

LANDSCAPE AND SOIL PROPERTIES AS PREDICTORS OF FINE ROOT TRAITS IN
THREE TROPICAL SPECIES

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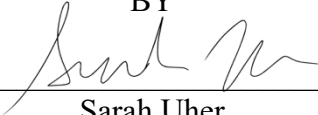
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BY




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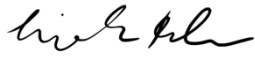
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Sarah Uher. Landscape and Soil Properties as Predictors of Fine Root Traits.

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Fine roots are important to plant physiology and nutrient cycling in ecosystems. Fine root function can be inferred from morphological and architectural traits, which vary across and within species and biomes. Environmental gradients have been studied in relation to fine root traits, however, our understanding of fine roots across soil and landscape gradients is limited. Here, we present fine root morphological and architectural data of three tropical tree species across landscape and soil gradients that expands our understanding of fine root plasticity across environmental gradients. We measured fine root specific root length (SRL), average diameter, root tissue density (RTD), and branching frequency of *Cordia alliodora*, *Handroanthus chrysanthus*, and *Iriartea deltoidea*. Soil pH, bulk density (BD), and moisture were measured to establish soil gradients. Measurements were taken in tropical pasture and primary forest landscapes to establish a landscape gradient. We found that landscape only predicted diameter, with slightly higher diameter in the pasture. Diameter and SRL were most consistently predicted by soil pH and BD. These interactions differed between species and landscape, potentially indicating different mechanisms of fine root determination within different species and landscape, although these results could be the effect of inadequate sampling size or variable consideration. Nonetheless, this study underscores our poor understanding of tropical fine roots, especially in landscape-change contexts and across soil property gradients. Future studies should investigate the role of soil nutrient fractions, organic matter content, and fungal symbionts in contributing to fine root plasticity across environmental gradients.

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INTRODUCTION

An important but understudied component of tropical rainforest productivity is roots. Fine roots are key to plant physiology, resource acquisition, nutrient cycling (Barley et al. 1970), and inputs to the belowground carbon sink (Cusack et al. 2018). Nutrient and water uptake is facilitated by fine roots (Barley et al. 1970), making fine roots critical to plant survival and growth. Since autotrophic plants are the foundation of food webs (Elser et al. 2000), fine roots are essential for the maintenance of trophic interactions. Furthermore, high rates of energy investment and turnover in fine roots account for a significant portion (~22-33%) of net primary productivity (NPP) in terrestrial ecosystems (Robinson et al., 2003; Vogt et al. 1986; Pregitzer 2008), representing a below-ground carbon input. The importance of fine roots highlights the need to characterize their traits and understand their function. Investigation of fine root traits will improve our understanding of plant survival strategies and their interaction with local landscapes and global nutrient cycles.

Traditionally, studies have defined acquisitive fine roots as having a diameter <2mm. However, fine root diameter differs between species (Pregitzer et al. 2002; Comas & Eissenstat 2009), rendering the 2mm cutoff as less accurate for interspecific studies. Instead, branching position of roots – quantified by root order, with the first order being the most distal roots – is a more accurate measure of root function, with lower order roots being more likely to be absorptive fine roots (McCormack et al. 2015). This renders some previous studies as potentially misleading and calls for a consistent definition of fine roots in future studies. Here, we define fine roots as roots belonging to the first three orders as determined by the morphometric classification protocol outlined by Fitter et al. (1982).

Fine root trait measurements include morphology and architecture. Root morphology describes structural characteristics of roots, and includes specific root length (SRL), average diameter, and root tissue density (RTD). These indices are reflective of the amount of energy invested in below-ground roots, i.e. NPP, which may contribute significantly to carbon sequestration (Rasse et al. 2005; Clemmenson et al. 2013). Root architecture refers to how root systems occupy space in soil and can be measured by branching frequency. Morphological and architectural traits can be indicative of root functionality. For example, acquisitive roots are characterized by high SRL, low diameter, and high branching, while conservative roots exhibit low SRL, high diameter, and low branching (Fort, Jouany, & Cruz 2013; Weemstra et al. 2016; 2020). Tradeoffs between these root traits represent a root economics spectrum (RES), and have been the subject of several studies (e.g. Freschet et al. 2017; Dallstream, Weemstra, & Soper 2023; Kramer-Walter et al. 2016; Iverson 2014; Reich 2014; Weemstra et al. 2016; 2020).

Between and within species and biomes, fine root morphology and architecture are variable, (Iverson 2014; Ma et al. 2018; Siefert et al. 2015), possibly reflecting differing needs of species and strategies of co-existence within landscapes (Dallstream, Weemstra, & Soper 2023). Furthermore, root trait variability is thought to be highest in the tropics (Ma et al. 2018; Chen et al. 2013). Morphology of fine roots, including SRL, diameter, and tissue density, differ between species and have been found to be phylogenetically conserved (Pregitzer et al. 2002; Weemstra et al. 2020; Kong et al. 2014; Valverde-Barrantes et al. 2017). Fine root architecture, characterized by branching patterns, also differs between species (Comas and Eissenstat 2009), but is weakly conserved across phylogenetic lineages (Kong et al. 2014), suggesting that root architecture may be a plastic trait influenced by environmental factors. Environmental conditions may also influence phylogenetically conserved morphological traits, as species-specific

morphological traits have been shown to vary across biomes (Iverson 2014; Ma et al. 2018; Addo-Danso et al. 2020) and environmental gradients (Robles-Aguilar et al. 2019; Li et al. 2020; Pierick et al. 2021; Pierret et al. 2021; Freschet et al. 2017; Pierret et al. 2007; Fort et al. 2017; Weemstra et al. 2021). If fine roots exhibit plasticity – i.e. vary across landscape gradients – plants may be able to alter their rooting strategies to cope with landscape and climatic shifts.

Few studies have examined root trait variation across environmental gradients, limiting our understanding of the role and relative importance of environmental determinants of fine root traits. Nonetheless, studies have investigated elevational gradients (Pierick et al 2021; Weemstra et al. 2021), water availability (Fort et al. 2017), nutrient concentration (Hodge 2004), and soil properties (Freschet et al. 2017; Li et al. 2020; Robles-Aguilar et al. 2019) in relation to root traits. Furthermore, most studies have looked at general patterns across species rather than species-specific relationships with environmental factors (Dallstream, Weemstra, & Soper 2023). Species-specific studies may reveal fine grain patterns otherwise undetectable in across-species studies and provide insight into how co-occurring species differentially invest in fine root production. This study will help us better understand species-specific fine root traits across an environmental gradient by looking at fine root traits of 3 tree species across variable soil conditions.

A limited number of studies have specifically examined the relationship between soil characteristics and root traits (e.g. Freschet et al. 2017; Li et al. 2020; Robles-Aguilar et al. 2019; Craine et al. 2001; Kramer-Walter et al. 2016). Soil moisture content and pH interact with nutrient availability and microbial communities (Chapin et al. 2002; Maire et al. 2015), which in turn have relationships with root traits (Hodge 2004). In part determined by soil pH, bulk density (BD), and moisture, soil fertility has been shown to have a negative relationship with RTD,

branching frequency, and SRL, and a positive relationship with fine root diameter (Craine et al. 2001; Kramer-Walter et al. 2016). Furthermore, Robles-Aguilar et al. (2019) revealed a negative relationship between pH and SRL in *Lupinus angustifolius*, while Freschet et al. (2017) found a negative relationship between pH and RTD at a global scale. Soil BD has had a positive relationship with RTD and diameter, and a negative relationship with SRL (Freschet et al. 2017; Clark, Whalley & Barraclough 2003). While studies have investigated these relationships, there are many unresolved questions as to how local soil properties interact with root systems. This study hopes to contribute data which will help resolve general and species-specific determinants of fine root traits.

Soil characteristics, such as pH, bulk density (BD), and moisture content, have been found to change upon land-use conversion. Specifically, deforestation of tropical rainforests have been shown to increase pH and BD, while decreasing soil organic carbon (Nye and Greenland 1964; Veldkamp 1994). If land use change affects soil properties, and soil properties can predict root traits, it can be expected that root traits will differ between land-use types, such as between primary forest and pasture. However, no studies to our knowledge have investigated how co-occurring land-use and soil changes might impact fine root traits. This question is especially pressing in tropical regions, which have been prone to land use conversion (Keenan 2015); the Chocó Rainforest, a diversity hotspot in Northwestern Ecuador, has been more than 90% deforested over the last several decades (Dodson & Gentry 1991). Furthermore, high soil variation exists in the tropics (Townsend, Asner, & Cleveland 2008), making tropical regions an ideal location to study the interaction between soil properties and fine root traits. As such, it is important to investigate the impact of tropical deforestation on fine roots, including the impact on the relationship between soil and root traits.

In this study, we aim to characterize fine root traits in three tropical species – *Handroanthus chrysanthus*, *Cordia alliodora*, and *Iriartea deltoidea* – across different landscapes – pasture and primary rainforest – in the Chocó Rainforest. We look at variation of root traits across landscape and soil gradients to investigate plant survival strategies and below-ground carbon input across taxa and land conditions. Fine roots of *H. chrysanthus* have been considered in studies investigating response to drought (Santos et al. 2022), while *C. alliodora* fine roots have been studied in the context of slash and burn deforestation and cacao plantations (Muñoz & Beer 2001). *I. deltoidea* fine roots were included in a carbon content analysis of the Arecaceae family (Cambronero et al. 2021). However, these studies defined fine roots as having a diameter <2mm, potentially misrepresenting fine roots as a functional group. Furthermore, these studies only looked at fine root biomass and surface area and did not include soil characteristics as predictors of these metrics. This study attempts to fill these gaps by providing the first thorough investigation of fine roots in these species. Specifically, we examined fine root RTD, SRL, and average diameter as metrics of root morphology, and branching frequency to measure fine root architecture. These traits were analyzed in relation to the soil properties of pH, BD, and percent moisture content to identify soil predictors of root traits.

It is expected that fine root traits will differ between species. Furthermore, morphology is expected to remain consistent within species across land use and soil types, while architecture is predicted to differ. If soil is not found to be a significant predictor of fine root traits, it may be the case that these species are unable to adapt to changing soil conditions, or that they do not need to. This study will uncover how, and when these species modify their rooting strategies and below-ground carbon investments to survive in a rapidly changing landscape.

METHODS

Study site

Roots were sampled from trees in the Chocó Rainforest on property owned by the Ecuadorian NGO, Fundación para la Conservación de los Andes Tropicales (FCAT). Samples were collected in June and July of 2022. The region varies in temperature from 23 to 27 °C and receives an average of over 2,000 mm rain annually. Elevation in this region ranges from 200m to 550m. Samples were collected from *H. chrysanthus*, *C. alliodora*, and *I. deltoidea* trees in old pasture and primary forest. Old pasture was used for cattle for over a decade, while primary forest had been minimally altered in the past 50 years. Local community members helped to identify and confirm tree species and land use age.

Tree species were selected based on their frequency in the old pasture; the 3 most common tree species were selected for sampling in this study. Six individuals from each species were sampled from each land use type. A total of 35 individuals were sampled; only five *H. chrysanthus* could be located in the primary forest.

Root sampling and processing

Approximately 2-3g of fine roots were collected from the first 10cm of soil. Collections were done within 5 meters from the base of *H. chrysanthus* and *C. alliodora*, and within 1 meter from the base of *I. deltoidea*. Roots were always traced back to the trunk of the individual of interest.

Traditionally, absorptive fine roots are defined as having a diameter <2mm. However, since branching position is more indicative of root functionality than is diameter (McCormack et al. 2015), we define absorptive fine roots as roots from the first three root orders. Roots from the

first three root orders were sampled, using the morphometric classification protocol as outlined by Fitter et al. (1982). Samples inclusive of only the first one or two orders were also included.

Samples were stored in a plastic bag and transported to the lab at the FCAT Research Station. Roots were stored in tap water and placed in the fridge within 7 hours of collection. Determination of root order and scanning of roots were done in the lab within 48 hours of sample collection. Some roots were broken while excavating in the field, in which case we could not definitively assign root orders. In cases where the root had clearly broken, we conservatively assigned root order by assuming that the first root order may have broken off in the field. What therefore would have been the first root order in a sample which appeared to be broken was assigned as the second order. Areas where roots were broken were often indicated by a hair-like 'root tip.' This pseudo-root tip was cut off prior to scanning as to not negatively skew average root diameter.

A random subset of sampled fine roots were selected for scanning using an Epson Perfection V850 Pro scanner. Scanned roots were dried and weighed for determination of SRL and RTD. Root scans were cleaned up using Adobe Photoshop. Edited scans were analyzed using RhizoVision to extract average root diameter, branching frequency, total root length, and root volume. To obtain SRL and RTD, total root length and volume as determined by RhizoVision was divided by dry root mass, respectively.

Soil sampling and processing

A soil corer was used to collect two soil cores within 1 meter from the tree base for pH, nutrient, moisture, and bulk density (BD) analysis. One core was used for BD and moisture analysis, while the other core was used for pH analysis. Both cores were from the first 10cm of soil.

The core used for BD and moisture analysis was cut to a volume of a 5cm high 5cm wide cylinder. The wet mass of this core was taken before allowing the core to dry at 150°C for 24 hours. The mass was taken again post-drying to determine moisture content and BD. BD was calculated by dividing the dry mass by the volume of the 5cm-high cylinder. BD was measured in units g/cm^3 .

For pH analysis, the soil sample was first homogenized by kneading in a plastic bag. A slurry was made using 10mL of DI water and 5g of soil sieved to 2mm. pH was measured using a glass pH probe, which was calibrated after every ~10 samples.

Statistics

Data analysis was performed in R. Linear mixed effects models, linear models, type III ANOVAs and t-tests were used to determine how soil characteristics and root traits differed between landscapes (forest vs. pasture). They were also used to evaluate how fine root traits differed among and within species between landscapes and soil properties. To account for any within-species root trait variance between forest and pasture, landscape was included as a fixed effect in linear mixed effect models evaluating differences in root traits between species. Species was included as a random effect when testing across species, and individual tree ID was included as a random effect when testing for within species differences in root traits across landscapes and soil properties.

Model residuals were checked for homoscedasticity and normality using functions *plot()*, *qqnorm()*, and *qqline()*. When residual variance was not homogenous, heterogeneous variances were modeled by specifying a correlation structure with function *varIdent()*. In some models, the response variable was log transformed to produce better normality. Plots were made in R using package *ggplot2*.

RESULTS

Root Traits Differ Between Species

Tree species showed significantly different morphological and architectural traits ($p < 0.0001$). PCA analysis showed that all species grouped along root trait axes, with 87% of the variation explained by PC1 and PC2. Branching frequency and RTD appear to be inversely correlated, and a similar relationship exists between SRL and average diameter (Figure 1).

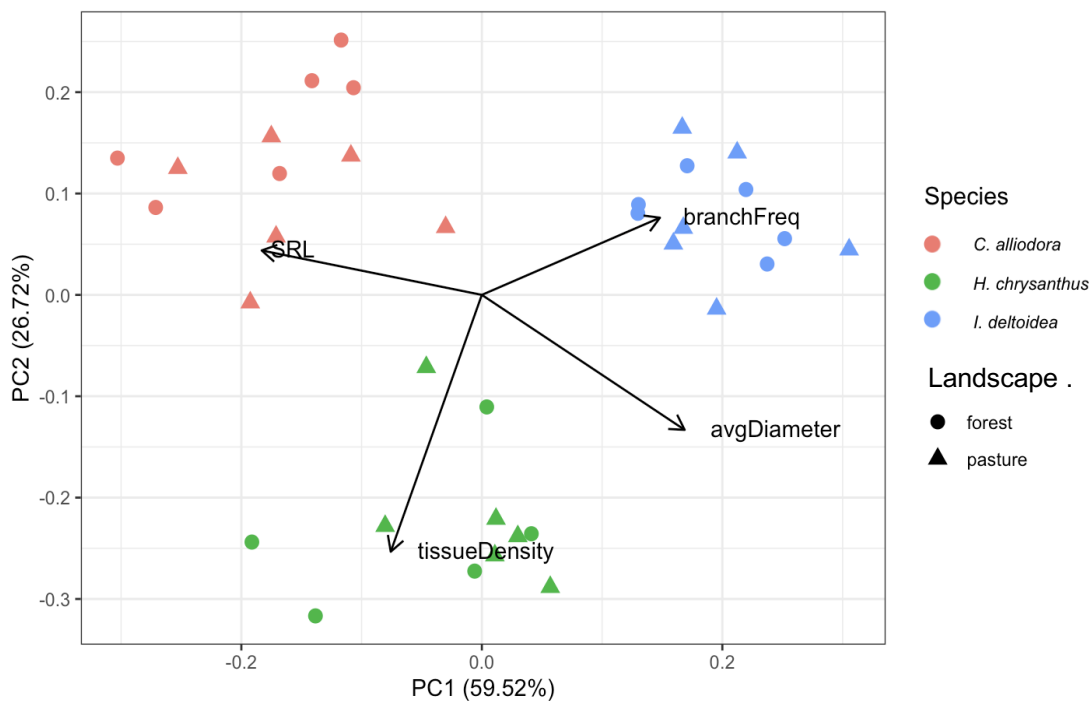


FIGURE 1. Fine root traits clustered by species along root trait axes. SRL = specific root length, tissueDensity = root tissue density, avgDiameter = average diameter, branchFreq = branching frequency.

Pairwise comparisons between species revealed that SRL significantly differs among all species pairings ($df=31$, $p < 0.005$). Average diameter was significantly lower in *C. alliodora* compared to both *H. chrysanthus* and *I. deltoidea* ($df=31$, $p < 0.0001$), but was not different between *H. chrysanthus* and *I. deltoidea* ($df=31$, $t=-1.652$, $p=0.24$). RTD was significantly higher in *H. chrysanthus* compared to both *C. alliodora* and *I. deltoidea* ($df=31$, $p < 0.0001$), but did not differ between *C. alliodora* and *I. deltoidea* ($df=31$, $t=2.044$, $p=0.12$). Branching

frequency did not differ between *H. chrysanthus* and *C. alliodora* ($df=31$, $t=-0.372$, $p=0.927$), but was significantly higher in *I. deltoidea* compared to both *H. chrysanthus* and *C. alliodora* ($df=31$, $p<0.0001$). (Figure 2). The range of each fine root measurement can be found in Table 1.

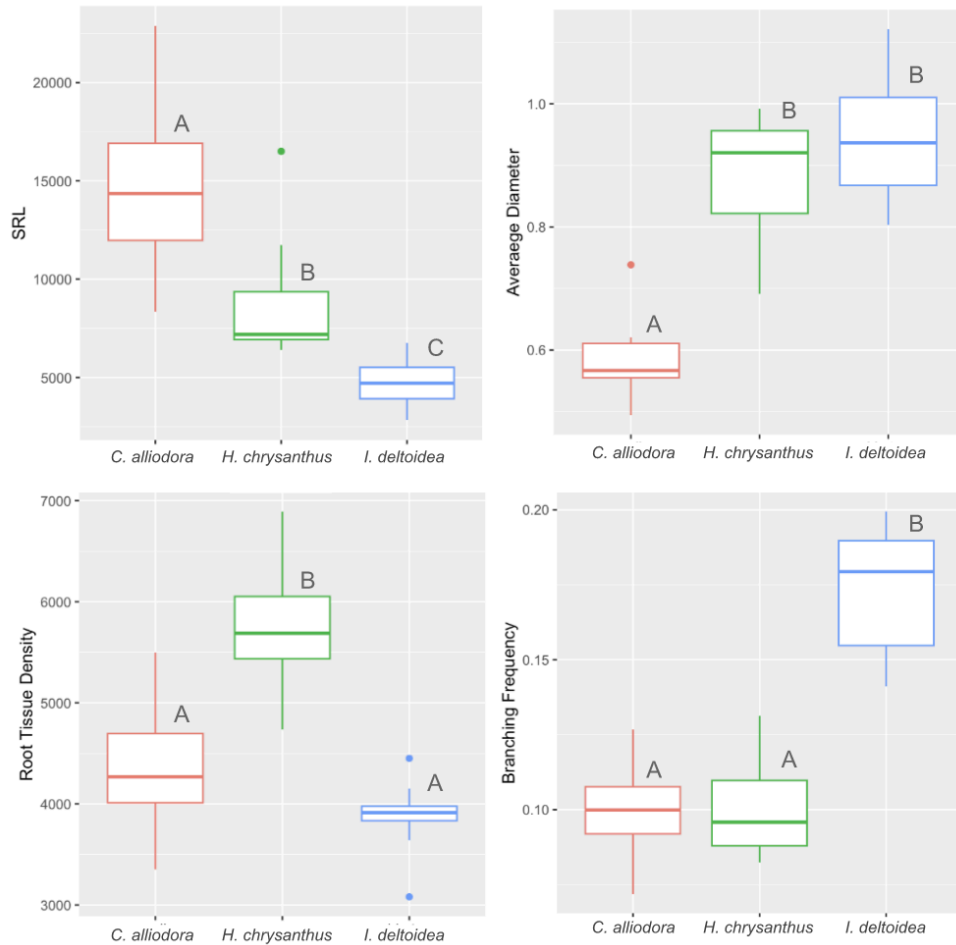


FIGURE 2. Fine root trait differences between species. SRL was significantly different across all species pairings; average diameter was significantly lower in *C. alliodora* than *H. chrysanthus* and *I. deltoidea*; RTD was significantly higher in *H. chrysanthus* than *C. alliodora* and *I. deltoidea*; branching frequency was significantly higher in *I. deltoidea* than *C. alliodora* and *H. chrysanthus*.

	Highest		Lowest		
	mean	SE	mean	SE	
SRL (mm/g)	4767.2	± 342.1	14583.3	± 1132.0	<i>C. alliodora</i>
Diameter (mm)	0.5855	± 0.0174	0.9429	± 0.0283	<i>H. chrysanthus</i>
RTD (g/mm)	3880.4	± 92.6	5749.9	± 193.8	<i>I. deltoidea</i>
Branching Freq(/mm)	0.099	± 0.005	0.172	± 0.006	

TABLE 1. Species with the highest and lowest measurements for each root trait. Highest and lowest values are reported as mean ± standard error (SE), and the species which the values occurred in are indicated by color.

Fine Root Trait Variation Across Landscape

Linear mixed models with species as a random effect found no differences between forest and pasture in SRL ($F=2.84$, $p=0.10$), RTD ($F=0.20$, $p=0.65$), or branching frequency ($F=0.48$, $p=0.50$) across all three species. Average diameter was significantly different between forest and pasture across all three species ($F=4.17$, $p=0.05$), with slightly greater diameter in the pasture (0.8316 ± 0.0428 mm) compared to the forest (0.7732 ± 0.0441 mm) (Figure 3).

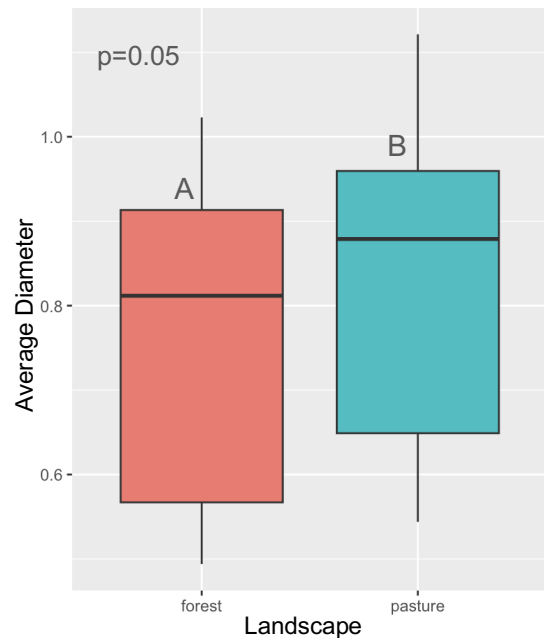


FIGURE 3. Changes in average root diameter between landscape. Average diameter was slightly higher in the pasture, although a high degree of overlap in diameter exists between landscape.

Within each species, linear mixed models with tree ID as a random effect revealed that no root traits differed significantly between pasture and forest.

Variation of Soil Properties

Variability in soil traits was found between and within landscapes. Figure 4 shows high variability of three soil traits and lack of clustering within landscapes. T-test analyses indicated that bulk density significantly differed between forest and pasture ($t=-2.6191$, $df=23.115$, $p=0.0153$), as did moisture content ($t=2.8489$, $df=30.506$, $p=0.007787$) (Figure 5). pH was not significantly different between forest and pasture ($t=0.23403$, $df=29.411$, $p=0.8166$), but still varied considerably within our population sample, with a range from 4.71 to 6.49. BD and percent soil moisture were also variable, with a range of 0.41-1.07g/mm³ and 22.40-54.39%, respectively.

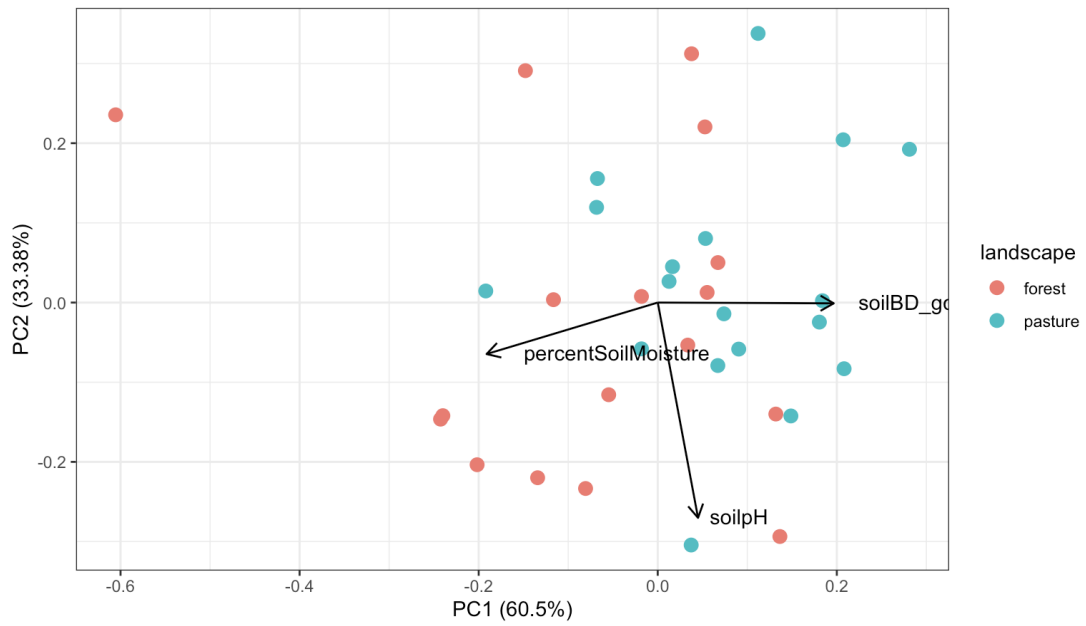


FIGURE 4. Soil property variation. Soil properties did not cluster by landscape. percentSoilMoisture = moisture content, soilBD = soil bulk density, soilpH = soil pH.

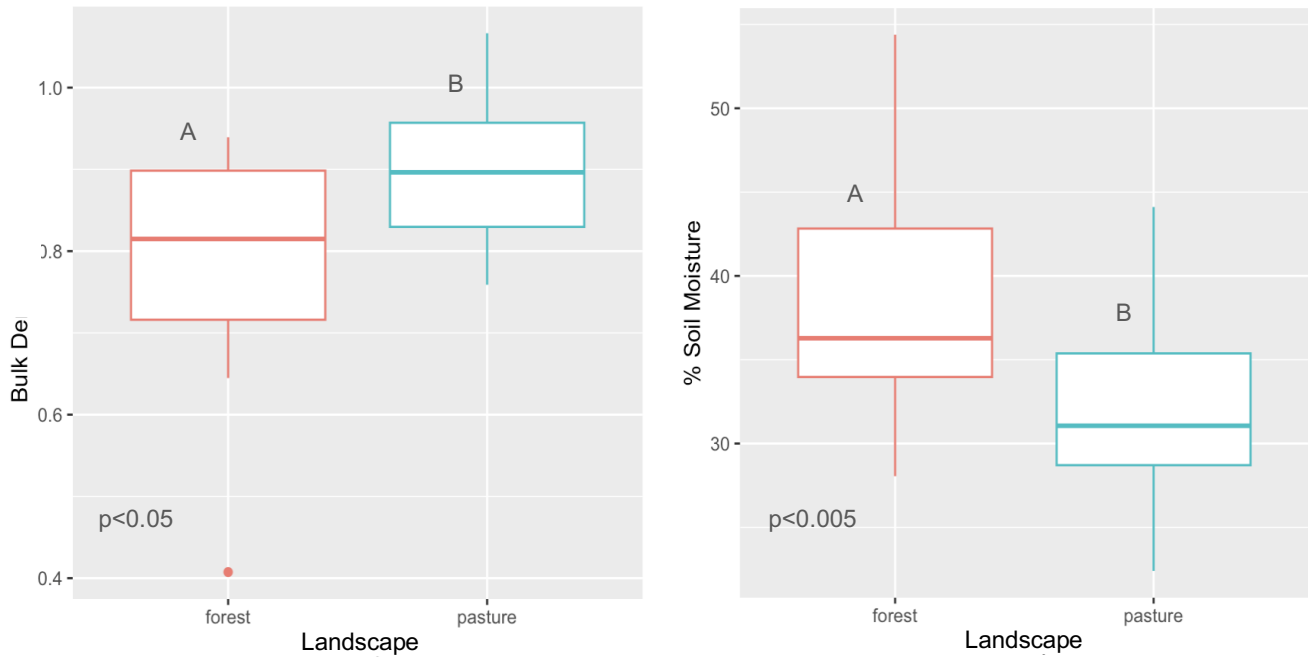


FIGURE 5. Soil property variation between landscape. BD was significantly higher in the pasture, while moisture was significantly higher in the forest.

Soil Properties as Predictors of Fine Root Traits

Across all species, pH predicted diameter ($F=4.51$, $p=0.04$), while bulk density predicted tissue density ($F=5.40$, $p=0.03$) (Figure 6a). Within the pasture, diameter and SRL were predicted in opposite directions by pH. Diameter was also marginally by BD across all species in the pasture (Figure 6b). In forest landscapes, diameter and SRL were marginally predicted by BD in opposite directions (Figure 6c).

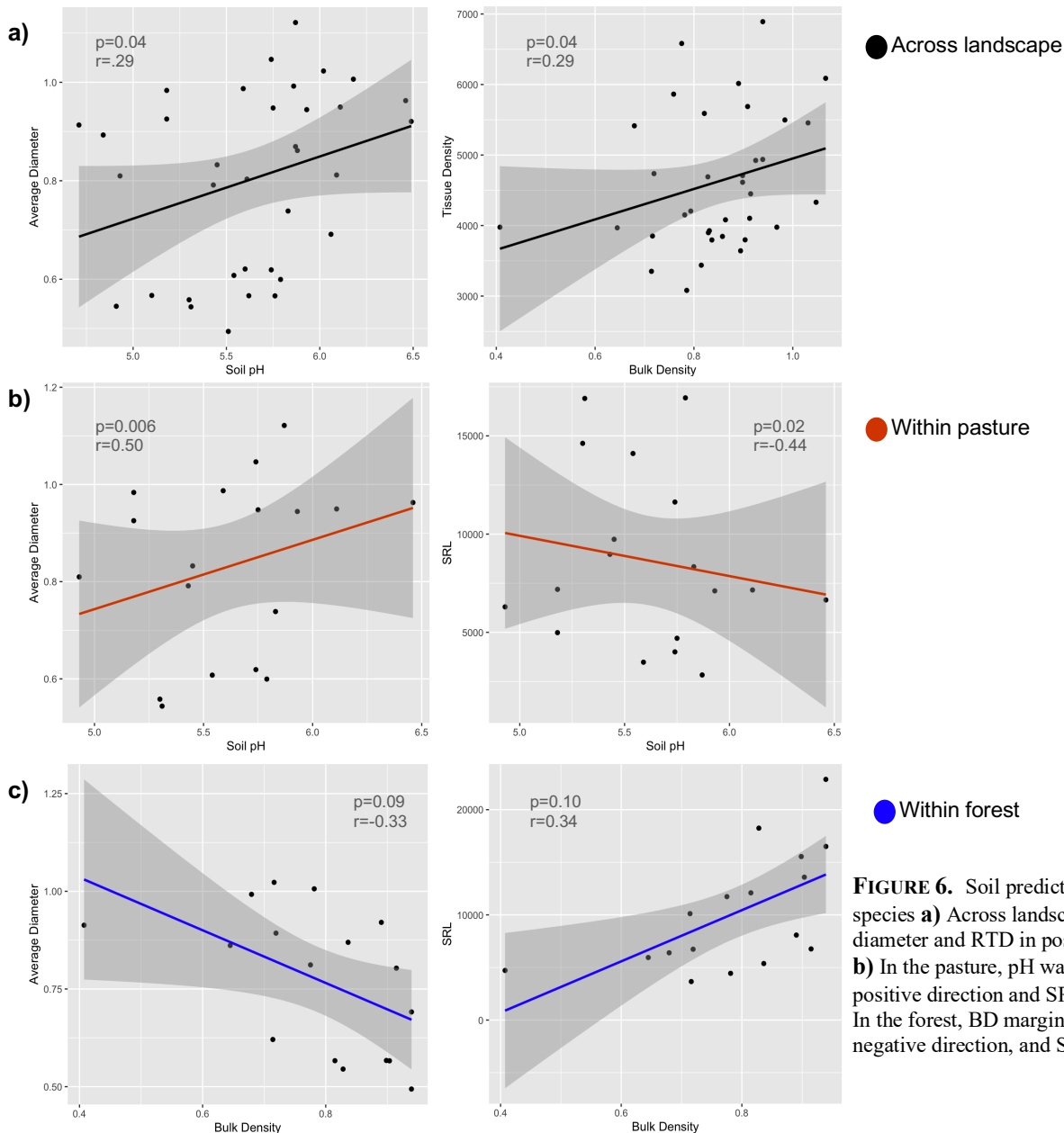


FIGURE 6. Soil predictors of fine root traits across species **a)** Across landscape, pH and BD predicted diameter and RTD in positive directions, respectively. **b)** In the pasture, pH was a predictor of diameter in a positive direction and SRL in a negative direction. **c)** In the forest, BD marginally predicted diameter in a negative direction, and SRL in a positive direction.

Soil predictors of root traits were variable between species. Within *C. alliodora*, pH significantly predicted SRL in a negative direction ($F=7.23$, $p=0.03$) and marginally predicted diameter in a positive direction ($F=4.815$, $p=0.059$) (Figure 7a). *C. alliodora* RTD was also positively predicted by BD ($F=5.42$, $p=0.048$) (Figure 7b).

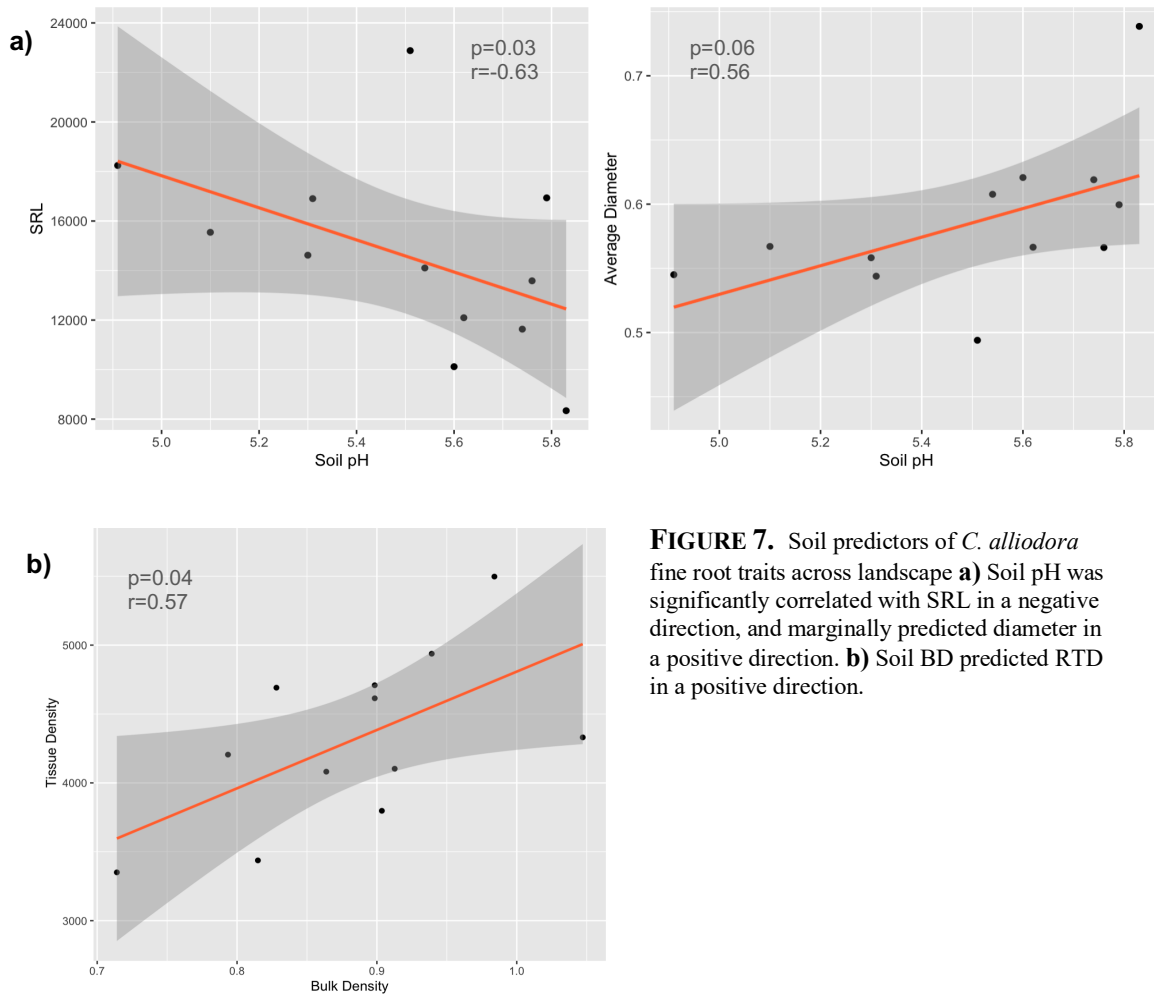


FIGURE 7. Soil predictors of *C. alliodora* fine root traits across landscape **a)** Soil pH was significantly correlated with SRL in a negative direction, and marginally predicted diameter in a positive direction. **b)** Soil BD predicted RTD in a positive direction.

No soil characteristics were significant predictors of *H. chrysanthus* or *I. deltoidea* across landscapes. However, when looking within pasture and forest individually, *H. chrysanthus* root traits are significantly predicted by soil parameters in the forest. BD significantly predicted both SRL in a positive direction and diameter in a negative direction (Figure 8).

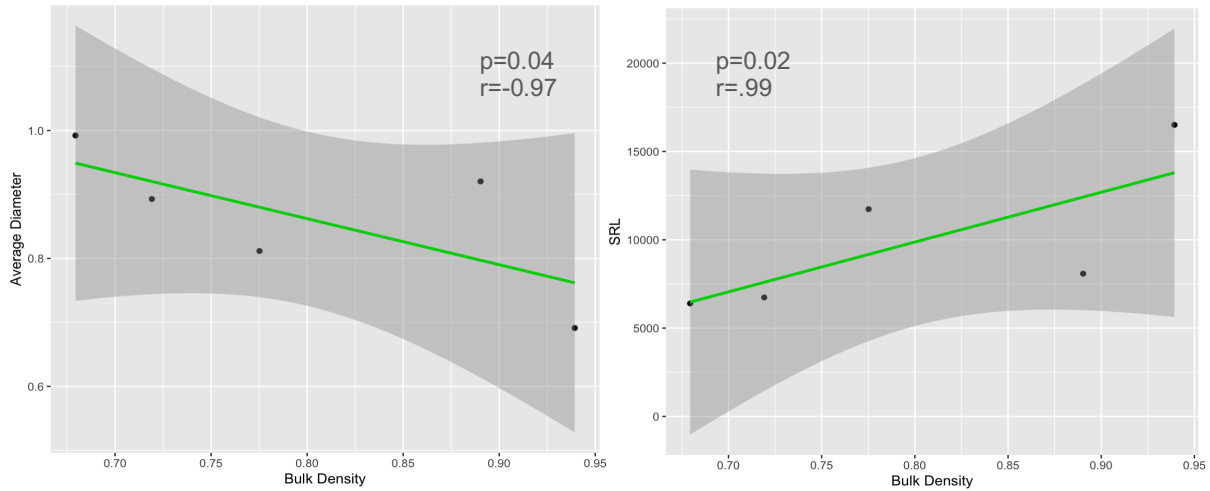


FIGURE 8. Soil predictors of *H. chrysanthus* fine root traits in the forest. Soil BD predicted diameter in a negative direction, and SRL in a positive direction.

SRL and diameter were consistently predicted in opposite directions (Figure 6b, Figure 6c, Figure 7a, Figure 8). Branching frequency was not predicted by any soil properties, and soil moisture content did not predict any root traits. *I. deltoidea* root traits were not predicted by any soil properties across and within landscapes.

DISCUSSION

Root trait differences between species

All fine root traits differed significantly across species. Pairwise t-tests revealed that SRL differed amongst all species, while diameter, RTD, and branching frequency had one species with significant differences from the other two. The species with significant differences was not consistent across root traits, highlighting the variation of root traits across all species, rather than one species as a consistent outlier.

Patterns of root traits in these species reveal a relationship between SRL and diameter. The species with the highest SRL – *C. alliodora* – also had the lowest diameter, while *I. deltoidea* had the lowest SRL and the highest diameter. These findings are consistent with the hypothesized tradeoff between SRL and root diameter (Fort, Jouany, & Cruz 2013; Weemstra et al. 2016; 2020). However, no clear relationship involving RTD or branching frequency emerged. High branching frequency has been hypothesized to co-occur with high SRL and low diameter to produce acquisitive fine roots (Weemstra et al. 2016). In this study however, the highest branching frequency appeared in *I. deltoidea*, which also had the lowest SRL and highest diameter. These findings suggest that *I. deltoidea* may utilize increased branching frequency to compensate for short, thick roots. This may also indicate that *I. deltoidea* is limited in producing long and fine roots, and therefore increases branching to acquire nutrients within its limited morphological plasticity.

Variation of fine root traits across these species is consistent with previous findings that phylogeny is the largest predictor of root trait variance across species (Valverde-Barrantes et al. 2017; Weemstra et al. 2020; Kong et al. 2014). Furthermore, the high degree of variation found in this study points to a potential mechanism that allows species to co-exist in the species-dense tropics, especially in relation to nutrient acquisition (Dallstream, Weemstra, & Soper 2023). Future work should focus on linking root morphological traits to specific root functions in order to better understand how the variability in root traits affects community and ecosystem-scale processes.

Landscape versus soil as predictors of root traits

While landscape did not robustly predict root trait differences across and within species, it is important to consider the non-uniformity of soil between and within landscapes. Variability

in soil traits was found between and within landscapes. This is highlighted in Figure 4, which shows high variability of three soil traits and lack of clustering within landscapes. Despite this lack of clustering, t-test analyses indicated that bulk density and moisture content significantly differed between forest and pasture, while pH was not significantly different. This is surprising, since previous studies have found soil pH changes after landscape conversion via slash-and-burn (Veldkamp et al. 2020). The lack of pH change may be an effect of long-term use, which may have allowed the initial pH change to balance out over time. Changes in BD and moisture in the pasture can be explained by long term occupation of cattle, which would increase the compaction of the soil (i.e. BD), thereby decreasing the ability of soil to hold water. These effects are shown in our results, with higher BD and lower moisture content in the pasture compared to primary forest.

The evident variability of soil pH, BD, and moisture content between and within landscapes merits an investigation of the relationship between soil properties and root traits. Across all species and landscapes, pH predicted diameter, and bulk density predicted tissue density, both with weak positive relationships. This signifies that soil pH and BD may generally influence diameter and tissue density respectively, although these weak relationships point to the presence of better predictors for these root traits. Nonetheless, the positive relationship between pH and diameter indicates that roots may become thicker and more conservative in increasingly basic soils. Root-trait conservatism has been found to be a stress response to soil conditions in some plants (Robles-Aguilar et al. 2018; Fort et al. 2017), meaning that basic soils may create a stressful environment for these tropical species, which are typically adapted to acidic soils (Fujii 2014). Moreover, higher RTD with increasing BD was found. This same relationship was

reported in Freschnet et al. (2017), and could reflect a need for denser roots in denser soil conditions (Bengough et al. 2006).

When examining soil-root interactions within pasture and forest landscapes, soil property predictors remained the same as across all landscapes: pH and BD significantly predicted fine root traits. However, the root traits predicted were different; Within the pasture, pH predicted SRL in addition to diameter, and BD marginally predicted diameter – not RTD, as found across all landscapes. BD also marginally predicted SRL in addition to diameter within the forest. The difference in root traits predicted by soil between landscapes highlights how the interaction between fine roots and soil properties might change with land-use, while the consistency of soil predictors highlights the potential importance of pH and BD in mediating root traits.

In the pasture, high pH was correlated with a conservative morphology, having increased diameter and lower SRL. Interestingly, the opposite relationship was found in Robles-Aguilar et al. (2019), where higher pH correlated with more acquisitive roots. This may be a result of differences in study species and sites: Robles-Aguilar et al. (2019) looked at *Lupinus angustifolius* – a non-tropical plant – in Australia, while this study looked at three species in the Neotropics. Neotropical plant species are well-adapted to acidic conditions, making an increase in pH potentially stressful for these plants. Conversely, *Lupinus angustifolius* is adapted to mildly acidic soils, meaning that an increase in pH might relieve the plant from acidic stress, especially that associated with low P levels (Robles-Aguilar et al. 2019). As such, it makes sense that fine root relationships with pH would differ between geographically distinct species, with higher pH being conducive to a conservative root trait response in acid-tolerant species while high pH correlates with acquisitive roots in non-acid-tolerant species. Indeed, species-specific root trait responses to environmental gradients were found in Weemstra et al. (2021).

In the forest, BD marginally predicted diameter and SRL. The presence of different soil predictors between landscapes indicates that there may be different environmental factors within each landscape that influence plasticity of fine roots. For example, variable competition in magnitude and type between pasture and forest may influence rooting strategies. Nutrient concentrations may also differ between landscape, and N and P availability has been shown to influence fine root traits (Wurzburger & Wright 2015). If N and P concentrations vary across the landscape sampled in this study, it is possible that these variables had influence on fine root traits. Indeed, available P can change with pH (Turner & Blackwell 2013; Penn & Camberato 2019), making it likely that P concentration contributed to fine root variation across a soil pH gradient. Predictions addressed here may be supported if other variables are taken into consideration, such as nutrient concentration, surrounding vegetation levels and types, and soil chemistry.

A point of interest is how BD predicted tissue density across all landscapes, but not within individual landscapes. This is potentially a result of small sample sizes within each landscape (pasture n=18, forest n=17). Alternatively, BD may have nearly significantly predicted tissue density within pasture and forest, but was cancelled out by other soil properties when looking at individual landscapes. Indeed, BD was the best predictor of tissue density in both pasture and landscape ($p < 0.2$).

It is important to note that when SRL and diameter were predicted by the same soil property, they were predicted in opposite directions. This is consistent with the hypothesis that SRL and diameter reflect a tradeoff within the RES (Reich 2014; Freschet et al. 2017).

Species-specific interactions between soil properties and fine root traits

The interactions between soil properties and fine root traits across all species are reflected within individual species. Within *C. alliodora*, pH predicted SRL and marginally predicted diameter across landscapes, with SRL and diameter being predicted in opposite directions to produce conservative fine roots with increasing pH. RTD was also predicted by BD across landscapes, likely contributing to the significance of the interaction between BD and RTD across all species. However, *C. alliodora* root traits were not predicted by any soil properties within either landscape, again reflecting our low sample size, or that soil properties become less significant predictors when examining each landscape individually. Inclusion of more environmental variables, such as nutrients, may help us answer why these interactions are significant across but not within landscapes.

H. chrysanthus root traits were not predicted by soil characteristics across landscapes. However, when looking at *H. chrysanthus* root traits within the forest, BD becomes a significant predictor of SRL and diameter, with higher BD favoring more acquisitive fine root traits. Other studies have found the opposite: higher BD has been correlated with thicker and denser roots (Freschet et al. 2017; Bengough et al. 2006; Clark, Whalley & Barraclough 2003), likely due to the ability of thick roots to better penetrate dense soil (Bengough et al. 2006; Materechera, Dexter & Alston 1991). It could be the case that BD does not affect fine root traits in the forest, and instead that BD is correlated to another variable that does impact fine root formation. Moreover, this interaction was only significant in the forest, reflecting how different variables between the pasture and the forest may be important in fine root trait determination.

Root traits of *I. deltoidea* were not significantly predicted by any soil properties. Differences in soil predictors of root traits between species emphasizes the need to further study species-specific root trait plasticity across environmental gradients. Furthermore, conflicting

results between this study and previous studies demand a further investigation into why these differences occur. It is possible that this is simply a result of inadequate sampling size, or that important variables were left out of this analysis. On the other hand, it is likely the case that different interactions exist between fine roots and soil properties between species and tropical landscapes, and that we are just beginning to understand these interactions.

Fine root plasticity across environmental gradients

We found that SRL and diameter variances were most consistently predicted by soil traits. This goes against findings by Kong et al. (2014), which found that SRL and diameter are phylogenetically conserved and therefore not predicted to change across environmental gradients. Furthermore, Freschnet et al. (2017) found that SRL and diameter were not correlated with any physical soil properties, and were instead related to climatic variables, nutrients, and soil chemistry. This highlights the importance of including more environmental variables in future studies. However, the analysis done by Freschnet et al. (2017) encompassed several species on a global scale, producing coarse-grain results. Here, we examine species-specific interactions at a local scale, revealing fine-grain differences in fine root traits across soil properties. These results reflect how species-specific investigations within tropical localities can reveal fine root plasticity.

Branching frequency was not significantly predicted by landscape nor any soil properties, suggesting a lack of plasticity in fine root architecture. However, Kong et al. (2014) predicted that root branching would be plastic across environmental gradients, and other studies have supported this claim (Pregitzer 2008; Liese, Alings, & Meier 2017; Kramer-Walter et al. 2016). However, these studies focused on soil chemical properties, including nutrient concentration,

while this study primarily focused on soil physical properties. This indicates fine root branching may be plastic over nutrient gradients, but not over soil physical property gradients.

CONCLUSION

Soil is an important factor to consider when investigating fine root variability. Soil pH and BD were significant predictors of fine root traits, while landscape was not a consistent predictor, although soil characteristics do differ between landscapes. This finding challenges generalizations about broad landscape classifications and reflects a reality where these broad categories are themselves heterogenous environments. This speaks to the complexity of understanding tropical environments and calls for fine-scale characterizations of plant-soil dynamics to better predict forest recovery after landscape conversion.

Soil predictors of root traits changed when looking across landscapes versus within pasture and forest. Difference in soil predictors across landscapes calls for a deeper investigation of why these differences occur. Furthermore, low r values in many of our models indicate that root trait variance is likely better predicted by variables not included in this study. Possible explanations include variable competition levels, vegetation types, nutrient concentrations, soil chemistry, and symbionts or other microbial factors. It is difficult to make solid conclusions in this study due to limits in study sampling. Inclusion of more replicates and variables might allow for robust conclusions about the relationships between soil characteristics and root traits across species. Including additional soil and environmental variables may reveal new patterns in fine root plasticity, while a larger sample size would increase the statistical power of such findings. Nonetheless, soil pH and BD did predict fine root SRL, diameter, and RTD. Soil predictors of root traits differed between species, highlighting the need to investigate root traits at a species-

specific level across environmental gradients to better understand root trait plasticity. Contrary to previous studies, this study reveals how morphological traits can be plastic across soil gradients, while branching remains consistent. Several results were the opposite of the results found in understudies, underscoring our lack of understanding of tropical fine root traits and the need for additional studies of fine root plasticity across environmental gradients, especially at species-specific levels in the tropics.

REFERENCES

- Addo-Danso, S.D., Defrenne, C.E., McCormack, M.L. et al. (2020). Fine-root morphological trait variation in tropical forest ecosystems: an evidence synthesis. *Plant Ecol* 221: 1–13.
- Barley, K.P. (1970). The configuration of the root system in relation to nutrient uptake. *Adv. Agron.* 22: 159–201.
- Bengough, A. G. et al. 2006. Root responses to soil physical conditions; growth dynamics from field to cell. – *J. Exp. Bot.* 57: 437–447.
- Brando P.M., Paolucci L., Ummenhofer C.C., Ordway E.M., Hartmann H., Cattau M.E., Balch J. (2019). Droughts, wildfires, and forest carbon cycling: A pantropical synthesis. *Annu Rev Earth Pl Sc* 47: 555–581.
- Cambronero, M., Avalos, G., Alvarez-Vergnani, C. et al. (2021). Allometric Models to Estimate Carbon Content in Arecaceae Based on Seven Species of Neotropical Palms, PREPRINT (Version 1) available at Research Square.
- Chapin, F. S., Matson, P. A., Mooney, H. A., & Vitousek, P. M. (2002). *Principles of terrestrial ecosystem ecology.*
- Chen W.L., Zeng H., Eissenstat D.M., Guo D.L. (2013). Variation of first-order root traits across climatic gradients and evolutionary trends in geological time. *Global Ecology and Biogeography* 22: 846–856.
- Clark, L., Whalley, W. & Barraclough, P. (2003). How do roots penetrate strong soil?. *Plant and Soil* 255, 93–104.
- Clemmensen K.E., et al. (2013). Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science*: 339(6127):1615-8
- Comas, L.H. and Eissenstat, D.M. (2009). Patterns in root trait variation among 25 co-existing North American forest species. *New Phytologist*, 182: 919–928.
- Craine, J.M., Froehle, J., Tilman, D.G., Wedin, D.A. and Chapin, III, F.S. (2001), The relationships among root and leaf traits of 76 grassland species and relative abundance along fertility and disturbance gradients. *Oikos*, 93: 274–285.
- Cusack, D.F., Markesteijn, L., Condit, R. et al. (2018). Soil carbon stocks across tropical forests

- of Panama regulated by base cation effects on fine roots. *Biogeochemistry*, 137: 253–266.
- Dallstream, C., Weemstra, M. and Soper, F.M. (2023), A framework for fine-root trait syndromes: syndrome coexistence may support phosphorus partitioning in tropical forests. *Oikos*: e08908.
- Dodson, C. H., & Gentry, A. H. (1991). Biological Extinction in Western Ecuador. *Annals of the Missouri Botanical Garden*, 78(2): 273–295.
- Elser, J., Fagan, W., Denno, R. et al. (2002). Nutritional constraints in terrestrial and freshwater food webs. *Nature* 408: 578–580.
- Fahey, T.J., and M.A. Arthur. (1994). Further studies of root decomposition following harvest of a northern hardwood forest. *Forest Science*, 40: 618–629.
- Fitter, A. H. (1982). Morphometric analysis of root systems: application of the technique and influence of soil fertility on root system development in two herbaceous species. *Plant, Cell & Environment*, 5(4): 313–322.
- Fort, F., Jouany, C., Cruz, P. (2013). Root and leaf functional trait relations in Poaceae species: implications of differing resource-acquisition strategies, *Journal of Plant Ecology*, 6(3): 211–219
- Fort, F., Volaire, F., Guillioni, L., Barkaoui, K., Navas, M.-L. and Roumet, C. (2017), Root traits are related to plant water-use among rangeland Mediterranean species. *Funct Ecol*, 31: 1700–1709.
- Freschet, G.T. et al. (2017), Climate, soil and plant functional types as drivers of global fine-root trait variation. *J Ecol*, 105: 1182–1196.
- Fujii, K. Soil acidification and adaptations of plants and microorganisms in Bornean tropical Forests. (2014). *Ecol Res* 29: 371–381.
- Hetrick, B. A. D. (1991). Mycorrhizas and root architecture. *Experientia*, 47(4), 355-362.
- Hodge, A. (2004), The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytologist*, 162: 9-24.
- Iversen, C.M. (2014). Using root form to improve our understanding of root function. *New*

- Phytologist, 203(3): 707–709.
- Keenan, R. J. et al. (2015). Dynamics of global forest area: results from the FAO Global Forest Resources Assessment 2015. *For. Ecol. Manag.* 352: 9–20.
- Kong D, Ma C, Zhang Q, Li L, Chen X, Zeng H, Guo D. 2014. Leading dimensions in absorptive root trait variation across 96 subtropical forest species. *New Phytologist*, 203: 863–872.
- Liese R, Alings K, Meier IC. (2017). Root Branching Is a Leading Root Trait of the Plant Economics Spectrum in Temperate Trees. *Front Plant Sci.* 8:315
- Li, F.L., McCormack, M.L., Liu, X. et al. (2020). Vertical fine-root distributions in five subalpine forest types shifts with soil properties across environmental gradients. *Plant Soil*, 456: 129–143.
- Kramer-Walter, K.R., Bellingham, P.J., Millar, T.R., Smissen, R.D., Richardson, S.J. and Laughlin, D.C. (2016), Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum. *J Ecol*, 104: 1299–1310.
- Ma, Z. et al. (2018). Evolutionary history resolves global organization of root functional traits. – *Nature*, 555: 94–97.
- Maire, V. et al. (2015). Global effects of soil and climate on leaf photosynthetic traits and rates. *Global Ecology and Biogeography*, 24(6): 706-717.
- Materechera, S.A., Alston, A.M., Kirby, J.M. & Dexter, A.R. (1992) Influence of root diameter on the penetration of seminal roots into a compacted subsoil. *Plant and Soil*, 144, 297–303.
- McCormack, M.L. et al. (2015). Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytologist*, 207: 505-518.
- Muñoz, F., Beer, J. Fine root dynamics of shaded cacao plantations in Costa Rica. (2001). *Agroforestry Systems*, 51: 119–130.
- Nye, P. H. & Greenland, D. J. (1964). Changes in the soil after clearing tropical forest. *Plant Soil*, 21: 101–112.

- Penn, C.J.; Camberato, J.J. A Critical Review on Soil Chemical Processes that Control How Soil pH Affects Phosphorus Availability to Plants. (2019). *Agriculture*, 9: 120.
- Pierick, K., Leuschner, C. and Homeier, J. (2021), Topography as a factor driving small-scale variation in tree fine root traits and root functional diversity in a species-rich tropical montane forest. *New Phytologist*, 230: 129-138.
- Pierret, A., Latchackak, K., Chathanvongsa, P. et al. (2007) Interactions between root growth, slope and soil detachment depending on land use: a case study in a small mountain catchment of Northern Laos. *Plant Soil*, 301: 51–64.
- Pregitzer, K. S. (2002). Fine Roots of Trees: A New Perspective. *The New Phytologist*, 154(2): 267–270.
- Pregitzer, K. S. (2008). Tree Root Architecture: Form and Function. *The New Phytologist*, 180(3): 562–564.
- Rasse, D.P., Rumpel, C. & Dignac, MF. (2005). Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant Soil*, 269: 341–356
- Reich, P.B. (2014), The world-wide ‘fast–slow’ plant economics spectrum: a traits manifesto. *J Ecol*, 102: 275-301.
- Robinson D, Hodge A, Fitter A.H. (2003). Constraints on the form and function of root systems. In: de Kroon H, Visser EJW eds. *Root ecology*. Ecological studies, Vol. 168. Berlin, Germany: Springer-Verlag, 7–18.
- Robles-Aguilar, A.A., Pang, J., Postma, J.A. et al. (2019). The effect of pH on morphological and physiological root traits of *Lupinus angustifolius* treated with struvite as a recycled phosphorus source. *Plant Soil*, 434: 65–78.
- Santos EA, Haro-Carrión X, Oshun J. Age-specific and species-specific tree response to seasonal drought in tropical dry forests. (2022). *Sci Total Environ*. 850:157908.
- Siefert, A. et al. 2015. A global meta-analysis of the relative extent of intraspecific trait variation in plant communities. *Ecol. Lett.* 18: 1406–1419.
- Townsend AR, Asner GP, Cleveland CC. (2008). The biogeochemical heterogeneity of tropical forests. *Trends Ecol Evol.*, 23(8): 424–31.

- Trejo, D., Barois, I. & Sangabriel-Conde, W. (2016). Disturbance and land use effect on functional diversity of the arbuscular mycorrhizal fungi. *Agroforest Syst.* 90: 265–279
- Turner, B.L. and Blackwell, M.S.A. (2013). Isolating the influence of pH on the amounts and forms of soil organic phosphorus. *Eur J Soil Sci*, 64: 249–259.
- Valverde-Barrantes, O. J. et al. (2017). A worldview of root traits: the influence of ancestry, growth form, climate and mycorrhizal association on the functional trait variation of fine-root tissues in seed plants. – *New Phytol.* 215: 1562–1573.
- Veldkamp, E. (1994), Organic Carbon Turnover in Three Tropical Soils under Pasture after Deforestation. *Soil Science Society of America Journal*, 58: 175–180.
- Vogt, K. A., Grier, C. C., Vogt, D. J. (1986). Production, turnover, and nutritional dynamics of above- and belowground detritus of world forests. *Advances in Ecological Research* 15: 303–307.
- Weemstra, M. et al. (2016). Towards a multidimensional root trait framework: a tree root review. *New Phytologist*, 211: 1159–1169.
- Weemstra, M, Kiorapostolou, N, van Ruijven, J, Mommer, L, de Vries, J, Sterck, F. (2020). The role of fine-root mass, specific root length and life span in tree performance: A whole-tree exploration. *Funct Ecol.* 34: 575–585.
- Weemstra, M, Freschet, GT, Stokes, A, Roumet, C. (2021). Patterns in intraspecific variation in root traits are species-specific along an elevation gradient. *Funct Ecol.* 35: 342– 356.
- Wurzburger, N. and Wright, S.J. (2015), Fine-root responses to fertilization reveal multiple nutrient limitation in a lowland tropical forest. *Ecology*, 96: 2137–2146.