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New Phytologist Supporting Information

Notes S1

Article title: **Toward a coordinated understanding of tropical forest hydro-biogeochemical root functions for application to vegetation models**

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SI Figure 2: Data Sources and Processing

Data source and processing details for Figure 2:

Data are from an updated version of Global Root Trait (GRooT) database, which combines root trait observations from the Fine Root Ecology Database (FRED, ver. 3, (Iversen *et al.*, 2017; Iversen *et al*., 2021), the Plant Trait Database (TRY ver. 5, Kattge *et al.*, 2011; Kattge *et al.*, 2020). Data were mobilized by the Tropical Root Trait Initiative (TropiRoot; https://tropiroottrait.github.io/TropiRootTrait/), and additional datasets (Guerrero-Ramirez *et al.*, 2021); https://groot-database.github.io/GRooT/) were joined with the World Checklist of Vascular Plants (WCVP, Govaerts *et al.*, 2021, POWO 2023) to obtain information about the species' climate description, described in the WCVP as "habitat type of the taxon, derived from published habitat information". The WCVP's climate description categories include desert and dry shrubland, desert or dry shrubland, montane tropical, seasonally dry tropical, subalpine or subarctic, subtropical, subtropical and tropical, temperate, temperate and tropical and wet tropical. In cases where species occur in multiple climate zones, the climate zone designed represents where most of the species occur naturally. Wet tropical includes species located in rainforests (lowland and highlands), seasonally dry tropical includes species located in pronounced wet and dry seasons, dry tropics where rainfall is less than 50 centimeters per year, and montane tropical represent species located in high-elevation mountains (above ~ 4000 m a.s.l) (personal communication Rafaël Govaerts). Species categorized as subtropical and tropical and temperate and tropical were not represented in GRooT.

SI Table 2: Root trait survey in leading vegetation models: Methods for constructing Table 2.

Methods for model survey: We tallied traits rather than dynamic state or rate quantities, except for nutrient uptake baseline rate rates (yellow and orange columns in Table 2), to give an idea of how many unique root variables would need empirical (e.g., field) quantification for executing the model in a tropical forest. In the tally of above and below-ground traits for each model, whole-plant traits and ecosystem-level parameters were not tallied and correlated or closely related (e.g., parameters in photosynthesis calculations) plant traits were counted once. We found that most root traits with a role in support, storage, distribution, longevity, or stoichiometry are typically not dynamic in response to resource (water or nutrient) changes. Root traits which are dynamic in some models include leaf:fine-root area ratio, which some models adjust in response to water availability, and to a lesser extent nutrients (note letters m or n in the table), maximum rooting depth, which can change with tree size in a couple models (an indirect response to increased resource availability via tree growth), and the remaining water uptake- and nutrient uptake-related traits, which generally concern functional root traits that respond to resource changes and to plant requirements.

We present a sample of current vegetation models based on a broad cross-section of land models at the forefront of representing how plant traits, climate, and community composition coevolve in forests within an Earth system model framework, while comparing to a sample of emerging trait-flexible approaches. We additionally include (as outgroups) two extremes on a vegetation parameter richness spectrum - an ESM without dynamic vegetation (E3SM-ELM) and two parameter-rich forest individual-based gap models (IBM) that have been applied to tropical forests (FORMIND, TROLL). ESM-capable refers to models that can be coupled to a global earth system model to simulate vegetation-climate feedbacks. Models are grouped into three main approaches (PFT, cohort, or IBM) for assigning plant traits. Within these groups trait values are held constant over the lifespan of an individual; we are not aware of any approaches that explicitly allow for trait plasticity over ontogeny. “PFT” is plant functional type represented by an average individual with characteristic trait values; “Cohort” is an average individual within a tree size class, functional type, and in most cases, a particular canopy layer or light environment; “IBM” is individual based

model modeling every tree within a patch. A subset of IBMs use spatially explicit locations of trees, termed gap models. NSC = non-structural carbohydrates in root exudates. P50 indicates 50% loss of hydraulic conductivity due to embolism.

Specific file or table source of trait information within each paper cited (identified by model # here and on Table 2):

#	**File and table within citation for source of information**
1	Table S1 in "ELM-CNP_Yang.etal.2016_Supp.doc", Notes S1 (search for 'root') in "ELM CNP_Thornton.etal.2007_S1.txt"
2	Table S6, S15 in "LPJmL4.0_Schaphoff.etal.2018_Supp.pdf"
3	default traits: Table S6, S15 in "LPJmL4.0_Schaphoff.etal.2018_Supp.pdf"; variable rooting depth traits: Appendices A1.3, A1.5 in "LPJmL4.0_Sakschewski.etal.2021.pdf"
4	Table 5 in "ED2.2_Longo.etal.2019_ED2.2-description_GMD_Supp.pdf"; Notes S1 in "ED2_Xu.etal.2016_Supp.pdf"
5	aboveground traits see Table D1-D4 Massoud et al. (2019); belowground traits search for 'root' here: https://github.com/NGEET/fates/blob/main/parameter_files/fates_params_default.cdl and https://fates-users-guide.readthedocs.io/projects/tech-doc/en/stable/fates_tech_note.html; also see "All FATES root traits (Joe's notes)"
6	Table S1 in "LM3PPA-TV_Martinez-Cano.2021_Supp.pdf"
7	Appendix C Tables C1 and C2, see also Appendix A2 & B4 in "LM3_Weng.etal.2015.pdf" (20 aboveground) /(10 belowground); Appendices A2, A2.1-A2.4, A4, A7, Tables D1 and D2 in "LM4_Kou-Giesbrecht.etal.2021.pdf" (1 new aboveground)/(21 new belowground)
8	Appendices B and C in Smith et al. (2014)
9	default root and aboveground traits: Appendices B and C in Smith et al. (2014); new root traits: Notes S2.1 and Table S1 in "LPJ-GUESS-NTD_DantasDePaula.etal.2021_supp.pdf"
10	Tables A1, A2 in "CABLE-POP_Haverd.etal.2018.pdf" + root traits that all LSMs have (water stress factor, root distribution) + CASA CNP root traits (same as ELM-CNP)
11	According to Thonicke et al. 2020 Section 2.1 "Each PFT is based on an earlier implementation of these PFTs in LPJmL-4 (Schaphoff, von Bloh, et al., 2018)". Therefore, use Table S6, S15 in "LPJmL_Schaphoff.etal.2018_Supp.pdf" + 1 additional trait Narea

Scheiter et al. 2013, Langan et al. 2017. The supplement of Langan et al. (2017) contains a full model description, which is the only published full description of aDGVM2.

Rius et al. 2023 Tables SM3, SM5

Appendices B through J in “FORMIND_Fischer.etal.2016_MARKEDsupplement.pdf”

Chave 1999, Maréchaux & Chave 2017

SI Boxes Methodological and Statistical Details and additional Results

SI Box 1. Details of Methods and Results for Fine-Root Morphology and Variance Partitioning

A key need for representing fine-root function in ecosystem models is to disentangle the effects of phylogeny versus local site conditions on commonly measured traits. These new data from Panama and Puerto Rico support recent literature showing low phylogenetic control over root traits in tropical forests, where root morphology might respond more to resource conditions.

Root Collection and Morphology

We collected two sets of data in Panama and Puerto Rico with the objective of assessing the sources of fine-root trait variation. We collected fine-root trait data within individuals for the first three or four root orders separately, and then assessed variance partitioning among root segments within individuals, among individuals within the same species, and among species. The Panama study was focused on intra- and inter-individual variation, separating up to the 4th order roots and combining 1st and 2nd orders (both considered absorptive), for three root segments per individual tree for 10 individuals each per two canopy species (*Trichilia tuberculata* and *Poulsenia armata*). These two species are relatively abundant on Barro Colorado Island, Panama. The Puerto Rico study was more focused on intra- and inter-specific variation. Fine roots were collected from six locally abundant species in the Luquillo Experimental Forest (*Dacryodes excelsa, Manilkara bidentata, Prestoea montana,* and *Ocotea leucoxylon* with three individuals per species, and *Drypetes glauca* and *Sloanea berteriana* with two individuals per species), separating roots into 1st, 2nd, and 3rd orders.

On BCI in Panama, we selected two relatively common tree species *(Trichilia tuberculata* and *Poulsenia armata)* as the focal tree species for this study. They have positive and negative phosphorus affinities respectively (Turner *et al.*, 2018) and are abundant species at the 50-ha plot in Barro Colorado Island (BCI) – Panama (abundances for the 2005 census data contributed by Dr. Joseph Wright). Beginning on 18 of July 2019, we identified ten mature individuals per species in BCI. We measured root morphological traits in all ten individuals per species on three root systems per individual and nutrient uptake in three individuals per species as described below.

We measured root morphology by excavating root systems from 10 individuals per species (*Trichilia tuberculata* and *Poulsenia armata*) and scanning three root systems per individual to assess variance within individuals, within species, and between species. We selected 10 individuals per species and three root systems per individual to measure root morphological traits. Roots from seven individuals per species were selected just to measure root morphological traits and the other three individuals were selected from the nutrient uptake experiment (described in the next section). For each individual just selected to measure root morphology, we gently excavated one coarse root per individual from the stem to 1st, 2nd, 3rd, 4th and 5th order and depths of ~ 20 cm. We cut the root at its 5th order, gently placed in a Ziploc and stored into the fridge for no more than a week. For each 5th order root, we selected three 4th order roots at the top, middle and end of the 5th order root to understand variability of traits within individual. These roots were separated in orders (1st, 2nd, 3rd and 4th order) and cleaned through a 0.25 mm mesh size to remove all the soil attached to the roots. Painting brushes were used to gently clean the roots when soil particles were still attached to them.

All root orders were scanned for morphology analysis and then dried at 60 ^{o}C until constant weight to determine dry root mass (mg). Roots were scanned using the 9800XL plus - Microtek - TMA 1600II scanner at 1200 dpi. All images (n=180) were analyzed using WinRHIZO (WinRHIZO Regular, Regent Instruments, Canada) to determine specific root length [root length per dry mass (SRL - cm mg^{-1})], specific root area [root superficial area per dry mass (SRA- cm^2 mg^{-1})], root tissue density [root dry mass per volume (RTD - mg cm^{-3})] and mean root diameter (Metcalfe *et al.*, 2008). For Panama, the total number of root systems was 180 (2 species x 10 individuals × 3 root segments × 3 root orders (Order 1-2 merged, Order 3 and Order 4).

In Puerto Rico, we collected at least two root samples from each tree by tracing roots until fine roots were found (following Yaffar et al. 2021). We separated the first three root orders and scanned them separately using WinRHIZO (version 12; Regent Instruments Inc., Quebec City, Canada) to obtain total root length (cm) and average diameter (mm). Root traits measured were specific root length, root diameter and root tissue density for the first three root orders of two to three individuals nested within six common tree species. *Dacryodes excelsa, Manilkara bidentata, Prestoea montana* and *Ocotea leucoxylon* species had three individuals per species, while *Drypetes glauca* and *Sloanea berteriana* had two individuals per species. Total number of root systems was 51 root systems (6 species × 2/3 individuals × 3 root orders).

Statistical methods

We performed a partition variation analysis to analyze the percentage of variance explained (%) by each level of root trait variation (species, individuals within species, root segments within individuals within species and residuals). These analyses were performed just on the traits from absorptive root orders that are usually considered to be related to soil resources uptake. Variance partitioning analysis of fine-root morphological traits was completed for two tropical forests: A) Panama (2 species × 10 individuals × 3 root segments × 3 root orders = 180 root segments); B) Puerto Rico (6 species × 2 or 3 individuals × 3 root orders = 51 root segments). The percent of variance explained by species (blue), individual (orange), and root segment within individual (yellow, Panama only) are shown, as well as the residual unexplained variance (gray). Individuals were nested within species, and root segments were nested within individuals.

Models used were mixed models (lme) from lme4 R package with the following code: trait per order model ~ lme (trait per order ~ 1, random = ~1 |Species/Individual, data = df, na.action = na.omit).

To ensure robustness and reliability of our variance partition analyses, outliers were identified using the Interquartile Range (IQR) method. This involved defining a 'reasonable' range of values based on the first and third quartiles (Q1 and Q3) of the data. Values that fell 1.5 times the IQR above Q3 or below Q1 were classified as outliers and excluded.

The variance partition analysis for Panama did not change too much after removing outliers for root diameter, SRA and SRL after removing outliers. On the other hand, RTD did have some changes, increasing the variance at species or individual tree scale and decreasing variance at the root segment scale. The Puerto Rico data had some more changes, especially with SRL and RTD.

Results

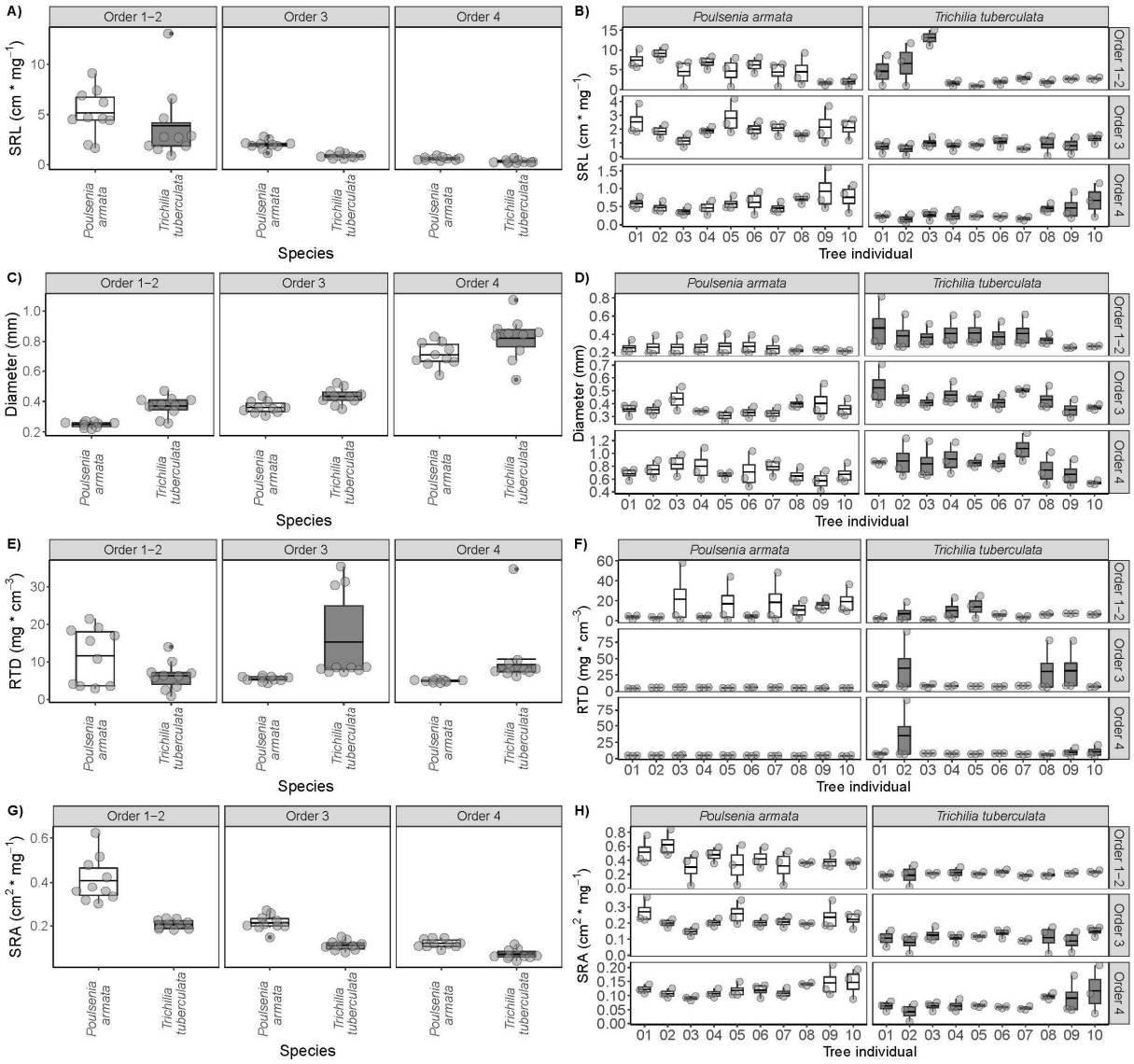
For Panama data, the largest source of variation among fine-root segments was within individuals for diameter and SRL, and lower variation among individuals or between the two species. For some traits, variation within individuals was general across all 10 individuals per species (e.g., SRL), whereas for other traits large variation within a few individuals drove the trend (e.g., RTD, see Box 1 Fig. SI1). Puerto Rico data exhibited substantial interspecific variation among the six species sampled for fine-root diameter and SRL, but not for RTD, and much lower

intra-specific variation than was apparent in the Panama data, but this data set lacked replication within individuals. This interspecific variation appeared driven primarily by one species in the Puerto Rico group (*D. excelsa*, see Box 1 Fig. SI2), and was strongest for fine-root diameter.

These data suggest that there can be large variation in fine-root morphology within individual trees and among individuals within species, pointing toward flexible responses to the soil environment at small scales. These data also indicate that some species in tropical forests likely have unique morphologies, which should be related to root function in future research for use in the development of tropical forest PFTs or trait groupings. Finally, these data point toward the importance of sampling across scales and combining morphology with direct functional measurements (see Box 3).

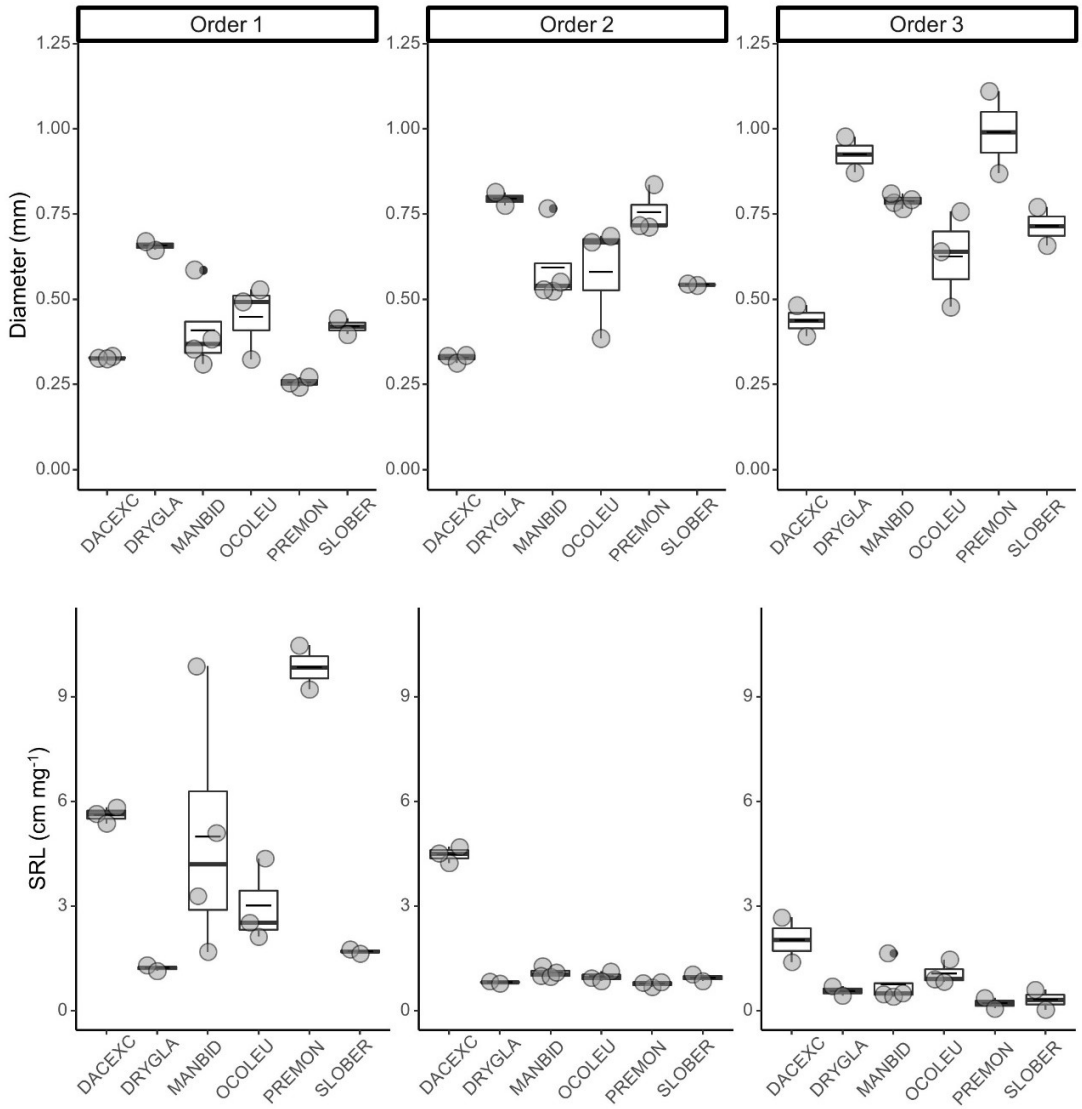
Box 1 Figure SI1: Comparison of fine-root diameter, SRL, SRA and RTD by species in Panama

Comparisons of root morphological traits for two species up to 4th root order are shown. Left panels show average comparisons by species (n = 10 individuals per species), right panels show comparisons by individual per species (n = 3 root segments per individual). Note that some traits had broad variation among individuals (e.g. SRL panel B, diameter panel D), whereas for other traits just a few individuals had large variation (e.g. RTD panel F). Traits shown include diameter, specific root length (SRL), specific root area (SRA) and root tissue density (RTD).



Box 1 Figure SI2: Comparison of fine-root diameter and SRL by species in Puerto Rico

Species include: *Dacryodes excels* (DACEXC)), *Manilkara bidentate* (MANBID), *Prestoea montana* (PREMON) and *Ocotea leucoxylon* (OCOLEU) with three individuals per species, and *Drypetes glauca* (DRYGLA) and *Sloanea berteriana* (SLOBER) with two individuals per species). Both diameter and SRL differences among species are driven primarily by DACEXC, which had particularly small diameter across root orders, and high SRL for 2nd and 3rd root orders.



SI Box 2: Details of Methods and Results for Fine-Root Nutrient Uptake

Methods

Panama Methods

We measured potential fine-root uptake rates of relatively abundant lowland forest canopy tree species in Gigante Peninsula in the Barro Colorado Nature Monument (BCNM), Panama. We included 33 individual trees of *Protium picramnioides* (previously *Tetragastris panamensis,* Daly & Fine, 2018)*,* and three individual trees each for *Trichilia tuberculata* and *Poulsenia armata* (one root system per tree). Across species, we used a nutrient-dilution method in the field on live fine-root tips still attached to parent trees, excavating, and placing intact, cleaned roots in vials of known nutrient solutions with bubbler aeration to maintain water oxygen. After field incubations of 4 to 24 hours we analyzed solutions to assess removal of nutrients by roots. A small subset of these roots was also measured for morphological characteristics.

For the Gigante Peninsula, Panama study, *P. pricramnioides* is a shade tolerant, mid and upper canopy species with heights ranging from 10-35 m, and root biomass accounts for roughly 25% of total plant C storage, as indicated in individuals from a Costa Rican lowland rainforest (Becker & Castillo, 1990; Uriarte *et al.*, 2004; Calvo-Alvarado *et al.*, 2008). We excavated live root systems from thirty-three individual trees and kept root intact and attached to the tree. Nutrient uptake rates for *P. picramnioides* were measured on 33 individual trees. Nutrient uptake was expressed in μmol of nutrient per g of dry root weight per hour. Each individual had up to 12 roots for each individual tree (n =14, 26, 22 and 26 for K^+, NH_4^+, NO_3^- and PO_4^{3-} respectively). We used twelve root tips from three separate root branches per tree. Each root tip was immersed for 3-4 hours in aerated vials of known nutrient concentration, after which depletion of N, P and K were measured, and average nutrient uptake rates were calculated for each tree on a root dry-weight (g) basis, as described below. Tree DBH was recorded as a covariate, and ranged from 84 – 564 cm across the 33 individuals.

On BCI, we selected two species (*Trichilia tuberculata* and *Poulsenia armata*) and three individuals per species to measure root nutrient and water uptake in the field (nutrient or water uptake per root mass per time) on July 2019. We placed clean roots to nutrient solutions in a 1-litre bottle with aeration changing solutions every day up to 5 days recording the time roots were inside solutions. We analyzed a subset of samples for ammonium (NH_4^+), nitrate (NO_3^-) and

phosphorus (PO_4^{3-}) in control solutions (without roots) and in solutions with roots and calculated nutrient uptake as μmol nutrient uptake per root mass per minute. We also recorded bottle weights before and after field collection to calculate water uptake. In the end of the experiment, we scanned and weighed all roots in solutions by root order.

We selected three individuals per species and three root systems per individual for nutrient uptake measurements. All 18 root systems were gently excavated to 1st order roots. We kept roots in-tact and attached to the main stem, cleaned them with distilled water until totally clean and then exposed the 5th order root with all subsequent orders (1st to 4th) to three different nutrient solutions in a pre-filled 1-litre Nalgene bottle with aeration apparatus using the nutrient dilution method in Lucash *et al.* (2007) and McFarlane and Yanai (2006) with modifications. Nutrient solutions consisting of distilled water, ammonium (NH_4^+), nitrate (NO_3^-), potassium (K^+), and phosphorus (PO_4^{3-}) were mixed together at three different concentrations (DI water, 5x and 10x) in order to assess nutrient uptake with variation in nutrient concentration. We covered the top of the Nalgene with parafilm to avoid any contamination and recorded the "start time". After approximately 24 hours, the solutions were replaced with a new Nalgene of fresh nutrient solution, or DI water. We repeated this procedure for additional time points up to 5 days and recorded all the start and end time that roots were placed and removed from solutions. We weighed all Nalgene vials before and after adding solutions and also after they were retrieved from the field to measure changes in solution mass and calculate water uptake. All solution samples were filtered in the lab through pre-rinsed Whatman Grade 1 filters (>11 μm particle retention) immediately after they were collected in the field then were frozen for posterior analysis. We also included a control vial (without roots) at each concentration. For this study, just a subset of the 10x solution concentration was analyzed for ammonium (NH_4^+), nitrate (NO_3^-) and phosphorus (PO_4^{3-}). The subset consisted of 10x treatment solutions with roots collected at two timepoints (day two and day three) from a total of 12 individuals from the two species and also two nutrient-control samples (10x solution without roots). There was no significant difference of nutrient uptake between timepoints (ANOVA: $F_{1,15}=0.34$; $P=0.57$), so they were pooled for statistical analysis. At the end of the final field incubation, we removed the roots from the vials by clipping at the point of entry to the solution, removed the root, and stored in a Ziploc to return to the lab for cleaning and scanning. We kept root samples in the refrigerator until they could be processed for no more than 1 week. In the lab,

we carefully rinsed all the roots to remove nutrient solution, separated them by orders (1-2, 3, 4 and 5).

For this experiment we used root biomass and root morphology as described in the SI for Box 1, using average morphology across orders 1-4. For morphological scans, orders 1 and 2 were scanned together because of the challenges to separate them, while higher orders were scanned alone. All the roots were scanned, dried and weighed using the same method described in SI for Box 1. All the roots from the three different treatments (DI, 5x and 10x) were considered for root morphological traits data in this study, and biomass was summed for each vial.

Table 1: Target simulated soil solution concentrations for *Trichilia tuberculata* and *Poulsenia armata* at Barro Colorado Island

Ion	**Fertilizer**	**Target solution Ion Concentration (umol L^{-1})**	
		5x	**10x**
NH_4^+	NH_4Cl	610	1220
NO_3^-	$NaNO_3$	85	170
PO_4^{3-}	$Ca_3(PO_4)_2$	40	80
Ca_2^+	$Ca_3(PO_4)_2$	60	120
K^+	K_2SO_4	160	320
Na^+	$NaNO_3$	85	170

Table 2: Target simulated soil solution concentrations for *Protium picramnioides* at Gigante

Ion	**Fertilizer**	**Target solution Ion Concentration (µmol L^{-1})**		
		1x	**5x**	**10x**
NH_4^+	NH_4Cl	25	125	250
NO_3^-	$NaNO_3$	15	75	150
PO_4^{3-}	K_2HPO_4	10	50	100
K^+	K_2HPO_4	20	100	200

Nutrient Uptake Calculations

Net uptake rates were calculated as the difference in nutrient mass content (µmol nutrient) between the nutrient-control (5x or 10x solution without roots) vial, and the experimental vial (5x or 10x solution with roots), divided by the dry root weight in grams (g dw) and experiment duration in hours (hr). The final units for net nutrient uptake are in µmol of nutrient uptake per gram dry weight of roots per hour (mg nutrient g^{-1}dw h^{-1}). For BCI nutrient uptake data we calculated nutrient uptake by root biomass using orders 1 to 3, for Gigante this was calculated just for order 1 (root tips). We considered the negative values as inactive roots, so we used just the positive values in our analysis to represent nutrient uptake.

Net nutrient uptake: µmol nutrient uptake per root mass per minute

$$\frac{(X\ Nutrient\ mass\ Blank - WaterBlank) - (X\ Nutrient\ mass\ RootVial - WaterBlank)}{RootWeight \times ExperimentTime}$$

Panama Statistical Methods

We first used analysis of variance (ANOVA) models with the two species from BCI to assess species (n = 2), time points (n = 4) and solution concentration (n = 3) effects on nutrient uptake or water uptake. The cleanest data (normal distribution of uptake rates with few zero values) was for the 5x concentration data *Protium picramnioides*, and the 10x concentration for the other two Panamanian species (not these were also similar to each other in each data set, Tables 1 and 2). We used data produced using these concentrations for all statistical analyses. We then used ANOVA to assess effects of the two BCI species and the type of nutrient (n = 3) on root nutrient uptake, including the interaction of species×nutrient type. We conducted this same analysis across all individuals (n = 33) the one species from Gigante for the four nutrients measured there (see figure in Box 3). For BCI data, nutrient uptake data were averaged across two time points because there were no differences among time points. For the BCI data, we also performed linear models to test for correlation between nutrient uptake or water uptake and environmental variables (soil moisture, soil temperature), as well as root morphological traits (SRL, SRA, RTD, root diameter, root weight, total root length), and tree diameter at breast height (dbh). All data were analyzed in R studio Version 1.4.1103 (R Core Team, 2021).

Singapore Methods

In Singapore, we measured fine-root uptake rates of ammonium (NH_4^+), nitrate (NO_3^-), and phosphate (PO_4^{3-}) of mature lowland forest canopy tree species in Bukit Timah Nature Reserve, Singapore. Bukit Timah is a coastal hill dipterocarp forest developed on highly weathered, low nutrient soils (Grubb et al., 1994; Ngo et al., 2013). We selected four individuals > 30 cm DBH in old-growth and four individuals > 30 cm DBH in secondary forests of two representative species found in both forest types, *Shorea curtisii* and *Campnosperma auriculatum*, to examine species and forest type differences in nutrient uptake rates. *Shorea curtisii* (Dipterocarpaceae) is the most common tree species in the Bukit Timah ForestGEO old-growth plot comprising 24% of the aboveground biomass of trees > 30 cm DBH in the 2-hectare plot (Ngo et al. 2013; Ngo et al. 2017) and is associated with ectomycorrhizal fungi. *Campnosperma auriculatum* (Anacardiaceae) is the most abundant tree species in the Bukit Timah ForestGEO secondary forest plot with 228 individuals >30 cm DBH occurring in the secondary 2-hectare plot (Ngo et a. 2013) and associated with arbuscular mycorrhizal fungi.

We used a simple hydroponic medium, Hoagland's No. 2 Basal Salt Mixture (hereafter referred to as Hoagland's solution) in 50 mL falcon tubes to estimate nutrient uptake rates of PO_4^3, NH_4^+, and NO_3^-. The hydroponic medium minimizes plant-microbe competition for nutrients to measure inherent tree physiological nutrient uptake capacity, allowing for standardized comparisons across trees (Zhu et al., 2021). Hoagland's solution (and modified versions of it) is widely used in the horticultural applications and studies (Reverchon et al., 2014), including studies on root exudates (Phillips et al., 2009) and ion uptake (Dickson et al., 2016). Two separate concentrations of Hoagland's solution will be used based on soil nutrient availability at Bukit Timah Nature Reserve (Ngo et al. 2013) and 10x the natural concentrations and a third treatment is pure Milli-Q water (Table 3). Uptake rates will be measured by submerging entire fine root sections in either high or low strength Hoagland's solution, then taking the difference in nutrient concentration from the initial stock. This allows for measurements independent of the heterogeneity of ambient soil nutrient and moisture conditions (Lucash et al., 2005).

Fine roots were excavated from under each tree to ensure species identification. Fine roots were defined as < 2mm and represented the first three root orders associated with nutrient uptake (McCormack et al. 2015). Sixteen trees were sampled in total, located, and identified by their tags from the Bukit Timah Big Tree Census (Ngo et. al., 2017). For each tree, three intact root segments

were identified by tracing coarse roots from the tree trunk until terminal roots ends are reached (Lucash et al., 2005; Pierick et al., 2021). Root segments were rinsed with deionized water and gently cleaned of soils. While cleaning roots may cause physical damage (Oburger & Jones, 2018), it is necessary to reduce the effects of any microbes or contamination. Each root segment was submerged in one of three tubes containing 50ml of one of the following: high concentration Hoagland's solution, low concentration Hoagland's solution, and Milli-Q water. After immersion, roots and tubes were wrapped in parafilm to reduce evaporation and contamination and incubated for 1 to 5 hours. After incubation, roots were excised at the water level mark, removed, and rinsed in a new tube containing distilled water to capture any remaining nutrients clinging on to the surface of the roots. Rinsing was not necessary for roots submerged in Milli-Q water for exudation measurements. All roots were then stored individually in airtight bags. The roots were collected for morphological characteristics, and the vials were taken to the Forest Ecology Laboratory at Nanyang Technological University for chemical analysis. Fine roots were rinsed with Milli-Q water, scanned for morphological traits, dried at 60°C for three days and weighed for root dry biomass.

Table 1: Target simulated soil solution concentrations for *Campnosperma auriculatum* and *Shorea curtisii* at Bukit Timah Nature Reserve, Singapore.

Ion	**Fertilizer**	**Target solution Ion Concentration (umol L^{-1})**	
		1x	**10x**
NH_4^+	NH_4Cl	8	78
NO_3^-	$NaNO_3$	32	316
PO_4^{3-}	$Ca_3(PO_4)_2$	3	33

Nitrate-N and Nitrate-N, and Ammonia-N were analysed through a segmented flow analyser (SEAL Analytical AutoAnalyzer 500). Ortho-phosphate-P was measured via spectroscopy using the TECAN Spark microplate reader at 880 nm using the molybdate method (Murphy & Riley, 1962; Vogt et al., 2015; Watanabe & Olsen, 1965). Each sample was measured as an average of three microplate well replicates.

Nutrient Uptake Calculations

Data post processing was done in R (R Core Team, 2019). Concentrations were adjusted for blanks, and negative concentrations measured were rounded off to zero. Net uptake rates were calculated as the difference in nutrient mass content (umol nut) between the nutrient-control (10x solution without roots) vial, and the experimental vial (10x solution with roots) plus the wash vial, divided by the dry root weight in grams (g dw) and experiment duration in hours (h). The final units for net nutrient uptake are in µmol of nutrient uptake per gram dry weight of roots per hour (mg nutrient g^{-1} dw h^{-1}).

Net nutrient uptake: umol nutrient uptake per root mass per minute

$$\frac{(X\ Nutrient\ mass\ Blank - WaterBlank) - ((X\ Nutrient\ mass\ RootVial \quad X\ Nutrient\ mass\ WashVial) - WaterBlank)}{RootWeight * ExperimentTime}$$

Singapore Statistical methods

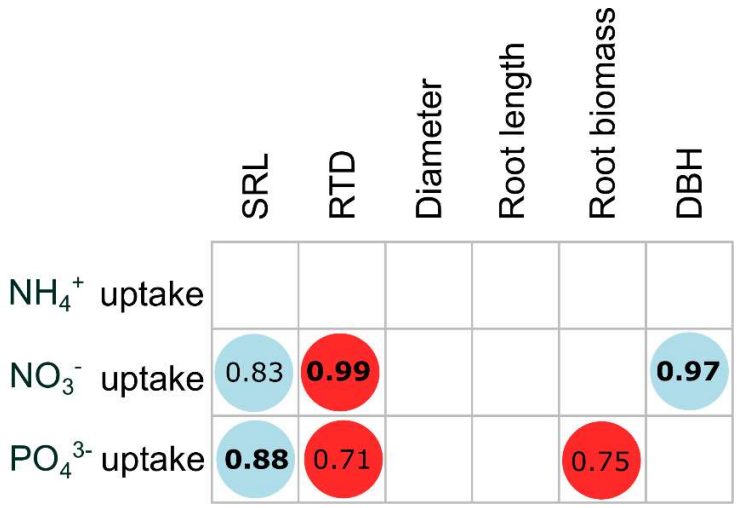
We used linear mixed models to determine whether uptake rates differed by the nutrient, species, forest type, and their combinations with focal trees as a random factor. We used the 10x dataset for all statistical analyses reported here. Pearson correlations were conducted to examine relationships between nutrient uptake rates and fine root morphological traits. Full methodological details, protocols, and data are available in Ng et al. (2022) and maintained on Github.

Results

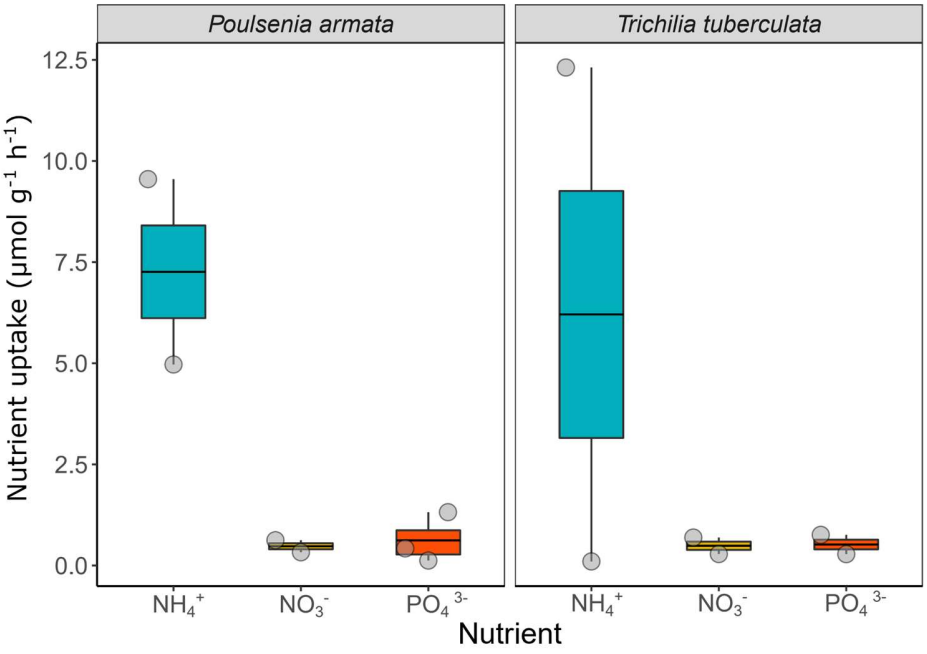
Panama Results

For *P. picramnioides* in Panama, there was generally high variation among individuals in K and NH_4^+ uptake rates, and less variation for NO_3^- and PO_4^{3-} uptake rates (see Box 3 in text). Box plot midlines are means, box edges are first and third quartiles, and whiskers are the minimums and maximums. Grey points represent data points. Letters at the top of box plots indicate significant ($P < 0.05$) differences among groups based on Tukey's HSD tests. Nutrient uptake was significantly different among nutrient types ($F_{3,87}=6.78$; $P=0.022$). We didn't measure morphological root traits for *P. picramnioides.* For the two Panamanian tree species measured separately (*T. tuberculata* and *P. armata*), uptake rates were correlated to some fine-root morphological traits (SI Figure Box3.1), and NH_4^+ uptake rates were higher than NO_3^- and

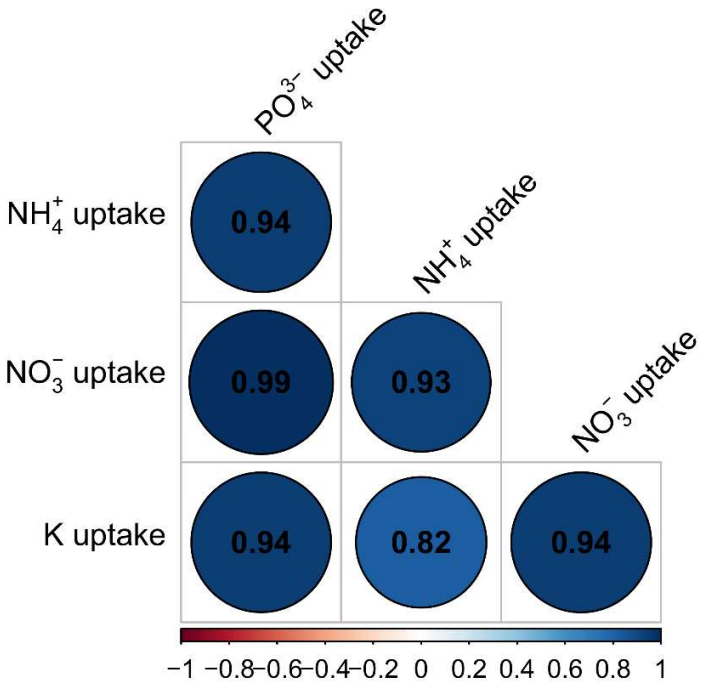
PO_4^{3-} uptake rates (K uptake was not measured for these, SI Figure Box3.2). Also, nutrient uptake rates were generally positively correlated with each other (SI Figure Box3.3).



SI Figure Box2.1 Correlations between nutrient uptake rate from absorptive roots (orders 1,2,3) and morphological root traits for each root system, as well as aboveground dbh of each of two tree species sampled on BCI in Panama (*T. tuberculata* and *P. armata*). Colors represent where there was significant correlation with $P \leq 0.1$. Boxes without colors do not show significant correlation between variables. Numbers inside the colorful balls are r^2. If r^2 are in bold it means that $P \leq 0.05$, and if r^2 are not in bold, it means that $P \leq 0.1$. Red balls represent negative correlation and blue balls represent positive correlation. n = 4 root systems for nitrate and ammonia and n = 5 for phosphate.



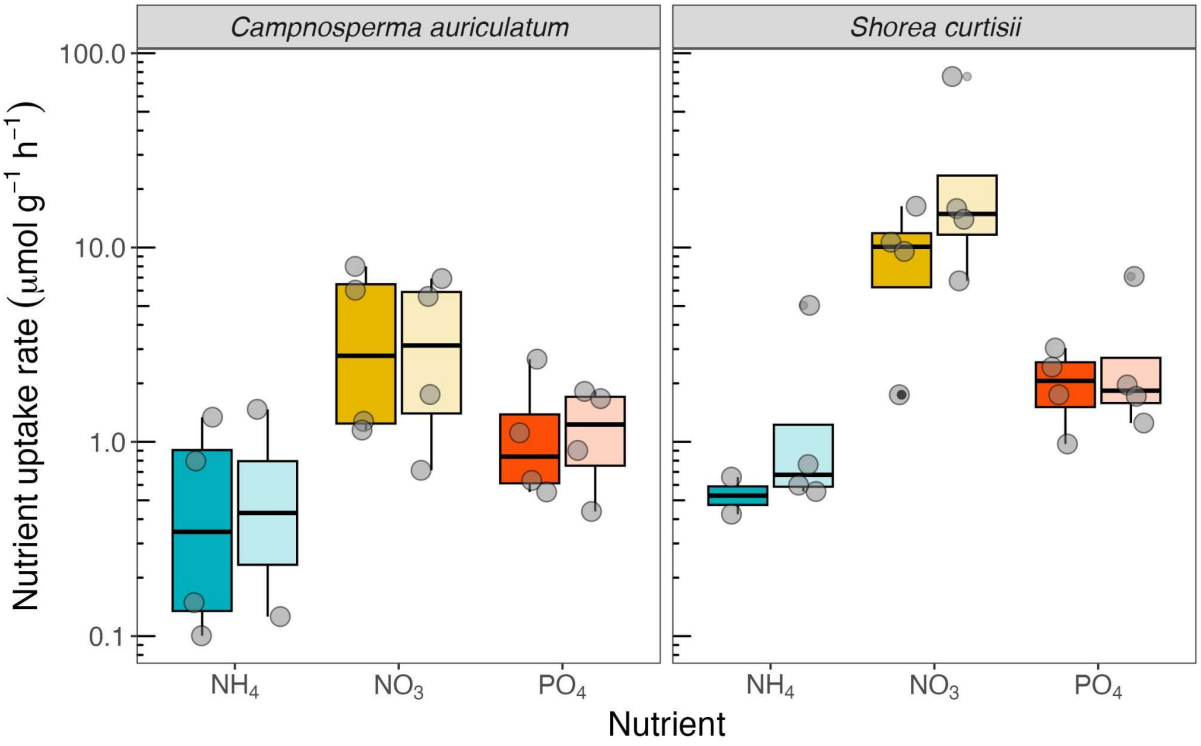
SI Figure Box2.2. Nutrient uptake for two different species (*T. tuberculata* and *P. armata*) were measured on BCI in Panama, using three mature forest individuals (n = 3 per species). Nutrient uptake is expressed in µmol of nutrient per g of dry root weight per hour. Box plot midlines are means, box edges are first and third quartiles, and whiskers are the minimums and maximums. Grey points represent data points. In ANOVA tests, there was no difference between species ($F_{1,9}$ =0.90; P =0.52) or between nutrient types ($F_{2,9}$ =2.34; P=0.15) and no interaction between nutrients and species.



SI Figure Box2.3. A correlation matrix is presented for the uptake rates of different nutrients for *P. picramnioides* in Panama (n = 60). Colors represent where there was significant correlation ($P \leq 0.05$). Numbers inside the balls are Pearson *r* coefficients. Blue symbols represent positive correlation. Nutrient uptake rates for PO_4^{3-}, NH_4^+, and NO_3^-, and K are in µmol per g dry root mass per hour.

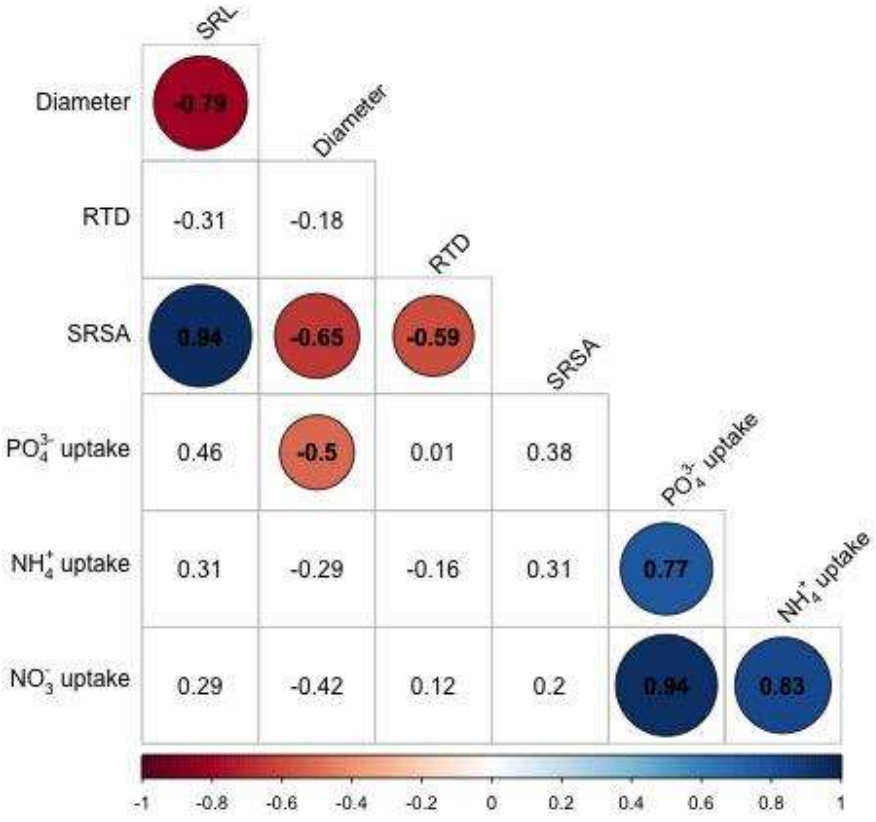
Singapore Results

In Singapore, we found a significant difference in uptake rates for the interaction between nutrient and species ($F2,26 = 10.94$; $P < 0.001$), as well as for the main effects of nutrient ($F2, 26 = 41.78$; $P < 0.0001$) and species ($F1,13 = 7.14$, $P < 0.05$) (SI Figure Box3.4). For both species, nutrient uptake rates were highest for NO_3^- and lowest for NH_4^+. *S. curtisii* had higher overall nutrient uptake rates compared to *C. auriculatum*, which was driven by the significantly higher NO_3^- uptake of *S. curtisii* compared to *C. auriculatum*. However, unlike the Panama data, we found little correlation between nutrient uptake rates and root morphological traits (only a negative relationship between diameter and PO_4^{3-} uptake rates, SI Figure Box3.5). Rather, there were strong correlations amongst morphological variables as well as strong positive correlations amongst nutrient uptake rates.



SI Figure Box2.4 Nutrient uptake rates for two coexisting tree species located in old-growth (darker shading) and secondary (lighter shading) forests in Bukit Timah Nature Reserve, Singapore. Boxes represent medians and 25th and 75th percentiles, with whiskers representing the data minimum and maximum excluding outliers. Grey symbols represent the nutrient uptake rate for four individual trees of each species per forest type. Linear mixed models demonstrated an

interaction between nutrient and species, where NO_3^- was highest for both species and *S. curtisii* had a significantly greater NO_3^- uptake rate than *C. auriculatum*. There were no effects of forest type or interactions with forest type for any nutrient.



SI Figure Box2.5 Correlation matrix for the Singapore nutrient uptake rates and root morphological traits for *S. curtisii* and *C. auriculatum*. Colors represent where there was significant correlation with $P \leq 0.05$. Boxes without colors do not show significant correlation between variables. Numbers inside the colorful balls are Pearson *r* coefficients. Red symbols represent negative correlation and blue symbols represent positive correlation. Note: SRL = specific root length; AvgDiam = mean diameter (mm); RTD = root tissue density; SRSA = specific

root surface area; Nutrient uptake rates for PO_4^{3-}, NH_4^+, and NO_3^- are in μmol per g dry root mass per hour.

Box 2 Summary

These data suggest different preferences among nutrients and species and suggest that some morphological traits might be valuable surrogates for nutrient uptake rates among individuals or species. The findings comparing uptake of different nutrients are consistent with other tropical N uptake studies, where species were found to have different preferences of various N forms across diverse plant forms (Andersen & Turner, 2013; Russo *et al.*, 2013; Andersen *et al.*, 2017; Templer et al., 2008). The relationship between soil nutrient availability and uptake rates at the root surface will require additional information describing the diffusion of nutrient from bulk soil to the root (e.g., McMurtrie & Näsholm, 2018).

SI Box 3: Details of Methods and Results for Rooting Depth

Here, we present a new analysis of data from a dry forest in Costa Rica where aboveground phenology has been linked to rooting depth (Smith-Martin *et al.*, 2020).

Root Depth Field Methods

Phylogenetically diverse species of tropical dry forest trees and lianas were planted in a common garden to be harvested as juveniles (1-year-old and 2-year-old), and mature individuals were harvested in surrounding forest. Plants were divided into functional groups based on aboveground deciduousness and life form: deciduous trees, evergreen trees, and deciduous lianas. Two cohorts of plants were sampled that differ in ontogenetic stage: juveniles and adults. We harvested a total of 47 juveniles and 33 mature individuals of common Costa Rican tropical dry forest species. Each individual was cut at ground level using a chainsaw, all belowground biomass was dug up with shovels and picks; all coarse roots down to diameters of 2–5 mm were excavated and maximum rooting depth of all of the excavated individuals was measured with a tape measure (methodological details in Smith-Martin *et al.*, 2020).

Results

Maximum rooting depth of: (a) one-year old, (b) two-year old, and (c) mature deciduous liana (orange), deciduous tree (yellow), and evergreen tree (blue) species from Costa Rican dry forests are shown in Box 2. Box plot midlines are means, box edges are first and third quartiles, and whiskers are the minimums and maximums. Grey points represent data points. Letters at the top of box plots indicate significant ($P < 0.05$) differences among groups based on Tukey's HSD tests. Raw data provided in SI.

SI Box4. Details of Methods and Results for Fine-Root Stoichiometry

Linking Fine-Root Stoichiometry to Functional Activity

We hypothesized that N fixing plants would separate from non-N fixing arbuscular and ectomycorrhizal root symbiont types based on root N and P content due to higher demands for P to fix N.

Methods

Tree roots were collected from Barro Colorado Nature Monument (BCNM), Panama. Nine co-existing species with known differences in root symbionts were chosen to explore the breadth of the root stoichiometry within different root symbiont types and to determine whether symbiont type could be related to differences in root N or P. These included (arbuscular mycorrhizal (AM), ectomycorrhizal (EM), and N-fixing (Nfix). Roots were traced from the base of focal trees, collecting only fine roots (<2 mm diameter). N was measured by combustion on an Elemental Analyzer, and P was measured after dry-ash acid digestion on a Lachat, both in the STRI soils lab. Species list and raw data are provided in Dataset S6. Linear models were conducted in R, with nutrient as the dependent variable, symbiont type the fixed factor, and species within symbiont type the replicate.

Results

Overall, root nutrient content did not vary by symbiont type. The mean root N content for the nine species co-occurring at BCNM was 16.45 (± 1.81) mg N g^{-1}, with no differences among the three root symbiont types ($F_{2,6}$ = 1.36, P = 0.33). Similarly, the mean root P content for the nine species co-occurring at BCNM was 0.53 (± 0.06) mg P g^{-1}, with no differences among the three root symbiont types ($F_{2,6}$ = 0.51, P = 0.62). It is worth noting the high interspecific variation in root nutrient content within each root symbiont type, particularly for the ectomycorrhizal species. Further investigation comparing root stoichiometry across root symbiont type and other functional groupings would be useful to understanding if the results for the nine species from Panama reflect general patterns across tropical forests.

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