant path coefficients in the final model (Fig. 1). Acknowledging the poor fit of models including the root length variable, this variable was replaced by two of its component traits, mean root diameter and specific root length. We used the *sem* function in the lavaan package of R (www.r-project.org) (Rosseel 2012). The data were fitted to the models using the maximum likelihood estimator with standard errors and χ^2 test statistic. We conducted all statistical analyses within the R statistical environment (ver. 4.0.5, www.r-project.org).

Results

Substantial interspecific variation among plant biomass and root functional traits

The eight species used in this study exhibited a wide range of plant biomass and root trait values (Supporting information). All 19 measured plant characteristics differed significantly among species ($p < 0.0001$, Table 1), except for two, root branching density and rhizosphere soil respiration. Across the eight species, there was a 3–45-fold variation in plant biomass, first-order root biomass showed the largest variation (CV = 100.6%), aboveground biomass had the lowest variation (CV = 40%). Root morphological traits showed 2–43-fold variation, and ranged from a CV of 27% for first-order root diameter to a CV of 82% for absorptive specific root length. Root branching density and root exudate-derived respiration showed similar variation (CV_{RBD} = 23.8% vs. $CV_{root\ exudate}$ = 21.9%). Also, a similar range of variation was found for composite (biomass-related) root traits (coefficient of variation ranging between 137 and 143%). By contrast, rhizosphere soil respiration had the lowest cross-species variation (CV = 5.9%) among plant biomass and root functional traits.

CO_2 efflux and primed SOC

The total CO_2 efflux of the plant–soil system showed a similar trend across all eight species, that is, an initial decrease in soil CO_2 efflux, then an increase up to the maximum value on day 120, then again a decrease over time (Fig. 2). The magnitude of plant-derived CO_2, the total CO_2 efflux and primed C were all significantly affected by sampling time (Supporting information). In addition, all of these soil parameters were influenced by species identity (Supporting information).

Over the 204 days of plant growth, the eight treatments with plants all exhibited higher soil CO_2 production relative to the unplanted control. Mean daily CO_2 production from the unplanted control was 1.08 mg C kg^{-1} soil day^{-1}, against 2.90 mg C kg^{-1} soil day^{-1} on average in planted pots. This ranged from 2.67 (*Schima superba*) to 4.13 mg C kg soil day^{-1} (*Cunninghamia lanceolata*) depending on the species (Fig. 3a). All species induced a positive RPE, corresponding to an acceleration of SOC decomposition compared to the unplanted control over the entire experimental period (Fig. 3a). There were significant differences in RPE among species with values ranging from 0.75 to 1.03 mg C kg soil day^{-1} during the entire experimental period (Fig. 3a). The magnitude of RPE was also significantly affected by sampling time and the interaction between sampling time and plant species (Fig. 3b). Over the 204 days of growth, compared to the unplanted treatment, the presence of plants stimulated the decomposition of SOC by 82% on average, with the lowest for *Triadica sebifera* (70%) and the highest for *Cunninghamia lanceolata* (96%) (Fig. 3b). However, there was no significant difference in the RPE between ECM and AM tree mycorrhizal type (Supporting information).

Plant biomass and root traits as potential drivers of the RPE

Three plant traits, including aspects of root morphology, architecture and physiology were significantly or marginally-significantly related to the RPE (Table 2, Fig. 4). This included positive relationships between the RPE and mean diameter of first-order roots (MRD_{1st}; $R^2 = 0.61$, $p = 0.02$) and root exudate-derived respiration ($R^2 = 0.42$, $p = 0.08$). The RPE was also negatively related to RBD ($R^2 = 0.52$, $p = 0.04$). Interestingly, there was no relationship between plant growth rate and the PRE ($R^2 = 0.00$, $p = 0.92$) and the type of mycorrhizal association did not influence the RPE ($p = 0.86$).

Considering all plant traits together, the combination of MRD_{1st} and RBD appeared most relevant to explain the RPE ($R^2 = 0.72$, $p = 0.043$; Table 3). Further, a path analysis of causal relationships between root traits and the RPE provided a more detailed picture of potential relationships occurring between root traits and the RPE ($R^2 = 0.79$, $p = 0.947$, CFI = 1.00, RMSEA = 0.00). This analysis revealed that part of the relationship between MRD_{1st} and the RPE was indirect (Fig. 1b). The main driver of RPE was MRD_{1st} with both a substantial direct effect and indirect effects via its influence on root exudate-derived respiration, specific root length of first-order roots (SRL_{1st}) and RBD.

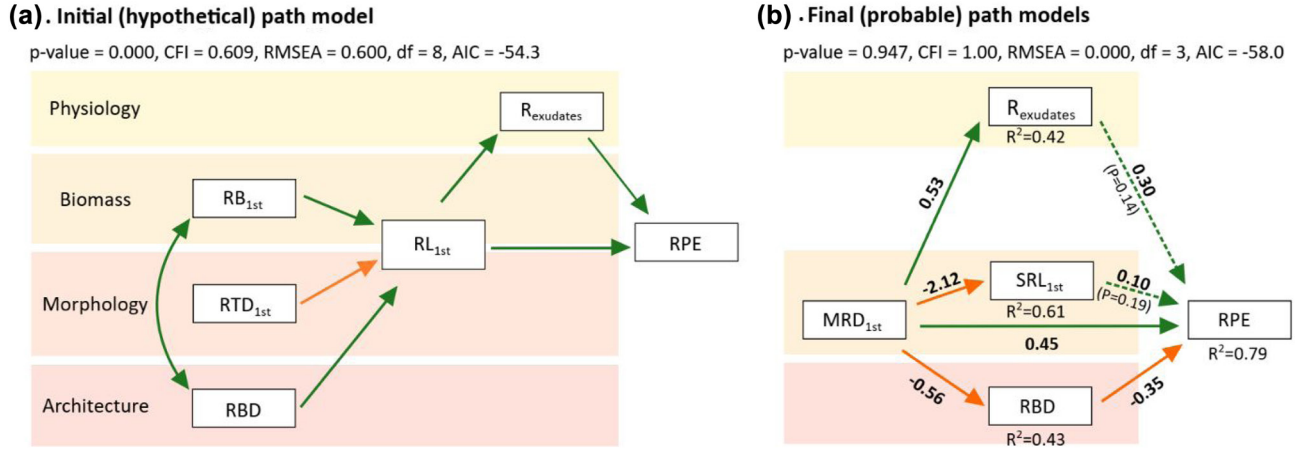

Figure 1. (a) Initial and (b) final path models of the influence of plant belowground traits on the rhizosphere priming effect (RPE). The initial path model shows a significant misfit, whereas the final most probable path model shows no significant misfit between the empirical data and the causal structure specified by the models. Boxes are measured variables and arrows represent hypothesized causal links. Regarding the final path model (b), values on the lines are the standardized path coefficients between the causal variable and the caused variable. All path coefficients are significantly different from zero (solid lines), or marginally significant where p-values are displayed next to path coefficients (dashed lines). The R^2 represent the percentage of variance explained by the causal variables. Green and orange lines indicate positive and negative relationships, respectively. Thickness of the lines is proportional to the strength of path coefficients. For all traits, we used the average value of five individuals per species. $R_{exudates}$ is root exudate-derived respiration; SRL_{1st} is specific root length of the first-order roots; MRD_{1st} is mean root diameter of the first-order roots; RBD is root branching density; RB_{1st} is biomass of the first-order roots; RTD_{1st} is root tissue density of the first-order roots; RL_{1st} is root length of the first-order roots.

Discussion

The main objective of this study was to investigate how plant traits, with a particular focus on root traits, affect SOC decomposition through RPEs. In contrast with most previous studies, either focusing on grass species (Shahzad et al. 2015, Wang et al. 2016, Henneron et al. 2019) or on few tree seedlings (Cheng et al. 2014, Yin et al. 2018, 2020), we used here a relatively larger pool of eight tree species featuring contrasting functional traits. Our findings provide preliminary evidence that the magnitude of RPEs is influenced by several root traits related to aspects of root morphology, architecture and physiology and illustrate how measuring comprehensive sets of plant traits is necessary to adequately capture the effects on plants on soil functioning.

Rhizosphere priming on soil C decomposition

The average stimulation of SOC decomposition by our eight tree species, by 82% on average, is higher than that the 59% observed by Huo et al. (2017) in a meta-analysis of 31 studies on RPE, but similar to the 77% recorded for tree species only. Overall, our and previous results on RPE suggest that RPE may be most positive in the presence of tree roots as compared to roots of other plant types. However, it remains unclear how this related to differences in the quantity and quality of root-derived organic matter between tree and herbaceous species (Wang et al. 2016, Girkin et al. 2018). Interestingly, this difference may not be simply linked to a difference in mycorrhizal association – trees typically showing either or both of ECM and AM associations as compared to herbaceous species typically associating with AM fungi only. Indeed, while previous studies have suggested a potential role of mycorrhiza and the type of mycorrhizal association in RPE processes (Paterson et al. 2016, Frey 2019, Yin et al. 2021), here we did not observe a difference in RPE between ECM and AM tree mycorrhizal type. This is despite ECM roots tend to provide more C to the rhizosphere and have a greater capacity to produce extracellular enzymes than AM roots, suggesting that ECM roots should typically induce higher RPE than AM roots (Brzostek et al. 2015, Kumar et al. 2020). Overall, the reasons behind a potentially stronger effect of tree species on RPE remain uncertain and call for renewed research in this direction.

In contrast to the relatively consistent RPE effect observed here among our eight tree species (from 70 to 96%), other reports of RPE found in the literature present highly variable values, ranging from a 79% decrease (Thurgood et al. 2014) to an increase of over 500% in SOC decomposition (Shahzad et al. 2015). Even when considering studies focusing on tree species only the observed variation in RPE was considerably higher than this observed here. For instance, among three tree species, Yin et al. (2018) and Bengtson et al. (2012) observed RPE ranging from 26 to 146%, and from 152 and 244%, respectively. It should be noted that in the present study the lack of light during the measure of total respiration (48 h) from plant–soil system, motivated by the need to stop plant absorption of soil respired-CO_2, also likely led to an underestimation of the

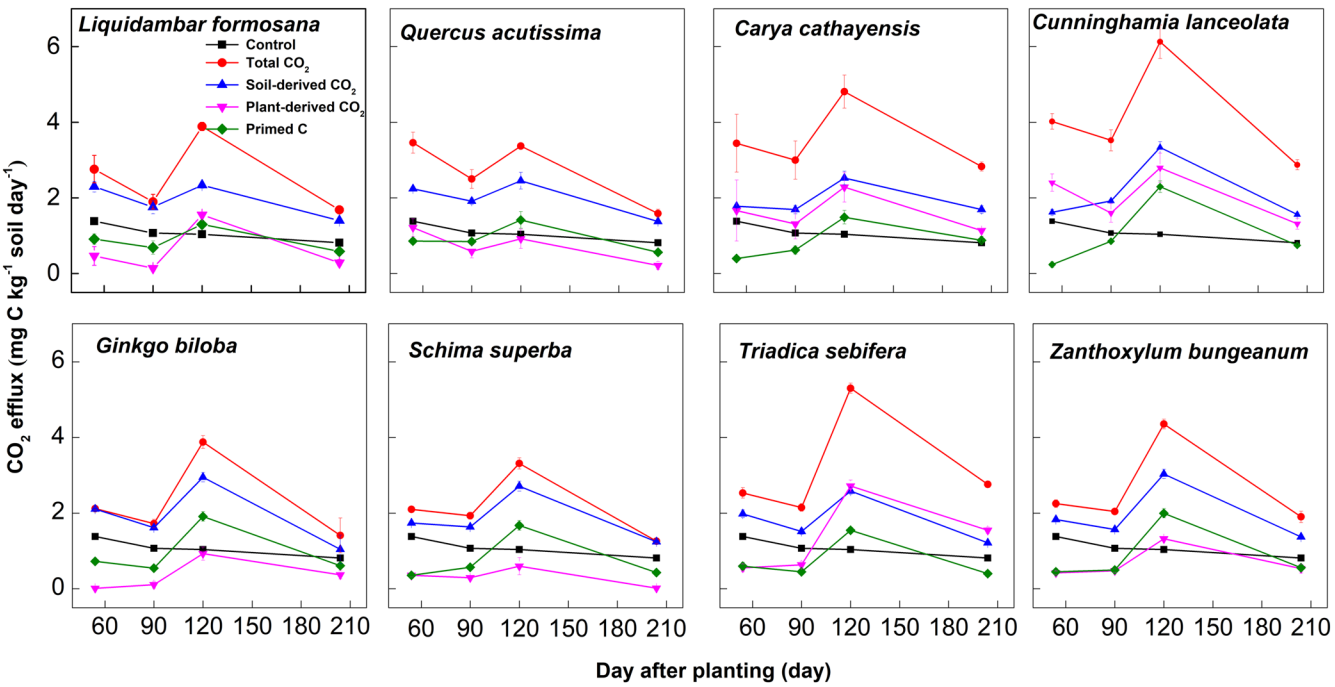

Figure 2. Change in CO_2 efflux from microcosms induced by eight plant species along 204 days of growth. CO_2 efflux from an unplanted control (black lines with squares), as well as the total CO_2 (red lines with circles), soil-derived CO_2 (blue lines with triangles), plant-derived CO_2 (pink lines with inverted triangles) and priming of SOC (green lines with diamonds) induced by plants are represented for each species. Values are mean ± SE.

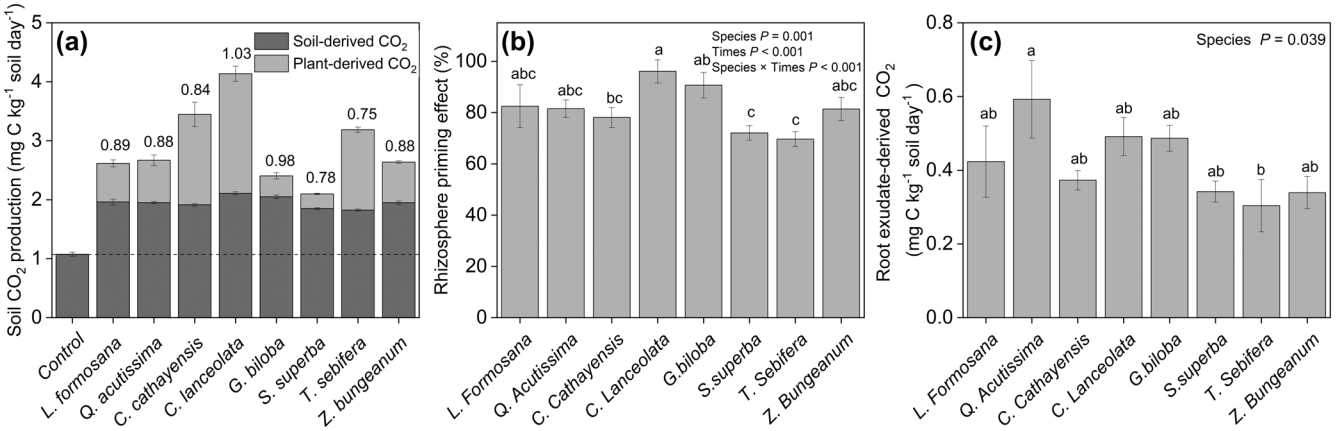

Figure 3. Mean daily (a) soil organic matter (SOM-)derived CO_2, plant-derived CO_2 and CO_2 associated to the primed C (data above the bars), (b) rhizosphere priming effect, and (c) root exudate-derived CO_2 along the 204 days of growth in planted treatments. Sub-legend shows ANOVA p values. Values are mean ± SE; n = 5 for all treatments except for n = 4 for *Quercus acutissima*, *Carya cathayensis* and *Schima superba* treatments.

true RPE. During prolonged darkness or shading, the amount of photosynthates progressively decreases, which negatively affects root exudation, and therefore potentially reduces the magnitude of the RPE as compared to natural light conditions (Kuzyakov and Cheng 2004, Tang et al. 2019). For example, the magnitude of the RPE under 40% of ambient full light was only 71% that observed under full light (Chang et al. 2020). Besides, there is some evidence that the effect of light intensity may be further modulated by plant species identity and traits (Li et al. 2020). Additionally, our method also likely slightly underestimates the true RPE due to its inability to detect primed C of very old organic matter dating from before the 23 years of C_4 plant cultivation.

We found that the magnitude of RPE was significantly affected by the sampling time, in line with previous studies (Zhu et al. 2011, Wang et al. 2016, Wang et al. 2020). However, the lack of destructive sampling at different stages does not allow to explore how the dynamics of plant above- and belowground traits influence the magnitude of RPE. Along the duration of the experiment, the similar trend observed across all species of a peak of RPE after 120 days of plant growth suggests that the initial phase of plant growth was key in stimulating the RPE. This points out to a potential coupling between plant root phenology and the RPE. The following decrease observed across all species could be further interpreted as a lower impact of plants on the RPE once the pot has been fully colonized by roots. Growing root tips are indeed known to be very active in increasing root C inputs to soil (Paterson et al. 2006) and may therefore contribute disproportionately to RPE as the plant initially expands dramatically its root system to explore the surrounding soil volume. While environmental conditions could have played a role in these temporal patterns by influencing plant relative growth rate, we did not observe an influence of relative growth rate on the RPE. Overall, it remains unclear whether that consistent trend of an early peak of RPE could represent an artefact of experimental manipulation or a realistic consequence of an actively growing root system and requires further testing.

Rhizosphere priming often has greater impact on the decomposition of soil C than the general priming effect induced by additions of substrates (e.g. glucose, plant residues) other than root exudates. Two meta-analyses have reported that on average, the general priming effect may speed the decomposition of SOC by ca 14% (Luo et al. 2015) or 27% (Zhang et al. 2013). By contrast, the average value of 82% RPE from our current study are 3–6 times higher than their values. In an experiment quantifying the priming effect of leaf litter additions from 15 tree species including some of the same species as studied here, Chao et al. (2019) also observed an average priming effect of 11%, that is, a value 86% lower than the RPE recorded here. This difference may have several causes. First, the rhizosphere is the hot spot of root exudation, and the total amount of C entering the soil via root exudation is likely higher than C inputs from plant yearly litter productions in this study would be higher than this of litter input realistic studies of litter addition. In addition, root exudation of carbohydrates, which could be preferentially utilized by soil microbiota, may arguably be able to alleviate energy limitation in microbial activity more consistently along time and therefore to stimulate SOC decomposition (Finzi et al. 2015, Soong et al. 2020) more efficiently than discrete litter inputs to soils. Third, the presence of plants generally increases SOC decomposition due to the destruction of soil aggregates by living roots, as compared the sole release of exudates (Bengtson et al. 2012), which leads to lower physical protection of SOM and a greater release of mineral-protected C for microbial decomposition (He et al. 2020, Wang et al. 2020). Fourth, plant roots release oxalic acid and other organic acids, which have strong metal chelators abilities and can further disrupt mineral-organic associations (Keiluweit et al. 2015). Finally, the uptake of nutrients by roots could induce microbial growth to be limited by

Table 2. Regression coefficient (β), strength (R^2) and significance (p) of univariate linear regressions between descriptors of plant biomass and traits and the rhizosphere priming effect. (+/−) represents the sign of the relationship. Variables explaining the highest variation in RPE ($R^2 > 0.4$) and subsequently retained for multivariate regression analyses are highlighted in bold.

	β-value	R^2 (+/−)	p-value
Plant biomass			
Plant total biomass	0.006	0.062 (+)	0.553
Aboveground biomass	0.006	0.022 (+)	0.726
Belowground biomass	0.020	0.135 (+)	0.370
Leaf biomass	0.023	0.091 (+)	0.467
First-order root biomass	−0.010	0.000 (−)	0.965
Absorptive root biomass	0.003	0.000 (+)	0.968
Morphological root traits			
Mean first-order root diameter	0.533	**0.607** (+)	**0.023**
Mean absorptive root diameter	0.036	0.022 (+)	0.726
First-order root tissue density	−0.071	0.027 (−)	0.699
Absorptive root tissue density	0.005	0.000 (+)	0.984
First-order specific root length	−0.001	0.213 (−)	0.250
Absorptive specific root length	−0.002	0.190 (−)	0.281
Architectural root traits			
Root branching density	−0.096	**0.521** (−)	**0.043**
Physiological root traits			
Root exudate-derived respiration	0.623	**0.424** (+)	**0.080**
Rhizosphere soil respiration	0.375	0.317 (+)	0.146
Plant-derived CO_2	0.041	0.072 (+)	0.562
Composite (biomass-related) root traits			
First-order root length	−0.007	0.003 (−)	0.895
Absorptive root length	−0.007	0.003 (−)	0.905
First-order root length density	−0.007	0.003 (−)	0.895
Absorptive root length density	−0.006	0.002 (−)	0.917

nutrients (rather than C or energy provided by rhizodeposition), thereby leading microbes to decompose nutrient-rich SOM to acquire nutrients: the mining hypothesis (Craine et al. 2007). Overall, the consistently high RPE observed here across eight contrasting species of trees and the much higher effect as compared to soil priming effects due to litter input suggest that several of these processes are likely to happen concomitantly to determine the major role of the RPE in global C cycling processes.

Root traits control rhizosphere priming

Our results only poorly support our first hypothesis that the RPE positively covaries with high biomass, fine root length, root branching density and root exudation rate. Taken individually, only high root exudate-derived respiration, considered as representative for root exudation rate, induced a marginally higher RPE. Since the role of root exudates in driving the RPE is well documented (Wang et al. 2016, Henneron et al. 2019), this result suggests that our estimate of CO_2 released during an incubation of rhizospheric soil may not have accurately reflected the effects of root exudates on the RPE (but see Henneron et al. 2019). One reason for this is that our rhizospheric soil, sampled at the end of the experiment, had already experienced substantial priming during the 204 days of plant growth and may not well represent what has happened during the experimental period. Second, the rhizospheric soil is incubated in Mason jar where no live root exudation is taking place, as compared to the pots with live plants. Therefore, the root exudate-derived respiration appears unable to capture the short-term effect of root exudation.

Whereas most of our hypothesized root trait effects were non-significant, we observed a negative relationship between the RPE and root branching density. We expected that higher

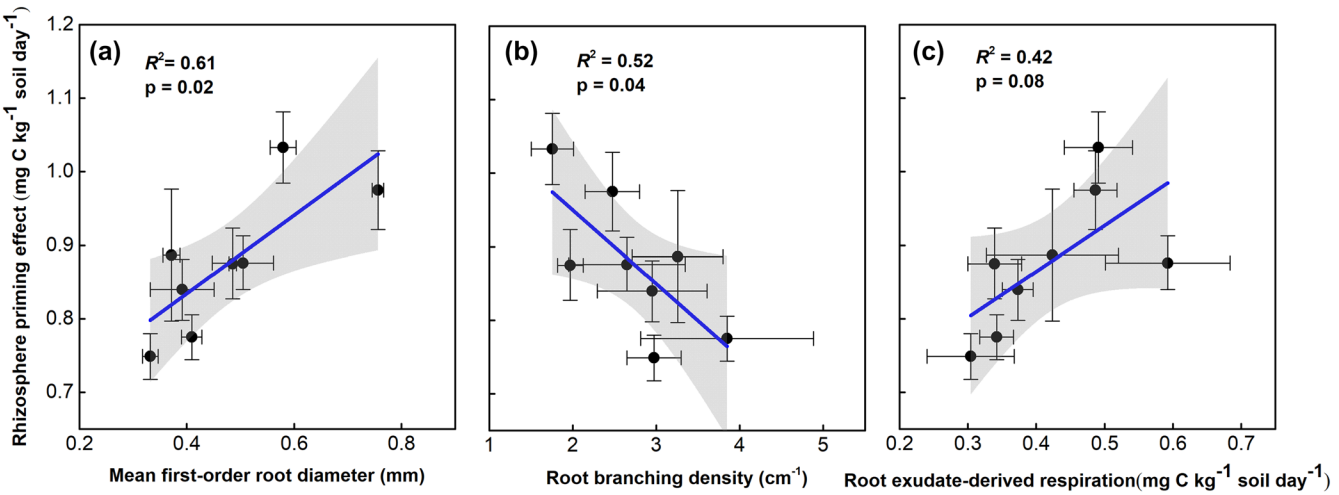

Figure 4. Relationships between the rhizosphere priming effect and (a) mean first-order root diameter, (b) root branching density, and (c) root exudate-derived respiration. The means of each species are plotted and error bars represent ± SE; n = 5 for all treatments except for n = 4 for *Quercus acutissima, Carya cathayensis and Schima superba* treatments. Strength (R^2) and significance (p) of linear regressions are displayed when (marginally-) significant. The filled areas indicate the 95% confidence interval.

Table 3. Best models (lowest AICc) of stepwise forward multiple regressions between plant functional traits and the RPE. The strength (R^2) and significance (p) of models are displayed.

Model	AICc	Plant functional traits	R^2	p-value
1	−44.90	mean first-order root diameter; root branching density	0.716	0.043
2	−44.47	mean first-order root diameter; root branching density; root exudate-derived respiration	0.767	0.094
3	−44.31	mean first-order root diameter	0.607	0.023

root branching density would be mainly representative of higher root tip numbers and therefore higher potential exudation capacities, but lower branching density might also enable larger soil volume exploration and thus be most effective for enhancing root effects on a larger soil volume (Freschet et al. 2021b). Additionally, a high root branching density contributes to greater enmeshment of soil particles and increases soil aggregate stability, which may play a non-negligible role in limiting SOC mineralization rates (Poirier et al. 2018).

In support for our second hypothesis, the three root traits that influenced the RPE to some extent (i.e. mean diameter of first-order roots, root branching density and root exudate-derived respiration) represented three complementary aspects of root structure and function (morphology, architecture and physiology, respectively). Mean diameter of first-order roots represents the type of economics strategy of the root (e.g. the reliance on mycorrhizal association; Bergmann et al. 2020), whereas root branching density is a key driver of the strategy of root to explore versus exploit the soil volume, and root exudate-derived respiration relates to the activity of the roots per unit mass of root deployed. However, surprisingly, we found no relationship between any measure of plant biomass (e.g. specific organ biomass, total biomass) and the RPE. This is in contrast to a recent meta-analysis showing that plant total and aboveground biomass were correlated with the RPE (Huo et al. 2017). Indeed, plants that have higher biomass may generally allocate more labile C into soil surrounding the root and thereby intensify the mineralization of native soil C (Dijkstra et al. 2006). Nonetheless, different species differ in a broad range of root traits (Sun et al. 2021, Freschet et al. 2021a) and in their nutrient use efficiency (Henneron et al. 2020), so that estimates of plant biomass may not always reflect well plant nutrient requirements and acquisition, and consequently their influence on soil C priming. Moreover, plant biomass and growth rate were relatively similar in this study, reducing therefore the potential for these parameters to influence the RPE.

Among our eight tree species, the strongest predictor of the RPE was the mean diameter of first-order roots, possibly due to its central position in the network of root trait relationships. Particularly, higher mean diameter of first-order roots negatively influences root branching density (Eissenstat et al. 2015), as also observed here, and this has positive consequences for the RPE, as revealed by our path analysis. Among our tree species, higher mean diameter of first-order roots also positively influences root exudate-derived respiration, indicating a potentially higher production of exudates, also with positive consequences on the RPE. This positive relationship may have several causes. First, higher mean diameter of first-order roots implies higher proportion of cortex tissues in the root, and therefore higher metabolic activity of the root with potential link to exudation processes (Ding et al. 2020, Freschet et al. 2021b; Kong et al. 2019). Second, higher mean diameter of first-order roots may imply higher reliance of thicker roots on mycorrhizal colonization (Kong et al. 2014, Paterson et al. 2016, Bergmann et al. 2020), increased transfer of carbon to the fungal symbiont and increased mycorrhizal fungi exudation. Part of the increased root exudate-derived respiration observed in thicker roots may indeed be attributed to the action of mycorrhizal fungi fed by plant carbon allocation as mycorrhizal fungi have an important role in enzyme exudation and soil carbon priming (Bradford 2014, Yin et al. 2020). Mycorrhizal associations are indeed an efficient way to increase the volume of soil under influence of the root system and have particular abilities to degrade SOC (Frey 2019, Yin et al. 2021), which will require further attention in future studies exploring the drivers of root effect on the RPE.

Conclusion

We demonstrated here that several aspects of root structure and function (i.e. mean diameter of first-order roots, root branching density and root exudate-derived respiration) have complementary influences on the magnitude of the RPE. This work illustrates how measuring comprehensive sets of plant traits, including aspects of plant root morphology, architecture and physiology, is necessary to adequately capture the effects of plants on soil functioning. However, future assessment will need to further include one potentially important aspect of root effect on the RPE, that is, plant reliance on mycorrhizal colonization and mycorrhizal traits. Nonetheless, we identified a central role for the mean diameter of first-order roots, potentially via its influence on several other traits with likely more direct link to soil carbon priming. As first-order root diameter can be routinely measured, the role of this trait in plant RPE could offer a rough but useful first estimation of the RPE, if confirmed over wider range of species and environmental conditions.

Overall, our results confirm the potential use of root traits to predict the integrated response of soil C dynamics to changes in species composition under future climate change (Cheng et al. 2014, Finzi et al. 2015, Henneron et al. 2020). Although our use of a non-native soil (the C_4 soil) and small tree seedlings planted in pots likely misrepresent the

influence of mature trees grown in field ecosystems (Mokany and Ash 2008, Freschet et al. 2017, Yin et al. 2018), they yield promising results that should open the way to further experiments with larger number of species and more realistic field conditions.

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Author contributions

Lin Chao: Writing – original draft (lead); Writing – review and editing (equal). **Yanyan Liu:** Data curation (equal). **Weidong Zhang:** Project administration (equal). **Qingkui Wang:** Formal analysis (equal). **Xin Guan:** Investigation (equal). **Qingpeng Yang:** Investigation (equal). **Longchi Chen:** Formal analysis (equal). **Jianbing Zhang:** Investigation (equal). **Baoqing Hu:** Methodology (equal). **Silong Wang:** Project administration (equal). **Zhanfeng Liu:** Resources (equal). **Grégoire T. Freschet:** Visualization (equal); Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.sbcc2frbh (Chao et al. 2023).

Supporting information

The Supporting information associated with this article is available with the online version.

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