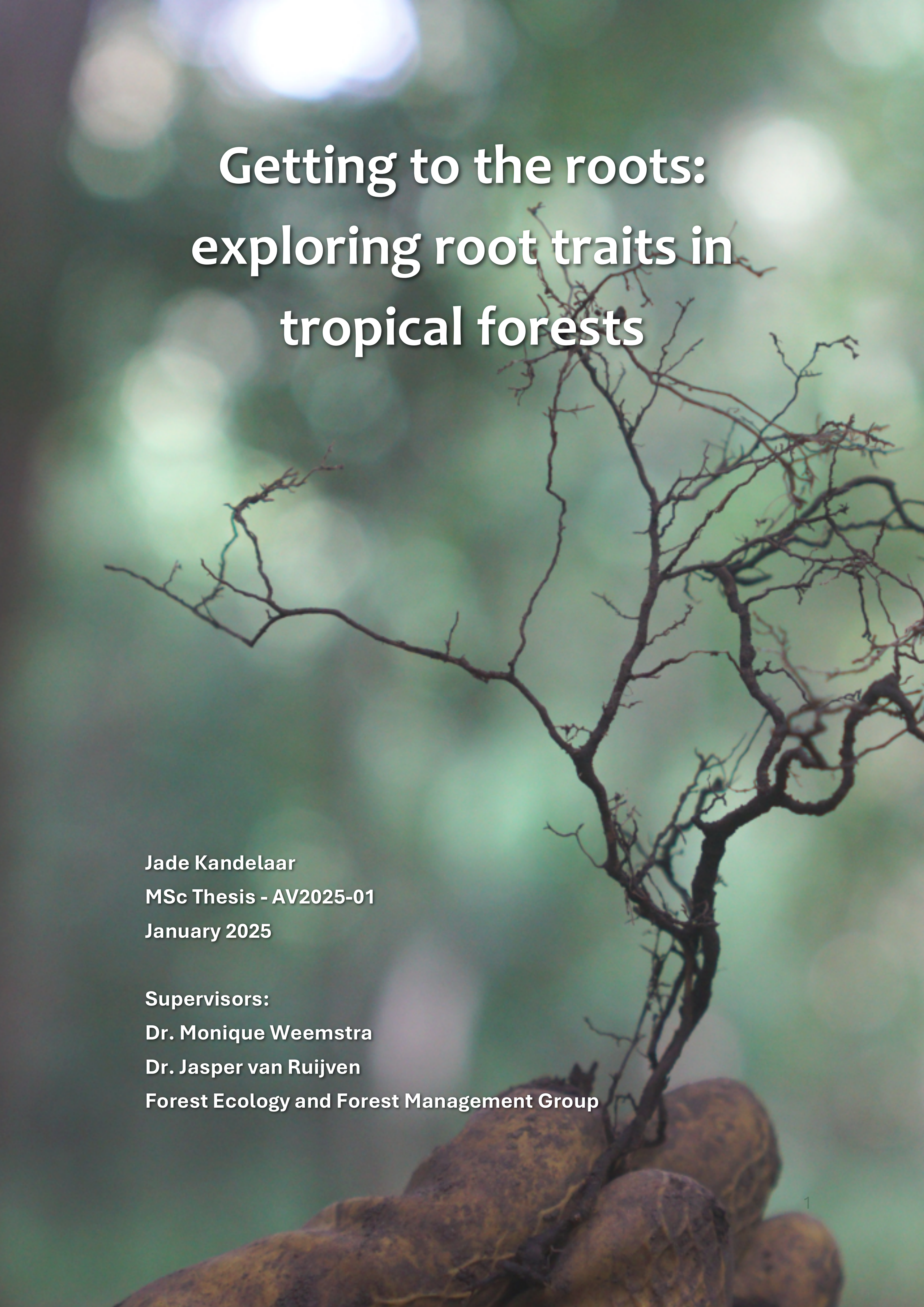

Getting to the roots: exploring root traits in tropical forests

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MSc Thesis - AV2025-01
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The author declares
1) that this work is her own and
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Abstract

Roots are a fundamental part of every forest ecosystem, but they are severely understudied due to their invisibility and complexity. Recent studies propose the Root Economic Space (RES) framework, which explains resource acquisition strategies through two key trade-offs: the conservation gradient (fast vs. slow strategies) and the collaboration gradient (interaction with mycorrhizal fungi). Although this framework appears to explain many underground processes, root research is dominated by trees from temperate regions. Tropical ecosystems remain understudied, despite the importance of these ecosystems for global biodiversity and their vulnerability to climate change.

This study examined the variation and universality of root traits across the globe, with a focus on tropical regions. Fine roots of 20 tree species were sampled in a dry tropical forest in Costa Rica and compared with existing fine root data from tropical rainforests and forests worldwide. Findings suggest that there are significant differences between tropical forests, but that this variation is not directly explained by wet and dry forest types. On the global root economic space, results indicate that tropical tree species have on average, thicker and denser roots than species from colder regions. Tropical forests show more variation along a conservation gradient, while colder regions show slightly more variation along the collaboration gradient. This could be explained by the longer growing season in the tropics, and more collaboration with (arbuscular) mycorrhizae. Overall, this research provides insight into the universality and differences of tree roots across biomes worldwide.

Keywords: root traits, tropical dry forest, root economic space, Neotropics

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1. Introduction

Tropical forests host a major portion of the world's tree species. In the Neotropics alone, it is estimated that forests are home to between 19,000 and 25,000 tree species—an enormous number compared to the mere 124 tree species found across Europe (Slik et al., 2015). This immense diversity results in a complex environment with countless ecological interactions. To understand the complexity of tropical forests, functional traits can offer insight in the patterns and similarities driving ecological processes (Swenson, 2013). Understanding these traits is an essential part of understanding plant strategies, species coexistence, and broader ecosystem functions (Violle et al., 2007).

The various ways in which plants interact with their environment can be studied through theories and frameworks, which organize functional traits along axes of trade-offs (Worthy & Swenson, 2019). An example is the Leaf Economic Spectrum, which reduces the wide range of above-ground traits to a simplified one-dimensional spectrum between growth and survival (Wright et al., 2004). Leaves with a relatively larger surface facilitate high light absorption, and a fast return of investment. This is referred to as the *acquisition/fast strategy* in the LES (Wright et al., 2004). However, this advantage comes at the cost of increased vulnerability to herbivory. Therefore, other plant species prioritize investing in higher tissue density and protective chemicals: the *conservation/slow strategy*. Abundant empirical evidence supports the universal existence of the LES throughout plant species and across biomes (Reich, 2014; Wright et al., 2004). While research in trait variation studies both leaves and (fine) roots, leaf traits are far better understood compared to their invisible and intricate below-ground counterparts: fine roots (Iversen et al., 2017).

1.1. The root economic space

Trees interact with their environment through their roots: with fine roots, trees absorb water and nutrients from the soil, and coarse roots transport the nutrients and stabilize the tree (Tobin et al., 2007). Initially, it was assumed that a similar framework as the LES applies to below-ground resource acquisition, with fast growing acquisitive roots on one side of the spectrum, and dense conservative roots on the other side of the spectrum. However, roots face a more complex environment than leaves: soil texture, fungal interaction, and unevenly distributed water and nutrients. Therefore, a one-dimensional spectrum fails to describe the diverse plant strategies to acquire resources from their environment (Weemstra et al., 2016).

Aiming to resolve this question, Bergmann et al. (2020) introduced a two-dimensional Root Economics Space (RES[1]), which proposes that root functional traits align along

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[1] In previous research (Mommer & Weemstra, 2012), the abbreviation 'RES' has also been used to describe the Root Economic Spectrum. However, since the current domain builds on the assumption that a multi-dimensional root spectrum better describes the belowground trait complexity, the root economic spectrum has been dismissed. Therefore, in this report RES will refer to the Root Economic Space.

independent key gradients: *a conservation gradient* and a *collaboration gradient* (see Fig. 1). The conservation spectrum considers the 'classic' trade-off between fast and slow acquisition strategies (Bergmann et al., 2020). Roots with a high nitrogen content (N) are successful in fast nutrient uptake but are less robust and thus have a shorter lifespan (Reich, 2014). On the other hand, species might invest in stronger and denser roots, with the benefit of resilience and a longer life span, but with the disadvantage that the denser roots slow down the transport of nutrients through the root cortex to the stele, and therefore is slower in nutrient uptake (Zhao et al., 2024). This is indicated by a high root tissue density (RTD), the root mass per unit root volume (Bergmann et al., 2020; Kramer-Walter et al., 2016).

While this "classic" fast-slow spectrum has proved to explain fundamental variation in aboveground plant traits (Reich, 2014), Bergmann et al. (2020) show that a second axis is the main dimension of root trait variation. This so-called collaboration axis considers a gradient between two forms of optimal resource acquisition: the *do-it-yourself strategy* whereby species develop relatively longer absorptive roots (high specific root length - SRL), or the *outsourcing strategy* in which species develop relatively thick roots (high root diameter - RD) to collaborate with mycorrhizal fungi to collect resources. Mycorrhizal fungi have evolved to effectively collect mineral nutrients, such as N and phosphorus (P), from the soil, which they can share with plants in exchange for photosynthate carbon (Brundrett, 2002).

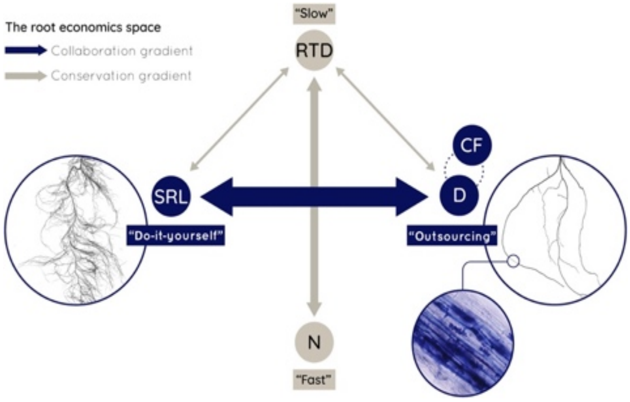

Figure 1. Conceptual framework of the Root Economic Space. Taken from Bergmann et al (2020).

1.2. Roots in tropical forests

While the RES has proved to be a promising framework to understand belowground strategies (Carmona et al., 2021; Weigelt et al., 2021; Zhang et al., 2024a), our knowledge of the RES continues to develop. Particularly, much remains unknown about root traits in tropical forest ecosystems. Trait data from these regions are limited compared to the abundant data that is available from temperate forests. Since tropical regions host an extraordinary diversity of species, our understanding of belowground patterns in the tropics is derived from only a small fraction of the species that exist there, which limits our knowledge of universal belowground strategies. In the development of the RES framework, Bergmann et al. (2020) used data from global databases, dominated by temperate forest species: only 16% of the species in the global dataset is from tropical forests. This bias towards temperate species may affect our understanding of resource acquisition strategies in different regions. While logically, it can be expected that tropical trees face similar tradeoffs when allocating their resources, there are biotic and abiotic factors that influence these tradeoffs (Laughlin et al., 2021). Tropical forests share a couple of characteristics, which may differ from temperate forests and could impact the pattern that is observed in the RES.

Firstly, in tropical regions, the majority of mycorrhizal fungi are arbuscular mycorrhiza (Sun et al., 2024), which require a larger cortex for colonization. A high RD correlates with a larger root cortex (Kong et al., 2014), which is why tropical tree species likely have a larger RD than temperate tree species. However, while the hot and moist conditions in tropical rainforests create a favorable environment for mycorrhiza, they also promote the growth of pathogenic fungi. Therefore, it is expected that some tree species may adopt a strategy of evolving thinner roots with a smaller cortex, which could help limit infection risks.

Secondly, tropical forest soils have low phosphate availability (P), a limiting nutrient for tree growth. Trees likely rely more on arbuscular mycorrhizal fungi to retrieve these nutrients and follow the *outsourcing strategy,* indicated by a larger RD (Cusack et al., 2021; Soudzilovskaia et al., 2019; Yaffar et al., 2022). Another possible strategy is that trees develop more explorative roots with a higher SRL and branching frequency to find the scarce and unevenly distributed soil minerals (Weemstra et al., 2023). The root trait branching frequency (BF) is not included in the RES, but a study on Amazonian tree species has pointed out the importance for resource acquisition in the tropics (Weemstra et al., 2023). A higher SRL and BF mean that roots have in total a larger absorbative surface per biomass investment, with which they can come in contact with nutrients (Zhang et al., 2024a).

Thirdly, the seasons in tropical regions are defined by rainfall rather than temperature: in most tropical regions there is a distinct wet and dry season and a longer growing season. This may allow trees to invest in denser roots with a high RTD (the slow strategy). In temperate regions where the growing season is shorter, roots are prone to have higher N, which can indicate the fast strategy (Chen et al., 2016; Laughlin et al., 2021). However, due to the competition for nutrients, it is equally plausible that some trees adopt a fast-strategy (indicated by high root N), optimizing for rapid resource acquisition to outcompete neighbors.

In short, the diverse ecological and environmental conditions in tropical forests may result in a wide range of belowground strategies. Several studies have shown that root morphological variation is greater in (sub)tropical regions compared to colder climates, due to environmental heterogeneity and high species diversity (Chen et al., 2013; Ma et al., 2018; Valverde-Barrantes et al., 2021).

Moreover, grouping all tropical forests together risks overlooking their nuanced ecological and functional differences. Tropical forests span across three contents, and include dry and wet forests, montane and lowland forests, and forests growing on fertile or nutrient-poor soils (Guerrero-Ramírez et al., 2024). A major tropical forests type, dry tropical forests, have been mostly overlooked in terms of fine root research. It is expected that dry forests were a large part of tropical forests, before they had fallen to desertification and deforestation (Allen et al., 2017). These forests are characterized by a harsh 4 - 7 months rain free period, in which the trees loose most of their leaves and the soil hardens (Janzen, 1988). The response of belowground strategies to environmental factors, such as participation, is complex (Allen et al., 2017). Yet, there is some evidence which suggests that drier areas lead to denser roots, to cope with long periods of drought (Sun et al., 2024), and that trees collaborate with mycorrhiza in both moist and dry conditions (Lovelock et al., 2003).

1.3. Research questions

To better understand the diversity of belowground strategies in the tropics, this research focuses on root traits in a dry tropical forest in Costa Rica. Thereby, this research aims to explore the variation and universality of the Root Economic Space (RES) globally, specifically focusing on how root traits in tropical ecosystems differ internally and in relation to other biomes. These objectives correspond with the following questions and hypotheses.

Q1. Does the root economic space (RES) exist among dry tropical tree species in Santa Rosa National Park, Costa Rica?

H1. I expect that the RES exists among dry tropical forest species. Based on allocating principles, trees cannot produce both expensive, dense, and well-protected roots and cheap roots with fast metabolic rates. These physiological constraints firstly lead to a tradeoff between RTD and root N, representing the conservation gradient, and secondly between SRL and RD, representing the collaboration gradient.

Q2. How do morphological and architectural root traits vary between dry and wet forests in the Neotropics?

H2. While most forest ecosystems host a variety of below ground strategies, I hypothesize that tree species in dryer forests have slightly thinner and denser roots, to cope with longer periods of drought. Additionally, trees in soils with a higher C/N ratio (inverse proxy for soil fertility) are expected to have a higher specific root length, to efficiently forage for scarce nutrients.

Q3. Where are tropical tree species placed on the global root economic space in comparison to temperate forests?

H3. I hypothesize that tropical forest species have on average a higher RD, because they collaborate more with arbuscular mycorrhizal fungi than species in colder climates. On the conservation axis, tropical species are expected to shift slightly more towards the slow strategy (high RTD), because the longer growing season allows investing in expensive dense roots. For an illustration of this hypothesis, see Fig 2.

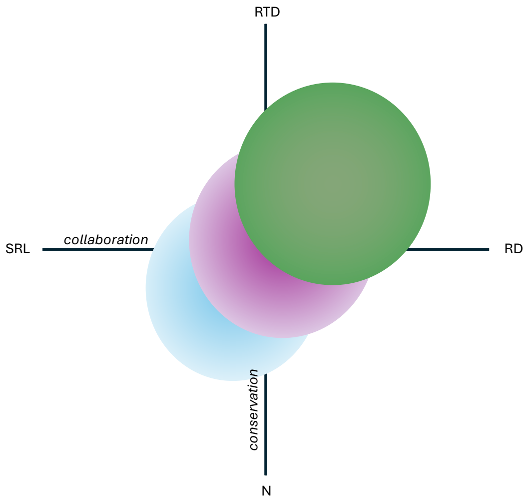

Figure 2. Visualization of hypothesis 3, The three circles indicate a simplified version of three biomes: green – tropical biome, yellow – sub tropical & temperate biome, blue – continental biome. For abbreviations, see table 1.

2. Methods

2.1. Study Location

Data collection took place in a forest plot called San Emilio, located in Santa Rosa National Park, which is part of the Área de Conservación de Guanacaste (ACG) in Costa Rica. The plot spans 15.64 hectares (ForestGEO, 2021) and is located at an elevation ranging from 240 to 300 meters above sea level (Enquist & Enquist, 2011). San Emilio is a seasonally dry tropical forest, with a well-defined dry season (January – May) and a wet season (June- December). The area has an average annual rainfall of 1500 mm, although this precipitation can vary significantly from year to year (Swenson et al., 2020). In this area, soil has been described as chromic luvisol (FAO & UNESCO, 1961).

The entire forest plot consists of secondary forests on former agricultural land (Fig. 3). In comparison to other dry tropical forests, it is relatively far in its successional development, with estimated ages ranging between 100 and 170 years (Swenson et al., 2020). In the last fifty years, this plot has been censused four times. Since 2021, San Emilio has been part of the ForestGEO (Forest Global Earth Observatory) network, a worldwide forest research organization. The most recent census was completed in 2021 by Chmurzynski (2024), during which a total of 176 tree species were identified and approximately 34,000 trees counted.

FIGURE 3. DRY FOREST HABITAT IN SANTA ROSA NATIONAL PARK

2.2. Species selection

From the 179 species present in the ForestGEO plot, a selection of 20 species was made based on the following characteristics:

- abundance: the most abundant species were selected, to ensure a representative sample of the local forest community

- size: species of which the majority of individual trees had a DBH larger than 50cm were excluded, to limit the scope of this study to relatively smaller individuals and because sampling from larger individuals is more challenging
- phylogeny: species were selected to represent the three main superorders: rosids, asterids and magnoliids (see figure 4)
- feasibility to sample: some tree species were omitted because their root systems made it difficult to sample. For example, *Luehea speciosa* trees are very large and their roots went deep in the soil without making shallow lateral roots. Therefore, this species was replaced by *Astronium graveolens*, another tree from the rosid superorder.

We went into the field with this list of 20 tree species, and labeled 5 individuals per species. Most labeled trees had a DBH between 5 and 20 cm, although some were slightly thicker or thinner.

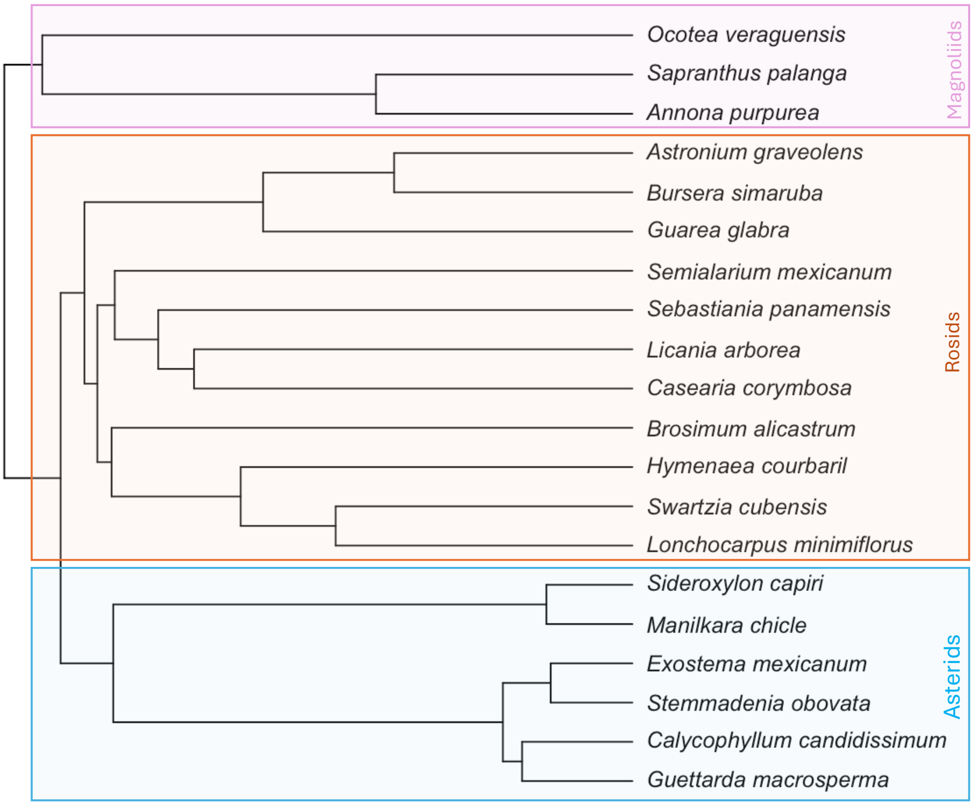

FIGURE 4. PHYLOGENETIC TREE OF THE 20 SPECIES SAMPLED IN SANTA ROSA NATIONAL PARK. GENERATED WITH PHYLOMAKER PACKAGE IN R (JIN & QIAN, 2022).

2.3. Sampling

Root samples were collected in June and July 2024. This is the wet season, therefore the soil was not too hard. The sampling protocol was as follows. First, the litter layer was removed around the tree trunk. After that, roots were manually traced from the base of the stem to the most lateral and finest roots in order to ensure we collected roots from the target species. Using small gardening tools and shovels, we carefully retrieved the smaller roots from a soil depth of maximum 20 cm. Around each tree, 15 to 30 pieces of roots were collected, which included both fine and coarse roots. It depended per species and per tree how much exactly was collected: we collected less from trees that were more difficult, such as those with deeper roots or hard-to-find fine roots, compared to easier trees. The root segments were temporary stored in a labeled plastic bag, with a wet paper towel to prevent dehydration. We noted our qualitative observations, which can be found in Appendix 1. In total, samples were taken from 100 trees.

2.4. Washing and processing samples

Upon return to the research station, samples were processed according to the guidebook from Freschet, et al., (2021). First, samples were soaked for at least an hour in separate buckets of water. When the dirt particles had loosened, the root samples were well rinsed under running water, using a fine sieve. Remaining dirt or litter particles were carefully removed by hand. Clean roots were stored in a cleaned and labeled plastic bag. This process was repeated until all roots were clean.

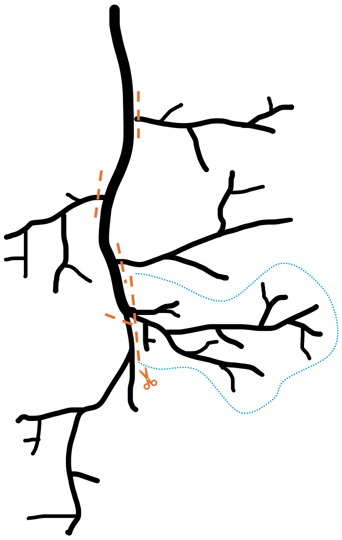

Figure 5. Illustration of a root sample. Orange lines show which fine roots would be collected. Blue line around an intact root segment for scanning.

After cleaning, the root samples per individual tree were spread out to select absorbative roots. Most samples contained both coarse and fine roots. The sorting process began with identifying the central coarse root. Lateral roots, characterized by softer tissue and smaller diameter (>2mm) compared to the central coarse root, were classified as absorptive roots and separated accordingly (see Fig 5). The coarse roots were discarded and all the absorbative roots were kept for analysis. From these absorbative roots, three to five of the more intact absorptive roots were selected for scanning, see the blue line on Fig 5. Remaining fine roots (between 0.2 and 0.4 gr per tree) were stored in paper envelopes for subsequent chemical analysis.

The 3 – 5 intact absorbative roots were individually scanned in grayscale with 600 DPI resolution, using a high-resolution flatbed scanner (Epson Scanner Perfection V700 Photo, USA). Root segments were placed in a low tray with a small layer of water. Using tweezers, roots were untangled and spread out as much as possible. Using a glass plate, roots were flattened to ensure that all root parts were emerged in the water. In total, 485 roots were scanned (for an example, see Appendix 1).

Scanned root images were edited in photo edited (Windows Photo’s Editor) to remove objects which were not part of the root. This included mostly hyphae and air bubbles. In order to quantify morphological traits, root scans were analyzed using RhizoVision Explorer (Seethepalli & York, 2020), with an image thresholding level of 195. We measured the root traits which are featured in the RES, and are commonly used to describe root systems (Table 1). RhizoVision provides morphological data per scan (5 scans per tree), which was averaged to calculate morphological data per tree.

Afterwards, the scanned roots were dried in an oven at 60 C° for 48 hours. The dried root samples are weighed on an analytical microbalance (Sartorius Quintix513-1S) to determine dry root weight.

TABLE 1. FINE ROOT TRAITS WITH DEFINITION AND MEASUREMENT

Abb	Trait Name	Unit	Definition	Measurements
SRL	Specific Root Length	m g^{-1}	Root length per unit of dry mass, indicating potential soil exploration versus biomass investment.	*length (m) / weight (g)* Total root length via image analysis (RhizoVision); dry mass after oven-drying.
RD	Root Diameter	mm	Average diameter of first to third-order roots.	Calculated using image analysis with RhizoVision.
RTD	Root Tissue Density	g cm^{-3}	Dry mass per unit of fresh volume, showing root density and construction cost.	*weight (g) / volume (cm)* Volume from image analysis (RhizoVision); dry mass after oven-drying.
N	Root Nitrogen Concentration	mg g^{-1}	Amount of nitrogen in root tissue, indicating metabolic activity.	Chemical analysis of powdered roots using a CN elemental analyzer.
BF	Branching frequency	px cm^{-1}	Amount of root tips per cm root length, referring to potential soil exploration	*Root tips (px) / length (cm)* Root tips and length calculated by Rhizovision

2.5. Data selection from databases

Field data that I collected at San Emilio was further complemented by existing data from GRooT, a global fine-root trait database (Guerrero-Ramírez et al., 2021). Root trait data from GrooT was selected to resemble the data collected in Costa Rica, from natural systems. More specifically, the following filters and criteria were used:

- Growth form: exclusively trees
- Vitality: living / unspecified
- Belowground entity: exclusively fine roots (FR)
- Root diameter: tree roots with a diameter >2mm were omitted
- Function: exclusively absorptive roots
- Type study: agricultural studies / plantations were omitted
- Treatment: studies with experimental treatments (added/removed nutrients) were omitted
- Trait data from tree species in desert ecosystems were omitted
- Traits: only species were selected when all the morphological traits SRL, RD or RTD were available
- Extreme outliers were inspected (checked whether their methods matched our methods) and otherwise omitted

Average trait values per tree species were calculated. Species were grouped per biome using the climatic information in the dataset (see table 2). The four biomes were inspired by the climate zones in Freschet et al. (2017) and by MET Office (n.d.). When climatic data was not provided, the latitudes of the study locations were used to determine the biome. When species existed across multiple biomes, the global plant database of Kew was used (POWO, 2025) to determine where they grow natively.

TABLE 2. SPECIES PER BIOME

Biome	***Köpen climate zones***	***Latitudes***
Continental	Dfa + Dfb + Dfc + Dwb	+/- > 60°
Temperate	Cfb +Cwa + Cwb	+/- 40 ° – 60 °
Mediterranean	Csa	
Subtropical	Cfa	+/- 23,5 ° – 40 °
Tropical	Af + Am	+/- 23,5°

Additional root trait data were sourced from two studies conducted in the Amazon region. Vleminckx et al. (2021) collected data in French Guiana, where annual rainfall ranged from 2150 to 3700 mm, while Weemstra et al. (2023) conducted their study in Brazil, where annual precipitation averaged around 2400 mm. Both studies were carried out in mature lowland rainforests with annual temperatures averaging 25°C. The studies focused on trees from three distinct forest habitats: terra firme, white-sand, and seasonally flooded forests. For the purpose of this analysis, only trees from the terra firme habitat were included, as the nutrient-poor white-sand soils and the seasonally flooded soils present fundamentally different environmental conditions compared to the forest ecosystem in Costa Rica.

2.6. Phylogenetic trees

To map evolutionary relationships among species, I constructed a phylogenetic tree per research question using the phylomaker function from the *V.Phylomaker* and *V.Phylomaker2* packages (Jin & Qian, 2019, 2022). These packages include a megatree (*GBOTB.extended.tre*) of 74,531 plant species across 10,587 genera, standardized using the updated LCVP nomenclature (Jin & Qian, 2022). Since not all species were represented in the megatree, I used scenario 3, the most commonly used method for generating phylogenetic hypotheses. The plot.phylo function from the *ape* package was used to visualize the phylogenetic trees (Paradis et al., 2002). See Appendix 2.

2.7. Data analysis

All data were processed using RStudio (version 2023.12.1+402) and Microsoft Excel. Data analysis focused on species-level mean trait values, of three datasets corresponding with the three research questions (see Table 3).

TABLE 3. SPECIES PER DATASET

Santa Rosa		**Neotropics**		**Worldwide**	
N trees	*N species*	*Countries*	*N species*	*Biomes*	*N Species*
100	20	Brazil	119	Continental	24
		Costa Rica	20	Temperate	151
		French Guiana	16	Mediterranean	14
				Subtropical	155
				Tropical	255
	20		*155*		*599*

On the level of Santa Rosa National Park (Q1), I performed an analysis on the three morphological root traits that (partly) constitute the Root Economics Spectrum (RES): root diameter (RD), specific root length (SRL) and root tissue density (RTD). Unfortunately, we did not receive the root chemical data in time for the analysis, which meant I could not include one of the key traits, root nitrogen (RN), in the study.

The data of the 100 trees was first inspected for outliers using a modified z-score. While some outliers were identified (based on a z-score of > 3 or < -3), they remained in the analysis as they reflected ecological variation rather than errors in the dataset. To visualize the data in a boxplot, I used the *ggplot* function from the *ggplot2* package (Wickham et al., 2007).

Next, I conducted a Principal Component Analysis (PCA) on the 20 species to examine the distribution of species along trait gradients. To account for phylogenetic dependencies, I used the phy.pca function from the *phytools* package (Revell, 2011). I used scale = TRUE, to standardize for the different trait units, and method = "BM" which is the default method. Correlations between root traits were tested with a Spearman's Rank correlation test.

In order to study the variation of root traits between dry and wet tropical forests (Q2), I used data from forests in three Neotropical countries: dry forest – Costa Rica, wet forests – French Guiana and Brazil. Data from French Guiana and Brazil was collected in wet tropical forests but analyzed separately to prevent the larger Brazilian dataset from disproportionately influencing the comparison. Consequently, country was used as a proxy for dry or wet forest conditions.

As an indication of environmental differences, I looked at average soil C/N ratio of all plots measured in each as an indicator for soil fertility. The average C/N ratio (indicating higher fertility) is lower in Costa Rica compared to Brazil and French Guiana (Table 4, Fig. 6). Annual rainfall is with 1500mm also lowest in Costa Rica, and with 2700 highest in French Guiana.

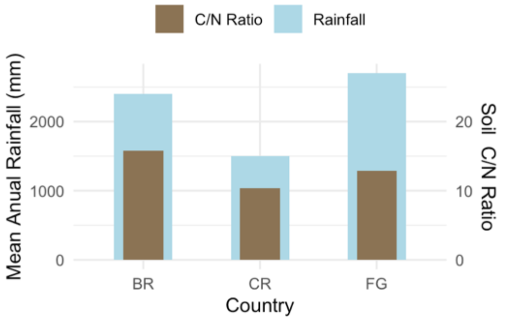

Figure 6. Mean annual rainfall and soil C/N ratio per country. BR is Brazil, CR is Costa Rica, FG is French Guiana.

Table 4. Environmental data per country.

	Rainfall	**C/N**
Brazil	2400 mm	15.75
Costa Rica	1500 mm	10.39
French Guiana	2700 mm	12.89

Note. Average annual rainfall in mm, C/N ratio in CR is average of 10 soil samples from the same plot, in BR and FG average of all Terra firme plots.

To test for differences in root traits between the Neotropical countries, I performed a phylogenetic analysis of variance (phylANOVA), from the *phytools* package (Revell, 2011). Since the data was not normally distributed, I used square root-transformed data of four morphological root traits (SRL, RD, RTD and BF) from Brazil, Costa Rica and French Guiana.

Country was considered as the independent variable, while root trait was dependent. The phylANOVA was followed by a pairwise posthoc test using method = "holm".

A PCA was performed using the same method as described in the previous paragraph. Differences between countries were tested using permutational multivariate analysis of variance (PERMANOVA) with the adonis2 function from the *vegan* R package (Oksanen et al., 2001). As a post hoc test, pairwise comparisons between groups were performed using pairwise.adonis from the *pairwiseAdonis* package (Martinez Arbizu, 2020), with 999 permutations.

To analyze variation in root traits across biomes (Q3), I conducted a PCA in the same way as described in previous paragraphs. Differences between groups and correlations were tested using the same method.

3. Results

3.1. Root traits in Santa Rosa National Park

In Costa Rica, the measured trees exhibited an average root diameter (RD) of 0.56 mm (± 0.19), an average specific root length (SRL) of 17.29 m/g (± 10.65), and an average root tissue density (RTD) of 0.31 g/cm^3 (± 0.09). Variation in root traits differed considerably across species. For instance, SRL varied by only 1.2-fold in *Sapranthus palanga*, whereas it exhibited a 2.7-fold variation in Annona purpurea (see Appendix 3).

Multivariate analysis of the three root traits shows that 82% of their variation across species is explained by PC1 and an additional 17% by PC2 (Fig. 7). RD loads strongly and negatively on PC1, and RTD and SRL both load positively on PC1 (see Table 5 in Appendix 4). Loadings on PC2 are marginal for RD, strongest and positive for SRL, and negative for RTD. Loadings on PC1 thus suggest a negative relationship between RD and SRL, which is confirmed by a correlation test (ρ= -0.851; $P < 0.001$). RTD was also negatively correlated to RD ($\rho = -0.472$, $P < 0.001$), but not to SRL ($\rho = 0.037$, $P = 0.71$).

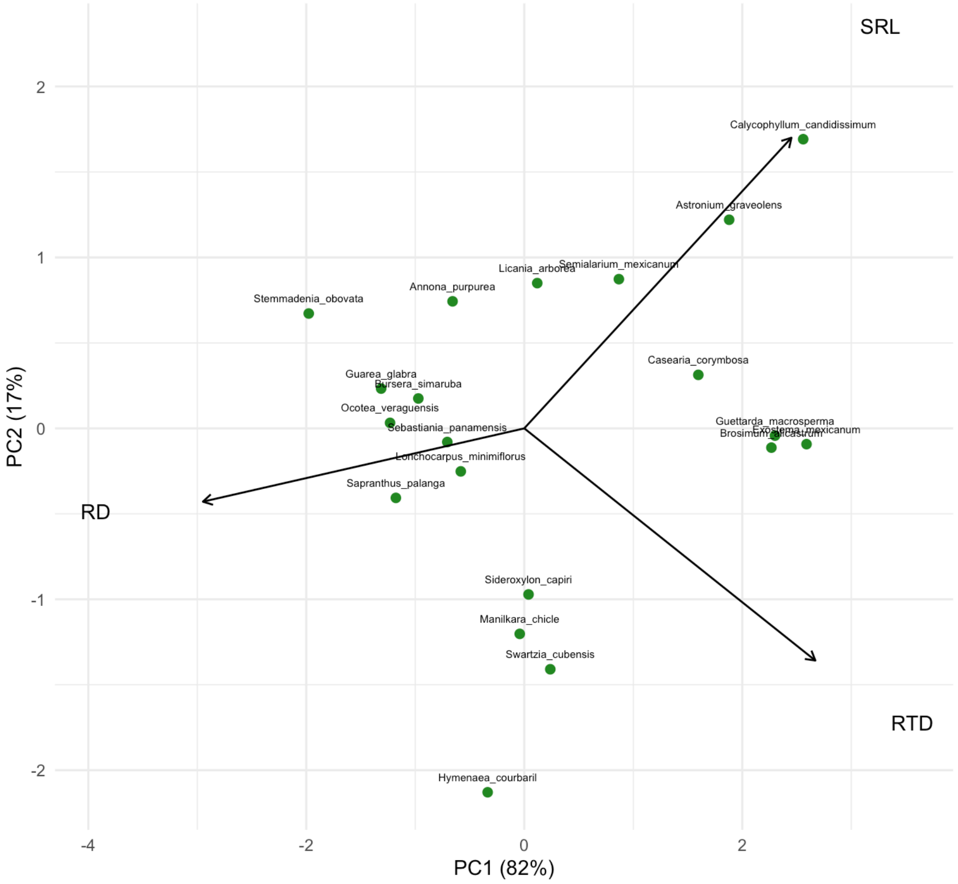

FIGURE 7. PHYLOGENETIC PRINCIPAL COMPONENT ANALYSIS. EACH GREEN DOT REPRESENTS 1 SPECIES, THE AVERAGE OF 5 TREES. FOR TRAIT ABBREVIATIONS, SEE TABLE 1.

3.2. Root traits in the Neotropics

Across the three Neotropical countries (i.e. Costa Rica – dry; Brazil & French Guiana – wet), root traits varied (See Fig. 8). Overall, specific root length (SRL) differed between countries (phylANOVA: F = 7.664, p = 0.001), such that SRL was significantly lower in French Guiana than in Costa Rica and Brazil ($p < 0.05$), but did not differ between Costa Rica and Brazil (p = 0.153). Root diameter (RD) was also significantly different across countries (phylANOVA: F = 6.963, p = 0.002). While RD in Costa Rica did not differ significantly from Brazil or French Guiana (p = 0.346 and 0.078, respectively), a significant difference was found between Brazil and French Guiana (p = 0.003). Similarly, root tissue density (RTD) differed significantly between countries (phylANOVA: F = 13.356, p = 0.001), with Costa Rica differing significantly from both Brazil and French Guiana ($p < 0.05$). Lastly, country also significantly influenced branching frequency (BF) (phylANOVA: F = 5.085, p = 0.011), where only Brazil and Costa Rica showed significant differences (p = 0.027).

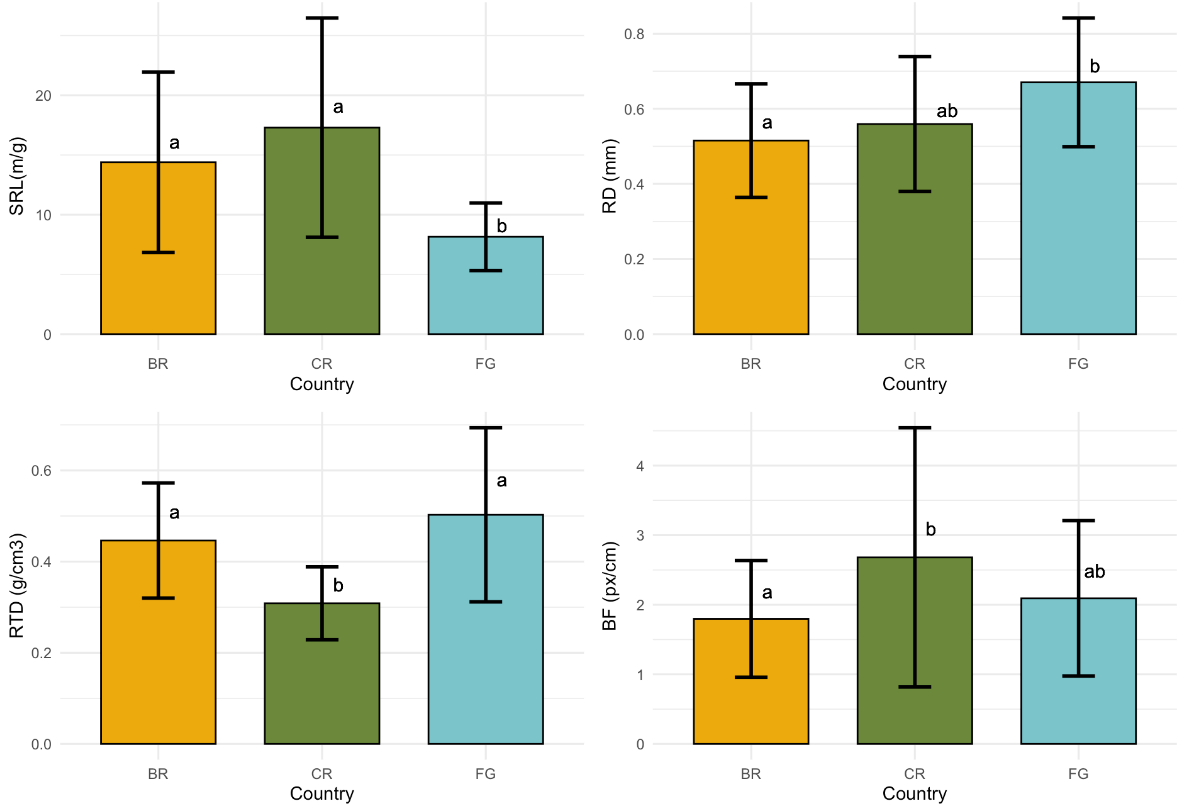

Figure 8. Bar graph of four root traits between three different countries: BR is Brazil, CR is Costa Rica, FG is French Guiana. Statistical differences calculated with phylogenetically corrected Anova. For trait abbreviations see Table 1.

A phylogenetic principal component analysis (PCA) indicated that PC1 explained 63.3% of the variation in root traits, while PC2 explained 22% (Fig. 9A). RTD and RD were positively associated with PC1, with RTD contributing more strongly to PC1, whereas RD loaded more strongly and positively on PC2. SRL and BF were negatively associated with PC1, with SRL contributing more to PC1 and BF more strongly, and negatively, to PC2. These loadings suggest a negative relationship between SRL and RTD ($\rho = -0.230$, P = 0.004) and between RD and BF ($\rho = -0.532$, $P < 0.001$). The negative loadings for both BF and SRL on PC1 and PC2 suggest these traits are correlated, as is further supported by a significant correlation (ρ = -

0.258, P = 0.001). The elongated ellipse along PC1 around species in Costa Rica (CR) indicates that most variation in CR occurs along an RD–SRL/BF gradient, with less variation observed for RTD.

When BF was excluded from the analysis, PC1 explained 74.4% of the variation, and PC2 explained 21% (see Fig. 9B). In this analysis, SRL and RTD were primarily loaded on PC1, while RD was more strongly loaded on PC2. Loadings on PC1 indicate a negative relationship between SRL and RTD. On PC2, the negative loadings for SRL and RD suggest a correlation between these two traits (ρ = -0.766, p < 0.001). A permutational multivariate analysis of variance (perMANOVA: $F_{2,152} = 8.065$, $p < 0.001$) revealed significant differences in root traits among the groups. Post hoc pairwise comparisons confirmed that all group differences were significant (p < 0.001).

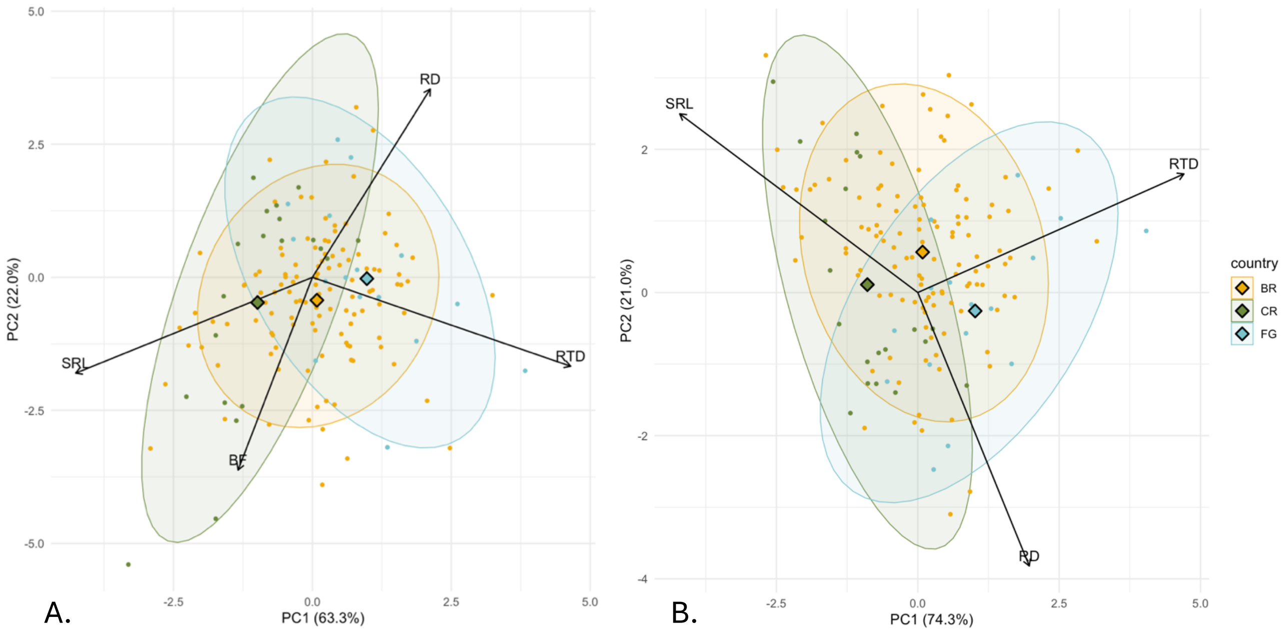

Figure 9. Phylogenetic principal component analysis. A) is with BF included, B) is without BF. Each dot represents one species from a different country: orange is Brazil green is Costa Rica, blue is French Guiana. Diamond represents the centroid of the species of each country. Ellipses show the 95% confidence intervals per country. For trait abbreviations see table 1.

3.3. Tropical trees in the worldwide RES

At the global scale, PC1 explained 76.0% of the variation in root traits, while PC2 accounted for 18.7% of the variation (Fig. 10). SRL loaded strongly and negatively on PC1, whereas RD showed a strong positive loading on PC1, indicate a negative relationship between SRL and RD (ρ = -0.733, p < 0.001). This suggests that species with thicker roots (high RD) tend to have lower SRL, while species with thinner roots exhibit higher SRL. RTD was more strongly associated with PC2, suggesting that variation in RTD is perpendicular to variation on PC1. A weak but significant negative correlation was also observed between RTD and both SRL (ρ = -0.235, p < 0.001) and RD (ρ = -0.208, p < 0.001).

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Species from tropical forests clustered higher along PC1 and PC2 compared to species from temperate, continental, and subtropical forests, but showed lower values on PC2 compared to Mediterranean species (Fig. 10). This pattern suggests that tropical forest species tend to exhibit, on average, higher RD and RTD values than species from colder biomes, but have lower RTD values than Mediterranean species. Additionally, the shape of the ellipses representing 95% confidence intervals, indicates different patterns of trait variation across biomes. The tropical forest ellipse is elongated along PC2, reflecting greater variability in RTD within this biome, whereas the ellipse for temperate species is more aligned with PC1, indicating greater variation in SRL and RD.

A permutational multivariate analysis of variance (perMANOVA: $F_{4,594} = 35.486$, $p < 0.001$) showed significant differences in root traits across biomes. Pairwise post hoc comparisons confirmed that all biome pairs differed significantly ($p < 0.05$), except for temperate vs. continental forests ($p = 0.864$) and subtropical vs. continental forests (p.adj = 0.31).

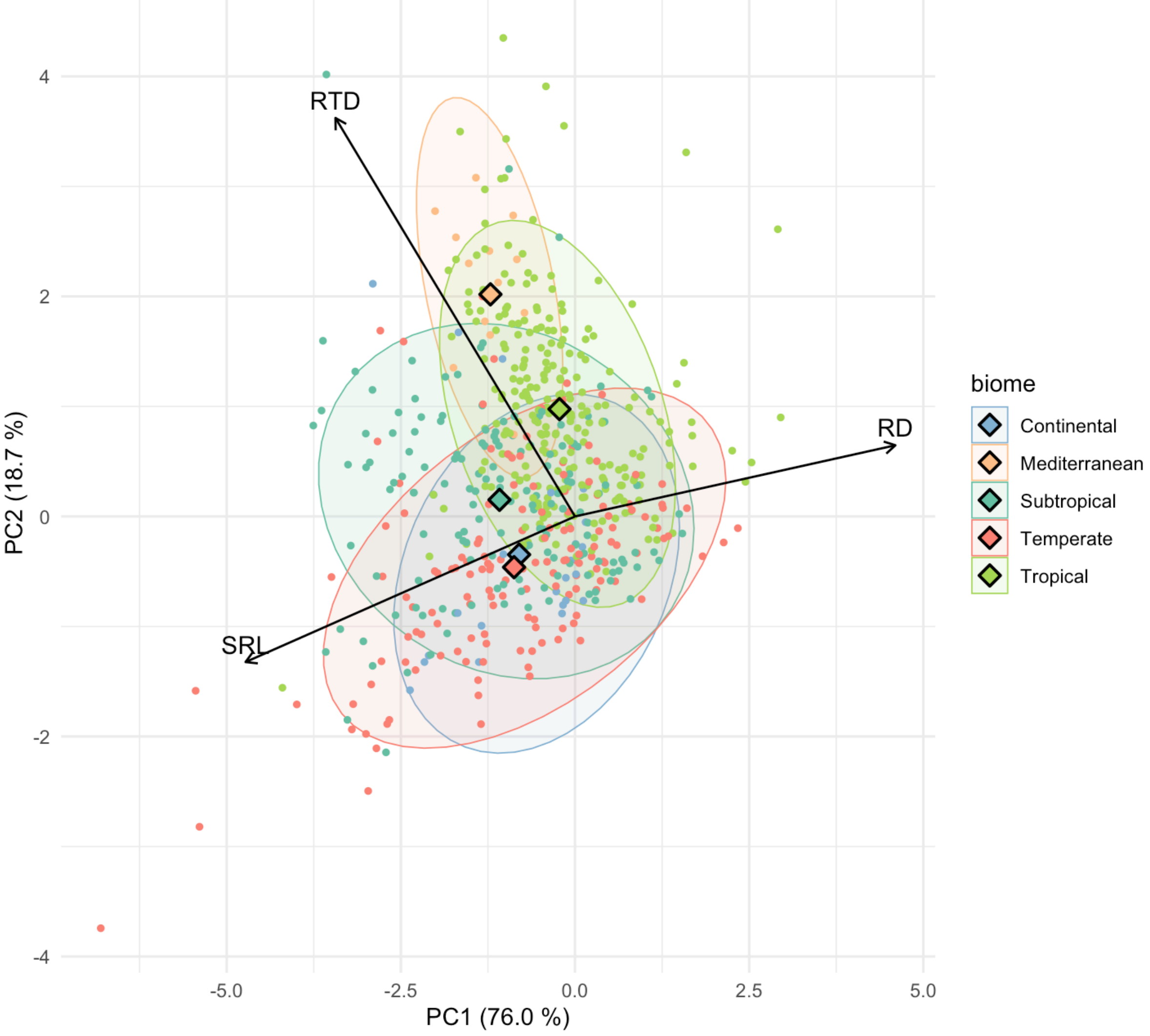

Figure 10. Phylogenetic PCA of tree species grouped in 5 biomes. Each dot represents one species. Diamond represents the centroid of the species per biome. Ellipses show the 95% confidence intervals per biome. For trait abbreviations see Table 1.

4. Discussion & Conclusion

Among the species in Santa Rosa National Park, my findings suggested that a tradeoff exists between specific root length (SRL) and root diameter (RD) and that root tissue density (RTD) is perpendicular to this tradeoff, indicating the existence of a multidimensional root economic space (RES). When I looked at the differences in root traits between dry and wet forests in the Neotropics, I did not find significant differences between the dry forest in Costa Rica and both wet forests in Brazil and French Guiana, except for RTD which was significantly lower among the species in Costa Rica. Finally, on the global root economic space, my findings suggested that tropical forests occupied a different position than temperate forests. Specifically, tropical trees have on average a higher RD and RTD in comparison to temperate forests.

4.1. Root traits in Santa Rosa National Park

The results showed a strong negative relation between specific root length (SRL) and root diameter (RD), suggesting a tradeoff between these traits. Similar tradeoffs have been observed in other tropical forests (Cusack et al., 2021; Kong et al., 2014; Valverde-Barrantes et al., 2021; Vleminckx et al., 2021), and across biomes worldwide (Bergmann et al., 2020; Freschet et al., 2017; Ma et al., 2018). This tradeoff was expected, because tree species face fundamental physiological constraints when allocating resources: they can either invest biomass in a higher SRL or in a greater RD. Mathematically, SRL is a function of root length per unit dry mass, which inherently depends on RD: $SRL = 4 / (\pi * RD^2 * RTD)$ (Bergmann et al., 2020; Ostonen et al., 2007).

Bergmann et al. (2020) referred to the SRL – RD axis as the collaboration gradient. Previous studies have linked high SRL and low RD to efficient soil exploration (Cusack et al., 2021; Freschet, et al., 2021) and high RD with a high mycorrhizal colonization rate (Kong et al., 2014; Ma et al., 2018). This is because thicker roots tend to have a larger root cortex, providing a greater potential habitat for fungal colonization (Brundrett, 2002). However, the SRL-RD gradient needs to be treated with caution when using it as a proxy for fungal collaboration, because thicker roots do not necessarily indicate a symbiotic relationship between the tree and mycorrhizal fungi, it might just mean a lower SRL (Zhao et al., 2024). Initially, my study aimed to include mycorrhizal colonization data, but we were unable to conduct the necessary qPCR analysis within our timeframe. Therefore, I cannot confirm that species with thicker fine roots had a larger mycorrhizal association.

In addition to the collaboration gradient, the RES framework includes a second axis that constitutes a tradeoff between RTD, and root N. The results showed that root tissue density (RTD) was almost perpendicular to both RD and SRL, suggesting that RTD is on a separate gradient. While I hypothesized RTD would be independent, I found a negative relation between RTD and RD and no relation between SRL and RTD. The negative relationship between RD and RTD may be explained by thicker roots often having a larger cortex (Kong et al., 2019), which has a lower tissue density than the stele (Bergmann et al., 2020). Yet, many studies found a negative relation between RTD and SRL (Laughlin et al., 2021; Valverde-Barrantes et al., 2021; Vleminckx et al., 2021; Weemstra et al., 2023), and a slight positive relation between RTD and RD (Kong et al., 2014). Other studies found a nonlinear and negative relation between RD and RTD (Kong et al., 2019) and a weak and ambiguous relation between SRL and RTD (Kong et al., 2019; Kramer-Walter et al., 2016). The different combinations of RTD and SRL/RD across empirical studies suggested that RTD occupies a separate axis from SRL and RD, which led to the framework of the RES with two dimensions (Bergmann et al., 2020; Zhang et al., 2024b). Therefore, although a negative relation between RTD and RD was observed, the RES seems to exist among the species in Santa Rosa National Park.

4.2. Root traits in Neotropics

The results showed variations in root traits among the three Neotropical countries (i.e. dry: Costa Rica; wet: Brazil and French Guiana). However, I expected that the difference in precipitation between these countries would select for thinner and denser roots, but I found no distinction in root traits between dry and both wet forests, apart from RTD which was lower among the species in Costa Rica.

A possible explanation is that fine roots are influenced by other abiotic factors, such as soil minerals, than by rainfall alone. Extensive research has pointed to phosphorus (P) as a shaping factor in root trait variation in the tropics (Cunha et al., 2022; Cusack et al., 2021; Ushio et al., 2015). Since P was not included in the current analysis, the potential impact is unknown. Therefore, I looked at the average soil C/N as a general indicator of the soil properties. Costa Rica had higher soil fertility (lower average C/N ratio) than Brazil and French Guiana. Contrary to my expectations, I did not find significant differences in SRL and RD between Costa Rica and Brazil. Possibly other unmeasured soil properties, such as acidity, texture and sand content could have been more important factors for shaping fine roots (Huaraca Huasco et al., 2021). Cusack et al. (2024) emphasize that soil and environmental factors do not operate in isolation but interact in complex ways to shape the diversity of root strategies in tropical forests. For example, phosphorus, nitrogen, and other nutrients may influence root morphology differently depending on the specific combination of local rainfall patterns and microbial communities. Therefore, the differences in belowground strategies between these regions cannot be explained solely by broad metrics such as annual precipitation or C/N ratios.

Moreover, species can differ significantly across regions, which could explain the variation in root traits between the two wet forests (i.e. Brazil and French Guiana). Fine roots are known to exhibit high plasticity (Ostonen et al., 2013), allowing trees to adjust root traits in response to varying climatic and environmental conditions. A handful of the same tree species were investigated in both countries, yet their root traits varied significantly. For example: *Licania micrantha* had an average SRL of 13.2 m g^{-1} in Brazil and 3.7 m g^{-1} in French Guyana.

To gain a more accurate understanding of the differences in root traits between dry and wet tropical forests, future comparative studies should incorporate detailed and localized soil characteristics, such as phosphorus content, pH levels, and mineral composition, to capture the nuanced interactions between soil conditions and root traits.

The only trait that clearly differed between wet and dry forests was RTD, which, contrary to my hypothesis, was lower in Costa Rica than in Brazil and French Guiana. I expected dry forest species to develop roots with higher RTD to protect against long periods of drought, because denser roots might be better protected against cavitation (Laughlin et al., 2021). However, Freschet et al. (2017) suggested that in drier environments, where soil moisture changes a lot over a year, species might grow roots with higher N and lower density to compete with other organisms when water and nutrients are suddenly available. During the dry season in Costa Rica, water availability is extremely limited (Swenson et al., 2020), which may increase the need to maximize resource acquisition in the wet season before conditions are unfavorable again. However, more research is needed to better understand the relation between RTD and seasonality in dry tropical forests.

Additionally, The PCA in Figure 10 suggests that dry tropical tree species vary more along the collaboration gradient (RD – SRL/BF) than along the RTD gradient. This pattern is influenced by the larger sample size of Brazilian species (n = 119) compared to those from Costa Rica (n = 20) and French Guiana (n = 16). I also observed a slight positive correlation between SRL and BF, which aligns with what I expected, because both root traits indicate the tree's capacity for soil exploration (Freschet et al., 2021).

4.3. Tropical trees in the worldwide RES

My analyses among root traits across different biomes indicated that tropical species generally exhibit higher root tissue density (RTD) and root diameter (RD) compared to species from colder climates. These findings align with previous studies comparing root traits across biomes (Guerrero-Ramírez et al., 2024; Ma et al., 2018). The higher mean RTD in tropical forests suggests that it might be beneficial for tropical species to invest in conservative roots (Bergmann et al., 2020) with a longer lifespan (Freschet et al., 2017). Laughlin et al. (2021) found that trees in colder climates tend to have a lower RTD, which is more efficient for fast nutrient uptake (Zhao et al., 2024). This can be explained by the freezing temperatures in winters in temperate and continental biomes. During the winter fine roots might partly freeze, more need for faster growth during the limited growing season.

Many studies found that roots in tropical regions have a significant larger RD than temperate species (Freschet et al., 2017; Gu et al., 2014; Guerrero-Ramírez et al., 2024; Ma et al., 2018; Wang et al., 2022). The thicker fine roots in tropical forests may indicate a stronger association with arbuscular mycorrhizal fungi. This might be partially explained by the evolutionary history of tropical tree species: genetically older lineages, such as those found in tropical forests, tend to have stronger associations with arbuscular mycorrhizal fungi (Ma et al., 2018).

Most global studies (Bergmann et al., 2020; Freschet et al., 2017; Guerrero-Ramírez et al., 2024; Ma et al., 2018) retrieve their data partly from the same databases (GrooT or

FRED), which probably contributed to the similarity in the observed patterns (higher RTD and RD in the tropics). However, in my analysis, I added additional data from 155 Neotropical species, which were not yet included in the GrooT database, resulting in a dataset in which half of the data came from tropical species. Thus, these findings further strengthen our understanding of global patterns in root traits.

Interestingly, tropical species appear to show greater variation along the density axis (PC2) than along the SRL - RD axis. This suggests that tropical trees may adopt a wider range of strategies on the conservation axes. Perhaps this variation could also be explained by the longer growing seasons in tropical forests and the high availability of photosynthetic carbon (Cusack et al., 2021). As a consequence, some species might gain a competitive advantage through less dense roots to allow more efficient nutrient uptake (Zhao et al., 2024), whereas others may invest in denser roots to enhance endurance and protection. These different strategies might explain the variation that is observed in RTD.

4.4. Conclusion: a global root economic space

This study investigated the morphological root traits of tree species from (Neo)tropical forests. Root traits were sampled from trees in a dry tropical forest in Costa Rica, an ecosystem that is underrepresented in root research. The findings indicate that these species have evolved to develop roots along a gradient between root diameter and specific root length, and a somewhat separate axis for root tissue density (RTD). This suggests the existence of the root economic space among dry tropical forest species.

Root traits differed among three Neotropical countries (Costa Rica, Brazil, and French Guiana), but – in contrast to my hypothesis – these differences were not related to variation in annual rainfall, nor in soil C:N ratio. Possibly, other unmeasured nutrients, like phosphorus, are more important, as well as the interactions with other biotic and abiotic factors. Finally, on the global root economic space, I found that tropical forests, on average, have a higher root diameter and greater tissue density compared to biomes in colder regions.

Overall, (despite the missing root nitrogen data) this study provides evidence for the existence of the root economic space (RES) among tree species from Neotropical countries and across tree species worldwide. In line with the theory of the RES, I found that the tradeoff between SRL and RD explains most of the variation among the species in the different datasets. Thereby this research shows that tropical trees make similar tradeoffs as temperate trees, but that tropical trees, on average, tend to exhibit higher RD and RTD values than species from subtropical, temperate and continental biomes. This suggests that tropical tree species might collaborate more with mycorrhiza.

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View over Santa Rosa National Park

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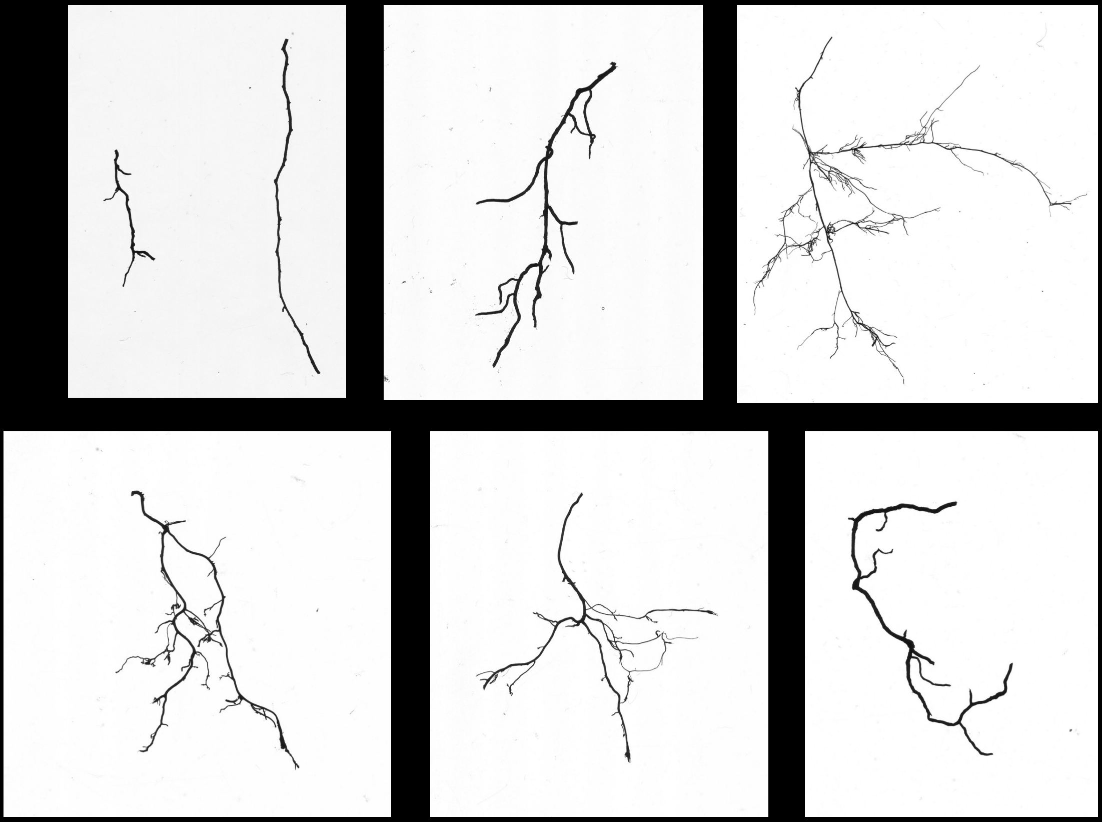
7. Appendixes

Appendix 1: Scans per species

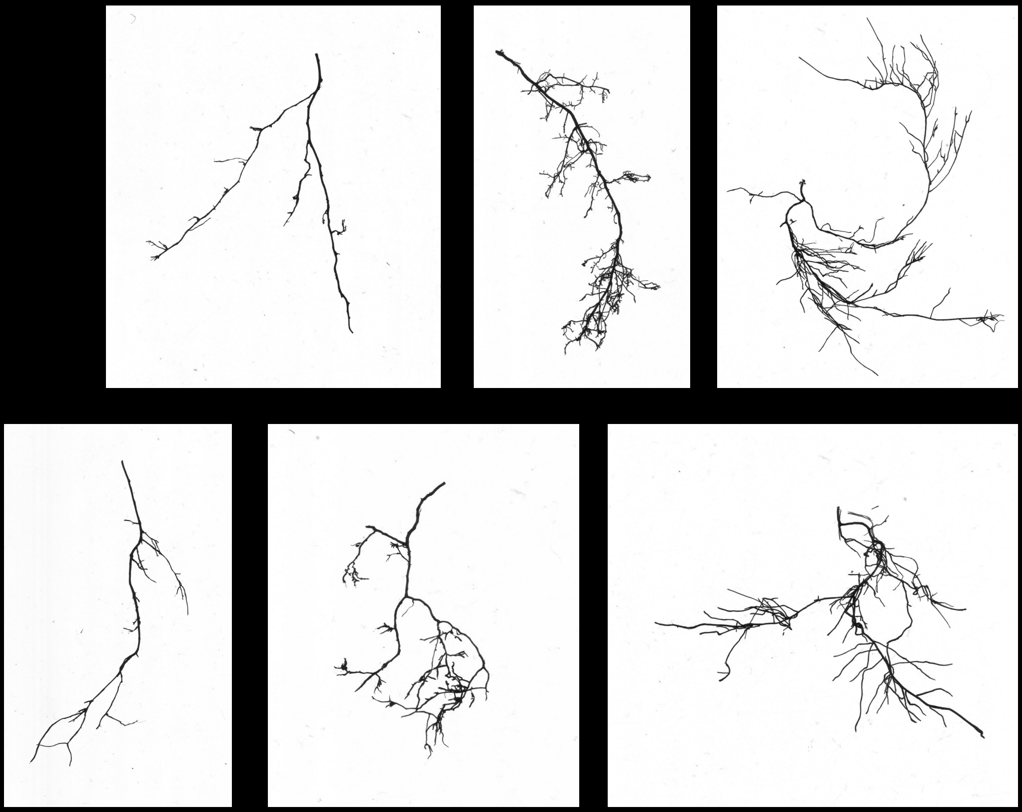
Annona purpurea
Family: Annonaceae
Clade: Magnoliids

Observation: roots are dark black.



Astronium graveolens
Family: Anacardiaceae
Clade: Rosids

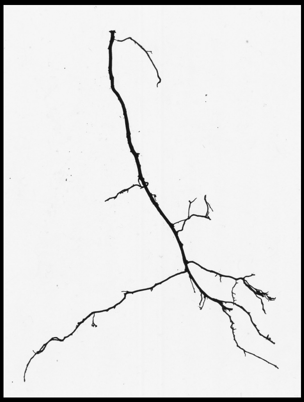
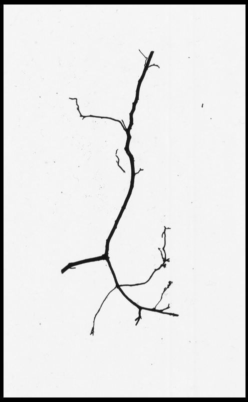
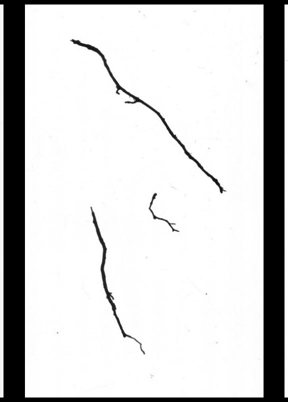
Observation: roots grow up from deeper in the soil and stay superficial. White fungi in soil.



Bursera simaruba
Family: Burseraceae
Clade: Rosids

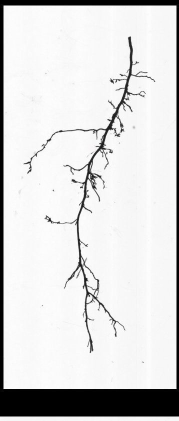
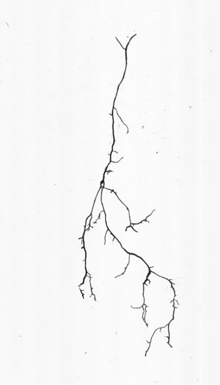
Observation: deep roots, roots are very red. Tree makes long cable like roots. Fine roots were hard to find.

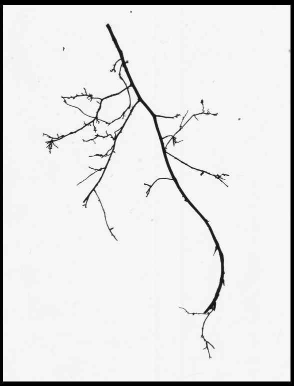
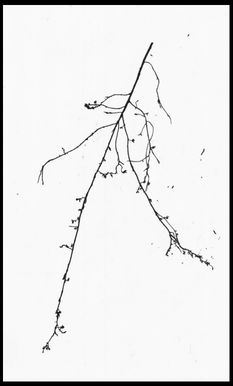


Brosimum alicastrum
Family: Moraceae
Clade: Rosids

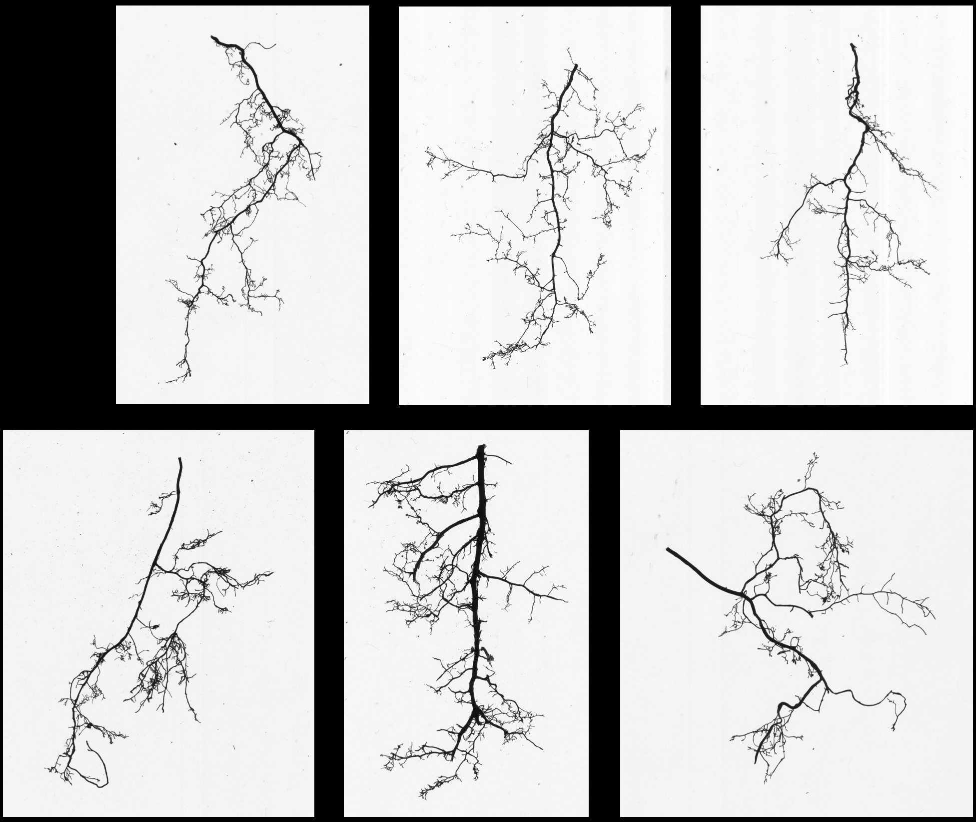
Observation: fine roots are dark red and strongly pigmented. When soaked in chloride, the chloride becomes green.

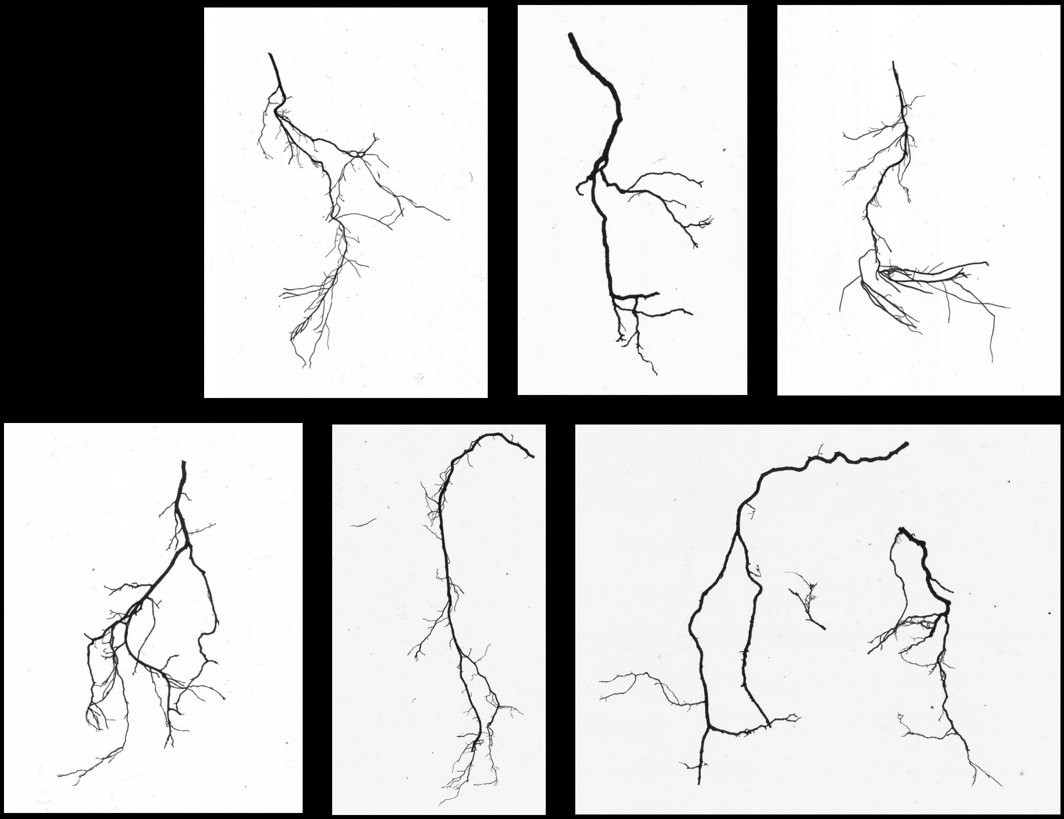

Calycophyllum candidissimum
Family: Rubiaceae
Clade: Rosids

Observation: tiny roots surrounding bigger roots, fine roots are highly branched.



Casearia corymbose
Family: Salicaceae
Clade: Rosids

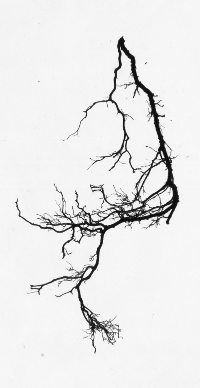
Observation: easy to sample, superficial roots.

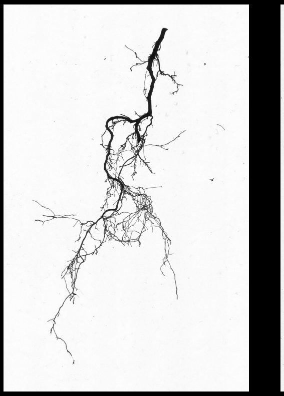
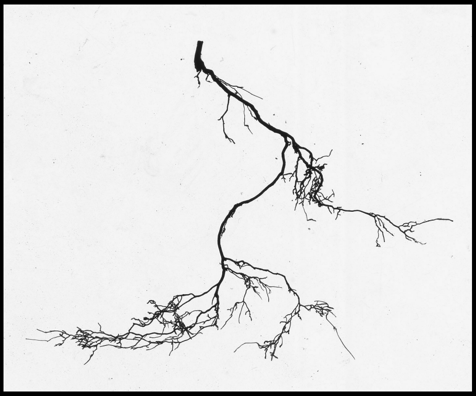


Exostema mexicanum

Family: Rubiaceae
Clade: Rosids

Observation: roots break easily. Many small roots directly on the stem, coming from the 'armpits' of the larger tree roots. Root tips are light.

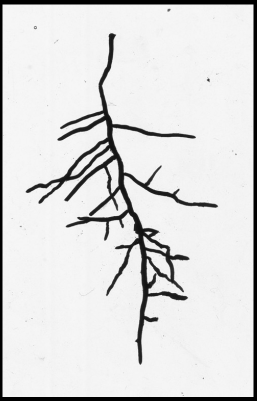
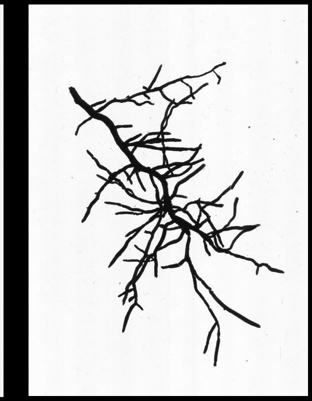
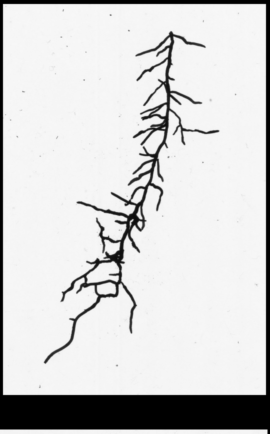
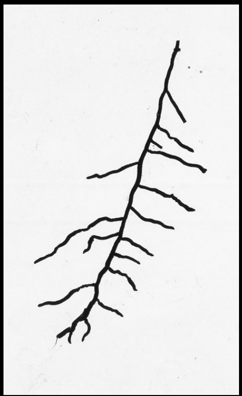
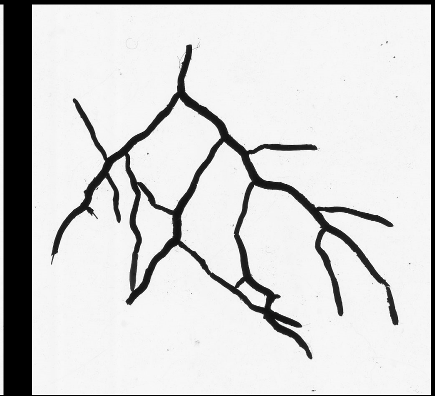
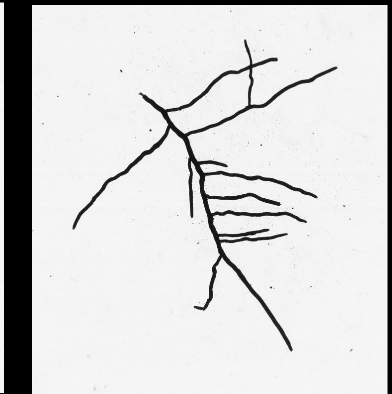



Guarea glabra

Family: Meliaceae
Clade: Rosids

Observation: easy sample, very similar to Ocotea veraguensis *but with dark red root tips.*

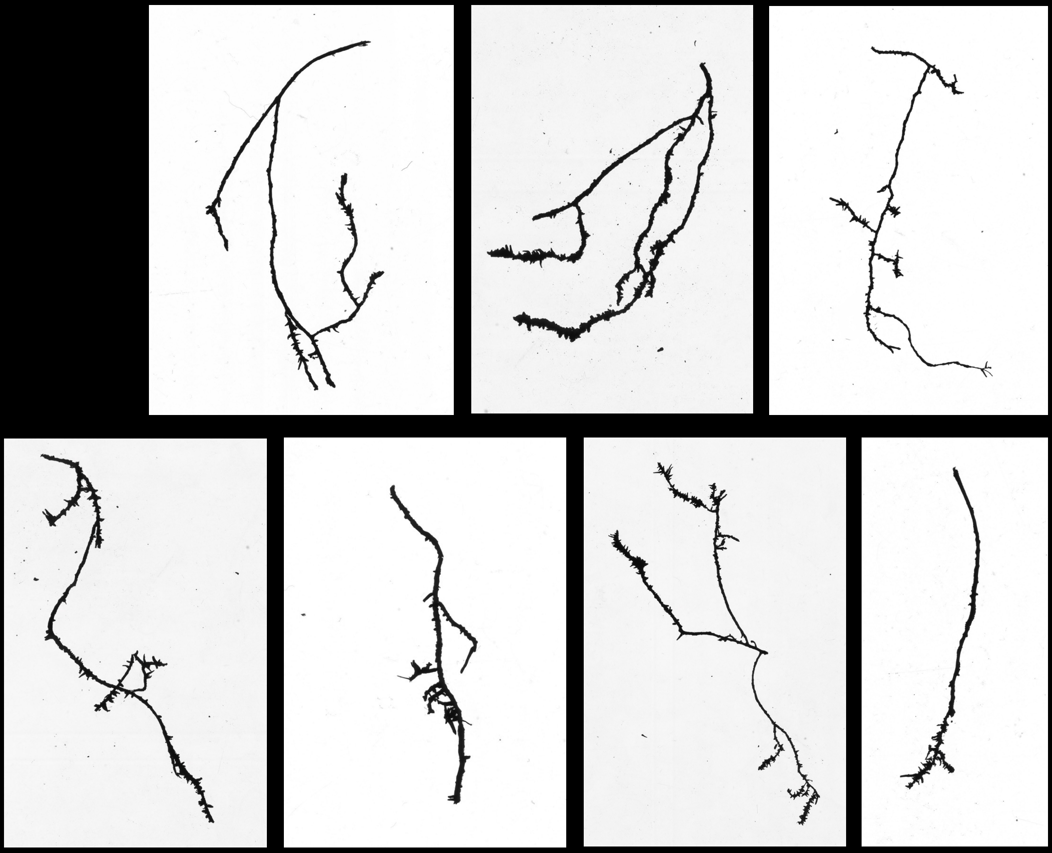







Guettarda macrosperma
Family: Rubiaceae
Clade: Rosids

Observation: fine roots grow from the armpit of the larger tree root, also smells like armpit. Similar to Exostema mexicanum, *but* Exostema mexicanum, *has white root tips.*

Hymenaea courbaril
Family: Fabaceae
Clade: Rosids

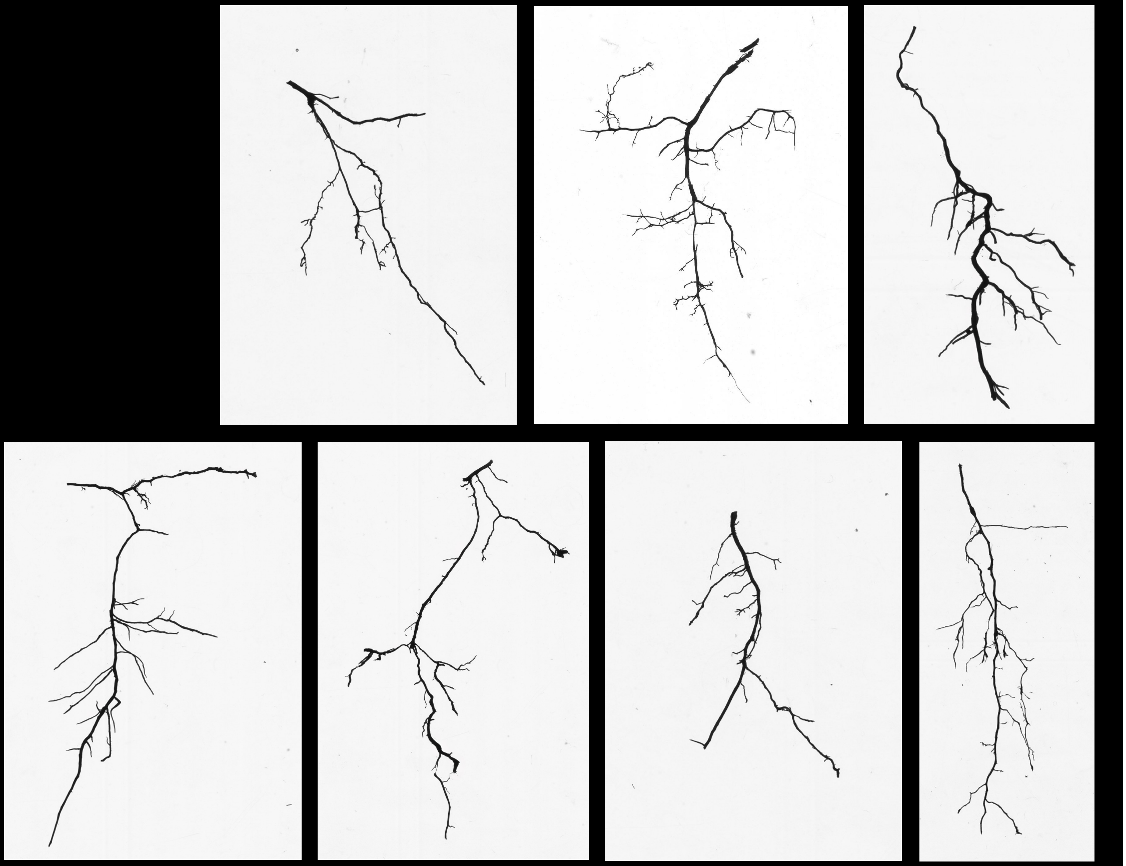
Observation: roots have many small spikes, which break off easily. Fine roots are noticeably thick.



Licania arborea

Family: Chrysobalanaceae
Clade: Rosids

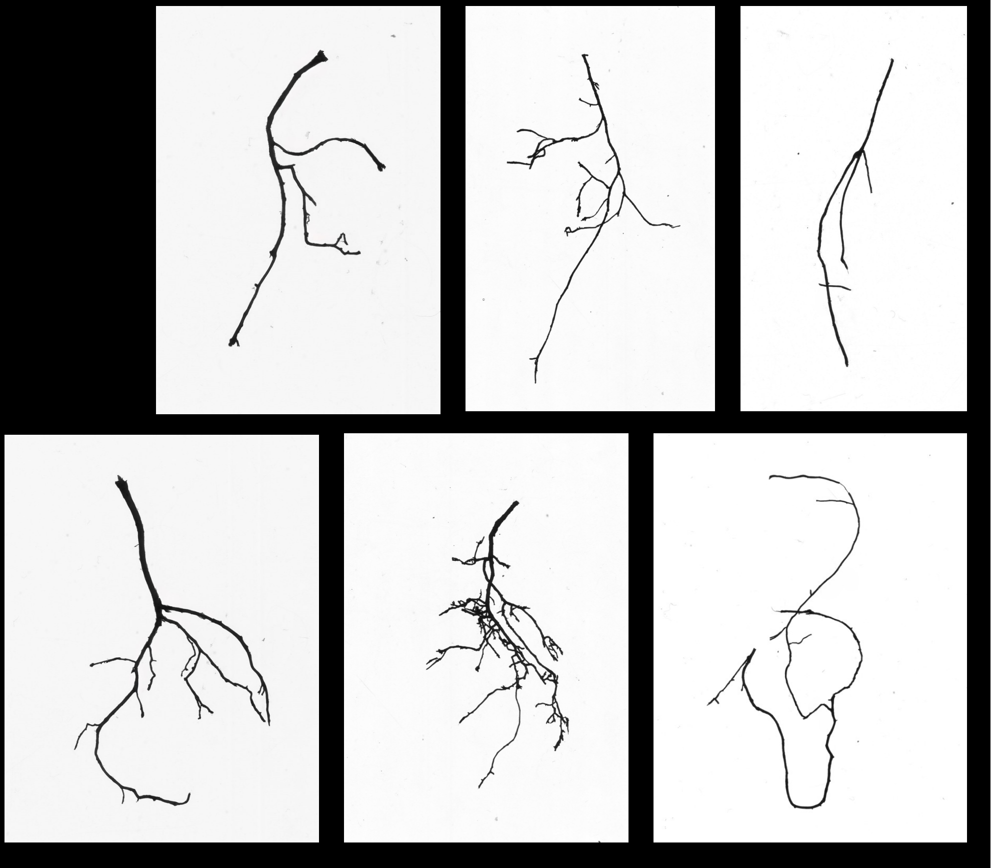
Observation: roots are spongy and release air on the scans.



Lonchocarpus minimiflorus

Family: Fabaceae
Clade: Rosids

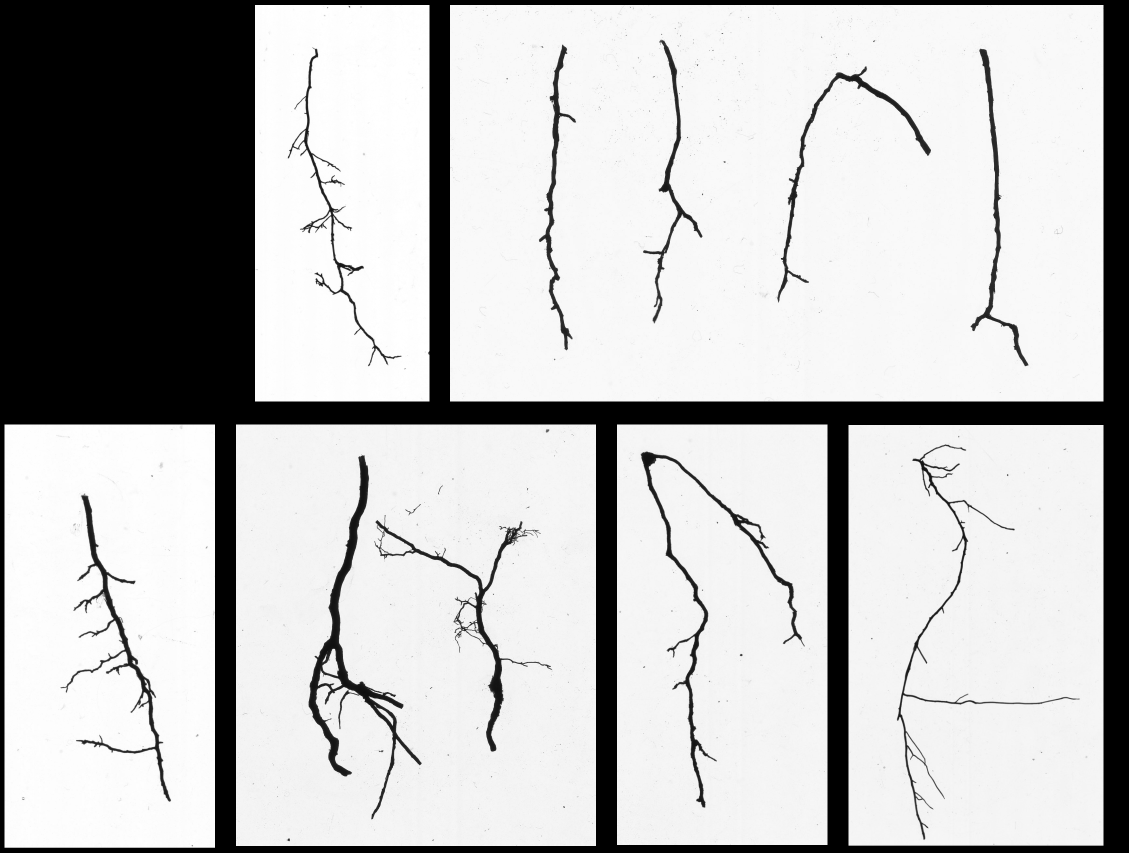
Observation: roots are white, sometimes abruptly splitting in smaller roots.



Manilkara chicle

Family: Sapotaceae
Clade: Asterids

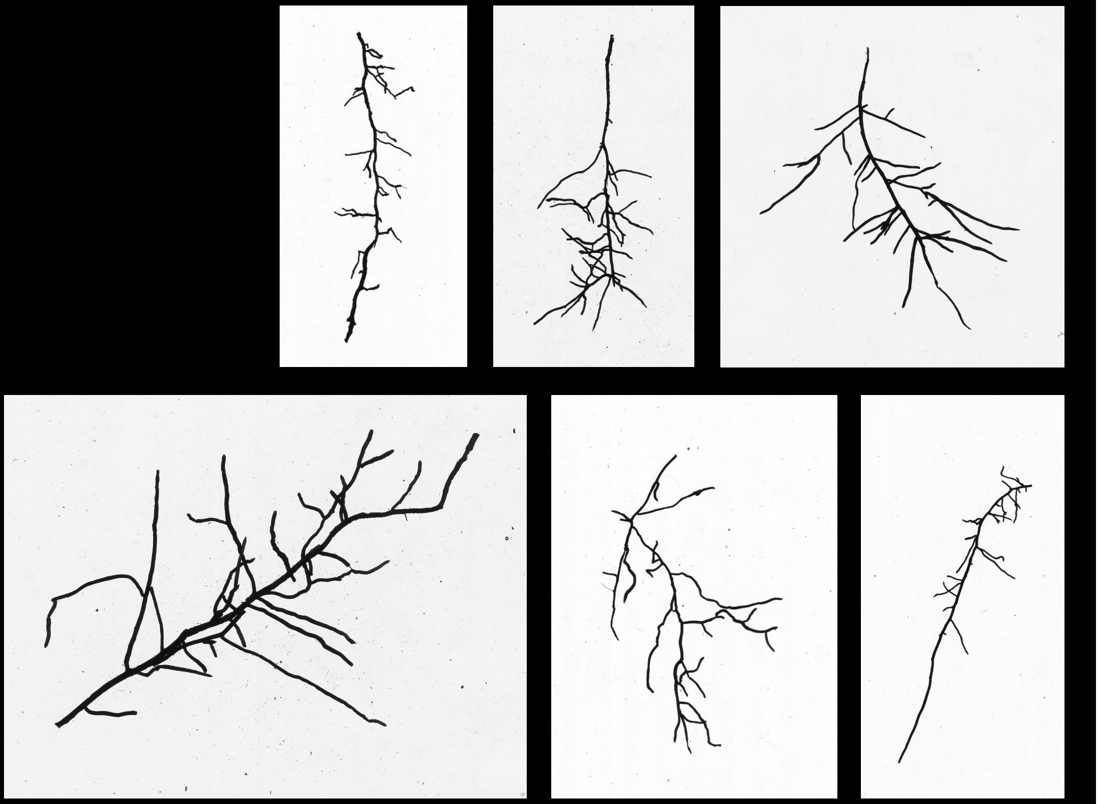
Observation: some fine roots have small black parts.



Ocotea veraguensis

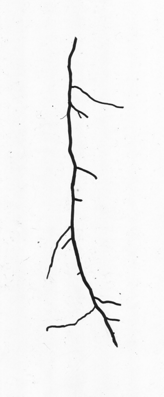
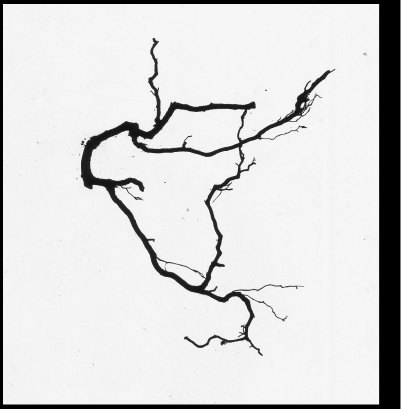
Family: Lauraceae
Clade: Magnoliids

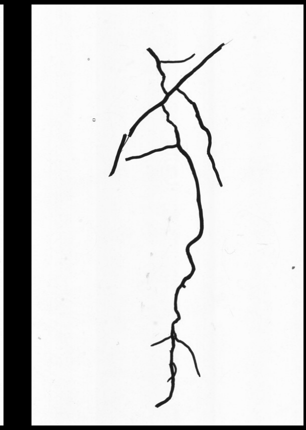
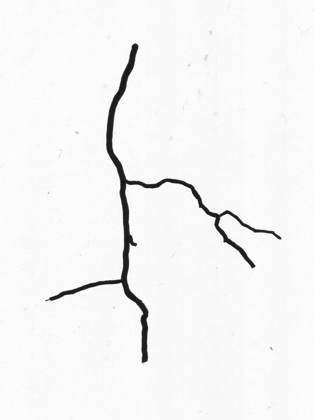
Observation: easy sample. Thicker black roots with light tips. Roots smell like pine needles.



Sapranthus palanga
Family: Annonaceae
Clade: Magnoliids

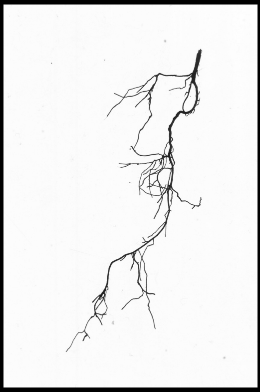
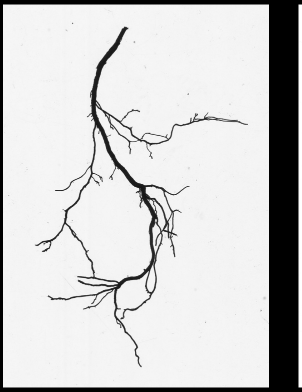
Observation: roots are dark black, and the tree makes long cable like roots.

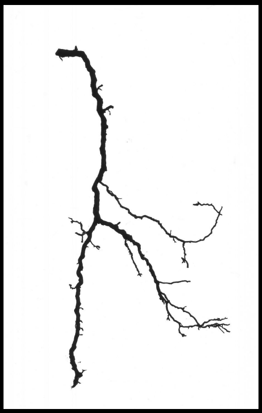




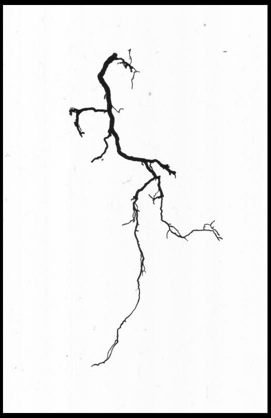



Semialarium mexicanum
Family: Celastraceae
Clade: Rosids

Observation: yellow coral like roots. Many small roots connected to a little larger one. They are quite soft and remind of liquorice.

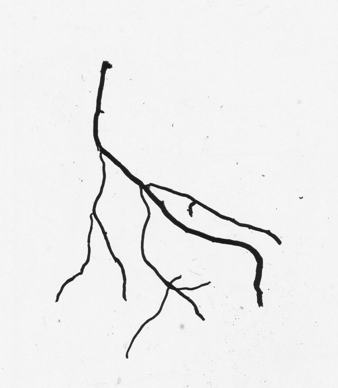



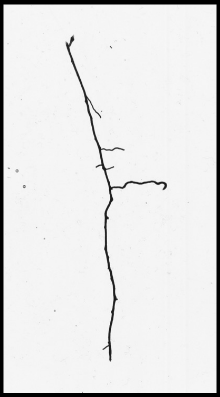


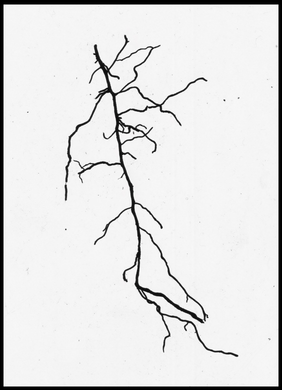


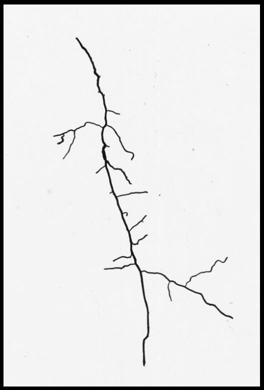
Sebastiania panamensis
Family: Euphorbiaceae
Clade: Rosids

Observation: when scratched, coarse roots are red. Fine roots are less red. When roots break, they release milky substance.

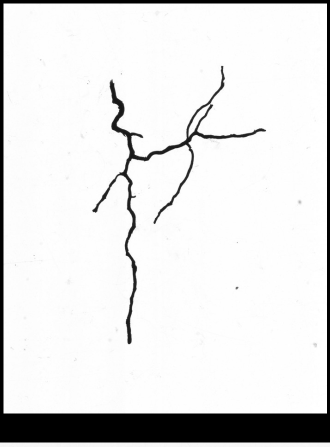


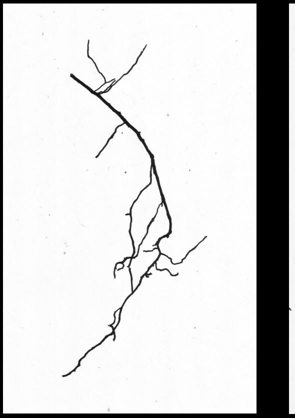


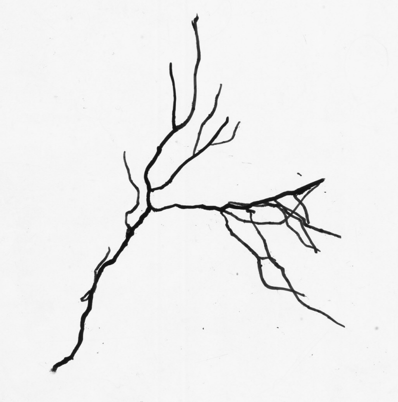


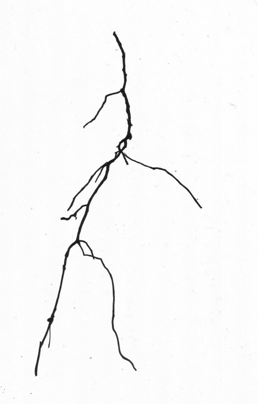


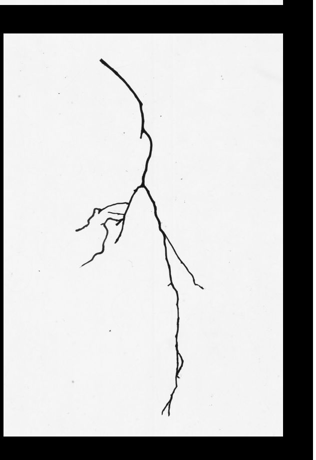
Sideroxylon capiri
Family: Sapotaceae
Clade: Asterids





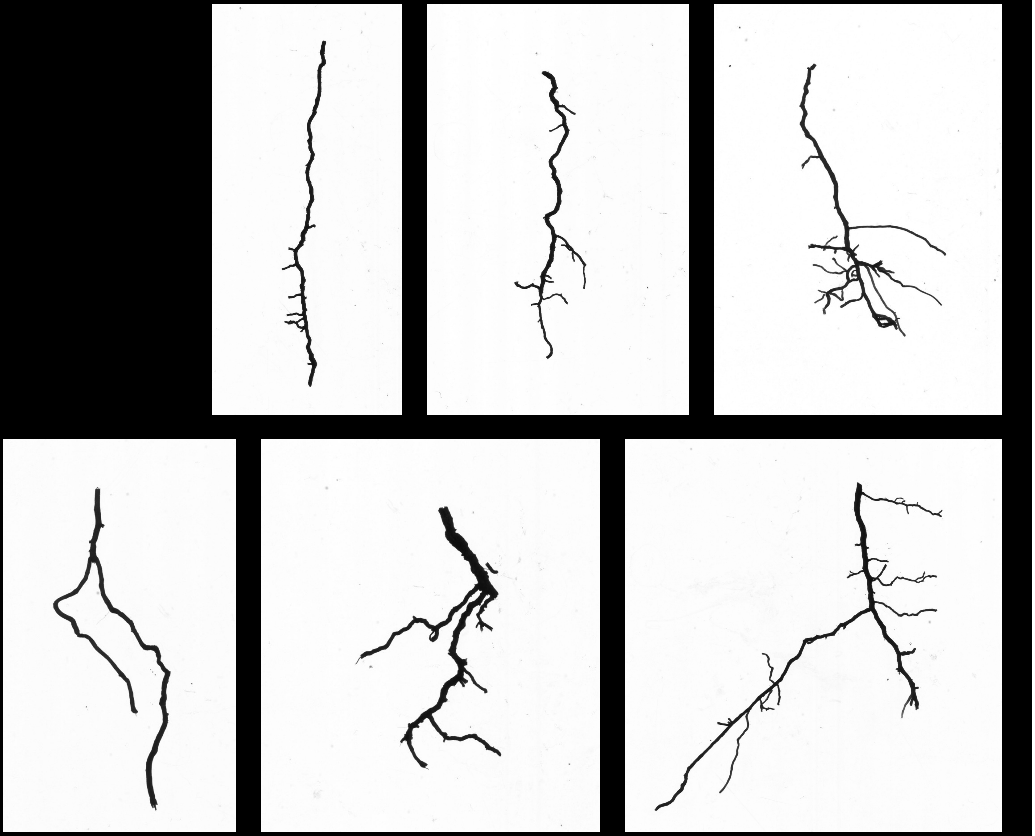






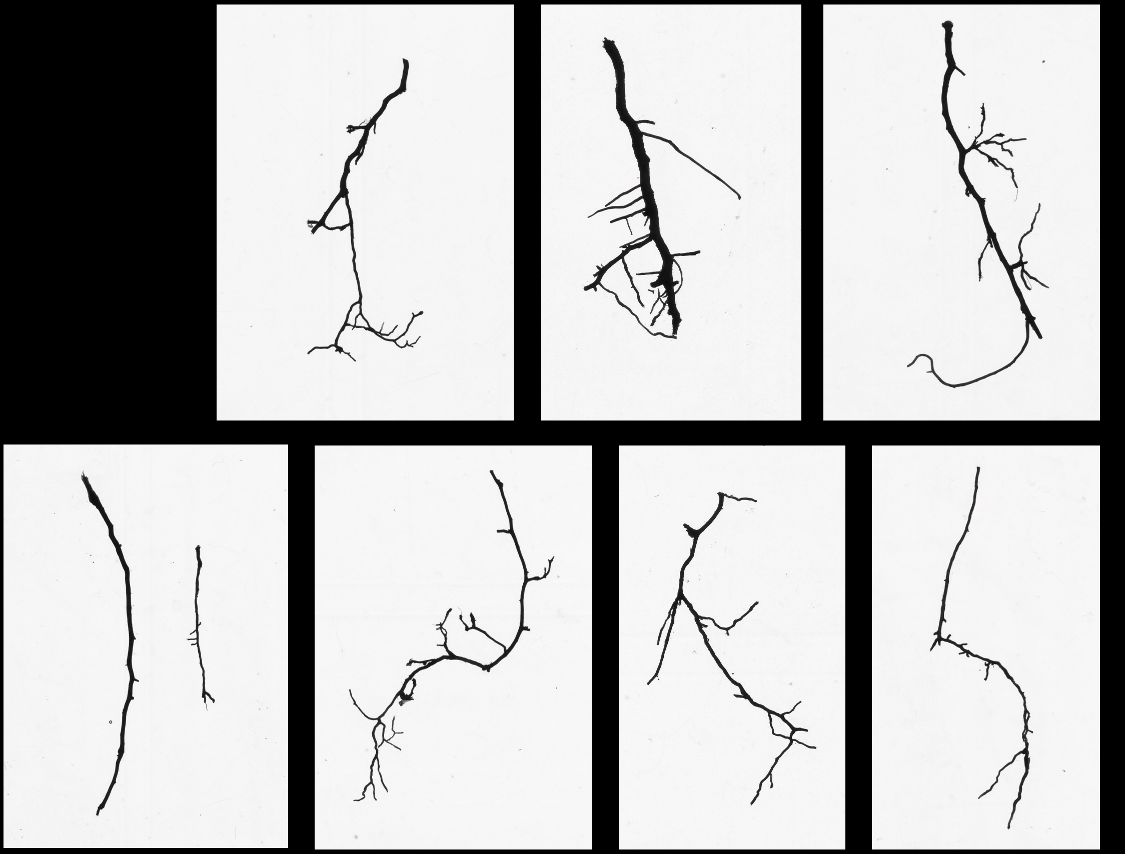
Stemmadenia obovate
Family: Apocynaceae
Clade: Asterids

Observation: roots break easily and thick spongy layer. Fine roots float and release air on the scan.



Swartzia cubensis
Family: Fabaceae
Clade: Rosids

Observation: roots are very deep and make root nodules.



Appendix 2: Phylogenetic trees

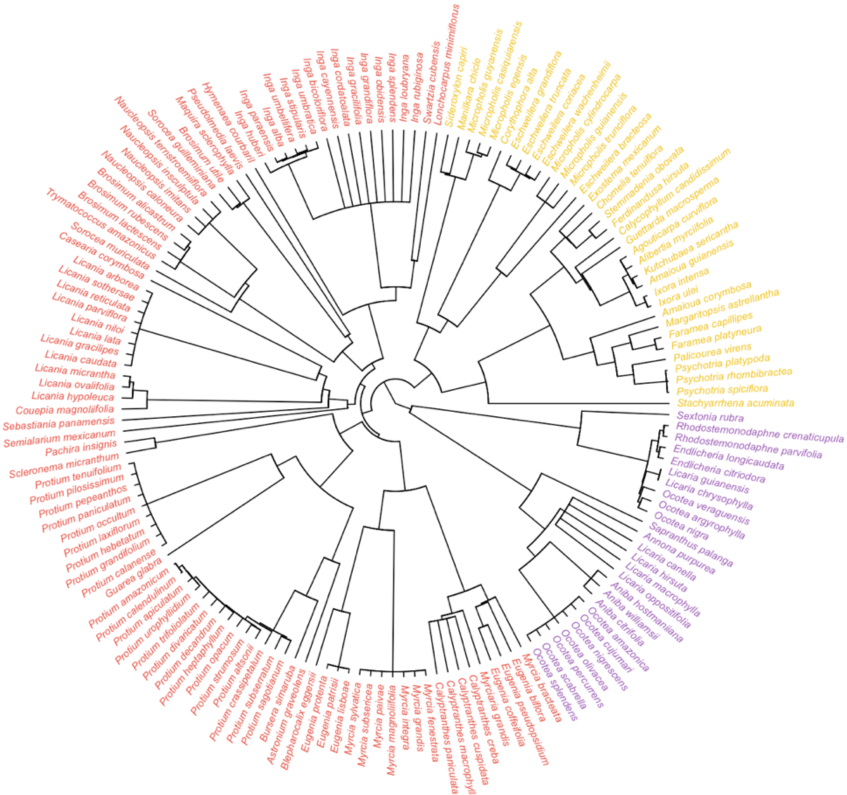


FIGURE 12. PHYLOGENETIC TREE OF THE 155 SPECIES FROM THREE NEOTROPICAL COUNTRIES, NAMES CORRESPOND WITH CLADES: RED NAMES ARE ROSIDS, YELLOW NAMES ARE ASTRIDS, URPLE NAMES ARE MAGNOLIIDS. GENERATED WITH PHYLOMAKER PACKAGE IN R (JIN & QIAN, 2022)

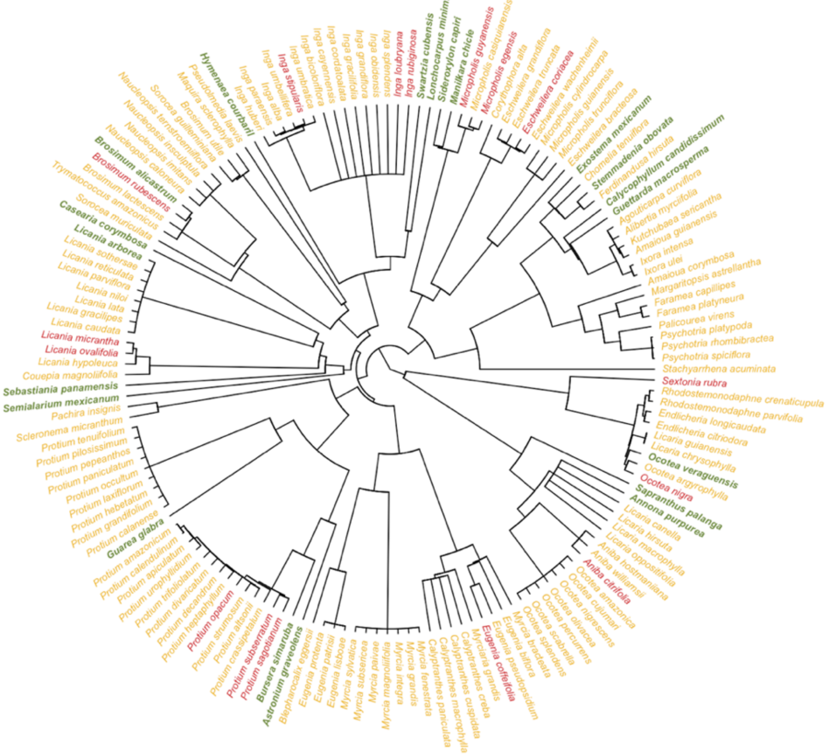


FIGURE 11. PHYLOGENETIC TREE OF THE 155 SPECIES FROM THREE NEOTROPICAL COUNTRIES: GREEN BINOMIALS ARE SPECIES FROM COSTA RICA, YELLOW NAMES ARE SPECIES FROM BRAZIL, GREE NAMES ARE SPECIES FROM FRENCH GUIANA. GENERATED WITH PHYLOMAKER PACKAGE IN R (JIN & QIAN, 2022).

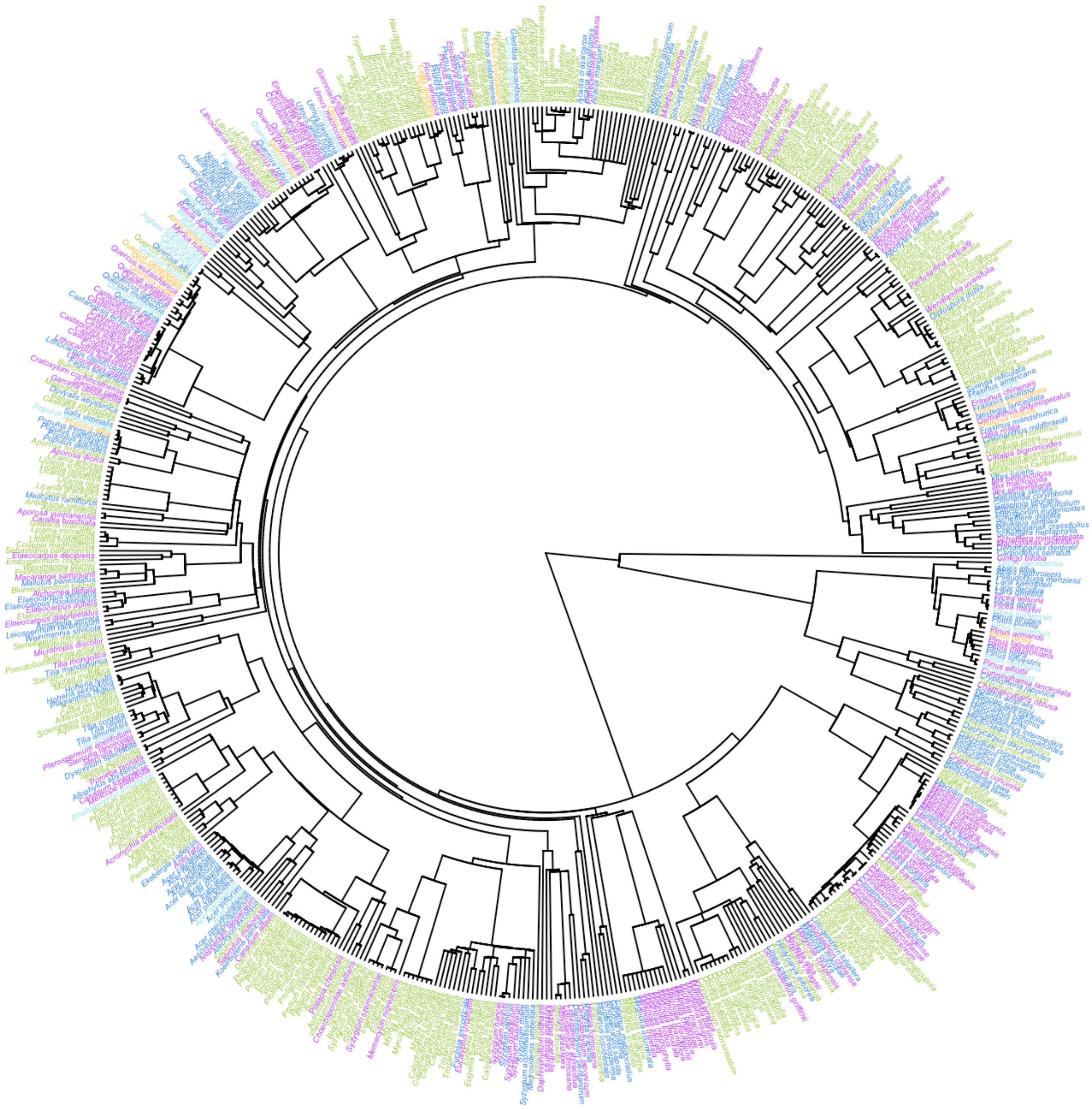


FIGURE 13. PHYLOGENETIC TREE OF THE 599 SPECIES FROM BIOMES WORLDWIDE, NAMES CORRESPOND WITH BIOMES: GREEN NAMES ARE TROPICAL BIOMES, PURPLE NAMES ARE SUBTROPICAL BIOME, BLUE NAMES ARE TEMPERATE BIOME, LIGHT BLUE NAMES ARE CONTINENTAL BIOME, YELLOW NAMES ARE MEDITERRANEAN BIOME. GENERATED WITH PHYLOMAKER PACKAGE IN R (JIN & QIAN, 2022)

Appendix 3: Boxplot of trees in Santa Rosa National Park

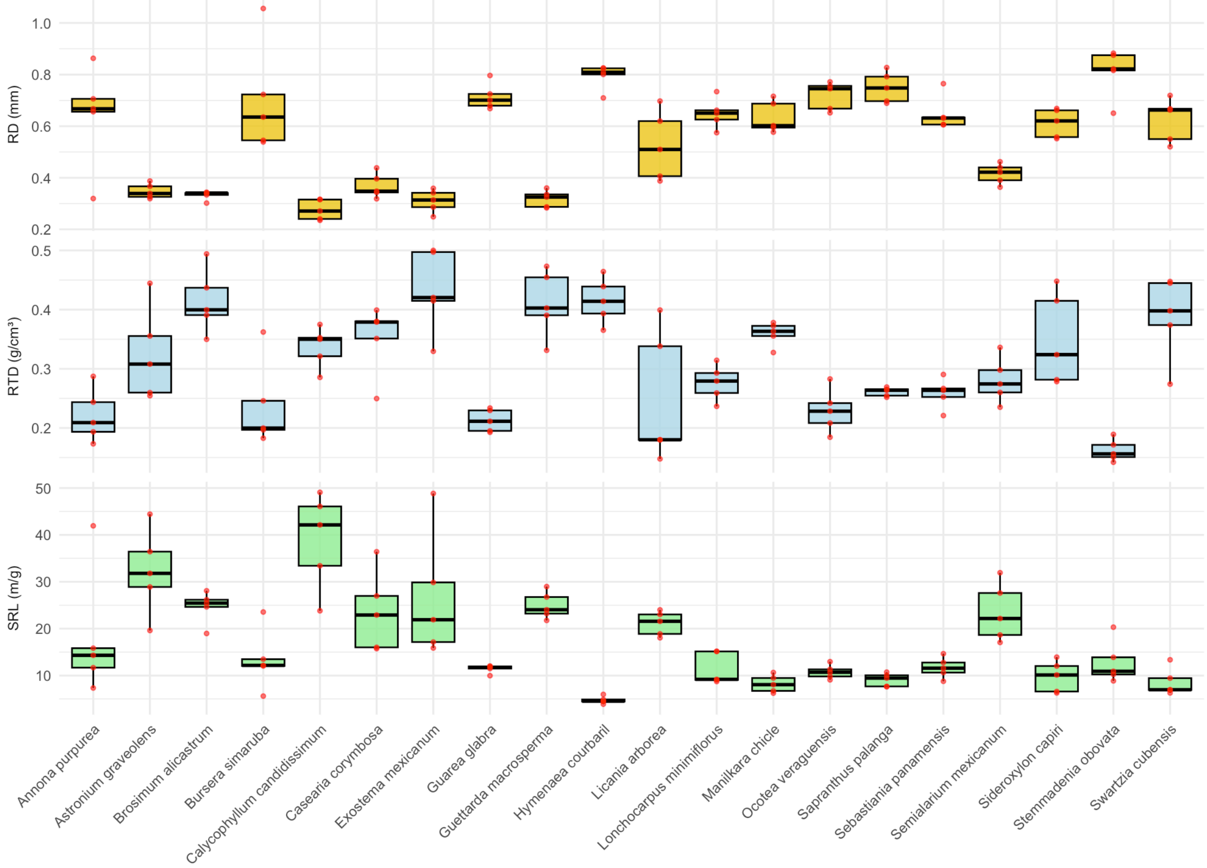


FIGURE 14. BOXPLOT OF ROOT TRAITS AMONG 20 SPECIES IN SANTA ROSA NATIONAL PARK. RED DOTS ARE INDIVIDUAL TREES, 5 TREES PER SPECIES. FOR ABBREVIATIONS, SEE TABLE 1.

Appendix 4: Loadings and correlations

TABLE 5. TRAIT LOADINGS PER DATASET, CORRESPONDING WITH THE THREE RESEARCH QUESTIONS

	Santa Rosa			Neotropics			Worldwide		
	PC1	*PC2*	*PC3*	*PC1*	*PC2*	*PC3*	*PC1*	*PC2*	*PC3*
RD	0.817	0.567	0.106	0.611	0.266	0.326	0.842	−0.324	0.432
SRL	0.890	−0.453	0.050	−1.000	0.003	0.000	−1.000	0.000	0.000
RTD	−0.983	−0.143	0.117	0.614	−0.095	−0.779	−0.481	0.812	0.331
BF				−0.294	−0.956	0.005			

TABLE 6. CORRELATIONS BETWEEN ROOT TRAITS PER DATASET, CORRESPONDING WITH THE THREE RESEARCH QUESTIONS. TESTED WITH SPEARMANS RANK CORRELATION TEST.

	Santa Rosa		Neotropics		Worldwide	
	rho	*P*	*rho*	*P*	*rho*	*P*
RD - SRL	-0.851	< 0.001	-0.766	<0.001	-0.777	< 0.001
RD - RTD	-0.472	< 0.001	-0.314	<0.001	-0.208	< 0.001
SRL - RTD	0.037	0.71	-0.230	0.004	-0.233	< 0.001

Appendix 5: The use of AI

For this thesis, I used the artificial intelligence (AI) platform ChatGPT. I am aware that ChatGPT is likely to making mistakes, so I never used it for tasks that I could not verify. I mainly used AI for coding, as I could easily check whether a code worked or not. I found it useful to have AI as a coding assistant, but I also learned not to overestimate the capacities. Often, the code did not entirely work, but I could use the AI outputs to improve my own codes. Prompts that I used for coding:

- *“Can you show me basic rules and concepts for coding in R?"*
- *"I want to make a phylogenetic tree using the PhyloMaker package. How should I structure the datasheet?"*
- *"How can I customize a ggplot?"*
- *"Why does this code give me an error?" (including the code)*
- *"My phylogenetic analysis does not work. Can you help me troubleshoot my phylogenetic PCA in phytools?" (including the code)*
- *"How can I color different biomes in my phylogenetic tree?"*

Besides coding, I used AI to improve my writing. I have been careful not to let the AI write anything, but only to review and refine what I had written. For example, I asked the AI to check my spelling and grammar. I sometimes asked it to improve the scientific language of a poorly phrased sentence, but I found that these suggestions often resulted in overly formal and pretentious language that did not feel authentic. In short, I asked the AI for corrections and selectively adopted the suggestions that I found useful. Prompts that I use for writing:

- *"How can I improve the flow of this sentence? Give three versions."*
- *"Can you improve the spelling and grammar in this paragraph? Highlight the changes in bold."*
- *"How can I improve the transition from paragraph X to paragraph Y?"*
- *"How can I say this more concisely?"*