The hidden life of tropical roots in Puerto Rico: functional root traits and their response to climatic disturbances

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Daniela Yaffar

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Dedication

To my husband, Benjamin Branoff, who helps me to be a better scientist, a better friend, and a better person every day.

To my family, that believed in me and supported my idea of living abroad so I could reach my professional goals.

To my advisor, Richard Norby, whose example as a person and a scientist, and his patience as an advisor helped me to develop and improve my scientific research.

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Abstract

Roots play a critical role in plant nutrition, and terrestrial carbon cycling. However, they are often understudied compared to their aboveground counterparts; especially in the tropics, where more carbon is cycled than in any other ecosystem. Some tropical forests, like in Puerto Rico, are more represented in scientific studies than others. However, this information is sparse, complicating the interpretation of root trait patterns. Trees in Puerto Rico have adapted mechanisms for withstanding hurricane disturbances, including in their roots. Additionally, as many tropical forests, some in Puerto Rico have low available phosphorus (P); thus, trees rely on root traits to enhance P acquisition. For example, higher root mycorrhizal colonization, branching, and exudation of phosphatase enzymes can each optimize P uptake. Yet, the high cost invested on these traits is a trade-off, which results in a diversity of traits among species. The aim of this dissertation was to synthesize root studies from Puerto Rico for the past 50 years, measure root response to warming and hurricanes disturbances, and test root trait strategies related to P acquisition from common species of the island.

I found from previous studies that rooting depth in Puerto Rico is shallow (<20cm), root nutrient concentrations do not vary much across forests, and there is under-representation of forests outside the Luquillo Experimental Forest. I measured a negative effect of warming on root production and biomass after 7 months of experimental warming. I captured an overall increase of root biomass after 10 months of the hurricanes (Irma and María) possibly due to plant composition change. Yet, the increase in root biomass was less in previously warmed plots than in control plots, suggesting a legacy effect of the warming treatment on the recovery of roots. When testing root traits related to P acquisition, I found that species with high mycorrhizal colonization also had high root phosphatase activity, but low branching ratio. This suggests a combined contribution of phosphatase enzymes from the plant and their fungal partners to obtain soil P, and that plants will either invest in more branched roots, or more mycorrhizal colonization and more phosphatase exudation.

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Chapter 1

Introduction

Plant roots, together with soil microbiota and invertebrates, compose the “hidden half” of the ecosystem, or the “black box” of carbon (C) budget (Waisel *et al.*, 1991; Hungate *et al.*, 1997; Norby, 1997). Despite its importance in plant nutrition, and C cycling, our understanding of the rhizosphere has been a struggle for centuries. Some recognized root ecologists still agree with the limitations of belowground studies. “Fine roots (..) are a royal pain to study” (Pregitzer, 2002), “our mechanistic understanding of many of the linkages between root form and function is still in its infancy” (Iversen, 2014), and **“**Sampling roots beneath the soil is much like sampling leaves blindfolded” (Norby, pers. conv., 2019) are some of the examples. Although, advancing technology (e.g. DNA sequencing) has facilitated species-specific root measurements and greatly improved belowground imaging (e.g. automated minirhizotrons and 3D magnetic resonance imaging; van Dusschoten *et al.*, 2016, Rahman *et al.*, 2020), equipment costs and sampling time and techniques remain a problem (Iversen *et al.*, 2017). This is especially true in tropical ecosystems, where there is greater species diversity combined with less accessibility to infrastructure than in any other biome.

In recent years, there has been a trend of more integrated belowground analyses that include the networking of empiricists with modelers (Matamala & Stover, 2013; Norby & Iversen, 2013; Sulman *et al.*, 2017; Koven *et al.*, 2019), microbiologists with ecologists (Tedersoo *et al.*, 2014; Cabugao *et al.*, 2017; Beals *et al.*, 2020), and a more centralized database (Iversen *et al.*, 2017), which has helped to better develop Belowground Ecology. However, belowground biological diversity and variability, combined with underrepresented biomes (e.g. tropics) entail a continuing effort to include belowground components in basic forest monitoring around the world, and thus, in global models (Warren *et al.*, 2015). This lack hinders predictions of root response to disturbances and their effect on ecosystem function. Further, the relationships between root structure (e.g. morphology) and function (e.g. nutrient uptake), is still under debate. Therefore, our understanding of the interactive soil-plant-microbe feedbacks in general and in response to disturbance is still limited.

Fine roots and fine root traits

Fine roots are commonly classified as any root that is less than 2 mm in diameter (McCormack *et*

al., 2015). Recently, however, a classification based on root function and root order, rather than root size, has been considered more useful (Pregitzer *et al.*, 2002; McCormack *et al.*, 2015). Following the stream-order description, first-order roots are those with no branching, second order roots are those in which first order roots originate, etc. Generally, only the first two root orders are capable of taking up nutrients, also called absorptive roots; whereas, the rest are called transportive roots (Iversen, 2014). Despite their great importance, fine roots, along with coarse roots, are the most poorly understood plant components in terrestrial ecology (Comas & Eissenstat, 2009).

Fine-root traits are any morpho-physiophenological characteristic that we can measure, and which can affect plant fitness directly or indirectly (Violle *et al.*, 2007). These traits are primarily dependent upon species phylogeny, soil biogeochemistry, and nutrient availability (Nadelhoffer & Raich, 1992). Root system (e.g. root standing stock), root morphology (e.g. root diameter, specific root length), and root chemistry (e.g. root macronutrients) are some root traits generally more studied than others (Iversen *et al.*, 2017; Yaffar & Norby, 2020). Architectural traits describe the root topology, and it includes branching ratio and branching intensity which are obtained by the difference between first and second order roots (Kong *et al.*, 2014). Dynamic traits include productivity, mortality, turnover, life span, and others which can be determined by different methods such as ingrowth coring and minirhizotrons (Neill, 1992; Yuan & Chen, 2012). Another important root trait is microbial association, which encompass mycorrhizal symbiosis with roots, and some of the measurements include counting the percent coverage of mycorrhizae fungal colonization inside fine-root tissue (Iversen *et al.*, 2017). Finally root physiological characteristics, such as enzymatic activity, and nutrient uptake are far less commonly studied yet very important for assessing root function (Iversen *et al.*, 2017). Additionally, root anatomy is another poorly studied trait, and was not measured it for this dissertation.

Root traits trade-offs

A basic principle in plant ecology is the trade-off between plant growth and survival (Grime, 1977), which has been successfully applied to plant leaf traits (Wright *et al.*, 2004), yet not as successfully to root traits (Weemstra *et al.*, 2016). The endeavor of mirroring the leaf economic spectrum on root systems has taken root ecology to the next level of complexity, exploring the

multidimensional variation of root traits worldwide (Norby & Iversen, 2013; Weemstra *et al.*, 2016; Bergmann *et al.*, 2020). This hypothesis of root traits trade-off has resulted in many recent studies seeking the relationships between root traits across species (Kong *et al.*, 2014; Lugli *et al.*, 2019). For example, it has been shown that there is a trade-off between root morphology (e.g. specific root length, root diameter), and some root traits related to nutrient acquisition (e.g. root phosphatase activity, mycorrhizal colonization, and root branching ratio); yet the direction of these trade-offs is still debated (Kong *et al.,* 2014, Eissenstat *et al.,* 2015, Weemstra *et al.,* 2016, Lugli *et al.,* 2019). Therefore, the Root Economics Spectrum (RES) might require more data to better understand the different spatial location of root traits in the current resource gradients, especially those related to P uptake, and to identify traits that are more responsive to environmental changes.

Some root traits are more responsive to environmental disturbances than others (Waisel *et al.,* 1991, Kong *et al.,* 2014, Ma *et al.,* 2018, Valverde-Barrantes *et al.,* 2020). For example, Kong *et al.,* (2014) found that root diameter, cortex thickness, stele diameter, and vessel diameter are less likely to change with environmental changes. However, traits like branching ratio, vessel density, and branch intensity are more responsive (Holdaway *et al.,* 2011, Kong *et al.,* 2014). These studies suggest that some traits are more sensitive to nutrient supply and competitive capacity. Yet, our knowledge of inter-specific, intra-specific, and inter-site root trait variation, and its influence on ecological processes is still scarce (Westoby & Wright, 2006; Defrenne *et al.*, 2019).

Root response to disturbances

Studies investigating the complexity of ecosystem responses to global change has greatly increased in the past few decades, and this has included, although in less proportion, the belowground component (Norby & Jackson, 2000; Wood *et al.*, 2012; Chung *et al.*, 2013; Cavaleri *et al.*, 2015). However, plant root responses to any environmental disturbance are tightly related to the type of disturbance, the magnitude, the plant community composition, and current environmental conditions; thus results are highly diverse (Norby *et al.*, 2004; Zhou *et al.*, 2012). This variability in results are also shown within warming experiments. For example, some experimental warming studies have shown that high temperature can increase root mortality

(Forbes *et al.*, 1997; Fitter *et al.*, 1999; Pritchard *et al.*, 2000; Majdi & Öhrvik, 2004; Wan *et al.*, 2004), increase root production (Majdi & Öhrvik, 2004; Wan *et al.*, 2004; Zhou *et al.*, 2012; Pugnaire *et al.*, 2019), or there could be no response of root dynamics (Dukes *et al.*, 2005). Further, most of warming studies have taken place in higher latitudes (temperate and boreal ecosystems).

Tropical ecosystems have little representation in root studies, and predictions of how tropical roots will respond to environmental disturbances are mostly empirical (Cavaleri *et al.*, 2015). Some of the environmental disturbances that tropical forests face are warming, droughts, hurricanes, flooding, and fires (Harris & Lugo, 2012; Malhi *et al.*, 2014). Studies on hurricane disturbances have shown that roots take up to 10 years to return to pre-hurricane conditions (Silver & Vogt, 1993), and that some trees can allocate old carbon to generate new roots when hurricanes result in widespread defoliation of the forest canopy (Vargas *et al.*, 2009). However, our understanding of the interactive effect of multiple disturbances on tropical roots is very limited.

Tropical forests and Puerto Rico

Tropical forests account for one third of global terrestrial primary production and stores half of terrestrial carbon in plant biomass (Field *et al.*, 1998; Beer *et al.*, 2010; Malhi *et al.*, 2014). Thus, small changes in carbon fluxes from these forests can have a considerable influence on the atmospheric CO_2 concentration (Nadelhoffer & Raich 1992, Wood *et al.*, 2012, Cavaleri *et al.*, 2015). Despite their importance in the C cycle and thus climate change, there are few available data of tropical forests compared to other biomes, and this is especially true for the belowground component (Cavaleri *et al.*, 2015; Iversen *et al.*, 2017).

There are some tropical sites that have been more represented than others, and this is the case of Puerto Rico. In the Fine-Root Ecology Database (FRED) (Iversen *et al.*, 2017), which holds around 315 root traits from more than 1213 species around the world, tropical data comprise 20%, of which 4% is from Puerto Rico (McCormack *et al.*, 2017). Nevertheless, root trait data from Puerto Rico are very representative of the tropics, considering its land surface area. However, differences in collection methods and fine-root classification makes hard to find

patterns across species and sites (Yaffar & Norby, 2020). Further, root phenology studies have been hardly studied in Puerto Rico.

Recently, great effort has been directed to sampling root phenology, and the response to warming treatment in Puerto Rico. The Tropical Responses to Altered Climate Experiment (TRACE) has developed a warming experiment in Sabana, Luquillo, where there are continuous measurements of aboveground and belowground components (Kimball *et al.*, 2018). Further, a multi-institutional project, Next Generation Ecosystem Experiment (NGEE) Tropics, started in 2015 and is dedicated to studying how tropical ecosystems will respond to climatic and atmospheric change. NGEE studies include above and belowground nutrient cycling and plant measurements emphasizing fine root traits and responses and the interaction between roots and soil.

As part of my doctoral dissertation, I worked with both, TRACE and NGEE Tropics, to understand root traits and root responses to environmental change in Puerto Rico. My overarching goal was to identify root traits and strategies in different environmental conditions and in response to warming in a wet tropical forest. This data ultimately could help fill some of the key knowledge gaps in belowground models of tropical forests, leading to better predictions of ecosystem responses to climate change.

The baseline of this dissertation is a review chapter (Chapter 2), where I made a compilation of all plant root studies that have taken place in Puerto Rico for the past 50 years. This chapter is organized by the root trait classification from FRED 2 (Iversen *et al.*, 2017; McCormack *et al.*, 2017), and provides over 1,000 data records from 46 published articles in both English and Spanish, and raw data shared by some researches. My next chapter (Chapter 3) is about how root-dynamic traits responded to two major disturbances: soil warming and hurricanes. In this study, I started measuring root response to experimental soil warming, but after the experiment was interrupted by two hurricanes ($\geq$ category 4), I had the opportunity to measure root response to the hurricane once the heaters were off, and the warming legacy interacting with the root recovery from the hurricanes. My next chapter (Chapter 4) brings a suite of root traits measurements (seven root traits) from some of the most common tree species in the Luquillo Experimental Forest (LEF), with the goal of expanding our knowledge of root traits from these

forests, and describing relationships between root traits across species and sites that could be suggesting a trade-off for phosphorus acquisition after the hurricanes. Further, I compared root traits before and after the hurricane disturbance to test their responsiveness. Finally, my conclusions chapter (Chapter 5), synthesizes all the outstanding results and patterns I found throughout this dissertation and provides a schematic approach of the belowground system and all the drivers that interacted with it during this study using an Odum System Diagram.

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Chapter 2

A historical and comparative review of 50 years of root data collection in Puerto Rico

My use of "we" in this chapter refers to my co-authors and myself. A version of this chapter was published in Biotropica: Yaffar D. & Norby R. J. 2020.

DY developed the concept for the project, collected and analyzed the data, and wrote the paper. RJN assisted in data analysis and reviewed and edited the paper.

Abstract

Fine roots play an important role in plant nutrition, as well as in carbon, water and nutrient cycling. Fine roots account for a third of terrestrial net primary production (NPP), and inclusion of their structure and function in global carbon models should improve predictions of ecosystem responses to climate change. However, studies focusing on underground plant components are much less frequent than those on aboveground structure. This is more marked in the tropics, where one third of the planet's terrestrial NPP is produced. Some tropical forests have been more represented in the literature than others, as demonstrated in the collective studies in Puerto Rico. This Caribbean island's biodiversity, frequency of natural disturbances, ease of access to forests, and long-term plots have created an ideal place for the study of tropical ecological processes. This literature review emphasizes 50 years of root research and patterns revealed around Puerto Rico. The data in this review were compiled from scientific publications, conference reports, symposiums, and raw data shared by some researchers. Emergent patterns include the shallow distribution of fine roots, the great variation in root biomass among different forest types, little variation in root phosphorus concentrations, the slow recovery of root biomass after Hurricane Hugo, and the fact that most data on roots come from the wet tropical Luquillo Experimental Forest, causing other habitat types to be under-represented. This review also shows the gaps in knowledge about fine roots in the island's ecosystems, which should be used to promote and guide future studies.

Introduction

Tree roots are an important carbon sink in plants representing approximately 26% of total plant biomass (Cairns *et al.,* 1997; Malhi *et al.,* 2011). However, coarse and fine roots are the most poorly understood plant components in terrestrial ecology, which is especially true in the tropics (Pregitzer, 2002; Comas & Eissenstat, 2009). This is problematic because tropical forests are among the most productive ecosystems in the world (Malhi *et al.,* 2011); thus, understanding global carbon cycling requires a thorough understanding of tropical forest components. From the little available data on tropical roots, the forests of Puerto Rico represent a disproportionally large amount relative to its land cover; however, many of these data are not readily available. Here, we assemble and summarize information on root systems from studies on tropical forests in Puerto Rico, including data from Spanish-language publications not previously published in English. We also discuss types of key research questions that could be addressed with these data.

Tree roots are categorized traditionally by their diameter. Roots bigger than 2 mm in diameter are called "coarse roots", and roots smaller than 2 mm in diameter are often called "fine roots" (Vogt & Persson, 1991). Fine roots can be further divided into adsorptive and transport based on their function related to nutrient and water uptake (Pregitzer, 2002; Iversen, 2014; McCormack *et al.,* 2015). Around 50% of soil respiration and 33% of net primary production (NPP) are attributed to fine roots (Jackson *et al.,* 1996; Hanson *et al.,* 2000; McCormack *et al.,* 2012; Kong *et al.,* 2014; McCormack *et al.,* 2015; Iversen *et al.,* 2017). Yet, despite their importance in plant survival, and global carbon and water cycling, fine roots are poorly represented in databases and Earth System Models (ESM; Warren *et al.,* 2015). One reason for this knowledge gap is the challenges associated with root sampling, which increase with higher plant diversity and poor infrastructure accessibility as is often the case when working in the tropics (Lamanna *et al.,* 2014; Siefert *et al.,* 2015; Iversen *et al.,* 2017).

Tropical forests account for around a third of terrestrial NPP (Field *et al.,* 1998), yet very little is known about fine-root traits (as defined by Violle *et al.,* 2007) in the tropics compared to temperate forests (Iversen *et al.,* 2017; Freschet *et al.,* 2017). Tropical trees represent around 20% of the data in the Fine-Root Ecology Database (FRED) (Iversen *et al.,* 2017), from which

4% is from Puerto Rico (Iversen *et al.,* 2017). In this review, we will first discuss the source of root data in relation to the geography and land-use history of Puerto Rico. We then describe the sources of data from the published literature and present the results of analyses organized by the root trait categories of FRED (Iversen *et al.,* 2017). We conclude with a discussion on root responses to environmental change and future research priorities.

Puerto Rico as a tropical research site

Puerto Rico is a Caribbean archipelago located within the geographic zone of the tropics (Figure 1). Puerto Rico has a high diversity of ecosystems (Harris *et al.,* 2012), and 10 of the 12 world orders of soils. The mean annual precipitation, air temperature, and elevation ranges from 254 to 5000 mm/year, 18-30^{o}C, and from sea level to 1338 m a.s.l., respectively (Frangi & Lugo, 1985; Murphy & Lugo, 1986a; Miller & Lugo, 2009a). According to the life zones of Holdridge, Puerto Rico's forests are considered "sub-tropical" (Ewel & Whitmore 1973). However, according to the climate classification of Köppen-Geiger, Trewartha, and Walter's zonobiomes, these forests are "tropical".

Up until the 1940's, more than 90% of today's Puerto Rican forests were agricultural fields. Beginning in the 1960's, most of these lands were abandoned following a transition of the island's economy from agricultural to industrial (Edel, 1962; Miller & Lugo, 2009a), allowing the unmanaged reforestation of the island (Miller & Lugo, 2009b). Currently, more than 60% of Puerto Rico is covered by forests (Brandeis & Turner, 2013).

Puerto Rico has a diverse fauna and flora that are greatly shaped by hurricane events on this island, which represent the greatest non-anthropogenic disturbance. The combination of the biological diversity, tropical climate, resilience to natural disturbances, and easy access to forest study areas make Puerto Rico an ideal tropical island for scientific studies. This is especially true for the Luquillo Experimental Forest (LEF), which has been managed by the USDA Forest Service (Forestry Division) and their antecedents since 1898.

Data sources and analysis

We assembled source material using Google Scholar, Web of Science, and Scopus for English-language reports, and Google Scholar for studies in Spanish. We looked at all papers returned when searching for the key words: “root”, “belowground”, “plant”, and “Puerto Rico” in English, and “raíz”, “raíces”, “planta”, “Puerto Rico”, “suelos” or “suelo”, árbol” or “árboles” in Spanish. Dr. Ariel Lugo, director of the International Institute of Tropical Forestry - Puerto Rico, provided raw data from Frangi & Lugo (1985), Murphy & Lugo (1986a), Cuevas *et al.,* (1991), and Lugo (1992). Colón & Lugo (2006) and Teh, Silver & Scatena (2009) also provided raw data for this review. Our database contains 1,091 records from 46 studies, from which eight studies are in Spanish.

To compare Puerto Rico’s data with other tropical and global studies, we used data from FRED (Fine-Root Ecology Database Version 2, https://root.ornl.gov). To compare root biomass by depth in both dry and wet forests, we defined fine roots as all roots less than or equal to 2 mm in diameter. When a study involved experimental manipulations, we used only the control data for biomass and chemistry comparisons. We modeled root vertical distribution based on Gale & Grigal (1987) asymptotic equation $Y = 1-ß^d$, where d is depth, Y is the proportion of roots from the surface to depth d, and beta is the numerical index of rooting distribution (Jackson *et al.,* 1996). High values of beta represent deeper rooting. For this, we used cumulative fraction of biomass for all studies that classified root biomass by depth, including fine roots and mixed roots. We calculated the beta for only the studies in which root biomass reached 70 cm or more, and we compared it with the beta when using all the studies regardless of the maximum depth.

To compare root:shoot ratio, we used data that were collected using the destructive method (whole tree sampling) and reported both belowground (fine + coarse roots) and aboveground biomass at the record level (trunk + branches + leaves) from species with at least three individuals. We calculated root:shoot ratio by dividing belowground biomass by aboveground biomass from each species. To compare Puerto Rico root:shoot ratio data with global meta-analyses from Cairns *et al*. (1997) and Mokany *et al.* (2006), we used the same formula as their

methods: root biomass = exp(-1.0850+(0.9256 × ln(shoot biomass)), and root biomass=0.18 × shoot biomass, respectively.

All statistical analyses were performed in R studio (RStudio Team, 2016). We used logarithmic regressions to compare root biomass and root nutrient concentrations with depth. We performed simple linear regressions to compare root vs shoot biomass, soil vs root N and P, and fine-root biomass vs soil N. We used an ANOVA to compare root:shoot ratio among species, and N and P among species. We performed an ANCOVA to test the effect of tree bole diameter (>5 cm, and <5 cm) and model source (i.e., meta-analyses) on the slope of our data regression. We ran multiple regressions between root biomass, depth, and mean annual precipitation from the book "Los Bosques de Puerto Rico" to find the best correlate of root biomass.

The story of "root digging" in Puerto Rico

For scientific purposes, root descriptions from Puerto Rico have been documented since the 1940's. Most of these studies encompass qualitative descriptions of roots (White & Childers, 1945; LaRue, 1952). From 1962 on, the study of roots became more quantitative. In 1967 began a study on the structure and function of various ecological compartments in LEF, including roots. The book that resulted from this study (Odum & Pigeon, 1970), particularly chapters by Odum and Ovington & Olson, reported root biomass, nutrient concentrations, and morphology of 42 species in LEF. However, this work classified fine roots as less than 5 mm in diameter, making it difficult to compare with recent studies that focused on a narrower definition of fine roots (<2 mm).

Following these studies, others have reported root biomass and nutrient concentration as part of their measurements in Puerto Rico (Figure 1). Most studies were based in LEF, including the tabonuco forest (dominated by *Dacryodes excelsa*; Kangas, 1992; Bloomfield *et al.*, 1993; Cusack *et al.*, 2011), the Sierra Palm forest (dominated by *Prestoea montana*; Frangi & Lugo, 1985), the Colorado forest (dominated by *Cyrilla racemiflora*; Cusack *et al.*, 2011), and the elfin forest (dominated by *Tabebuia rigida*; Cordero, 1999). The most studied species from these forests that included belowground measurements were *D. excelsa*, *Cecropia schreberiana*, and

Manilkara bidentata, from which only *C. schreberiana* is within the 10 species with greatest importance value on the island (Marcano Vega, 2019). The species with greatest importance value of Puerto Rico, *Spathodea campanulata* and *Guarea guidonea*, are underrepresented in these studies. The dry forest biome in general has less representation of root studies in Puerto Rico (Murphy & Lugo, 1986a; Murphy & Lugo, 1986b; Colón & Lugo, 2006; Cusack *et al.*, 2009). No whole-tree excavation was performed in forests outside of the LEF; thus, dry forest species (e.g., *Leucaena leucocephala*) are underrepresented for underground studies. Even fewer studies have measured roots in other ecosystems of the island (Parrotta, 1999; Viera *et al.*, 2008; Marin-Spiotta *et al.*, 2009; Ostertag *et al.*, 2008; Lugo *et al.*, 2011).

From all the studies considered here, 18% focused primarily on roots; the rest included roots as a secondary measurement. Following the FRED classification of fine-root traits (McCormack *et al.*, 2017), 25 studies considered in this review measured root system traits, 13 measured chemical traits, nine studied root dynamics, six looked at microbial association, four measured root physiology, two measured root architecture and morphology, and none studied anatomical traits (Figure 1). Here, we summarize these studies, assigning them by the different root traits categories of FRED.

Root system

Root biomass

Fine-root classification varied among the studies that measured fine-root biomass, making it difficult to synthesize across results. Ovington & Olson (1970) reported, using whole-tree removal method, an average biomass of 64.8 Mg ha^{-1} for coarse roots only (described as more than 5 mm in diameter), and Odum (1970a) reported 3.52 Mg ha^{-1} for small roots (described as less than 5 mm in diameter). Together, coarse and fine roots represent 25% of total tree biomass from these studies. Golley *et al.* (1962) measured root biomass (fine + coarse) from a red mangrove (*Rhizophora mangle*) forest on the southern shores of Puerto Rico. Prop-root (adventitious aerial roots) biomass was taken by whole-tree removal; the total prop-root biomass was 14.37 Mg ha^{-1}. Fine roots (<5-10 mm in diameter) plus peat and coarse roots (> 2 cm in diameter) were taken by cores and weighed 40 Mg ha^{-1} and 9.97 Mg ha^{-1} respectively. Coarse

root biomass is less than that reported by Ovington & Olson (1970) in the LEF, which may reflect differences in vegetation, root diameter classification, and the method of collection. Prop and coarse roots represent 46% of total tree biomass, which is more than in Ovington & Olson (1970). Although the diameter selected in this study for fine roots (5-10 mm) is greater than the rest of the studies presented in this review, and the biomass included peat biomass, we can nevertheless conclude that mangroves present especially high fine-root biomass in Puerto Rico. Frangi & Lugo (1985) found that in a primary wet palm forest at LEF total root biomass was 61.7 Mg ha^{-1} up to 95 cm deep, which represents 21.5% of total tree biomass, similar to what Ovington & Olson (1970) showed. Total root biomass was also obtained by using the coring method. Fine-root biomass (<1 mm) was 24.6 Mg ha^{-1} within 30 cm of depth and 27.2 Mg ha^{-1} at 95 cm of depth.

In the book "Los Bosques de Puerto Rico" (Lugo, 1983), seven studies reported root biomass by depth but not by species or diameter classification (Table 1). The variability in plant composition, precipitation, and soil type made root biomass distribution variable as well. We performed multiple regressions between root biomass, depth, and mean annual precipitation, but no pattern was found. The floodplain in Patillas (Alvarez *et al.,* 1983) has the highest root biomass in the book (35.5 Mg ha^{-1}), which could be related to the species composition (mangrove forest), high organic material accumulated in that horizon, and the anoxic conditions. This biomass is 15 Mg ha^{-1} less than the one reported by Golley *et al.* (1962), who included peat in the biomass calculations. The lowest root biomass was from a mature forest in Maricao with 6.27 Mg ha^{-1} in 30 cm of depth (Rivera *et al.,* 1983) where the soils were very dry and poor in calcium (Whittaker, 1954).

In the 1990's more studies began to consider fine roots following the "less than 2 mm in diameter" classification and using coring methods instead of total tree removal. Lugo (1992) reported 2.4 Mg ha^{-1} of fine-roots biomass (<1 mm dimeter) within 30 cm of depth in the tabonuco mature forest at LEF, which is around 10% of what was reported by Frangi & Lugo (1985). McGorddy & Silver (2000) found that fine-root biomass decreased in an elevation gradient at LEF (from 4.7 Mg ha^{-1} at 1000 m.a.s.l to 1.5 Mg ha^{-1} at 180 m.a.s.l, in 10 cm of depth). For a secondary forest in LEF, Cuevas *et al.* (1991) and Lugo (1992) measured 3.6 Mg

ha^{-1} of fine-root biomass (<2 mm diameter, to 30 cm depth) and 2.7 Mg ha^{-1} (<1 mm diameter, to 20 cm depth), respectively, which are slightly greater than the reported for a mature forest (2.4 Mg ha^{-1}; Lugo, 1992).

Fine-root biomass in a pine plantation in LEF was 0.7 Mg ha^{-1} (Cuevas *et al.*, 1991) and 0.9 Mg ha^{-1} (Lugo, 1992). A mahogany (*Swietenia macrophylla*) plantation in LEF had an average fine-root biomass of 1.1 Mg ha^{-1} (Lugo, 1991), which is similar to the pine plantation and less than the secondary forest. Fine-root biomass (<1 mm) in this same study represents on average around 20% of total root biomass, and total root biomass did not exceed 20.6 Mg ha^{-1} (Lugo, 1992), which is a third of what Ovington and Olson (1970) reported.

In a dry mature forest, Murphy and Lugo (1986a) measured a total fine-root biomass (<1 mm diameter) of 1.5 Mg ha^{-1} up to a depth of 100 cm, and 1.25 Mg ha^{-1} up to 30 cm of depth, which is less than in the wet mature forest (Frangi & Lugo, 1985; Lugo, 1992). Total root biomass was also less than what Ovington & Olson (1970), and Frangi & Lugo (1985) reported for the wet mature forest 45 Mg ha^{-1}, but higher than the secondary forests and plantations (Lugo, 1992).

Colón & Lugo (2006) reported a fine-root biomass (<2 mm diameter) of 5.39 Mg ha^{-1} at only 10 cm of depth in a dry mature forest, which is more than three times higher than Murphy & Lugo (1986a). The difference in fine-root biomass between Murphy & Lugo (1986a) and Colón & Lugo (2006) might be explained by the root diameter size, and the depth of collection.

Fine-root biomass from different land uses in the dry forest of Puerto Rio was also reported by Colón & Lugo (2006). In the previous land-use types of former human settlements (houses), a baseball park, an agricultural field, and a charcoal production area, fine-root biomass was 0.2 Mg ha^{-1}, 0.3 Mg ha^{-1}, 0.28 Mg ha^{-1} and 0.47 Mg ha^{-1} of fine-roots, respectively. These urban forests had less root biomass in the top 10 cm than any other site of the island.

Vertical root distribution

Studies that have separated root data by different soil depth layers show a declining fine-root biomass with depth. This pattern is clear for both, wet and dry forests of Puerto Rico (Figure 2a and 2b). Data points taken by Colón & Lugo (2006) and Murphy & Lugo (1986a) from the dry forest show less root biomass than in the rain forest.

The vertical root distribution of the dry forest of Puerto Rico is slightly deeper than the wet forest, based on the beta coefficient (Gale & Grigal, 1987; Jackson *et al.,* 1996). However, Puerto Rico's rooting distribution is shallower than other tropical evergreen forests globally. On average, a tropical evergreen forest has a beta of 0.96–0.97 (Jackson *et al.,* 1996; Schenk & Jackson, 2002), whereas beta in the wet tropical forest of Puerto Rico is 0.90 and 0.91 in the dry forest (Figure 3). A smaller beta value describes a greater proportion of roots closer to the soil surface. We obtained similar beta when we only used studies with depths greater than 70 cm (Frangi & Lugo, 1985; Murphy & Lugo, 1986a; Lugo, 1992). Although we need more studies that consider deeper sampling, we can conclude that more than 80% of root biomass in the island is in the first 20 cm of depth. Further, Odum (1970b) study showed that less than 1% of roots were found deeper than 80 cm at LEF.

Root:shoot ratio

Some studies reported on belowground and aboveground biomass of common species by removing whole trees from seedlings to adult trees up to 35 cm in diameter (Ovington & Olson, 1970; Fetcher *et al.,* 1996; Parrotta, 1999; Cordero, 1999; Stone *et al.,* 2013). Ovington & Olson (1970) studied total below- and aboveground biomass of 42 species in LEF. Fetcher *et al.* (1996) measured below- and aboveground biomass from two pioneer species (*Phytolacca rivinoides, C. schreberiana*) and two non-pioneer species (*P. riparia, M. bidentata*) in LEF under fertilization treatments. Cordero (1999) reported root biomass of *C. shcreberiana* in the dwarf forest of LEF from an experiment looking at wind exposure on plant form. Parrotta (1999) describes root biomass from *Casuarina equisetifolia, Eucaliptus robusta*, and *L. leucocephala* from experimental plantations at Toa Baja. Stone *et al.* (2013) measured above and belowground biomass of *Tabebuia heterophyla* planted in a greenhouse with soil from LEF and different

fertilization treatments. We used these data (with no treatment) to compare root:shoot ratio among species.

On average using all diameter roots, the root:shoot ratio from these studies is 0.45, ranging from 0.78 in *C. shreberiana* to 0.19 in *Ocotea leucoxylon*, but variation within species was large, and there was no statistically significant difference among species. Root:shoot ratio is greater in trees with bole diameter > 5 cm ($P<0.01$) compared to smaller trees (diameter < than 5 cm). However, we also found no differences among species within each size category. *C. borinquensis* and *C . shreberiana* have the highest root shoot ratio for the trees > 5 cm in diameter, and *S. berteroana* has the lowest root shoot ratio in this size class. However, *S. berteroana* has the highest root:shoot ratio for trees < 5 cm in diameter, and *O. leucoxylon* has the lowest ratio. Thus, we can conclude that tree diameter plays an important role in root:shoot ratio.

The root vs shoot biomass regression for trees of all diameters shows a significant difference ($P<0.05$) of the slopes between our model and the models reported in two meta-analyses for tropical evergreen forests (Crains *et al.,* 1997; Mokany *et al.,* 2006; Figure 4). Trees > 5 cm diameter have greater root biomass than predicted in the two global meta-analyses (Cairns *et al.,* 1997; Mokany *et al.,* 2006), and trees <5 cm diameter have a shallower slope than that of the larger trees (Figure 4). These meta-analyses considered more samples from around the world and a wider range of tree size class. However, these studies also used root data from different methods of collection, such as soil cores for fine roots, whole tree excavation (direct) and/or tree diameter (allometry: indirect), which could lead to the differences of total root biomass compared to using whole large tree excavation only (Waring & Powers, 2017).

Root chemistry

A few studies measured root nitrogen (N) and phosphorus (P) concentrations in Puerto Rico, one of which (Pett-Ridge & Silver, 2002) did not considered tree roots and was thus dropped from the analysis. Some authors reported N and P from only fine roots (Murphy & Lugo, 1986a; Cuevas *et al.,* 1991; Lugo, 1992; Parrotta, 1999; Scatena *et al.,* 1993), whereas others reported from a mixed sample of fine and coarse roots (Ovington & Olson, 1970; Lugo *et al.,* 2011). Only

Parrotta (1999), Lugo *et al.* (2011), and Ovington & Oslon (1970) reported root nutrient concentration by species. The rest of the studies used coring methods, and root nutrient concentration was not species-specific (Table 2). We compared these data separately based on the method of collection. Nevertheless, considering that root nutrient concentration changes with root diameter (Luse, 1970; Iversen *et al.*, 2008; Xia *et al.*, 2010; Jia *et al.*, 2011), soil nutrient availability (Gower, 1987; Vogt *et al.*, 1995; Li *et al.*, 2015), and whether the tree is an N-fixer (Valverde-Barrantes *et al.*, 2007), this comparison can suggest only broad patterns.

From the studies that used the coring methods, Cuevas *et al.* (1991) and Lugo (1992) reported very similar P (0.03%) and N (0.6–0.8%) concentrations in fine roots (<2 mm) from a pine plantation and its adjacent secondary forest (Table 2). However, fine-root N and P concentrations were higher in the mahogany plantation (0.06% P, 1.0% N) and its adjacent secondary forest (0.06% P, 1.3% N; Lugo, 1992; Table 2). Scatena *et al.* (1993) showed similar P concentration (0.05%) but higher N concentration (1.57%) for fine roots (<5 mm in diameter) in a mature forest of Bisley, LEF (Table 2). Sliver & Vogt (1993) found even higher fine root N concentration (1.8%) in the same forest of Bisley, but still similar P concentration (0.05%; Table 2). In the dry forest, Murphy & Lugo (1986a) reported similar P and N concentrations to what was found for Bisley (0.06% and 1.4%, respectively; root diameter <\1 mm; Table 2). Both fine-root N (Figure 5a) and fine-root P (Figure 5b) concentrations decrease exponentially with depth.

Fine roots reported in Puerto Rico have a similar range in root N concentration compared to other species from the tropics, ranging from 0.3% to 1.9% (Gijsman *et al.*, 1997; Collins *et al.*, 2016). Gijsman *et al.* (1997) reported N concentration in fine roots (<2 mm) from Panamá (1.25%), which is higher than the average for roots less than 2 mm in Puerto Rico (0.9% N). The lowest root N concentration is from the pine plantation and secondary forest of Guzmán, which skewed the total root N average. Puerto Rico has a slightly lower mean P concentration (0.04%) compared to other studies. A study in Barro Colorado, Panamá (Collins *et al.*, 2016) and a study in Maui-Hawaii (Schuur, 2001), reported a P average for roots less than 2 mm in diameter of 0.06 and 0.05%, respectively. However, the average root P concentration in Puerto Rico is highly influenced by the low P concentration in the pine plantation. Further, species composition plays an important role in fine-root nutrient concentrations, as well as root diameter, and soil nutrient

availability as reported in other studies (Luse, 1970; Gordon & Jackson, 2000; Valverde-Barrantes *et al.*, 2007; Iversen *et al.*, 2008; Xia *et al.*, 2010; Jia *et al.*, 2011 Li *et al.*, 2015).

Using available data for root nutrient concentration where species identity was known and tree diameters varied (Ovington & Olson, 1970; Parrotta, 1999; Lugo *et al.*, 2011), we found that there is a significant difference in N concentration ($P<0.01$) among the 38 studied species (Figure 6a), but no statistically significant difference in P concentration. The legumes *Ormosia rugii* and *Inga vera* had higher N (1.32% and 1.07%, respectively), and *L. leucocephala* had the lowest N concentration (0.13%) (Figure 6b).

We correlated fine-root nutrient concentration to soil nutrient concentration using raw data from Lugo (1992) and Cuevas *et al.* (1991). There is a positive linear correlation between soil N and fine-root N (Figure 7a). The same pattern is shown for fine-root P and soil P, although there is an outlier that drives much of the regression (Figure 8). Using only Lugo (1992) data, fine-root biomass is also positively correlated to soil N (Figure 7b), but not with soil P.

Root dynamics

Root production

Two studies analyzed root production among Puerto Rico's forests (Cuevas *et al.*, 1991), and one measured root regrowth (Kangas, 1992). Cuevas *et al.* (1991) used in-growth cores to measure root production (<2 mm in diameter) collected every 6 months in plantation plots and a paired secondary forest plot at LEF. They found that a secondary forest produced on average 8.54 Mg ha^{-1} $year^{-1}$ of fine roots in the first 30 cm of soil depth, while that of a pine plantation was 1.15 Mg ha^{-1} $year^{-1}$. The differences were explained by species richness, litterfall decomposition rate, and a combination of conditions that promote root production. Templer *et al.* (2008) reported a root production (<2 mm in diameter) of 1.6 Mg ha^{-1} over 11 months in 10 cm of soil depth in a mature forest of LEF. If this production is maintained constant over a year, root production would be 1.74 Mg ha^{-1} $year^{-1}$ which, despite the soil depth differences, is five times less than the one reported by Cuevas *et al.* (1991) for a secondary forest.

Kangas (1992) measured fine-root regrowth using pit excavation and re-excavation in 10 sites of a mature forest at LEF after 1 year and 4 years. However, due to the long inter-collection intervals, only the biomass accumulation was measured, not production. Kangas (1992) showed a root accumulation of 2.89 Mg ha^{-1} $year^{-1}$after a year, and 4.77 Mg ha^{-1} after 3 years.

Root decomposition

Six studies measured root decomposition in Puerto Rico, most of them in the wet forest of LEF and some in the dry forest (Table 3). Silver & Vogt (1993) used trench plots to measure fine-root decomposition in LEF (tabonuco forest). They found that 65% of fine root biomass remained after a year (decay constant *k* of 0.4 per year), which resembles the global pattern of root decomposition for broadleaf trees (Silver & Miya, 2001; Table 3). In the same forest, Bloomfield *et al.* (1993) used litterbags to compare root and leaf decay. They found no difference in root decay between riparian and upper-slope sites, which means that moisture was not as important as substrate quality for decay rate. In this same study, they found that root decay was slower for roots than for leaves in *D. excelsa* and *P. montana.* Leaves had more Ca than roots, whereas Al and Fe were higher in roots than in leaves. Further, they found that root decay for *D. excelsa* had a slower rate than roots of *P. montana*. This was explained by the N found within its roots for microbial decomposition.

In southeastern Puerto Rico (Sierra de Cayey), Ostertag *et al.* (2008) measured root decomposition for a forest chronosequence. Root decomposition was fastest in the 60-year-old sites and slower in the 10- and 30-year-old sites (*k* constant: 0.76, 0.48, and 0.46 per year, respectively). The total final mass remaining ranged from 26% to 39% after 22 months. Similar patterns in decomposition with forest age were reported by Silver & Miya (2001); thus, older forests seem to have faster fine-root decomposition.

Cusack *et al.* (2009) analyzed root and leaf decomposition of *Andropogon gerardii, Drypetes glauca,* and *Pinus elliotti* from LIDET (Long-term Intersite Decomposition Experiment Team) data, which included Luquillo wet forest and Guánica dry forest, Puerto Rico. Fastest decomposition of root biomass was found in Luquillo compared to Guánica (k constant: 1.06 and

0.26 per year, respectively). From the three species, *A. gerardi* had the fastest root decomposition, and *P. elliotti* had the slowest. Corroborating this, Harmon *et al.* (2009) reported, based on the same database from LIDET, that *P. elliotti*, had the largest root remaining of all the studied species (40.28%).

Root physiology

Templer *et al.* (2008) measured fine-root nitrogen uptake in a $^{15}NH_4^+$ and $^{15}NO_3^-$ addition experiment at LEF. They used ingrowth cores which were harvested sequentially up to 7 days. Fine roots took up 28% of the inorganic N during the first 24 hours, especially from $^{15}NH^+_4$ (~80%). Roots represented a significantly greater sink for N from $^{15}NH_4^+$ compared to microbial biomass, but not for $^{15}NO_3^-$.

Another physiological trait measured in Puerto Rico is root phosphatase activity. Luse (1970) found that when applying ^{32}P in the litter layer of a plot in LEF, fine roots from saplings had higher amounts of ^{32}P (20 times higher) than soils, and he attributed this to phosphatase activity and fungal diversity. Stone *et al.* (2013) used seedlings of *T. heterophylla* in a fertilization experiment and showed that, when adding P in the soil, phosphatase activity decreased significantly. Cabugao *et al.* (2017) corroborated this when they found a negative correlation of root phosphomonoesterase (PME) activity with P availability increase on adult trees up to 20 cm in diameter. However, Cabugao *et al.* (2017) also found that tree species play an important role in modulating root and bacterial PME activity even in the same P conditions. Both studies support the negative correlation pattern between phosphatase activity and soil P availability (Treseder & Vitousek, 2001).

Microbial association

Root microbial associations have rarely been studied in Puerto Rico but have become more common in the past 5 years. The first time that root mycorrhizal colonization was mentioned in a Puerto Rico study was in 1950 by the Puerto Rican Forest Service (Briscoe, 1959), when *Pinus*

species (*P. elliottii* and *P. caribaea*) were successfully stablished only after been inoculated with mycorrhizal fungi.

Due to Puerto Rico's large diversity in endemic orchids and their mycorrhizae specificity, fungal mycorrhizae and endophytes in these plants were also studied on the island. Bayman *et al.* (1997) described fungal endophytes from seven species of orchid roots and leaves from Carite State Forest. They found that root endophytes *Xylaria* and *Rhizoctonia* were more commonly found in roots (29 % and 45%, respectively). Further, the same authors found that the naturalized orchid *Oeceoclades maculata* is highly specific to the mycorrhizae fungi *Psathyrella* cf. *candolleana* during seed germination, but promiscuous as an adult (Bayman *et al.,* 2016).

Bachelot *et al.* (2017) and Bachelot *et al.* (2018) studied the diversity of mycorrhizal fungi in the wet forest of Puerto Rico. Bachelot *et al.* (2017) found that at the local scale, arbuscular mycorrhizae fungi (AMF) diversity in soil counteracted negative effects of leaf damage on seedling mortality. At the community scale, only rare tree species seedlings benefited from soil AMF diversity. Bachelot *et al.* (2018) showed that early-successional plant species are less dependent on the diversity of AMF than mid- and late-successional plant species, which contradicts other findings in tropical studies (Fischer *et al.,* 1994; Kiers *et al.,* 2000; Matsumoto *et al.,* 2005).

The only study that has related the microbiota with root architecture and morphology is described in Irizarry & White (2017). They germinated wild cotton (*Gossypium hirsutum*) seeds that were inoculated with bacteria isolated from non-cultivated Malvaceae plants from various parts of Puerto Rico, including Rincón, Guayama, and LEF. They found that the bacteria *Bacilus amyloliquefaciens* enhanced primary and lateral root growth by three times in comparison to those without the bacteria. Further, roots from an inoculated seed had thinner roots and higher root branching ratio than with no inoculation.

Root response to environmental changes

Fine-root responses to environmental changes have not been well documented in the tropics compared to temperate forests (Cuevas & Medina, 1988; Silver & Vogt, 1993; Fetcher *et al.,* 1996; Wright *et al.,* 2011). However, since hurricanes are the major non-anthropogenic disturbance in Puerto Rico, a few studies measured root response to hurricanes. Soil fertilization, and drought are other environmental changes that were less studied in the island. Human disturbance, specifically soil compaction, was also measured in Puerto Rico.

Root response to hurricanes

Root grafting is a common morphological root trait in hurricane prone areas (LaRue, 1952; Basnet *et al.,* 1993; Lugo & Scatena, 1995). LaRue (1952) found more tree genera exhibiting root grafting in Puerto Rico than in any temperate forests he studied. Lugo & Scatena (1995) suggested that tree unions may offer a more secure foundation when exposed to hurricane winds. For example, tabonuco (*D. excelsa*) trees on ridges tend to have more root grafting than on slopes and are known to be more successful in surviving and resprouting after hurricanes than trees without these unions (Lugo & Scatena, 1995). However, only bigger trees (>5 cm in diameter) present unions (Basnet *et al.,* 1993). Further, root grafting also forms an organic bench which can provide better conditions for root aeration and nutrient accumulation (Basnet *et al.,* 1993).

Studies showed a decrease in root biomass almost immediately after a hurricane disturbance (Parrotta & Lodge, 1991; Silver & Vogt, 1993). Root recovery rate varied depending on the hurricane intensity, precipitation during the recovery period, litter decomposition rate, and species-specific root traits such as root length (Parrotta & Lodge, 1991; Beard *et al.,* 2005; Lodge *et al.,* 2016).

Silver & Vogt (1993) simulated a hurricane disturbance by removing all aboveground biomass. After 2 months from the removal, root biomass declined 40%. This same study measured root biomass after Hurricane Hugo, where root biomass declined for the next 8 months. Silver &

Scatena (2009) continued measuring root biomass on the same sites and showed that it took more than 10 years to recover root biomass after Hurricane Hugo (Figure 9). Recovery was even slower for plots with aboveground removal. Root nutrient concentrations (P and K) decreased after biomass removal, and root P decreased even more after the hurricane.

Parrotta & Lodge (1991) measured fine root (<3 mm) biomass before and after Hurricane Hugo at El Verde Field Station area (LEF). They showed that live fine-root biomass decreased from 4.23 Mg ha^{-1} to 0.02 Mg ha^{-1} 2 months following the hurricane. Fine-root biomass recovery was slow, reaching to 0.49 Mg ha^{-1} after 8 months (Parrotta & Lodge, 1991). This slow recovery was attributed to physical disturbance, moisture stress (low rainfall after Hugo), and changes in non-structural carbohydrates in coarse roots.

Beard *et al.* (2005) showed that root mortality increased immediately after Hurricane Hugo. They also noted that each species had different decay rates and capacity to conserve nutrients. For example, tabonuco (*D. excelsa*), showed a faster decay rate compared to other common species, resulting in faster recovery time. Beard *et al.* (2005) concluded that the post-Hugo drought might have influenced the root recovery, causing high root mortality and emphasizing the importance of considering multiple disturbance responses. Ongoing investigations are measuring the effect of previous warming treatments in the TRACE experiment (Kimball *et al.*, 2018) on root responses after Hurricanes Irma and María in 2017 (Yaffar, 2020).

Lodge *et al.* (2016) correlated fine-root length to coarse woody debris from Hurricane Hugo and Hurricane Georges (Lodge *et al.*, 2016). Root length was used to indicate nutrient hotspots. They found that root length was greater away from dead logs in the dry season, and greater under logs during the wet season. Despite root length being significantly positively correlated with soil microbial C, the latter did not differ between dry and wet season, which is inconsistent with a competitive exclusion hypothesis. They hypothesized that soil P may have contributed to the rooting patterns, or the differences in the secondary compounds of the decaying logs might resulted in fine-root length differences. The Torres (1994) study suggested that aboveground adventitious roots from *Cyrilla racemiflora* extracts nutrients from dead wood from the same tree or nearby trees, allowing its recovery in LEF.

Root response to soil chemical and physical changes

Fetcher *et al.* (1996) measured the response of tree seedlings, two pioneers (*C. schreberiana and P. rivinoide*) and two non-pioneers (*M. bidentata and riparia*), to fertilization in a landslide at LEF. Across all four species there was more allocation to roots in the N + P treatment than in the N or P treatments alone. The pioneer species responded more to nutrient addition than the non-pioneer species. This was explained by the high potential growth and photosynthetic rates of pioneer species, as well as the mycorrhizal colonization.

Stone *et al.* (2013) found that seedlings of another early successional native species, *T. heterophylla,* in LEF increased its root biomass only with P addition. Contrary to Fetcher *et al.* (1996), Stone *et al.* (2013) showed no changes in root biomass when increasing soil N+P. Additionally, they measured changes in five extracellular enzyme activities, which helped correlate soil P deficiency with root growth when P was added. Therefore, multiple species-specific root traits, such as enzymatic activity, root morphology, architecture, root hair density, and mycorrhizal colonization should be taken in consideration in future studies to correlate with soil nutrient concentrations and better understand root uptake.

Soil compaction is another environmental factor, usually human-driven, that affects root growth. Tirado-Corbala & Slater (2010) measured root biomass from seedlings of planted trees of Puerto Rico in different soil types and compaction levels. Compaction caused a significant decrease in root mass for *Tecoma stans* but not for *Tabebuia rosea* or *Callistemon citrinus*. The three tree species presented higher root mass grown in sandy clay loam soils compared to trees grown in clay soil. Future studies should measure soil texture and compaction in relationship to different root physiological traits to better understand root response to soil physical changes.

Conclusions

The studies mentioned in this review gathered important pieces of information regarding root traits from different forest types in Puerto Rico over the past 50 years. Some of the conclusions

we gathered from this collection are: (a) Rooting depth distribution in the wet forest is shallower (above 20 cm) than presented in other tropical studies, yet only three studies considered rooting distribution further than 30 cm in depth. Thus, we suggest that more future studies confirm this pattern in the different forest types. The dry forest has slightly deeper distribution than the wet forest, but there are not enough data on vertical distribution of roots in the dry forest for a strong conclusion. (b) Total root biomass is greater in the wet forest than in the dry forest. (c) Fine-root biomass is much greater in the palm primary forest (Wet forest-LEF), followed by the secondary wet forest (LEF), the dry mature forest (Guánica), a mahogany plantation, a pine plantation, and urban forests in the dry area of the island. (d) There is a positive correlation between fine-root biomass and soil N concentration in the secondary forest and plantation. (e) Root N and P concentrations are species-specific and vary with root diameter. (f) Root:shoot ratio varies depending on the tree bole diameter (smaller trees have lower root:shoot ratio than larger trees), and it is higher in Puerto Rico (for trees >5 cm in diameter) compared to other tropical sites. (g) The diversity of mycorrhizal fungi is correlated with plant successional type, where early-successional plant species are less dependent on AMF diversity than mid- and late-successional plants. (h) Root grafting is an advantageous morphological trait in response to hurricane winds. (i) Root recovery after multiple disturbances (hurricane + drought) takes up to 10 years.

Studies including root data in Puerto Rico are very representative for the tropics, considering its landcover. However, there are many fine-root functional traits that have not been fully explored. There have only been two studies that have established long-term research to quantify the plasticity of root traits and their response to environmental changes. The nutritional advantage (if any) of root grafting has not been evaluated. Further, different root morphological, architectural, and chemical traits have not been directly correlated to the physiological traits. These are some of the understudied areas that could lead to future studies. Our synthesis can be used to enrich root database representation of the tropics, as well as provide more conclusive evidence for important hypotheses in root ecology that will ultimately better inform Earth System Model.

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Appendix

Table 1. Total root biomass (fine+coarse) by depth, forest type, and site. These data comprise a compilation of studies (each chapter/author) from Lugo (1983).

Forest	MAP[a] (mm)	Site	Type	Dominant species	Author	Depth	Root biomass Mg ha^{-1}	SE[b]
Wet	3870-4700	LEF	Mature	*Prestoea montana*	Berrios & Perez	0-25[c]	3.42	0.8
						0-30[d]	12.78	4.34
						0-30[e]	29.18	2.47
Wet	2520	Maricao	Mature	Mixed	Rivera *et al.*	0-10	6.86	2.94
						10-20	2.04	1.08
						20-30	1.11	0.79
						0-10	1.58	0.9
						10-20	2.89	0.94
						20-30	1.8	1.3
Wet	4700	LEF	plantation	*Switenia macrophylla*	Perez *et al.*	0-10	8.03	2.74
						10-20	4.74	0.21
						20-30	5.03	0.46
Wet	3920	LEF	plantation	*Switenia macrophylla*	Perez *et al.*	0-10	8.04	2.55
						10-20	4.62	0.27
						20-30	6.19	1.31
Karst	1411	Cambalache	Secondary	*Coccoloba diversifolia*	Serrano *et al.*	0-5	6.25	2.25
						5-15	0.4	0.12
						15-25	0.13	0.06
						0-5	6.13	1

Table 1 (Continued)

Forest	MAP[a] (mm)	Site	Type	Dominant species	Author	Depth	Root biomass Mg ha^{-1}	SE[b]
						5-15	1.07	0.31
						0-5	7.87	1.25
						5-15	2.8	0.62
						15-25	1.2	0.5
Dry	1413	Sabana grande	Mature	Mixed	Alvarez	0-5	6.2	1.89
						5-15	2.78	0.78
						15-25	1.11	0.22
Wet	1596	Caguas	Secondary	*Albizia procera*	de Rubio *et al.*	0-10	2.67	1.18
						10-20	1.78	78
						20-30	2.08	1.15
Wet	1915	Rio Piedras	Secondary	*Hymanea courbaril*	de Rubio *et al.*	0-10	13.74	2.46
						10-20	7.99	0.57
						20-30	0.84	0.21
Flood	1800	Patillas		*Laguncularia racemosa*	Alvarez *et al.*	0-10	11.67	1.84
						10-20	7.91	4.18
						20-30	15.93	13.37
Wet	2000	Rio blanco Naguabo		*Pterocarpus officinalis*	Alvarez *et al.*	0-10	4.93	1.04
						10-20	4.56	1.77
						20-30	6.57	2
Wet	1500	Dorado		*Pterocarpus officinalis*	Alvarez *et al.*	0-10	18.26	7.99
						10-20	5.12	2.23
						20-30	2.62	1.39

a. Mean annual precipitation, b. Standard Error, c. Elevation 400, d. Elevation 700, e Elevation 1000

Table 2. Root N and P average concentration at an ecosystem scale from different sites, forest type, and using different root diameter

Author	Forest type	Site	Root diameter	N %	SE[a]	P %	SE
Cuevas *et al.* (1991)	Secondary (Pine)	LEF	<2mm	0.6	0.1	0.03	0.005
Cuevas *et al.* (1991)	Pine plantation	LEF	<2mm	0.8	0.2	0.03	0.01
Lugo (1992)	Secondary (Pine)	LEF	<2mm	0.8	0.2	0.03	0.01
Lugo (1992)	Pine plantation	LEF	<2mm	0.7	0.3	0.03	0.01
Lugo (1992)	Secondary (Mahagony)	LEF	<2mm	1.3	0.5	0.06	0.02
Lugo (1992)	Mahagony plantation	LEF	<2mm	1	0.3	0.06	0.01
Silver & Vogt (1993)	Mature	LEF	<2mm	1.8	0.1	0.05	0.01
Scatena & Silver (1993)	Mature	LEF	<5mm	1.6	-	0.05	-
Murphy & Lugo (1986)	Dry primary	Guanica	<1mm	1.4	0.3	0.06	0.05

a. Standard Error

Table 3. Fine-root decomposition rate (k value) in Puerto Rico by site, author who reported, and forest species dominance

Site	**Precipitation (mm)**	**Author**	**Species**	***k* (per year)**
LEF	4000	Bloomfiled *et al.* 1993	*Prestoea montana*	0.6
LEF	4000	Bloomfiled *et al.* 1993	*Dacryodes excelsa*	0.83
LEF	3500	Silver & Vogt 1993	Mix	0.4
LEF	3363	Cusack *et al.* 2009	Mix	1.06
Sierra de Cayey -10 year old site	2000	Ostertag *et al.* 2008	Mix	0.48
Sierra de Cayey - 30 year old site	2000	Ostertag *et al.* 2008	Mix	0.46
Sierra de Cayey - 60 year old site	2000	Ostertag *et al.* 2008	Mix	0.76
Guánica - PR	508	Cusack *et al.* 2009	Mix	0.26
Global	-	Silver & Miya 2001	Mix	0.46

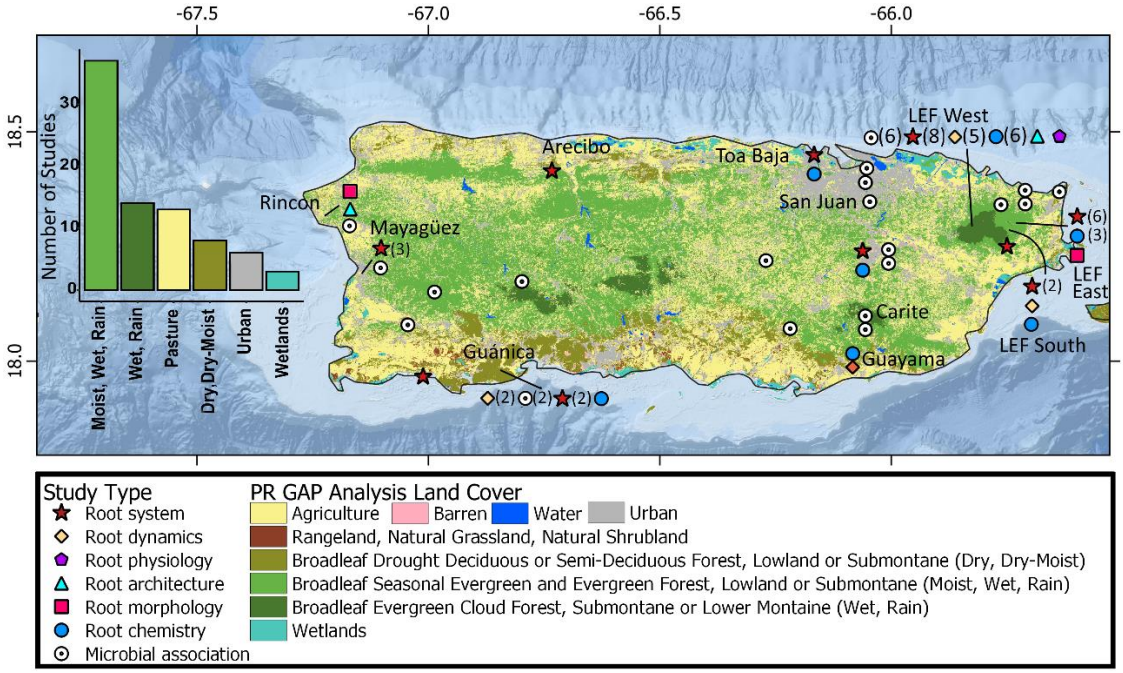


Figure 1. Map of the land cover of Puerto Rico from the USDA Gap Analysis Project (https://www.treesearch.fs.fed.us/pubs/38430/), showing where study sites were located. Symbols represent root traits according to FRED classification. The bar plot shows the number of studies per site.

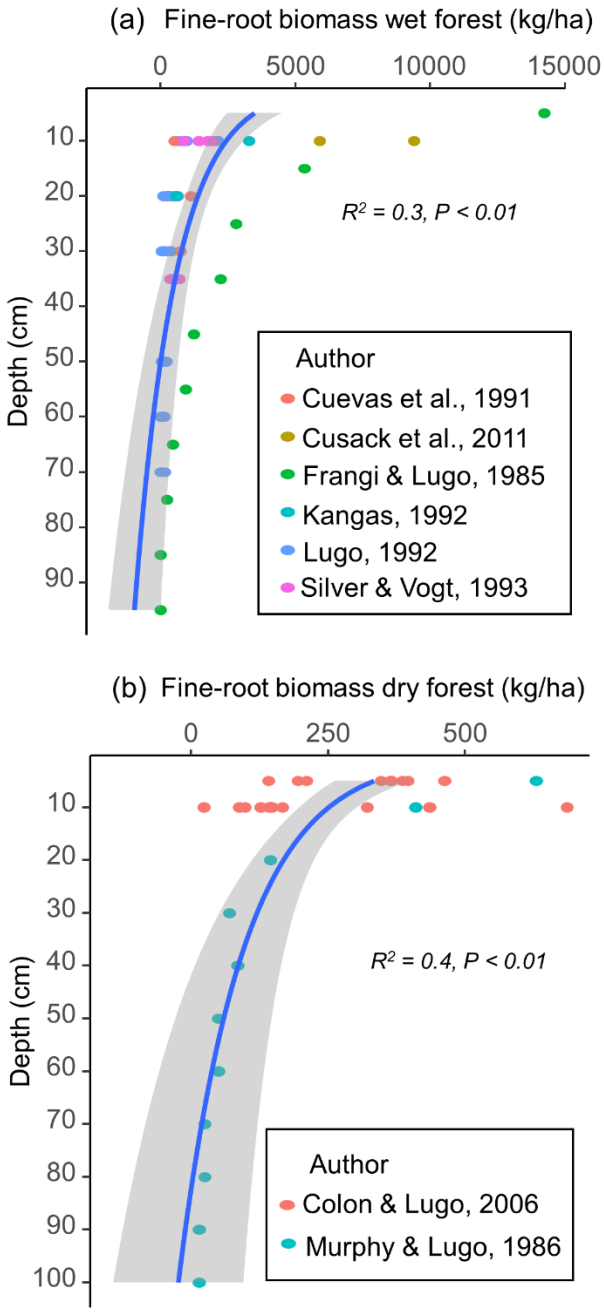


Figure 2. Logarithmic regression of root biomass in kg ha^{-1} by depth in (a) a wet forest and (b) in a dry forest (Studies are represented by authors, which are organized by colors)

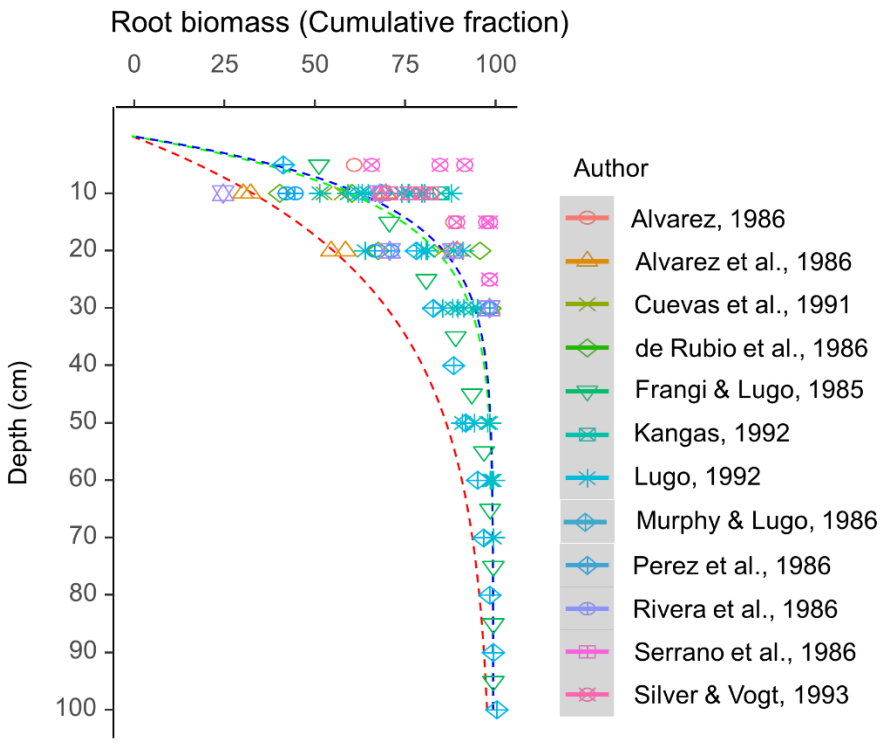


Figure 3. Comparison between the vertical rooting distribution of studies in Puerto Rico from wet forests (blue line), dry forests (green line), and the generalized beta distribution of the tropics (Jackson et al., 1996; red line)

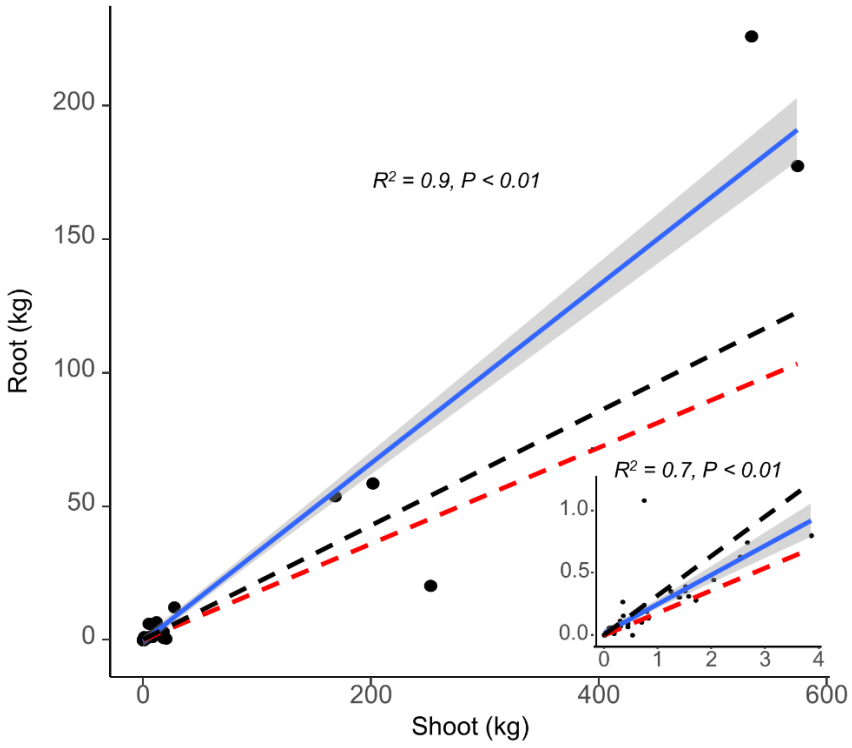


Figure 4. Linear correlation between root and shoot biomass from Puerto Rico data in blue, a world-wide meta-analysis (Cairns et al., 1997) in black, and another global meta-analysis only for forests (excluding shrublands, grasslands, Mokany et al., 2006) in red. The large graph shows all trees collected with various bole diameters, and the inset graph shows the correlation using only trees < 5 cm bole diameter

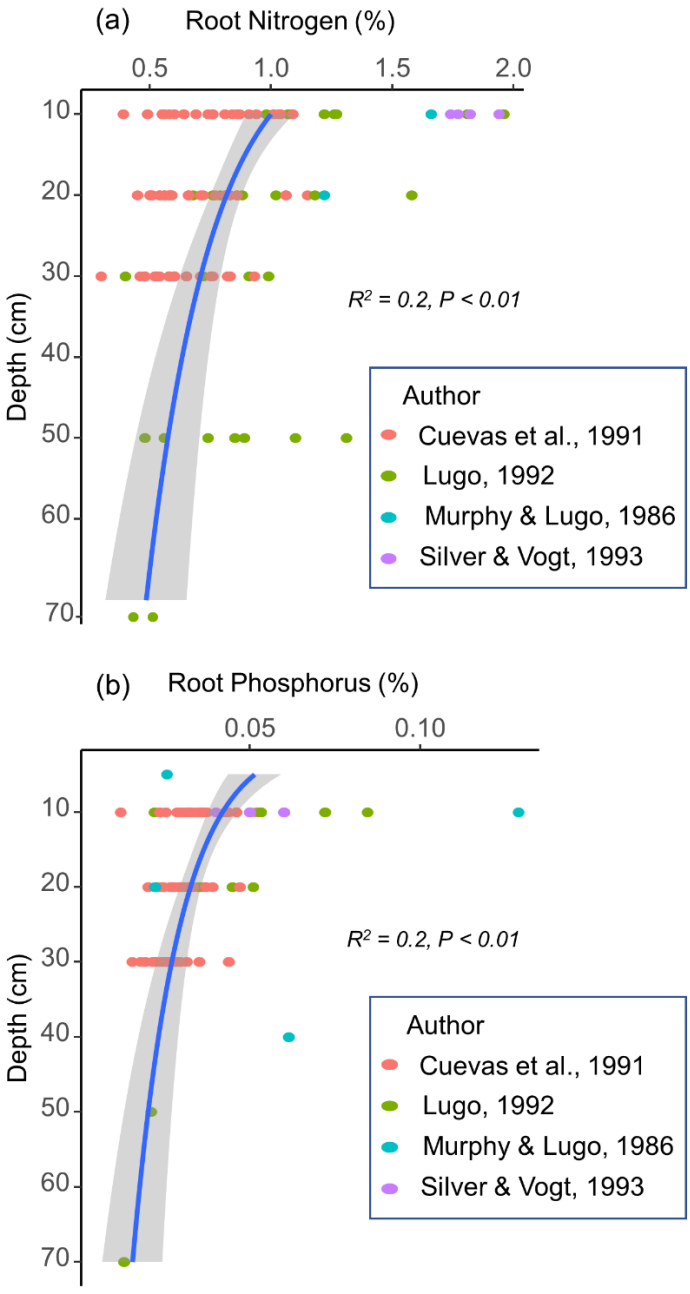
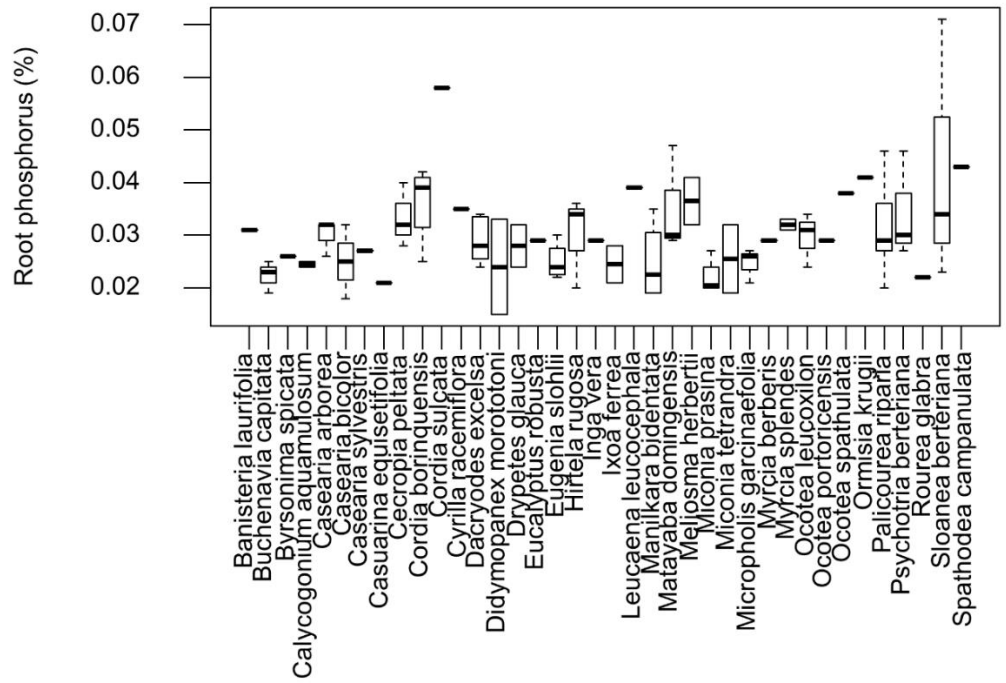


Figure 5. Logarithmic correlation between (a) fine-root nitrogen (%) and soil depth, and (b) fine-root phosphorus (%) and soil depth, using data from four studies in Puerto Rico that used the coring method

(a)



(b)

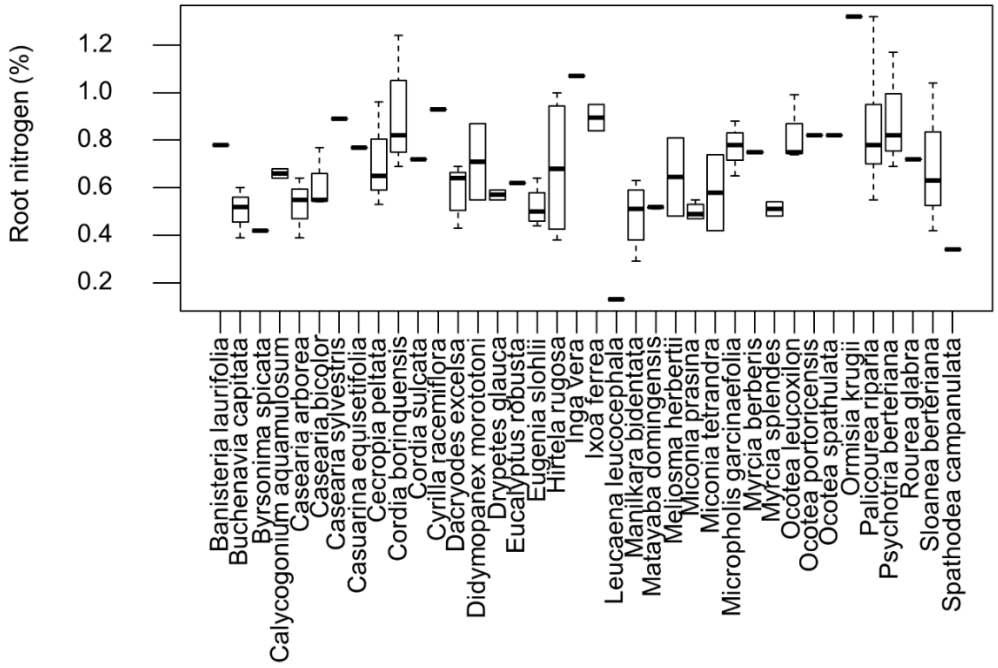


Figure 6. Root a) P and b) N concentration by species. All species roots diameters are mixed, except for Leucaena leucocephala, Eucalyptus robusta, *and* Casuarina equisetifolia, *which only fine roots were sampled for P concentration.*

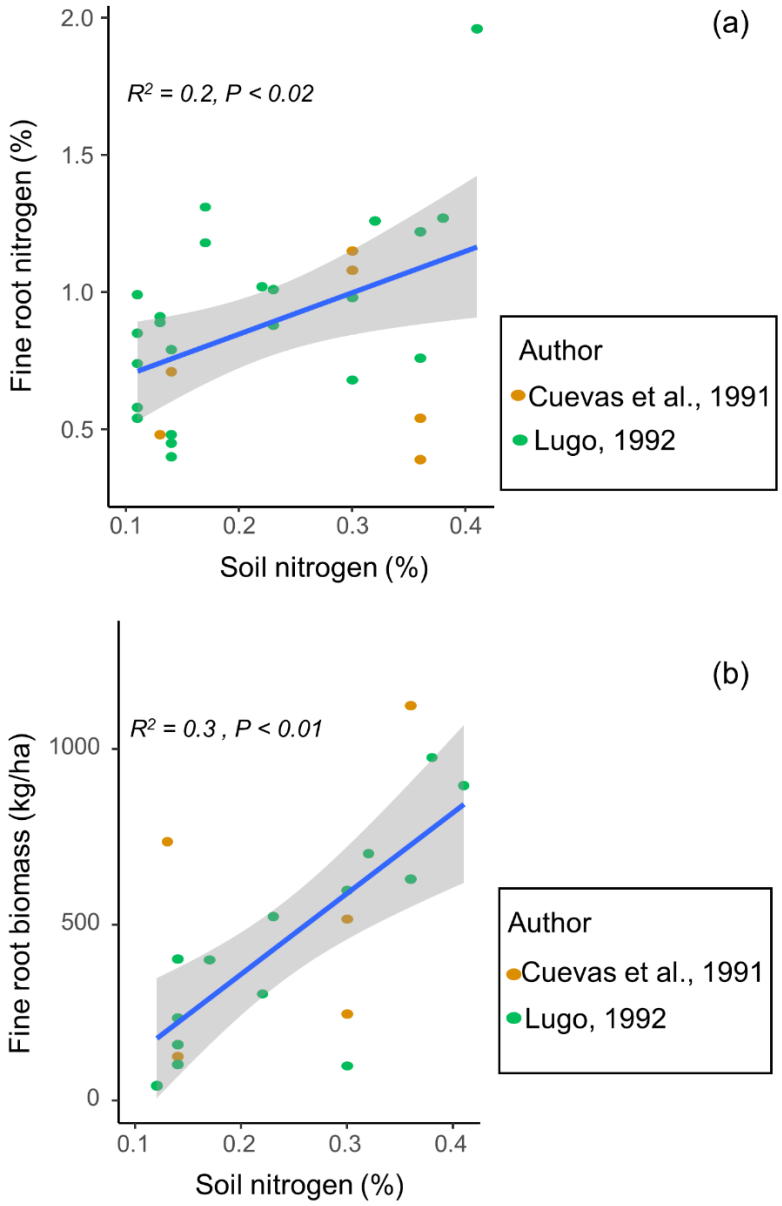


Figure 7. Linear correlation between (a) fine-root nitrogen (%) and soil nitrogen (%) and (b) Linear correlation between fine-root biomass and soil nitrogen (%) from secondary forests, pine plantations, and mahogany plantations in LEF

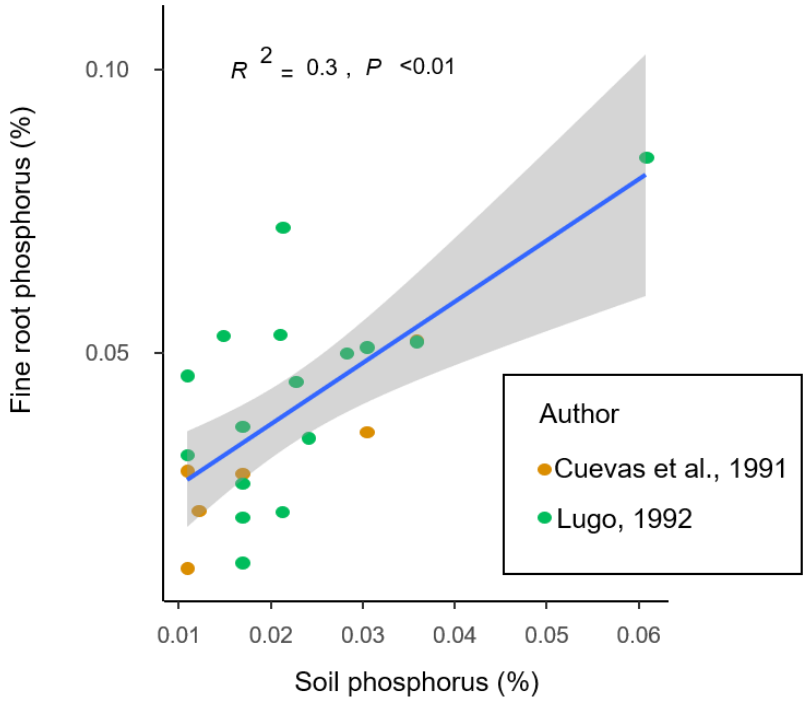


Figure 8. Linear correlation between fine-root phosphorus (%) and soil phosphorus (%) from secondary forests, pine plantations, and mahogany plantations in LEF.

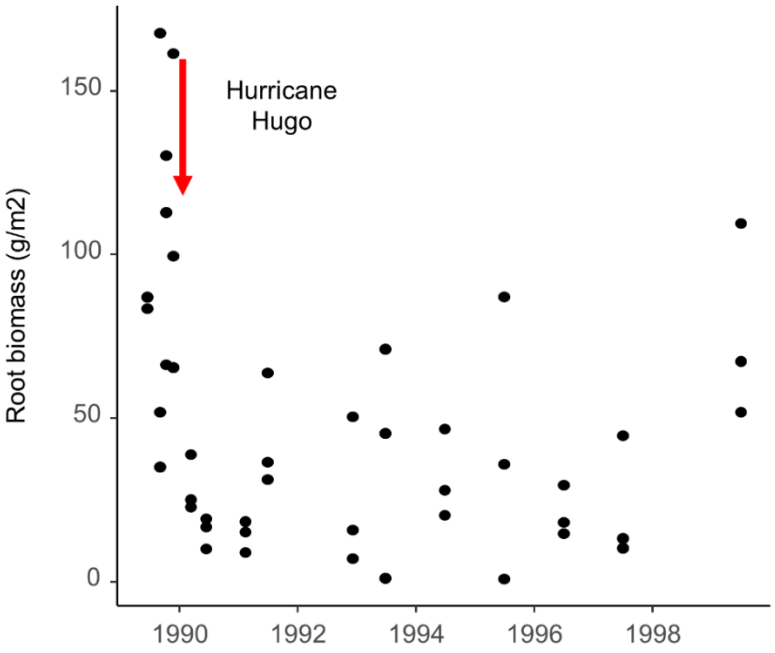


Figure 9. Ten years of root biomass from LEF, before and after hurricane Hugo, since 1990

Chapter 3

Soil warming and its legacy effects on root dynamics following hurricane disturbance in a wet tropical forest

My use of "we" in this chapter refers to my co-authors and myself.

A shorter version of this chapter was submitted to Nature Climate Change on October 30th:

Yaffar D., Wood T., Reed S. C., Branoff B. L., Cavaleri M. A. & Norby R. J.

DY participated in the field equipment installation, took the field and laboratory measurements, analyzed the data, and wrote the paper. TEW, SCR, and MAC developed the concept for the project, installed field equipment, provided environmental data, reviewed, and edited the paper. BLB assisted in data analysis, reviewed, and edited the paper. RJN installed the field equipment, helped developed the concept for the project, reviewed, and edited the paper.

Abstract

Tropical forests are forecast to experience unprecedented warming and increases in hurricane disturbances; yet, our understanding of how these productive systems, especially their belowground component, will respond to the interacting effects of varied environmental changes remains empirically limited. Increased temperatures in already-warm tropical ecosystems can generate stressful conditions, which could, in turn, alter plant root dynamics through multiple direct and indirect pathways of carbon, water, and nutrient cycling. We used minirhizotron, multi-time point root harvests, and root in-growth core to evaluate root dynamics responses to experimental warming ($^{+}4\ ^{o}C$) and two consecutive tropical hurricanes in an *in situ* warming experiment in a tropical forest: Tropical Responses to Altered Climate Experiment (TRACE) in Puerto Rico. We collected bi-weekly images from minirhizotron tubes in each of three 12 m^2 warming plots and three control plots. Following Hurricanes Irma and María in September 2017, the warming treatment was suspended for a full year to explore potential legacy effects of prior warming on forest recovery. Overall, we found that warming significantly reduced root biomass production and root standing stock over time. Following hurricane disturbance, root standing stocks increased ~4-fold in control plots and ~ 3-fold in warmed plots; yet, the net increase in production was 58% less in previously warmed plots, suggesting antecedent warming conditions suppressed roots' capacity to recover following hurricane disturbance. This pattern held true for both herbaceous and woody roots, suggesting that the consistent antecedent warming conditions reduced root capacity to recover following hurricane disturbance. Soil temperature and moisture were the main factors that explained root dynamics. Our findings provide an unprecedented look at the complex interactive effects of disturbance and climate change in a tropical forested ecosystem, and suggests a high potential for the trajectory of post disturbance recovery of key ecosystem processes to be significantly altered in a warmer world.

Introduction

In the anthropocene, changes to ecosystems rarely occur in isolation and the interactive occurrence of multiple interacting disturbances create a complex suite of drivers that influence ecosystem ecology in novel ways when compared to individual disturbances alone (Beard *et al.*, 2005; Ratajczak *et al.*, 2018). Biota are typically adapted to their historical disturbance regimes (Paine *et al.*, 1998); however, changes in the disturbance intensity or frequency, or the interaction with other drivers (e.g., changes in temperature) can alter how ecosystems respond to and recover from disturbance (Holling, 2013; Ratajczak *et al.*, 2018). For example, hurricanes can affect forest production by altering plant community composition and structure (Miller & Lugo, 2009). Species adapted to this type of disturbance recover after these events and eventually return to their pre-hurricane state (Peters *et al.*, 2011). Nevertheless, the interaction of multiple disturbances (e.g., hurricane and climate change) can alter ecosystem thresholds and could fundamentally change recovery patterns (Ratajczak *et al.*, 2018). The causation and consequences for such interactions are difficult to predict and measure; yet, human society is significantly altering multiple global change drivers (Rocha *et al.*, 2015; Steffen *et al.*, 2015), with strong potential to abrupt changes that transform ecosystem trajectories (Barnosky *et al.*, 2012). Accordingly, it is critical to understand the effects of and mechanisms influencing an ecosystem through multiple disturbances to better predict future scenarios of ecosystem change, as well as feedbacks to future climate.

Net primary production (NPP) is an important ecosystem function that can be altered by multiple disturbances. For example, in a CO_2 enrichment experiment at the Kennedy Space Center, Cape Canaveral, Florida, USA, although CO_2 increased NNP compared to the control plots, Hurricane Frances (September 2004; Category II) increased NPP even more and the differences between CO_2 enriched plots and control plots were even greater, especially in belowground production (Hungate *et al.*, 2013). Drivers shaping plant recovery after a disturbance can be related to soil biogeochemistry and the pulse of nutrient availability post-disturbance (Lodge *et al.*, 1991; Hungate *et al.*, 2013), species composition and susceptibility (Foster & Boose, 1992; Heartsill Scalley, 2017), and water availability (Beard *et al.*, 2005), among others. For example, plant root biomass following Hurricane Hugo took up to 10 years to return to pre-hurricane values (Teh *et*

al., 2009); however, this relatively slow recovery was partially due to low water availability during a drought period right after Hurricane Hugo (Beard *et al.*, 2005). In the case of Hurricane Iniki in Hawai'i, fine-root recovery took only 2 years to return to pre-hurricane values, and phosphorus fertilized plots exhibited a faster recovery than unfertilized control plots (Herbert *et al.*, 1999). since plant roots present a very dynamic response to disturbances as well as a great contribution to total plant NPP, they are ideal for studying the drivers and mechanisms involved in ecosystem responses to multiple disturbances. Further, the reestablishment of the root system after disturbance is necessary to support aboveground recovery.

Despite their importance in biogeochemical cycling (Norby & Jackson, 2000), Coarse and fine roots are the most poorly understood plant components in terrestrial ecology (Pregitzer *et al.*, 2000; Comas & Eissenstat, 2009). Fine roots account for one third of global annual NPP (Jackson *et al.*, 1997a), are essential for water and nutrient uptake, and they transport carbon and other nutrients into deeper soil layers, which contributes greatly to soil carbon budgets (Jobbágy & Jackson, 2000; Schenk & Jackson, 2002). Currently, most global change studies do not explicitly assess the effects on roots, and the belowground studies that do exist often do not consider roots at soil depths greater than 30 cm (Jackson *et al.*, 1997b; Iversen *et al.*, 2017), meaning our understanding of root dynamics is limited and biased to superficial soil layers (Yaffar & Norby, 2020). This is especially true in the tropics, which makes up two thirds of global terrestrial plant biomass (Pan *et al.*, 2011).

Tropical forests account for a disproportionate amount of global terrestrial primary production. Because of the large exchanges between tropical forests and the atmosphere (Foley *et al.*, 2003; Beer *et al.*, 2010), small relative changes in carbon fluxes from these forests can have a considerable influence on atmospheric CO_2 concentration (Raich & Schlesinger, 1992; Wood *et al.*, 2012; Cavaleri *et al.*, 2015). Further, tropical ecosystem carbon balances are affected by multiple climate disturbances such as hurricanes, droughts, floods, and fires (Harris & Lugo, 2012; Malhi *et al.*, 2014). Hurricanes, which originate in tropical waters, are considered one of the most intense weather disturbances affecting forest ecosystems, and the eastern Caribbean is one of the most susceptible land areas to strong hurricanes (McDowell, 2011). Recovery of forest carbon budgets and fluxes, including plant roots, take from 10 to 60 years following a Category 4

hurricane disturbance (Parrotta & Lodge, 1991; Silver & Vogt, 1993; Lugo *et al.*, 2000; Teh *et al.*, 2009). Yet, these studies have not considered the interacting effects of antecedent warming on the pace, magnitude, or trajectory of tropical forest recovery from hurricanes.

Increasing temperatures can affect root production directly or indirectly through alterations to metabolic processes, nutrient mineralization, water input, and carbon assimilation (Fitter *et al.*, 1999; Pregitzer *et al.*, 2000; Burton & Pregitzer, 2003; Tierney *et al.*, 2003; Piatek & Allen, 2010; Zhou *et al.*, 2011). The effect of climate warming, though, is not consistent across tree species or forest type. For example, some studies show that high temperature can increase root mortality rates due to higher evapotranspiration, leading to water shortage effects (Forbes *et al.*, 1997; Fitter *et al.*, 1999; Pritchard *et al.*, 2000; Majdi & Öhrvik, 2004; Wan *et al.*, 2004). Others show an increase in root production due to enhanced nutrient availability from decomposition (Majdi & Öhrvik, 2004; Wan *et al.*, 2004a; Zhou *et al.*, 2012; Pugnaire *et al.*, 2019), or no response in root dynamics to soil warming (Dukes *et al.*, 2005). However, most manipulative warming studies have focused on single-factor experiments with highly variable results (Norby *et al.*, 2004; Zhou *et al.*, 2012).

Given the uncertainty surrounding climatic influences on root dynamics, we developed study on an *in situ* warming experiment in a tropical forest (Puerto Rico), the Tropical Responses to Altered Climate Experiment (TRACE). The warming treatment in TRACE was applied for one year (root images were collected for 7 months) before hurricanes Irma and María passed over the island in September 2017. The hurricane events provided an extraordinary opportunity to explore how forests will recover following hurricane disturbance under a warmer future climate. We followed the root system response for 10 months following hurricane disturbance with no warming applied, so that we could evaluate the potential legacy effect of prior warming on the recovery process. The initial objectives of this study were to investigate how root standing stock, root biomass production, and root biomass mortality responded to $^{+}4\ ^{o}C$ warming treatment. After the hurricanes, our additional objectives were to measure how these root dynamics responded to hurricane disturbances and to assess if and how prior warming had legacy effects. Finally, we assessed how these responses changed with soil depth and environmental variables that have the potential to affect root dynamics (i.e., soil microclimate and soil nutrient status).

Materials and methods

Study site

The experimental site is located within the Luquillo Experimental Forest, at the USDA Forest Service Sabana Field Research Station, in northeastern Puerto Rico (LEF; 18°19'48"N, 65°43'48′W). The area is considered a wet "sub-tropical" forest according to the life zones of Holdridge, and "tropical" according to the climate classification of Köppen-Geiger, and Trewartha (Ewel & Whitmore, 1973; Kottek *et al.*, 2006; Jacob *et al.*, 2012). The site is in a secondary forest that has been recovering from deforestation for approximately 70 years at the time of this study, it is approximately 100 m above sea level, receives on average 3,300 mm of rainfall annually, and has a mean annual temperature of 24 ºC, with little variation throughout the year (García-Martino, Warner, Scatena, & Civico, 1996; Kimball *et al.*, 2018). The soils, derived from volcanoclastic sediments are Ultisols and are clay-rich and high in aluminum and iron (Brown *et al.*, 1983; Scatena, 1989). Prior to hurricanes Irma and María, common mature tree species in this forest were *Prestoea montana, Psychotria brachiata, Syzygium jambos,* and *Sloanea berteriana,* with an average diameter of 8 cm and a mean canopy height of 20 m (Cook *et al.*, 2013) and canopy openness of ~10% (Reed *et al.*, 2020). The vertical rooting distribution in Puerto Rico typically shows > 80% of fine root biomass is found in the first 20 cm of soil depth (Yaffar & Norby, 2020).

The warming experiment

The experiment has six plots, three control and three warmed, that are 12 m^2 and hexagonal in shape (Kimball *et al.*, 2018). The heated plots have an array of six infrared (IR) heaters each (Model Raymax 1010, Watlow Electric Manufacturing Co., St. Louis, MO) at approximately 3.6 m from the ground that, prior to the hurricanes, warmed the understory and soils to 4 ºC above the ambient temperatures. Plot locations were selected in the understory to avoid canopy trees such that heaters were above the canopy layer. The warming experiment started in September 2016. Each plot has soil temperature and moisture sensors at three depths (0–10, 20–30, and 40–50 cm), in addition to air temperature and relative humidity sensors. Soil temperatures were ~3.6 °C warmer than controls in 0–10 cm depth, and ~3 °C warmer than control at 20–30 cm and 40–

50 cm in depths after one month of warming (Kimball *et al.,* 2018). The design and detailed description of the plot installation can be found in Kimball *et al.* (2018). Prior to and during the year of warming, the TRACE team collected climate and soil microclimate data (Kimball *et al.*, 2018a), soil biogeochemistry data (Reed *et al.*, 2020), aboveground (Bachelot *et al.*, 2020; Carter *et al.*, 2020; Kennard *et al.*, 2020), and belowground data, including root dynamics (present study).

We used three different methods to measure root traits: minirhizotron tubes, soil core collections, and root in-growth cores. In each plot we installed two acrylic minirhizotron tubes (Pena-Plas, Pittsburgh, Pennsylvania, USA) of 5.1 cm inner diameter and 1.8 m long with a water-tight plug at the bottom, at 45° angle from the horizontal (as described in Norby, Ledford, Reilly, Miller, & O'Neill, 2004). The installation took place in February 2015, more than a year before the warming treatment was applied, allowing soil and roots to acclimate. We collected soil cores in March 2016, before warming began (three per plot using cores that were 5.1 cm in diameter and 10 cm in length), and we confirmed no difference in root biomass between plots assigned to warming treatment (328 $\pm$32 g m^{-2}) and those assigned for control (322 $\pm$44 g m^{-2}) in the top 10 cm of soil. We started taking root images with the minirhizotron camera in February 2017 to measure root length and diameter, as described below. We also installed three root in-growth cores (5 cm in diameter and 10 cm in length) per plot on April 2017, and these cores were harvested every 4 months until June 2018 to measure root morphological traits.

The hurricane events

The warming treatment was stopped after 11.5 months of heating on September 6th, 2017, due to Hurricane Irma passing 97 km north of Puerto Rico (Category 5) and Hurricane María passing from southeast to northwest across the island on September 20th 2017 (Category 4). Hurricane María had sustained winds up to 250 km hr^{-1} (among the strongest on record for the island; Lugo, 2020); rainfall averaged 500 mm in just 24 hours (Pasch *et al.,* 2019). Instantaneous tree mortality rates were twice that of Hurricane Hugo (~15%; Uriarte *et al.* 2019a). The energy dose dissipated from the combined winds of Hurricanes María and Irma was 232 PJ m^{-1} of vertical air

(Van Beusekom *et al.*, 2018), which is equivalent to the energy released by an explosion of 55 megatons of TNT over Puerto Rico (Lugo, 2020).

The hurricane events resulted in reduction of canopy cover at our experimental site from ~90% to 30%, and there was no difference in canopy loss among the six plots (Reed *et al.*, 2020; Figure 10). Immediately following the hurricanes, the forest floor was no longer visible due to the resulting input of branches and leaf litter (Figure 11). Only one out of 12 minirhizotron tubes was broken by a tree fall, and another was slightly shifted upwards by an uprooted tree. These two tubes were thus not considered in our analysis. The understory recovery was dominated by graminoid species during the first 4 months following the hurricanes, especially *Ichnanthus pallens* (Kennard *et al.*, 2020). After 9 months the understory was denser, with pioneer woody species like *Cecropia schreberiana* being more abundant (Figure 11).

Root measurements

Images were taken with a wireless manual minirhizotron camera (RhizoSystems, LLC, Idyllwild, California, USA) from continuous windows of soil area (8.3 mm wide, 6.4 mm tall) along the upper surface of the tube and for the entire tube length. There were 220 windows (images) per tube. We collected and analyzed 78,760 images in total, 36,960 of which were from the 7 months of the warming experiment, and 41,800 images from the 10 months after Hurricanes Irma and María. We analyzed all sessions (measurement collection day) using RootFly software version 2.0.2 (Clemson University, SC, USA, 2005-2011; https://www.quantitative-plant.org/software/rootfly). The same person, DY, processed all images. The minirhizotron data reported here are freely available from the NGEE-Tropics data archive (Yaffar, 2020).

We measured length and diameter of each root segment seen on the images. We used changes in root length and diameter to report root dynamics in this study. The appearance or growth in root length and diameter was considered root production. Root disappearance, without subsequent reappearance, was considered root mortality, as in Johnson *et al.* (2001). We calculated root surface area using root length and diameter and applying the formula of the area of a cylinder. We calculated root biomass by using a correlation between root diameter and root mass per

length (RML) from the soil core samples, as described by Iversen *et al.* (2008). To calculate root biomass by soil surface area, we first calculated the volume of soil observed by adding a depth of field to every window. The depth of field was calibrated by comparing the root biomass of the first 10 cm of depth from the minirhizotron tubes with the root biomass of cores collected on the same dates, following the approach of Cordeiro *et al.* (2020). Our depth of field was 1 mm, which is close to the 0.78 mm reported by Taylor *et al.* (2014). Finally, we calculated root turnover by dividing annual root production by the mean standing stock (Aber *et al.*, 1985; Aerts *et al.*, 1992) from the 7 months before the hurricane and 10 months after. We separated roots by their diameter class (< 1 mm, 1-2 mm, > 2 mm), and more than 90% of roots had a diameter of less than 2 mm. We performed the analysis considering all root diameters for before and after the hurricanes. A second analysis after the hurricanes focused just on roots >2mm diameter to distinguish woody roots from herbaceous roots, following Ma *et al.* (2018).

Soil cores (5 cm diameter and 10 cm depth) were collected using a PVC core. We manually picked all visible roots from each core after sorting the soil samples twice, and separated them into coarse (> 2 mm in diameter) and fine ($\leq$ 2 mm in diameter), live and dead, and cleaned gently with distilled water. We then scanned live fine roots using the WinRHIZO root-scanning software program (Regent Instruments, Inc., Québec, Canada) to measure total root length, average root diameter, volume and root surface area. All roots were oven dried by diameter class at 65 °C for at least 48 h and then weighed to obtain root biomass and calculate root biomass per volume. These data plus data from additional 30 individual roots that were collected from outside the plots (to increase sample size), that were also scanned, dried, and weighed were used to determine root mass per unit length (RML, mg cm^{-1}) before and after the hurricanes, and then used to calculate root biomass of minirhizotron measurements from root length and diameter. Measurements of soil Bray-extractable phosphate (PO_4^{3-}), KCL-extractable inorganic nitrogen (N; ammonium: NH_4^+, and nitrate: NO_3^-), total dissolved carbon (C) and N, and microbial biomass N, phosphorus (P), and C concentrations in these cores are described by Reed *et al.* (2020).

Root in-growth cores were made using a rigid mesh of polypropylene and had a 5.5 cm inner diameter and 14 cm length (5 mm × 3 mm hole size). The in-growth cores were filled with

homogenized, root-free soil that was collected adjacent to the plots. In-growth cores were first placed in the plots in April, 2017. All roots in-growth cores were collected every 3 months from installation, and roots were manually separated from the soil following each collection. Fine and coarse roots were divided into live and dead components. Live fine roots were scanned to measure length, diameter, and volume as described from soil cores. All roots were oven dried and weighed. After every in-growth core was removed, we installed a new in-growth core in the same hole. We used these data to measure morphology (root specific length and root diameter) before and after the hurricane.

Statistical analysis

For all statistical analyses, we used R 3.4.4 (R Core Team and contributors worldwide). To assess changes in root morphology through time using in-growth core data, we used a repeated measure mixed effect model ANOVA (Littel *et al.*, 2006; Zuur *et al.*, 2009), and tested the differences in specific root length (SRL) and fine-root diameter over time and the treatment effect. We included the interaction of treatment and time as a fixed effect and treatment within plot as a random effect (See Table 7), we used the lmer() function from the lme4 package (Bates *et al.*, 2015). To determine the relationship between root mass and root morphology, we log transformed the power function described by Iversen et al. (2008) to best fit the regression between root mass per length (mg cm^{-1}) and diameter from individual root samples collected from the soil cores before and after the hurricanes.

We analyzed the minirhizotron data using two hierarchical models for each independent variable: total root standing stock, root biomass production, and root biomass mortality for before and after the hurricanes separately using lme4() function from the lme4 package (Bates *et al.*, 2015). The first mixed effect model intended to answer whether treatment affected root dynamics and included the following independent variables: soil temperature and moisture, treatment, and session. We only included soil temperature and not air temperature due to their strong correlation. The second mixed effect model aimed to answer if there was an effect of treatment over time in root dynamics; thus, it included the independent variables mentioned above and the interaction between treatment and session. Soil moisture and temperature were taken as the

average value for the period of time since the previous session. The random grouping of independent variables was by plot for every model. We checked collinearity in each model using the check.collinearity() function (Lüdecke *et al.*, 2020). Temperature presented high collinearity with session and treatment before the hurricanes, and it was removed from the pre-hurricane models. We combined all data from each tube and averaged by plot since soil moisture and soil temperature data were plot-level variables. We also averaged soil temperature and moisture across different depths for this analysis. We employed a top-down strategy for model selection, and we built two-level mixed models. We considered the model with the lowest BIC as the best fit model. All models with a ΔBIC value < 2 were considered to have equivalent levels of support (Burnham & Anderson, 2004), otherwise we considered the simplest model to be the best fit. Non-normal data were log-transformed before analysis, and differences were considered significant at $P < 0.05$. We used QQ plots and Shapiro-Wilk test to evaluate all the assumptions for normality. Additionally we ran these two models on only coarse root standing stock for after the hurricanes to assess the potential influence of post-hurricane plant community (herbaceous vs. woody) on our dependent variables (Ma *et al.*, 2018).

We performed multiple linear models using the lm () function from the stats package (Chambers, 2017) to test the relationship of soil parameters with total root production and mortality during the warming treatment pre-hurricanes (September 2017) and after 10 months post-hurricanes (June 2018). Soil parameters included soil extractable and microbial nutrient concentrations (NH_4^+, NO_3^-, PO_4^{3-}; microbial N, P, and C) from core samples (from Reed *et al*. 2020), and soil temperature and moisture from the soil sensors. We also tested relationships of canopy openness (found in Reed *et al.* 2020), and total leaf area (Table 5) per plot with root dynamics using data collected in March 2017 and March 2018. We first tested the collinearity between the fixed factors, and removed microbial C from the model, due to high collinearity with microbial N and microbial P. Then we ran the models with each factor separately. We started with a model that included the nutrient/aboveground factor, the treatment, and the interaction of the factor with treatment. However, since we found no significant effect of the interaction we dropped it. We also dropped treatment for those models where treatment was not significant (see table 6).

We modeled root vertical distribution using Gale & Grigal (1987) asymptotic equation described in Jackson *et al.* (1997), in which low beta values represent shallower rooting. We used cumulative fraction of biomass from our minirhizotron data by treatment and before and after the hurricanes.

Results

Root dynamics in response to the warming treatment

Root standing stock in control plots increased during the 7 months of the warming experiment prior to the hurricanes. In contrast, root standing stock in the warmed plots declined over the same 7-month period (Figure 12a). The initial standing stock (February 2017) was 649 $\pm$ 143 g m^{-2} in the control plots and 526 $\pm$ 237 g m^{-2} in the warmed plots; final values before the hurricanes affected the treatments (September 2017) were 760 $\pm$ 212 g m^{-2} in control plots and 481 $\pm$ 81 g m^{-2} in the warmed plots. Through the mixed effect model we were able to test the difference in root stock in control plots compared to warming plots over time (Figure 12d; treatment × time, $P < 0.06$) and it was primarily a reflection of the difference in productivity (Figure 12e, time × treatment, $P < 0.08$, Table 4) rather than mortality (Figure 12f). Production (Figure 12b) and mortality (Figure 12c) were highly variable through time (CV of 63%, and 130%, respectively). There was no significant time × treatment interaction on root mortality (Table 4). The best fit model from the mixed effects model analysis included treatment (warming), time (session), and their interaction (Table 4); soil moisture was not included in the best fit model and temperature was removed due to collinearity. Root turnover was 1.97 $year^{-1}$ for the 7 months of the warming experiment (1.93 $year^{-1}$ for control plots and 2.07 $year^{-1}$ for warmed plots). Analyses by root surface area presented a very similar pattern to root standing stock.

From the soil core data, the annual aboveground measurements, and the soil sensors from before the hurricanes, we found that soil NH_4^+ was positively related to root production only when considering treatment in the model (P<0.01, R^2=0.95; Table 6, Figure 13a) across the 7 months (Table 6). We also found a positive relationship between root production and microbial N throughout all plots not considering treatment in the model (P=0.07, R^2=0.50; Figure 13b, Table

6). Microbial N was also positively related to root mortality throughout all plots not considering treatment in the model (P=0.07, R^2=0.50; Figure 13c, Table 6). No aboveground variables showed a relationship with root production before the hurricanes.

Root dynamics response to the hurricane and the warming treatment legacy

Root standing stock increased almost four-fold from 774 $\pm$223 g m^{-2} to 2918 $\pm$819 g m^{-2} in control plots and three-fold in previously warmed plots from 569 $\pm$102 g m^{-2} to 1465 $\pm$369 g m^{-2} (Figure 12a) during the 10 months after the hurricanes (Figure 12a). This increase over time was greater for the control plots than the previously warmed plots (time x treatment, $P < 0.01$, Table 4). Soil moisture and root stock were significantly negatively related (p= 0.01; Table 4). The largest difference in the root standing stock between control and previously warmed plots was attributable to increased production in controls from 4 +0.4 g m^{-2} day^{-1} right after the hurricanes (October 2017) to 19 $\pm$3 g m^{-2} day^{-1} after 10 months of the hurricanes (June 2018, Figure 12b, 12e) compared to 4.3 $\pm$2 g m^{-2} day^{-1} to 5 $\pm$0.6 g m^{-2} day^{-1} in the previously warmed plots. However, there was a large variation of production by time (session) after the hurricanes (Figure 12b, 12e). Soil temperature was included in the best fit model and influenced root production; there was greater production at lower temperatures (P<0.01; Table 4). Root mortality also increased with time (P<0.01) yet none of the other variables affected root mortality (Table 4). Treatment affected coarse root stock over time as well (P<0.01; Table 4). Root turnover was 3.6 $year^{-1}$ for the 10 months after the hurricanes (3.59 $year^{-1}$ for control plots and 3.65 $year^{-1}$ for previously warmed plots).

Root production after the hurricanes was again positively related to soil NH_4^+ throughout all the plots, not considering treatment in the mixed effect model (P<0.01, R^2=0.88; Figure 13d, Table 6). Microbial N was positively related to root production only when treatment was considered in the model (P<0.01, R^2=0.94; Figure 13e, Table 6). Soil NH_4^+ was positively related to root mortality throughout all plots, not considering treatment in the model (P=0.03, R^2=0.64; Figure 13f; Table 6). No aboveground variables showed a correlation with root production after the hurricanes.

Root dynamics by soil depth

Fine-root biomass was predominantly in the top 30 cm of soil (Figure 14). The cumulative root biomass distribution beta coefficient was on average 0.92. Although the control plots had a slight deeper root distribution (beta=0.95 before hurricanes and beta=0.96 after hurricanes) compared to warmed plots (beta=0.84 before hurricanes and beta=0.90 after hurricanes; Figure 14), this was mainly influenced by existent thicker roots in one control plot. Root production was detected as deep as 90 cm, and root mortality was observed at 100 cm (Figure 15). Overall, depth distribution of root standing stock, root biomass production, and root mortality did not different with treatment before the hurricanes (Figure 15 a-c). Root standing stock increased twofold after the hurricanes in the top 10 cm of soil. However, the greatest increase was in the next two depth ranges (10-20 and 20-30 cm), where standing stock was fivefold greater after the hurricane than before (Figure 15d). Root production increased after the hurricanes, especially for the control plots where production was augmented twofold in the first 10 cm of depth, and more than 50 times that of the next 20 cm of depth (10-30 cm), compared to values before the hurricanes (Figure 15e). Root mortality also increased after the hurricane, with mortality rates ranging from 4-32 times higher compared to those observed before the hurricane, although not in the first 10 cm of depth (Figure 15f).

Root morphology from soil in-growth cores

Fine-root morphological traits measured in in-growth cores changed over time. Specific root length (SRL) was significantly greater 10 months after the hurricanes compared to before the hurricane disturbance events ($P<0.01$; Fig. 16, Table 7), and was not related to the warming treatment (Table 7). Fine-root diameter decreased significantly after the hurricanes ($P<0.01$; Figure 16, Table 7), and this change was again not related to the warming treatment.

Discussion

Understanding forest responses to multiple disturbances is not straightforward, especially in tropical ecosystems, where more carbon is cycled than in any other biome and where field-based experimental studies are less common than in higher latitudes (Saugier *et al.*, 2001; Cavaleri *et*

al., 2015; Wood *et al.*, 2019). Although changes to the aboveground structure of a tropical forest following a major tropical hurricane can be seen with the naked eye, the untangled response of the rhizosphere is not easily perceived. The combined interactions of nutrient, water, and carbon cycling, as well as the microbial feedbacks in the rhizosphere, can lead to different responses for plant root dynamics facing multiple disturbances compared to a single disturbance (Forbes *et al.*, 1997; Zak *et al.*, 2000; Anderson *et al.*, 2003; Dukes *et al.*, 2005; Björk *et al.*, 2007; Zhou *et al.*, 2012; Arndal *et al.*, 2014; Ratajczak *et al.*, 2018).

Our study measured root dynamics in response to experimental warming ($^{+}4$ °C), the effects of two consecutive hurricane events, the legacy effects of the prior warming on root recovery, and the environmental factors that may help predict these responses. We found that, prior to the hurricanes, root standing stock and root production were negatively affected by the warming treatment through time. Following the hurricanes, root standing stock increased greatly across both treatments, and the increase in net root production was 58% less in the previously warmed plots than in the control plots, despite similar climatic conditions for the year of hurricane recovery. These patterns suggest that warming can substantially reduce roots' capacity to respond to hurricane disturbance if those plants were previously exposed to warmer conditions.

Response to warming

The values we obtained for root stock overall and root production pre-hurricanes were within the ranges of values reported in other tropical studies (Berish, 1982; Cuevas *et al.*, 1991; Jackson *et al.*, 1997b; Yaffar & Norby, 2020). Rooting depth was similar to what other studies found previously in Puerto Rico (30 cm; Frangi & Lugo, 1985; Yaffar & Norby, 2020). No deeper distribution pattern was observed in the warmed plots versus the control plots possibly since there was no secondary drought effect of the warming (Lynch & Wojciechowski, 2015; Wood *et al.*, 2019).

We found significant effects of warming on root production and root standing stock throughout the 7-month measurement period prior to the hurricane disturbance. We found that root production was positively related to soil NH_4^+ and microbial N concentrations. According to

Reed *et al*. (2020), control plots had greater NH_4^+ than warmed plots, before the hurricanes. This difference in NH_4^+ concentration in the soil could have been related to greater decomposition from organic N to NH_4^+ in the control plots (Roes *et al*., forthcoming) compared to the warmed plots. Previous warming studies have suggested a positive response in root production as a result of increased nutrient availability (Wan *et al.*, 2004; Majdi & Öhrvik, 2004; Zhou *et al.*, 2012; Pugnaire *et al.*, 2019). However, since we did not measure fluxes of this mineral, we cannot describe cause and effect between root production and soil NH_4^+.

Higher temperatures have been found to positively affect root dynamics via an increase of photosynthesis, respiration (Johnson, 1990; Apple *et al*., 2000; Atkin *et al.*, 2000), microbial activity, nutrient mineralization (Shaver *et al.*, 2000; Rustad *et al.*, 2001), and directly through metabolic and physical processes (Wan *et al.*, 2004). In other cases, an increase in temperature has reduced soil moisture, which triggered a decrease of root production and changes in rooting distribution (Pregitzer *et al*., 2000; Wan *et al.*, 2004; Björk *et al*., 2007). However, all the results from previous *in situ* warming experiments have been based in temperate and boreal biomes (Rustad *et al.*, 2001; Cavaleri *et al.*, 2015) where temperatures reach below freezing and precipitation is usually no greater than 9.5 mm day^{-1} (Whitaker, 1975). In the case of our study site in Puerto Rico, where air temperatures range between 23 ^oC (February) and 27 ^oC (October), and rainfall is generally not limiting to plant growth (8 mm day^{-1} minimum and 12 mm day^{-1} maximum in a year; Harris, Heartsill Scalley, & Scatena, 2012), the temperature to plant-soil feedbacks are distinct from higher latitude ecosystems (Balser & Wixon, 2009). Identifying the direct effects of warming treatment on root dynamics is a complex task because temperature affects virtually all chemical and biological processes (Shaver *et al.*, 2000), and the effect varies among ecosystems.

The initial environmental conditions in our site might have played an important role in the root dynamics response to warming. It is known that the upper canopy strata in our site is already exceeding their photosynthetic temperature optima (Topt; Mau, Reed, Wood, & Cavaleri, 2018; Miller *et al*., *in review*). Although the understory is usually well under its Topt (<30^oC; Miller *el al., in review*; Carter *et al.*, 2020), during some hours of summer days surface temperature exceeded this Topt only in the warmed plots (3% of the summer period). However, control plots

surface temperature did not exceed temperatures over 27 ^{o}C. Assuming that most of roots measured in our study were from plants inside the plots, the difference in surface temperature due to treatment might have had an effect in photosynthesis rates, potentially leading to less C allocated to the roots from the warmed plots. Carter *et al*. (2020) found that from 2 species sampled in the TRACE plots, one species reduced its photosynthetic rate when daily temperature increased and maintained its respiration rate without acclimating to the warming treatment previous to the hurricanes.

Although root dynamics were not directly related to soil moisture in this study, soil moisture values were highly variable at our site and could have been related to other soil and topographic properties (Reed *et al.*, 2020; Burt and Butcher, 1985). High soil moisture can also increase the biological demand for oxygen (O_2), or nutrient mobility (Silver *et al.*, 1999; Wood & Silver, 2012), either of which could have indirect cascading effects on root production and root mortality. These forests can reach very low levels of O_2 availability under conditions of high precipitation (Silver *et al.*, 1999), which has been shown to have negative consequences for plant survival (Uriarte *et al.*, 2018). However, we did not measure soil O_2 concentrations or soil nutrient fluxes during this study to test these effects on root dynamics.

Root mortality was also positively related to microbial N concentrations. This may be due to increased cell death and lysis, and the concomitant utilization of the low C/N necromass, which can result in greater N mineralization and higher soil NH_4^+ content (Aerts *et al.*, 1992b; Jama *et al.*, 1998; Urquiaga *et al.*, 1998; Hawkes *et al.*, 2007) We recognize that the direction and interaction of these effects might be more complex and include other mechanisms, such as competitive interaction among plant species, and the activity of herbivores and pathogens (Mooney *et al.*, 2012). Yet, our results suggest that the warming treatment had effects on soil nutrient concentrations, and possibly on plant metabolism, that were associated with changes in root dynamics over time.

Hurricane response

During the 10 months after Hurricanes Irma and María, root production, mortality, and stock increased significantly compared to values before the hurricanes. Root biomass production after the hurricanes was greater than what has been recorded for Puerto Rico (Cuevas *et al.*, 1991). This shift in root dynamics can be explained by the changes in the aboveground plant community, which we detected both in belowground measurements of morphological changes and visual qualitative cues. Grass first and then other herbaceous plants and woody seedlings dominated the forest floor after 3-12 months from the disturbance event (Kennard *et al.*, 2020). Roots from before the hurricane were thicker but shorter than roots observed after the hurricane. This change, according to an analysis of morphological differentiation of tree roots and herbaceous roots (Ma *et al.*, 2018) implies a change in dominance from woody trees to herbaceous plants, corresponding to aboveground observations (Kennard *et al.*, 2020). High values of root surface area after the hurricane (45.4 m^2 m^{-2}) were also consistent with values obtained from tropical grassland/savanna (42.5 m^2 m^{-2}; Jackson *et al.*, 1997).

Considering the change in plant community, our finding that root biomass standing stock and biomass production differed between control and previously warmed plots after the hurricanes could reflect changes in the dominance of herbaceous and graminoid plants. Thus, we separated roots into woody roots and herbaceous roots based on diameter size. We recognize this assumption is very broad, but this approach provides us some ability to differentiate based on root "type" (Ma *et al.*, 2018). From our ingrowth cores and soil cores, we knew that most roots belonging to herbaceous plants were thinner than 1 mm in diameter. Therefore, we assumed that roots greater than 2 mm belonged exclusively to woody plants. Since 70% of the understory plant composition after the hurricane were from herbaceous species (Kennard *et al.*, 2020), and due to visual observations, we infer that woody roots (>2mm) belong to woody plants that existed before the hurricane and thus responded to the combined interaction of warming and hurricane effects. When running the mixed effects model using only roots greater than 2 mm in diameter, we still found a significant effect of treatment on root standing stock through time (interaction treatment:session; table 1) after the hurricane events. Thus, both new herbaceous plants and trees responded similarly to the warming treatment legacy.

After the hurricanes, there was a slight difference (non-significant) in soil temperature between control plots and previously warmed plots in June 2018, which could be due to differences in the understory coverage. Although there was no difference in canopy openness or percent bare ground between warmed and control plots after the hurricanes. Total leaf area per plot showed a slight (but not significant) difference between control and previously warmed plots before and after the hurricanes, where control plots had more total leaf area than the warmed plots. Although we expected the aboveground recovery to mirror the legacy effect of warming after the hurricanes, the measurements taken for total leaf area, and canopy openness do not show strong evidence of the differential effect. We encourage future studies to account for the integrated measurements of aboveground/belowground carbon allocation in response to warming in greenhouse settings, and to consider interactive responses in root dynamics to seasonal changes in long-term plots in Puerto Rico.

The decrease of photosynthetic rate with temperature (Carter *et al.,* 2020) could have played a role in C allocation to the roots and C storage in plant tissue of the species located in our plots. Especially since leaf respiration did not acclimate to the warming treatment (Carter *et al.,* 2020). This could have had an effect on root recovery after the defoliation of the vegetation caused by the hurricane disturbance. Thus, the warming experiment could have had a legacy effect on the carbon storage available to produce root biomass after the major disturbance through slight decreases in species photosynthetic rate previous to the hurricanes.

The warming treatment had legacy effects on soil nutrients after the hurricanes, with greater soil NO_3^- and PO_4^{3-} concentrations in warmed plots and possibly greater NH_4^+ (not significant) in control plots (Reed *et al*., 2020). The observed positive relationships between root production and nutrient concentrations after the hurricanes could reflect a stimulatory effect of greater nutrient availability on plant (including root) production, or conversely, a stimulation of nutrient mineralization by increased root activity. Resolution of any cause-and-effect relationships will require more detailed analysis of nutrient fluxes in addition to pool sizes and analysis of nutrient cycling between plant and soil. Thus, the complexity of the biogeochemical response to

hurricane disturbances versus the plant response on previously warmed plots and the direction are not yet evident, and can be happening as parallel, or as concomitant non-additive processes.

Some other mechanisms could have been influencing the difference in root dynamic response to previous treatment after the hurricanes. For example, warming can modify plant soil feedbacks by altering belowground pathogen pressure and root associations with mutualistic symbionts (Van Der Putten *et al.*, 2010). Changes in soil microbial communities can potentially modify the microbial-mediated feedbacks (de Vries *et al.*, 2018; Pugnaire *et al.*, 2019). We did not account for the change in the rhizosphere microbial communities before or after the hurricanes, but we recognize that possible shifts in microbial communities due to warming and hurricane disturbances can affect plant density dependence (Bachelot *et al.*, 2020), and therefore affect root dynamics.

The relationships between soil nutrient, microbial nutrient, aboveground plant physiological response, and root dynamics are likely to have been bi-directional after the hurricane. The LEF was exposed to hurricane force winds combined with 500 mm of precipitation over 24 hours which resulted in a thick layer of high nutrient litter, significant changes in forest structure with the increase of cropped and downed trees, combined with near complete defoliation of the vegetation and 15% immediate mortality of large trees (Uriarte *et al.*, 2019). Thus, these hurricane events increased both the spatial and temporal variation in environmental conditions, which affected plant response directly and indirectly. Adding to these major hurricane disturbances, our studied plots were previously exposed to a warming treatment that had showed a significant decrease in root production and root standing stock. Thus, the biogeochemical processes that interacted with the climatic stress, and the physiological plant responses to both warming and hurricane disturbances are tightly intertwined and might have been affected in many different ways.

Conclusions

The recovery of the forests from disturbance is a critical aspect of forest dynamics, and it is important for societal wellbeing. Hurricane disturbances in Puerto Rico are common; current

plant species are adapted accordingly to these disturbances (Miller & Lugo, 2009). The forest canopy is shaped by strong winds (Basnet *et al.*, 1992), but the responses of root systems are not as evident. It is estimated that the return interval of a hurricane the size and power of María is 86 years (Lugo, 2020). However, the intensity and frequency of these hurricanes are shown to be changing due to climate change (Stocker *et al.*, 2013; Lin *et al.*, 2016). Without sufficient time to replenish plant energy reserves, ecosystem development could be affected (Lugo, 2020). Further, the interaction of natural disturbances, such as hurricanes, and climate warming can alter thresholds of the ecosystem (Ratajczak *et al.*, 2018).

We found in this study that experimental warming reduced root production over time, decreasing root standing stock. The warming also had a legacy effect on root standing stock after the hurricane disturbance, when overall rot production increased, but it increased less in previously warmed plots than control plots. Resilience and recovery of root systems are essential in the aboveground recovery after a disturbance (Vargas *et al.*, 2010; Uriarte *et al.*, 2019b). Root system responses can also affect the residence time of carbon in the soils (Kell, 2012); and soil stability (Gyssels *et al.*, 2005), with additional implications for the forest community, the C cycling, and the human society. Our study showed that warming can negatively affect the belowground plant component in a tropical forest and the effects of warming can further reduce the trajectory of recovery from hurricane disturbance. Inferring causation will help us anticipate future and multiple disturbances. The study presented here provides valuable empirical data that can be used to model future scenarios and help elucidate the key variables interacting on the belowground ecosystem.

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Appendix

Table 4. Mixed effect models; dependent variables; fixed effects; random effects; P values; R^2 marginal and R^2 conditional; coefficient estimates

Time	Model (lmer)	Dependent variable	Fixed effects	Random effect	P value	R^2m/ R^2c	Estimate
Before	Root stock~treatment + session + (1|plot)	Root stock	Treatment Session	Plot	0.57 0.02	0.05/ 0.89	8.32 -154.7
Before	Root production~treatment + session + (1|plot)	Root production	Treatment Session	Plot	0.7 <0.01	0.07/ 0.5	-0.6 -0.17
Before	Root mortality~treatment + session + (1|plot)	Root mortality	Treatment Session	Plot	0.9 <0.01	0.16/ 0.5	0.12 0.46
Before	Root stock~treatment+ session+ treatment:session + (1|plot)	Root stock	Treatment Session Treatment :session	Plot	0.57 0.02 0.05	0.06/ 0.9	-46.7 15 -13.5
Before	Root production~ treatment+ session+ treatment:session+ (1|plot)	Root production	Treatment Session Treatment :session	Plot	0.7 <0.01 0.08	0.08/ 0.5	0.9 -0.07 -0.19
Before	Root mortality~ treatment+ session+ treatment:session+ (1|plot)	Root mortality	Treatment Session Treatment :session	Plot	0.9 <0.01 0.86	0.16/ 0.5	0.36 0.48 -0.03
After	Root stock~treatment + session +moisture + (1|plot)	Root stock	Treatment Session Moisture	Plot	0.1 <0.01 0.01	0.5/ 0.8	-702.1 83.9 -0.95
After	Coarse root stock~treatment+ session+ (1|plot)	Coarse root stock	Treatment Session	Plot	0.18 <0.01	0.2/ 0.7	-659.1 37.9

Table 4 (Continued)

Time	Model (lmer)	Dependent variable	Fixed effects	Random effect	P value	R^2m/ R^2c	Estimate
After	Root production~ treatment + session+ temperature + (1|plot)	Root production	Treatment	Plot	0.6		-0.1
			Session		<0.01	0.25/	0.08
			Temperature		<0.01	0.27	-0.3
After	Root mortality~ treatment + session + (1|plot)	Root mortality	Treatment	Plot	0.1	0.15/	-1.24
			Session		<0.01	0.3	0.14
After	Root stock~treatment+ session+ treatment:session + (1|plot)	Root stock	Treatment	Plot	0.1		126
			Session		<0.01		142
			Treatment: session		<0.01	0.5/ 0.8	-86.78
After	Coarse root stock~treatment+ session+ treatment:session + (1|plot)	Coarse root stock	Treatment	Plot	0.2		825.3
			Session		<0.01		68.8
			Treatment: session		<0.01	0.3/ 0.8	-61.8
After	Root production~ treatment+ session+ treatment:session+ temperature +(1|plot)	Root production	Treatment	Plot	0.6		-0.2
			Session		<0.01		0.07
			Treatment: session		0.7	0.25/	0.01
			Temperature		<0.01	0.27	-0.31
After	Root mortality~ treatment+ session+ treatment:session+ (1|plot)	Root mortality	Treatment	Plot	0.1		-1.93
			Session		<0.01		0.1
			Treatment: session		0.3	0.15/ 0.3	0.07

Table 5. Total leaf area (m^2 m^{-2}) of understory by assigned treatment plots on conditions of pre-warming (2016), warming (2017), post-hurricane but unwarmed (2018), up to the re-start of the warming treatment after the hurricanes (2019)

Conditions	year	Plot ID	Assigned treatment	Total leaf area (m^2 m^{-2})
Pre-warming, pre-hurricane	2016	1	Control	0.29
		2	Unwarmed	0.17
		3	Control	0.31
		4	Unwarmed	0.24
		5	Control	0.43
		6	Unwarmed	0.14
Warming, pre-hurricane	2017	1	Control	0.39
		2	Warmed	0.26
		3	Control	0.50
		4	Warmed	0.41
		5	Control	0.77
		6	Warmed	0.11
Post-warming, post-hurricane	2018	1	Control	0.33
		2	Unwarmed	0.51
		3	Control	0.93
		4	Unwarmed	0.36
		5	Control	0.98
		6	Unwarmed	0.42
Warming, post-hurricane	2019	1	Control	0.43
		2	Warmed	0.37
		3	Control	0.52
		4	Warmed	0.32
		5	Control	0.84
		6	Warmed	0.63

Table 6. Linear models for before and after the hurricane events. Model; dependent variables; independent variables; P values; R^2; coefficient estimates.

Time from hurricanes	Model (lm)	Dependent variable	Independent variable	P value	R^2	Estimate
Before	Root production~ NH4+ treatment	Root production	NH4 Treatment	<0.01 <0.01	0.95	13.49 -10.52
Before	Root production~ microbial N	Root production	Microbial N	0.07	0.50	0.17
Before	Root mortality~ microbial N	Root mortality	Microbial N	0.07	0.50	0.28
After	Root production~ NH4	Root production	NH4	0.02	0.88	18.58
After	Root production~ microbial N+ treatment	Root production	Microbial N	<0.01	0.94	1.72
After	Root mortality~ NH4	Root mortality	Treatment NH4	<0.01 0.03	0.64	65.64 4.93

Table 7. Mixed effect models for specific root length (SRL) and root diameter; model; dependent variables; fixed effects; random effects; P values

Root trait	Model (lmer)	Dependent variable	Fixed effects	Random effect	P value
SRL	SRL~Time+ treatment+ time:treatment+ (1|Plot)	SRL	Time	Plot/treatment	<0.01
			Treatment		0.18
			Time:treatment		0.37
Root diameter	Root diameter~Time+ treatment+ time:treatment+ (1|Plot)	Root diameter	Time	Plot/treatment	<0.01
			Treatment		0.97
			Time:treatment		0.71

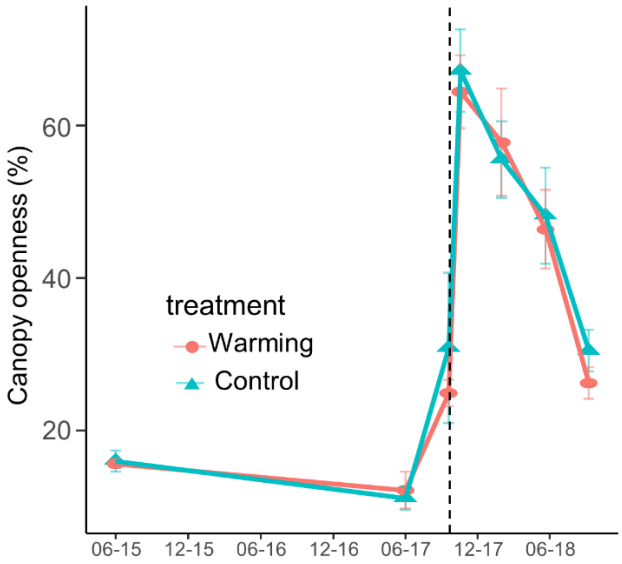

Figure 10. Circles represent the average canopy openness (%) per treatment (red circles warming blue triangles control) in undisturbed conditions (pre-treatment, 2015), after the warming treatment started (2017), and post hurricane (2018). The error bars represent the standard error of plots per treatment. The dates in x axis are expressed in mm-yy.

Figure 11. One plot from the warming experiment when the project began (left, April 2015). The same plot after a month from the two hurricanes disturbances (middle; Oct 2017), and nine months after (right; Jun 2018). Photo by TRACE team.

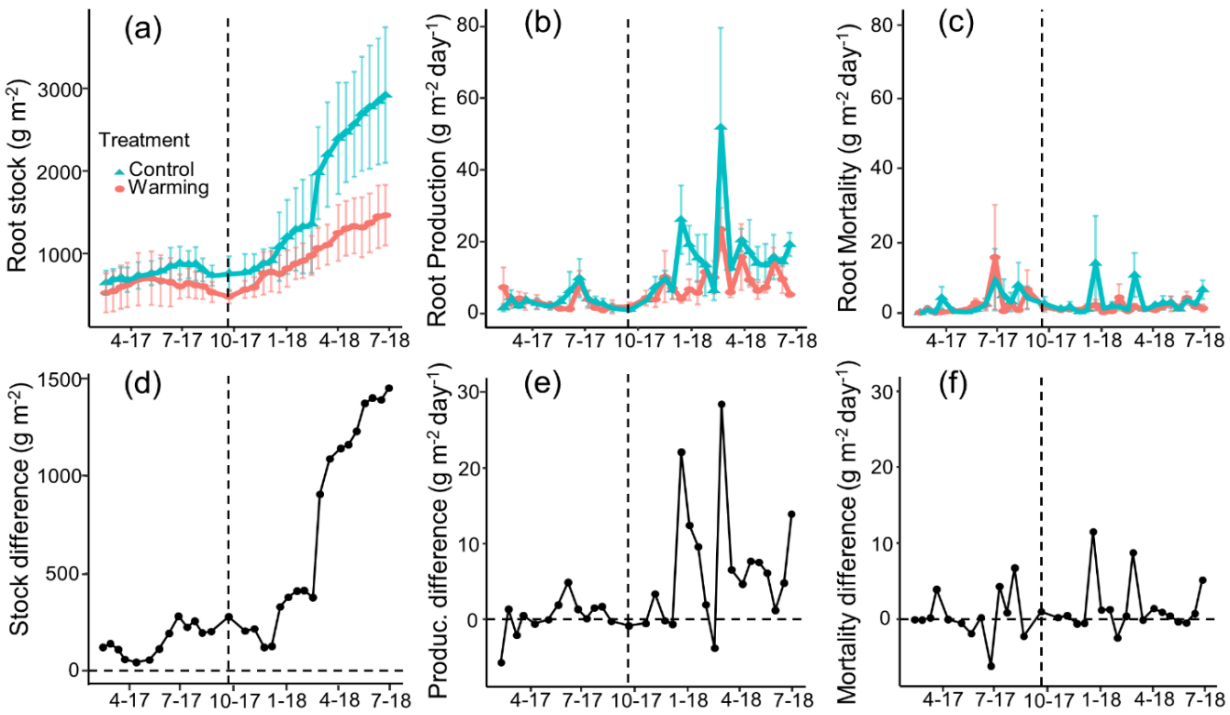

Figure 12. The top graphs represent a) Root biomass standing stock, b) root biomass production, and c) root biomass mortality through time. The dates represented by the points are the middle dates between one sampling and another. Dashed line determined when hurricane María hit Puerto Rico. The bottom graphs represent the difference of d) Root biomass standing stock, e) root biomass production, and f) root biomass mortality between control plots and warmed plots though time.

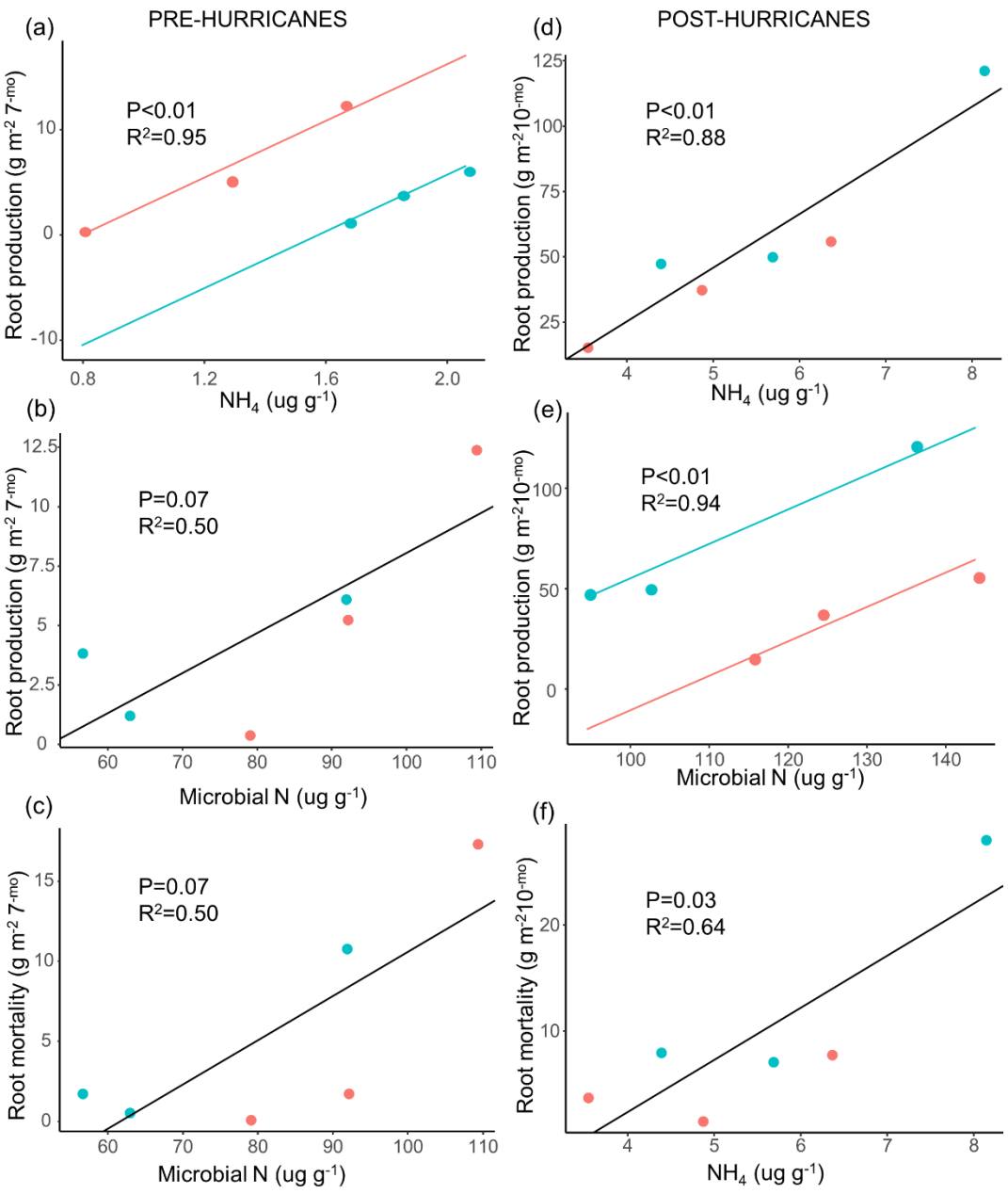

Figure 13. Significant regressions between root production and soil and microbial nutrient concentrations. Colors represent the treatment (red=warmed plots, blue=control plots), and the lines represent the linear model. Black lines represent models that did not include treatment, and the two lines (red and blue) represent the models that included treatment.

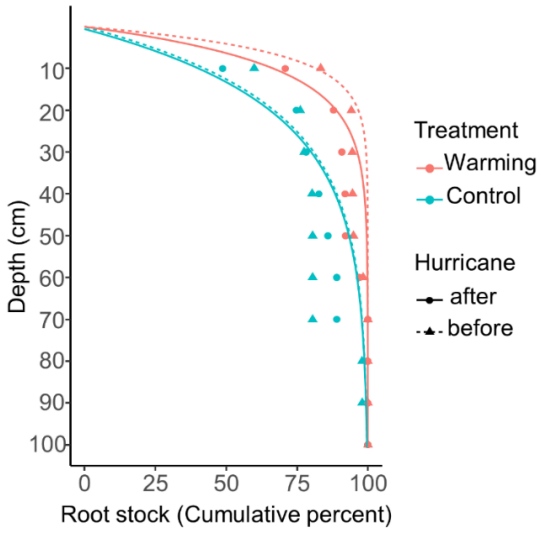

Figure 14. Averaged cumulative root biomass from all plots and time frame through the whole profile depth.

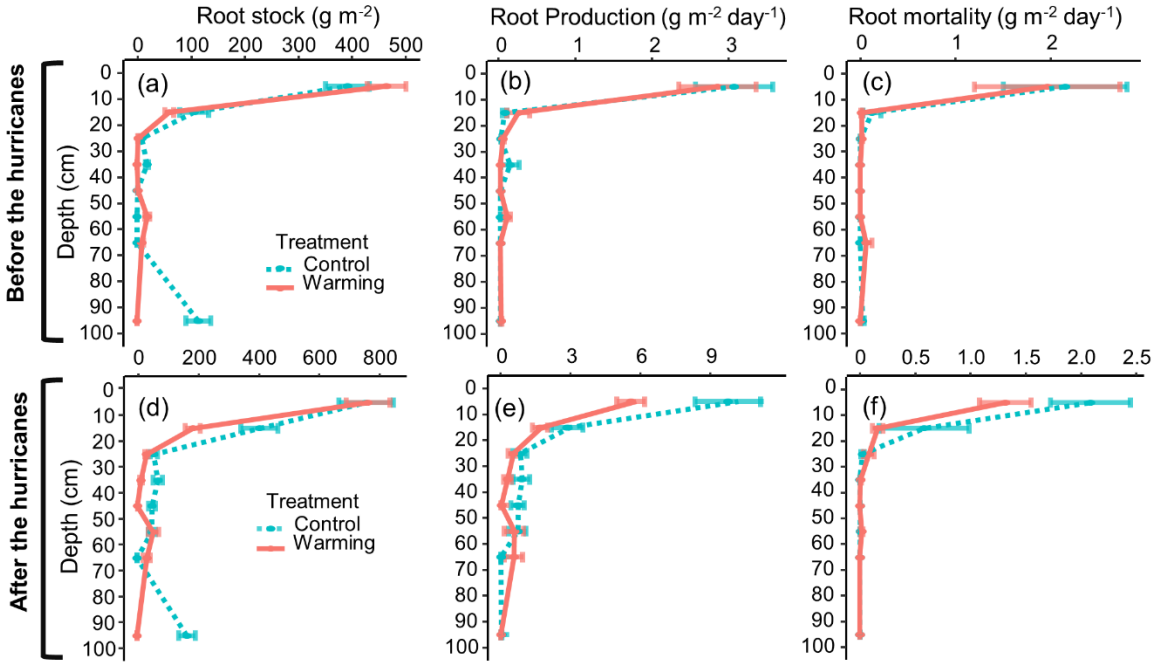

Figure 15. a-c) Root stock, root production, and root mortality before the hurricanes and d-f) after the hurricanes, as measured by the minirhizotron method by depth. The circles are the mean and the error bars represent the standard error. Blue circles with dashed lines depict control plot data and red circles with solid lines depict data from the warmed plots.

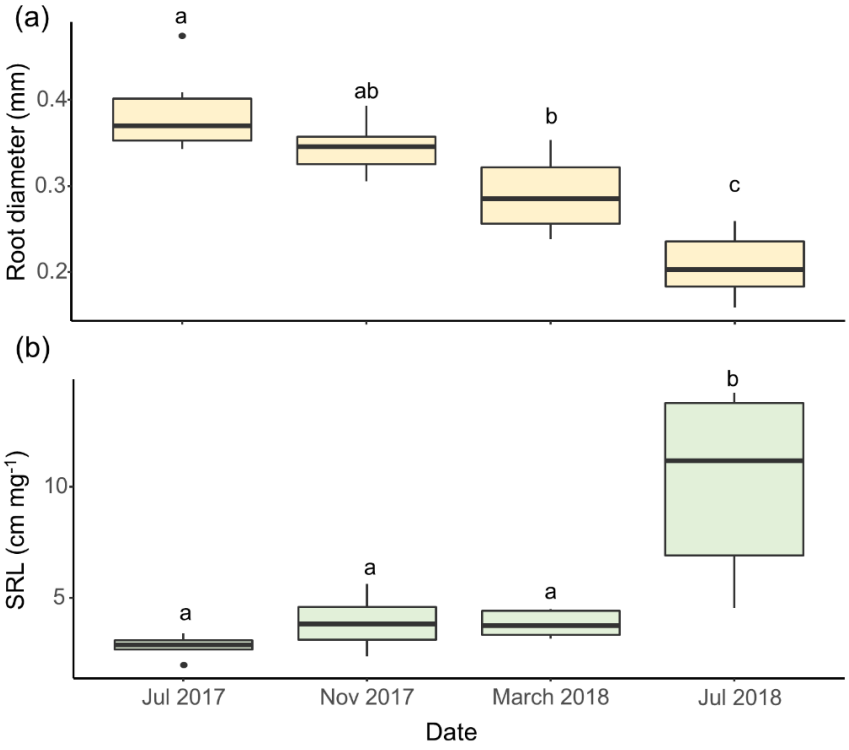

Figure 16. Boxplots showing a) specific root length with time, and b) fine-root diameter with time, using new roots collected from the in-growth cores. The x axis represents the date of root in-growth core collection. The vertical dashed line depicts when Hurricane María struck the site. Significant differences from the mixed model ANOVA coefficients are designated with different letters.

Chapter 4

Tropical fine-root trait strategies for phosphorus acquisition and their response to hurricane disturbance in a wet tropical forest of Puerto Rico

My use of "we" in this chapter refers to my co-authors and myself.

A version of this chapter will be submitted in Frontiers in Forests and Global Change: Yaffar D., Cabugao K. G., Child J., Carvajal N. & Norby R. J.

DY developed the concept of the study, participated in the field and laboratory measurements, analyzed the data, and wrote the paper. KGC participated in developing the concept for the study, participated in the field and laboratory measurements, reviewed, and edited the paper. JC assisted in the field and laboratory measurements. RJN participated in developing the concept of the study, helped in taking field and laboratory measurements, reviewed substantially, and edited the paper.

Abstract

Tropical trees commonly present adaptive functional traits to live in low available phosphorus (P) ecosystems, including root traits that optimize P acquisition from soils. These root traits include mycorrhizal colonization, root phosphatase activity (phosphomonoesterase; PME), and root branching. However, due to the high cost associated with an investment in any one of these root traits, different species may rely on one more than the others, creating a gradient of strategies for resource acquisition. At one end of this gradient, species have an "outsourcing" strategy, where they invest in their fungal symbiotic partners to obtain P. At the other end, species use the "do it yourself" strategy, which results in thinner roots for nutrient exploration. These strategies are part of the collaboration gradient dimension of the new root economics spectrum framework. Our study aimed to describe seven root traits from common species within the Luquillo Experimental Forest in Puerto Rico, evaluate the position of mycorrhizal colonization percentage, root branching ratio, and root PME in the collaboration gradient, and test their relationships between each other and with other root traits accounting for site and species variation. Additionally, we tested the responsiveness of these root traits to two consecutive hurricanes that passed by the island. We found that species with greater mycorrhizal colonization had a smaller root branching ratio, which would place greater investment in these two traits at opposite ends of the collaboration gradient. We also found that species with greater root mycorrhizal colonization presented greater root PME, which suggests a combined contribution of root PME by the root and their mycorrhizal symbiont partners, and that both root traits would be closer to the "outsourcing" end of the gradient. Root branching ratio, which was located in the "do it yourself" end of the gradient, was positively related to specific root length (SRL) when accounting for species and site variation. We further recognize the effect of species variation on broader patterns and suggest that future studies account for interspecific variation separately from intraspecific variation. Finally, we found that root PME decreased after hurricane disturbance, suggesting this trait is more responsive than others to abrupt environmental changes.

Introduction

Fine roots account for up to one third of global net primary production (NPP), and their contribution to carbon and nutrient cycling is driven by their functional traits (Jackson *et al.*, 1997; McCormack *et al.*, 2015). However, fine and coarse roots are the most poorly understood plant components in terrestrial ecology (Comas & Eissenstat, 2009) and the extent to which plant performance correlates to their root traits remains unresolved (Kong *et al.*, 2014; Weemstra *et al.*, 2016). Even less evidence comes from the tropics, where 35% of global terrestrial NPP originates (Phillips, 1998), and phosphorus (P) in soils often limits productivity (Vitoussek & Sanford, 1986). Our study aimed to explore root traits of common tropical trees, and the relationships of these root traits, especially those related to P acquisition, in order to test root trait trade-offs from the multidimensional root economic spectrum, of which one dimension represents the collaboration gradient (Kramer-Walter *et al.*, 2016; Bergmann *et al.*, 2020). Additionally, we tested the responsiveness of these root traits to a disturbance by comparing them before and after two consecutive hurricane disturbance events in the island.

Like their leaf counterparts, fine-root functional traits are characteristics that can be quantified and measured, and that affect plant fitness (Violle *et al.*, 2007). Unlike the hypothesized leaf economics spectrum, root traits do not match with a one-dimensional root economics spectrum, in which fast growing plant species would have acquisitive root traits (e.g. root nitrogen content) and slow growing species would have conservative root traits (e.g. root tissue density) (Wright *et al.*, 2004; McCormack *et al.*, 2012; Chen *et al.*, 2013; Kong *et al.*, 2014; Van-der-Heijden *et al.*, 2015; Weemstra *et al.*, 2016). Instead, root economics research considers not only the resource conservation "fast and slow" gradient as one dimension but also a resource "collaboration" gradient as another dimension (Bergmann *et al.*, 2020; Figure 17). The collaboration gradient takes into account mycorrhizal symbiosis, where thicker fine roots rely on mycorrhizal fungi for resource acquisition ('outsourcing' strategy), while thinner fine roots with high specific root length (SRL) are more independent in obtaining resources ("do it yourself' strategy). There are other root traits that we could deduce their spatial position in this new root economics dimension, for example root branching ratio for the "do it yourself" strategy (Figure 17) due to its strong relationship with SRL (Liu *et al.*, 2015). Yet, there are other functional traits such as root

phosphatase activity (phosphomonoesterase: PME), for which the position in the gradient is not as clear.

In the tropics, where more C and energy are exchanged with the atmosphere than in any other terrestrial ecosystem (Field *et al.*, 1998; Roy & Saugier, 2001; Townsend *et al.*, 2008; Beer *et al.*, 2010), trees have adapted mechanisms, including root trait trade-offs, to survive in generally low P availability (Vitoussek & Sanford, 1986; Kitayama *et al.*, 2000; Turner *et al.*, 2018). These trade-offs follow the collaboration gradient, investing C to have greater root mycorrhizal symbiosis ("outsourcing" strategy) (Smith & Read, 2008), or invest C in longer roots ("do it yourself" strategy) for a given diameter with greater branching (Liu *et al.*, 2015), which in turn could have greater exudation of PME to mobilize organic forms of P in soils (Olander & Vitousek, 2000; Lambers *et al.*, 2008; Cabugao *et al*., *in press*).

Each of the root traits related to P acquisition has an important contribution to P uptake by the plant (Spiers & McGill, 1979; Sinsabaugh *et al.*, 1993; Hodge, 2004; Burns *et al.*, 2013; Kong *et al.*, 2014; Dalling *et al.*, 2016). For example, arbuscular mycorrhizal fungi (AMF), which represents the most abundant plant mycorrhizal symbiont, are responsible for up to 80% of total P uptake in land plants (Douds *et al.*, 2000). However, the C cost to maintain this symbiosis is high; around 25% of C assimilated by plants is allocated to AMF (Jakobsen & Rosendahl, 1990). Although there is some evidence of a positive relationship between mycorrhizal colonization rate and root diameter (Kong *et al.*, 2014; Bergmann *et al.*, 2020), the commonly used method to measure colonization rates are not expressed in root area or volume (McGonigle *et al.*, 1990), which would lead to an overestimation of mycorrhizal presence based on root area. In this study, we will replace root diameter by root mycorrhizal colonization percent in the collaboration gradient (Figure 17).

Although root branching, which is a structural trait, is not as competitive as fungal hyphae in obtaining nutrients, they are still sensitive to patchy and pulsed nutrient supply, and at a lower C cost (Hodge, 2004; Kong *et al.*, 2014; Liu *et al.*, 2015; Cockerton *et al.*, 2020). The relationship between root branching ratio (or SRL) and AMF colonization is still inconsistent (Kong *et al.*, 2014; Liu *et al.*, 2015; Lugli *et al.*, 2019). Furthermore, the trade-offs between investing in P

mineralizing enzymes versus mycorrhizal fungi partners, or in more branched roots to acquire soil P is debatable (Treseder & Vitousek, 2001; Nasto *et al.*, 2017, 2019).

Root phosphatase activity is also a root trait related to P acquisition, and it directly represents root function. Phosphatase enzymes are released to the rhizosphere by both microbes and plant roots (Treseder & Vitousek, 2001; Smith & Read, 2008). These enzymes, phosphodiesterase (PDE) and phosphomonoesterase (PME), hydrolyze organic P compounds and then mineralize them into orthophosphates which are directly absorbed by either microbes or plant roots (Rejmánková *et al.*, 2011; Stone *et al.*, 2014). Theoretically, root phosphatase activity in the rhizoplane should mostly be a function of enzymes exuded from the roots, but it is not clear what fraction of these enzymes originates from the mycorrhizal fungi partners, especially AMF (Bartlett & Lewis, 1973; Gianinazzi *et al.*, 1979; Dodd *et al.*, 1987; Smith & Read, 2008). Thus, the relationships of these root traits and the strategies plants have to optimize P acquisition, in terms of C investment versus the nutrient output, have not been fully explored (Smith & Read, 2008), especially in tropical ecosystems. Finally, the responsiveness of these root traits to major disturbances that can alter soil P concentrations within the same trees have also been poorly explored.

In this study we aimed to describe morphological, chemical, and nutrient acquisitive root traits from common tree species in the Luquillo Experimental Forest (LEF) in Puerto Rico. We accounted for species and site variation while exploring possible patterns in root traits from the resource collaboration gradient dimension across species and sites. Specifically, we measured root morphology (SRL and diameter), architecture (root branching intensity and root branching ratio), root PME, mycorrhizal colonization, and root P concentration from eight of the most common species in three wet tropical forest sites of LEF. Additionally, after two consecutive hurricanes ($\geq$category 4) passed by the island, we tested the responsiveness of these root traits from three species before and after these major disturbances.

We hypothesized that higher root PME and higher root branching ratio would be associated with the "do it yourself" end of the collaboration gradient (Brundrett, 2002; Comas & Eissenstat, 2004; Liu *et al.*, 2015a; Weemstra *et al.*, 2016; Lugli *et al.*, 2019; Cabugao *et al.*, *in press*), while

higher mycorrhizal colonization would be on the "outsourcing" end of the gradient (Treseder & Vitousek, 2001; Nasto *et al.*, 2017; Bergmann *et al.*, 2020; Fig. 1). Further, we hypothesized that the hurricane disturbance would change root branching ratio, root PME, and mycorrhizal colonization due some environmental changes, including changes in soil P concentration.

Methods

Study site

Our three forests sites were located at the Luquillo Experimental Forest (LEF) in the northeast of Puerto Rico (18°18' N; 65°49'W). The LEF ranges in elevation from 150 to 400 m asl (Lodge *et al.*, 2016). The mean annual rainfall is 3500 mm, and the mean annual temperature is 24ºC. Two of the studied sites (EVS and EV1) occur at El Verde, at around 550 m asl. El Verde soils are Oxisols, with lower-Cretaceous volcaniclastic parent material (Stone *et al.*, 2014). The most common species of EVS is *Dacryodes excelsa* (Brown *et al.*, 1983; Stone *et al.*, 2014); whereas the most common species of EV1 is *Prestoea montana* (Uriarte & Zimmerman, 2016; Picón Ruiz, 2019). The difference between the two El Verde sites is the forest age. EV1 is a secondary forest of 62<76 years old and EVS is a mature forest of ~100 years old (Uriarte & Zimmerman, 2016). The Sabana site (SB2) is in a secondary forest of 35<62 years old trees with an elevation of approximately 150 m asl. Soils at Sabana sites are Ultisols, clay-rich, and high in aluminum and iron content, also with lower-Cretaceous volcaniclastic parent material (Scatena, 1989; Brown *et al.*, 1983). Some of the most common species at the Sabana site are *Psychotria brachiata, Syzygium jambos, and P. montana* (Uriarte & Zimmerman, 2016; Picón Ruiz, 2019). Both, weathered Oxisols and Ultisol soils are mostly P-poor (Yang *et al.*, 2013).

Hurricane events

Most of the analyses in this study are from root collections made 6 months after two consecutive hurricanes passed through Puerto Rico. However, we also collected roots for root traits analysis 6 months prior to the hurricanes, which we used to compare root trait responsiveness to the hurricane disturbance. On September 6th, 2017 Hurricane Irma passed 97 Km north of Puerto Rico as a Category 5 and Hurricane María passed from southeast to northwest across the island

on September 20th 2017 as a category 4. Sustained winds reached 250 km hr^{-1} (among the strongest on record for the island; Lugo, 2020) and precipitation over 500 mm in just 24 hours from María (Pasch *et al.*, 2019), which had a strong effect on the forest structure as evident when we performed sample collection 6 months following the storm. Tree mortality as a consequence of the disturbance was twice that of Hurricane Hugo (~15%; Uriarte *et al.* 2019a), and it included some of the trees we previously measured. There was a high canopy cover reduction (from ~90% to 30%, Chapter 3) and a change in soil nutrient concentration (Reed *et al.*, 2020).

Species and root voucher collection

We collected root vouchers from the most common species at El Verde and Sabana, including 1) native pioneer species: *Cecropia schreberiana Miq.*, 2) native non-pioneer species: *Dacryodes excelsa Vahl., Prestoea acuminata var. montana, Manilkara bidentata, Ocotea leucoxilon Allen, Calophylum calaba L,* 3) non-native pioneer species: *Spathodea campanulata P.Beauv.*, and 4) nitrogen fixer species: *Inga laurina Willd.* Only *P. montana* was a common shared species among the three sites; whereas *C. schreberiana, C. calaba*, and *I. laurina* were shared species between SB2 and EV1.

Phylogenetically our species are very distant from each other (Fig. 18). All species in this study were from different orders and families. *P. montana* is the only species from the monocot clade considered in this study. *Spathodea campanulata* and *M. bidentata* are from the asterids clade, and the rest belong to the rosids clade. We tested phylogenetic signal and conservatism for root traits from the species presented in this study, but we found no significance due the small number of species and great phylogenetic distance between them. Therefore, it was not necessary to account for phylogenetic relatedness in any of the statistical analyses.

We sampled three trees from each species, and at least two root clusters from each tree. Root system vouchers were taken by finding individual trees from each species with an above ground identification system, and then tracing roots from the bole until first order roots were found. Only root systems that contained at least 1st, 2nd and 3rd order roots intact were used as root system vouchers. From each voucher taken from the field, we took subsamples of only the 1st

and 2nd order roots and submerged them in ethanol (75%) for preservation before counting mycorrhizal colonization in the Center of Applied Tropical Ecology and Conservation of the University of Puerto Rico (CATEC, UPR). Another subsample of the first two orders was taken for measuring root phosphatase activity (phosphomonoesterase: PME), and was kept cold during transport to Oak Ridge National Laboratory for further analysis (ORNL; Cabugao *et al.*, 2017). The rest of the samples were stored in plastic bags in a refrigerator (for no more than 1 week) before analyzing them for morphology and architecture at the USDA Forest Service Sabana Field Research Station Puerto Rico. Due to collection restrictions, we were only able to measure mycorrhizal colonization from six species and root PME from four species.

Root morphological trait measurements

We measured root architecture by counting root branching ratio (the number of first order roots for each second order root) and root branching intensity (the number of first order roots for every 1 cm of second order root). Once roots were separated by order, we scanned them separately using WinRhizo software (Version 12) to obtain root length and diameter. We then dried all samples in an oven at 65^{o}C for 48 hours. Once dried, we weighed the samples separately. Specific root length (SRL) was calculated by dividing root length by dry root weight.

Root P concentration

Dried samples used for morphological and architectural traits were shipped to ORNL where we used them to measure phosphorus (P) concentration of the first three orders of each species separately. When there was not enough first order roots to perform a P digestion, we combined 1st and 2nd orders, since we have tested that these root orders do not present significant different P concentration and are classified as "absorptive" roots (Yaffar & Norby, dataset). We only report concentrations of the first two orders. We ground the samples in 50 mL falcon tubes using a Geno/Grinder 2010 (Spex Sampler Prep, Metuchen, New Jersey, USA) and we weighed 100 mg of ground root samples (some cases tissue sample was less, and we used the same weight for the standard to verify the results). We analyzed total root P using a Lachat BD40 block digestor for the high temperature digestion, followed by colometric analysis on a Lachat QuikChem 8000

series for the flow injection analysis (Lachat instruments, 2005). We follow the protocol described in Cabugao *et al.* (*in press*).

Mycorrhizal colonization analysis

Samples previously submerged in ethanol (75%) were cleared, stained and de-stained following the protocol from Kormanik and McGraw (1982) to measure the percentage of mycorrhizal fungal colonization. This process consisted of first clearing root cytoplasm and nuclei with potassium hydroxide (KOH) to allow staining penetration. To allow faster clearing, we placed the KOH container with the samples to a water bath at 40^{o}C. We then stained the roots with acid fuchsin and de-stained them in glycerol, so fungi could be observed in the roots. We transferred the samples to a slide placed lengthwise parallel to the long side of the slide. There was at least three slide-lengths of roots per slide to reach roughly 100 counts per slide. We scored mycorrhizae with a compound microscope set to 200X magnification following the McGonigle *et al.* (1990) protocol. We did not differentiate mycorrhizal structures (e.g. hyphae, arbuscle, vesicle), and we counted all structures only as "presence". We reported logit transformed percent of proportion of non-negative intercepts and total intercepts.

Root phosphatase activity

Subsamples of 1st and 2nd orders (absorptive) roots were analyzed for phosphomonoesterase (PME) activities using a modified version of the colorimetric para-nitrophenyl phosphate (pNPP) assay (Tabatabai & Bremner, 1969; Cabugao *et al.*, 2017; Png *et al.*, 2017). This assay focused on capturing the enzyme activity from exuded PME enzymes bound to the root surface (rhizoplane) as a response to the presence of organic P substrate (para-nitrophenyl phosphate). However, some of this activity might come from microbial cells due the difficulty of removing the microbial component completely.

For this essay we took a fine-root (≤ 1 mm) sample (~1g) and washed it in milliQ water multiple times to remove as much soil as possible. We added this root sample to 9 mL of 50 mM sodium acetate-acetic acid (for 1 L: 2.88 g sodium acetate; pH = 5.0 using acetic acid). Next, we added 1 mL of the substrate, 50 mM pNPP (1.856 g pNPP in 100 mL sodium acetate buffer), before

incubating the samples in a shaker at 27 °C for 1 hour. We terminated the reaction by taking 0.5 mL and adding it to 4.5 mL of 0.11 M NaOH. The concentration of the end product, para-nitrophenol, was measured through its absorbance at 405 nm on a Spectrophotometer 1100 (Cole Parmer, East Banker Court Vernon Hills, Illinois). We made a standard curve using 0, 100, 200, 400, 1000 µM pNP concentrations.

Soil measurements

We collected 5 cm diameter soil cores up to 5 cm depth, adjacent to every tree we sampled (within 1 m from the trunk). We measured gravimetric water content (GWC) for each sample to express soil available P on a per unit soil dry mass basis. We measured orthophosphate available in the soils using resin strips and followed the method described in Cabugao *et al.* (2017). We incubated 5 g of root-free fresh soil subsamples with distilled H_2O and resin strips for 24 hours on a shaker at the USDA Forest Service Sabana Field Research Station. We then removed the resin strips, rinsed them free of soil, and shipped them in plastic bags to ORNL for further analysis. We removed phosphate ions adsorbed on the resin strips by shaking resin strips in 50 mL of 0.25 M H_2SO_4 for 1 hr and quantified the concentration using a Lachat QuikChem 8500 (Hach, Loveland, Colorado, USA, see Cabugao *et al.*, 2017).

We measured organic P in soil subsamples from each core as the sum of acid and alkali extractions (Bowman, 1989; Condron *et al.*, 1990; Cabugao *et al., in press*). We added 2 g of wet soil, 3 mL of 18 M H_2SO_4, and 4 mL of deionized water in a 50 mL falcon tube, vortexing constantly to mix the sample. We then added deionized water for a total solution volume of 48 mL and centrifuged the sample at 2500 rpm for 10 minutes. Using a Whatman No. 1 filter paper, we separated the supernatant and saved the filtered solution as the acid extract. Next, we cleaned the remaining soil by washing it with deionized water and centrifuging the slurry. We then added the filtered paper (from the acid extraction) to the soil and shook this mixture on a shaker with 98 ml of 0.5 M NaOH at room temperature for 2 hours. We obtained the alkali extract by centrifuging and filtering the sample. The acid and the alkali extracts were analyzed by a Lachat QuikChem 8500.

Statistical analysis

We used R studio 3.3.4 for all analyses (R Core Team and contributors worldwide). We compared soil P concentrations (organic and available P) by site using an analysis of variance (ANOVA) aov() function and a post hoc HSD Tukey's test via tukeyHSD() (Chambers *et al.*, 2017; R Core Team and contributors worldwide). We also performed an ANOVA for each species to test root trait differences by site, as well as root trait differences between species in each site. For all multiple testing, we corrected the P value with the Bonferroni correction (McDonald, 2014). We used Non-metric Multi-dimensional Scaling (NMDS) to visually represent the traits that explain the variation between species and sites (Kruskal, 1964). We tested the effect of root branching ratio, root mycorrhizal colonization, and SRL, on root PME separately. We also tested the effect of root PME, root branching ratio, and root mycorrhizal colonization on root P percent, and the relationship of root branching ratio and root mycorrhizal colonization between each other and to SRL. We used two mixed effect regression models for each of the pairwise root trait mentioned above. One of the models considered site as the random effect, and the other considered species within site as the random effect. We used the lmer() function from the lme4 package (Bates *et al.*, 2015). We also used a mixed model ANOVA to test for differences in root traits and soil P concentrations before and after the hurricane considering tree within species within site as the nested random effect. We use the lme() function from the nlme package (Pinheiro & Bates, 2020), and added an autoregressive order 1 (ar1) covariance structure to improve the fit of the model (Littell *et al.*, 2000). We did not consider soil organic P or root mycorrhizal colonization in this analysis due the low number of samples with these measurements before the hurricanes. We used the estimated marginal means with the emmeans() function from the emmeans package for comparing and graphing the traits differences before and after the hurricane (Lenth *et al.*, 2020).

Results

Soil phosphorus concentrations between sites

The three studied sites varied in soil P (organic and available; Figure 19). Soil organic P was greater in EVS (0.19 mg g^{-1}) than both EV1 (0.12 mg g^{-1}) and SB2 (0.14 mg g^{-1}; Figure 19a;

F=10.2, P<0.01). Soil available P was greater in SB2 (0.36 µg g^{-1}) than EV1 (0.10 µg g^{-1}; Figure 19b; F=3.82, P=0.06).

Root traits by species and sites

For most root traits from first order roots, there was more difference among species within a given site than within the same species across sites (Table 8). Exceptions for this were root PME and root branching ratio, for which some species had significant differences by site, and root P concentration, which did not differ among species (Table 8). The differences in root traits by species is also visible in the non-metric multi-dimensional scaling (NDMS; Figure 20b), in which separation is mostly between species rather than sites (Figure 20a). Root branching ratio, root PME, and mycorrhizal colonization were the traits that explain most of the species variation in the NMDS (Figure 20b).

Overall, mean root diameter was greatest in *O. leucoxylon* (0.75 mm) followed by *C. calaba* (0.46 mm) and the thinnest first order roots were from *P. montana* (0.23 mm; Figure 21). Pioneer species *C. schreberiana* and *S. campanulata* had the greatest SRL (10.31 cm mg^{-1}, 10.64 cm mg^{-1}), and *C. calaba* had the lowest values (0.79 cm mg^{-1}; Figure 21). *P. montana* had the greatest root branching ratio (5.91), and together with *D. excelsa*, had one of the greatest root branching intensities (2.80 and 4.28, respectively; Figure 21). However, *P. montana* together with *I. laurina* had the lowest mycorrhizal colonization (12.1% and 0.91%, respectively), and the two pioneer species, *C. schreberiana* and *S. campanulata*, had the greatest values of colonization (70% and 75%, respectively; Figure 21). The pioneer species *C. schreberiana* also had the highest root PME, and *P. montana* had the lowest values for root PME (272.90 µmol $pNPg_{root}^{-1}hr^{-1}$ and 16.41 µmol $pNPg_{root}^{-1}hr^{-1}$, respectively; Figure 21). Finally, both pioneer species, *C. schreberiana* and *S. campanulata*, had the greatest root P concentrations (0.08% and 0.10%, respectively) and *P. montana* had the lowest P concentration (0.06%; Figure 21).

Root trait regressions across species and sites

Most root trait relationships were driven by species variation (Table 9; Figure 22). When considering the variation of the site in the mixed model as a random effect, mycorrhizal

colonization was positively related to root PME (Table 9; Figure 22a). For every one percent increase in mycorrhizal percent colonization, there was an increase of 25.9 μmol $pNPg_{root}^{-1}hr^{-1}$ of PME (Table 9). In contrast, root branching ratio had a significant negative relationship with root PME when considering only site variation in the random effect (Table 9; Figure 22b). For every increase in root branching ratio, there was a decrease of 10.8 μmol $pNPg_{root}^{-1}hr^{-1}$ on PME (Table 9). However, when considering species variation in the random effect, there was no significant relationship between these root traits (Table 9; Figure 22). Also, the negative relationship between root branching ratio and mycorrhizal colonization was only significant when site variation was considered, but became insignificant when considering species within site (Table 9, Figure 22d). Thus, the differences in root PME, root branching ratio, and mycorrhizal colonization was due the differences in species combined with the different community compositions among sites (Figure 22). Species like *P. montana* had the lowest mycorrhizal colonization percent, the lowest root PME, but the highest root branching ratio, contrary to *C. schreberiana* (Figure 22).

Soil organic P and available P did not show a relationship with root PME (Table 9). Further SRL had no significant effect on root phosphatase activity (Table 9). When analyzing root PME, root branching ratio, and mycorrhizal colonization effect on root P percentage, we found that only mycorrhizal colonization had a positive effect on root P percent, even when site and species variation was included in the model (Table 9; Figure 22c). We found that SRL had a positive effect on root branching ratio when considering species and site variation in the model (Table 9; Figure 22e). We found no relationship between SRL and root mycorrhizal colonization (Table 9).

Root traits before and after the hurricane disturbances

Hurricanes Irma and María had a negative effect on root PME and on soil available P (Table 10, Figure 23). Soil available P reduced three-fold from 0.66 mg g^{-1} to 0.22 mg g^{-1} after the hurricane disturbance when accounting for site, species, and individual tree variation. Root PME reduced also ~3 fold from 222 μmol $pNPg_{root}^{-1}hr^{-1}$ before the hurricanes to 79 μmol $pNPg_{root}^{-1}hr^{-1}$ after

the hurricanes. No other root trait significantly changed with the disturbance (Table 10; Figure 23).

Discussion

Soil P concentration among sites

We found differences in soil organic P concentration and available P concentration among the sites. The mature forest had the greatest concentration of organic P, and the youngest forest had the greatest available P. Phosphorus availability and its concentration depend on different factors such as soil age, climate, parent material, biota composition, and topographic position (Mage & Porder, 2013). Our three study sites had the same parent material, similar mean annual temperature, and mean annual precipitation, but different forest age, plant composition, and elevation (Scatena, 1989; Stone *et al.*, 2014; Uriarte & Zimmerman, 2016; Picón Ruiz, 2019). Thus, forest land use and forest age were an important difference among these forests that could have had affected soil P concentrations (Stevens & Walker, 1970; Walker & Syers, 1976; Filippelli, 2008; Uriarte & Zimmerman, 2016; Picón Ruiz, 2019) based on previous studies (McDonald *et al.*, 2012).

From recent land use change processes (from timber plantations to secondary forest; Picón Ruiz, 2019), SB2 and EV1 had greater abundance of pioneer species, such as *C. schreberiana* in SB2 and EV1, and *S. campanulata* only in SB2. These species produce high-quality leaf litter that decomposes rapidly and leads to high nutrient mineralization rates (Grime, 1974; Pastor *et al.*, 1984; Uriarte *et al.*, 2015; Lugo & Abelleira Martínez, 2018), and ultimately to the greater available P concentration that we measured in the soils. In contrast, in the mature forest at LEF ($\geq$100 years old; EVS), there was less available P due to P occlusion through pedogenesis, less high-quality leaf litter, and potentially more P inmobilization by microbes resulting in greater soil organic P (Stevens & Walker, 1970; Liptzin & Siver, 2009; Uriarte *et al.*, 2015). However, despite the differences in soil P concentrations among sites, root trait differences were mostly driven by species rather than sites differences.

Root traits by species

We found that root traits varied more among species than among sites. Interspecific root trait variation has been widely explored in the literature, and some root traits have been shown to be phylogenetically constrained, therefore they do not change much with site. This is the case for SRL and root diameter (Ryser & Eek, 2000; Kong *et al.*, 2014; Ma *et al.*, 2018; Wang *et al.*, 2018b; Valverde-Barrantes *et al.*, 2020). However, other root traits such as mycorrhizal colonization, and root branching have shown variation with different environmental conditions (Kong *et al.*, 2014; Weemstra *et al.*, 2016). Cabugao *et al.* (2017) found that root PME varied among both tree species and site in the LEF. Our study also confirmed that root PME and root branching ratio can vary among sites within the same species (Table 8). However, we did not see differences in root mycorrhizal colonization between sites. Powers *et al.* (2005) showed how site differences did not play an important role in AM hyphae length on different tropical forests. Further, some studies suggest that AM can potentially colonize root cortical tissue independently of soil P availability from each site, due to a lack of efficient mechanisms from the plant to control AM colonization (Johnson, 2010; Valverde-Barrantes *et al.*, 2016). Thus, the relationship between the mycorrhizal percent colonization and the soil P availability does not rely exclusively on soil P availability, but also other factors, including the evolutionary history of the plant species and its fungal partner (Valverde-Barrantes *et al.*, 2020).

We found that the pioneer species *C. schreberiana and S. campanulata* had greater mycorrhizal colonization rates than non-pioneer species. However, Bachelot & Lee (2018) showed that pioneer species in the same forests were more "independent" of their fungal mycorrhizal partners than non-pioneers. Our results also contradict with those presented in Myster *et al.* (2013), where mycorrhizal colonization percent was lower for the native pioneer *C. schreberiana* and greater for *D. excelsa* in the LEF. Yet, these differences in results might be attributed to differences in tree age since previous studies considered seedlings for these measurements, and we considered mature trees.

Root traits relationships and strategies across sites and species

When considering only site variation in our mixed effect model, we found that mycorrhizal colonization had a positive effect on root PME, but when we added species variation in the model, this relationship was no longer significant (Table 9; Figure 22). Thus, we conclude that root trait differences were mostly explained by species differences. It is possible that if we chose different species to test these same root trait relationships, we could find different patterns. Therefore, we recognize the importance of expanding this study to more tropical tree species. We hypothesized that greater root mycorrhizal colonization is part of the "outsourcing" end of the collaboration dimension (Bergmann *et al.*, 2020) and opposite to root PME as part of the "do it yourself" strategy. Both strategies require an energy cost as either investing in P mineralizing enzymes or in supporting mycorrhizal symbionts to maximize P acquisition from soils (Treseder & Vitousek, 2001; Nasto *et al.*, 2017, 2019). In contrast, in this study and with the species we considered, we found that species with greater mycorrhizal colonization also presented greater phosphatase exudation. Although root PME can be a combined contribution of root and fungi phosphatase enzymes, this has been explored mostly on ectomycorrhizal fungi (Bartlett & Lewis, 1973; Gianinazzi *et al.*, 1979; Dodd *et al.*, 1987), and ericoid mycorrhizae, but less so in AMF (Antibus *et al.*, 1992; Koide & Kabir, 2000; Smith & Read, 2008; Spohn & Kuzyakov, 2014).

Both AMF and ECMF are capable of exuding phosphatase enzymes, yet the ability to use organic P by AMF is still unclear (Koide & Kabir, 2000). Further, the hypothesis of a combined phosphatase enzymes exudation from roots and AMF, or the complementary strategy of the mineralization of organic P by root phosphatase and the concomitant exploitation of the readily inorganic P by AMF, is important to consider (Turner, 2008). Future corroboration of these results by increasing the species sampled is encouraged, as is measuring how much of the phosphatase enzymes is contributed by roots in comparison to the fungi.

We hypothesized that root branching ratio was an opposite strategy to mycorrhizal colonization, and on the same end ("do it yourself") as root PME in the collaboration based on previous studies (Pregitzer, 2002; Comas & Eissenstat, 2004; Xia *et al.*, 2010; Weemstra *et al.*, 2016). However, although we found no significant relationship between any of these root traits when

considering site and species variation in the model (Table 9), species that had greater root branching ratio did show less mycorrhizal colonization, but also less root PME (Figure 22). We also visualized this pattern at the NMDS (Figure 20), where we showed that *P. montana* presented the greatest root branching ratio, but the lowest mycorrhizal colonization and root PME, in contrast to *C. schreberiana*. Thus, we placed the species from this study where they might fit in the new Root Economics Spectrum space (Figure 24).

The root trait relationships that were significant, even when accounting for site and species variation in the model, were the positive relationships between root branching ratio with SRL, and root mycorrhizal colonization with root P. The SRL - root branching ratio relationship supports the hypothesis of a "do it yourself" strategy, in which plants will have thinner roots and more branching to better explore the soil and obtain nutrients (Hodge, 2004; Kong *et al.*, 2014; Liu *et al.*, 2015; Cockerton *et al.*, 2020). The positive relationship between root mycorrhizal colonization and root P concentration could potentially also reflect concentrations of leaf P (Schreeg *et al.*, 2014) and the contribution of mycorrhizal fungi in P uptake (Treseder, 2013; Liu *et al.*, 2018; Wang *et al.*, 2018a). We agree there are root traits that are species-specific that can determine species strategies along the collaboration gradient, but we argue that the root traits we studied here might not all fit under the proposed collaboration and conservation gradients. Further, we suggest considering the relationships of these traits accounting for species and site variation to test relationships of root traits regardless of other species differences.

We recognize the potential caveats of performing most of our root trait analyses on data post-hurricanes; thus, we compared our data to a subsample taken pre-hurricanes, where we considered only three species and did not account for mycorrhizal percent colonization. With these data, we found very similar patterns in the NMDS (Figure 25). Root branching ratio opposite to root PME. Therefore, we conclude that root PME is in the "do it yourself" end of the collaboration gradient along with root branching ratio, but on the opposite end at the "outsourcing" strategy is root mycorrhizal colonization. Considering our small sample size and the disturbance effects of the hurricanes, we still encourage further studies to include these root traits from more tropical species and to consider the differences between species while also accounting for intra-specific variation.

Testing the relationship of soil P concentrations and PME, we did not find a negative effect of soil available P on root PME or a positive effect of soil organic P on root PME in contrast to previous studies (Dakora & Phillips, 2002; Dalling *et al.*, 2016; Cabugao *et al.*, 2017). However, phosphatase production also depends on the P demand from plants, which we did not measure. Soil disturbance can also influence root phosphatase activity (Margalef *et al.*, 2017), and the changes in soil environmental conditions during the recovery from Hurricanes Irma and María could have played a role. This disturbance had an influence in nutrient concentrations from the litterfall (Reed *et al.*, 2020), which in turn could have changed the patterns seen in Cabugao *et al.* (2017). Further, there is still contradicting results in the literature, where soil available P does not affect root phosphatase activity (Margalef *et al.*, 2017; Cabugao *et al, in press*).

Hurricane impact on root traits

We found that root PME decreased after hurricane events, but the other root traits did not (Table 10; Figure 23). This suggests that root PME responds to environmental changes and it is not a fixed trait. According to previous studies, the decrease in soil available P and the increase in soil organic P could potentially increase the exudation of phosphatase enzymes from the root (Dakora & Phillips, 2002; Burns *et al.*, 2013; Dalling *et al.*, 2016; Margalef *et al.*, 2017). However, although previous studies have shown that soil organic P and available P increased in the LEF immediately after Hurricane Hugo (Sanford *et al.*, 1991; Teh *et al.*, 2009), we found that soil available P collected in this study decreased 6 months after the hurricanes. Thus, other factors might have affected the root enzymatic exudation. Hurricanes Irma and María might affected the vegetation in different ways: partial to total defoliation, increase in soil moisture (SB2 was flooded for several months), and the increase in understory vegetation (Kennard *et al.*, 2020; Reed *et al.*, 2020). Thus, the production of PME could have been compromised by a low N and P demand from the plant, or low C allocation to produce enzymes due the reduction of foliar cover. Further microbial composition change, potential changes in mycorrhizal colonization rates, and resource competition with new vegetation could have played a role in the response of root PME after the hurricanes.

Conclusions

Throughout this study, we have seen that species differences account for most of the variation in root traits. Within this diversity of root traits, we found that species with greater root branching ratio had less mycorrhizal colonization, as we hypothesized in the collaboration gradient. However, we found that species with greater root PME also exhibited less root branching ratio, contrary to our hypothesis. Thus, we suggest that both, roots and the arbuscular mycorrhizal fungi could be potentially contributing to P acquisition by a combined or complementary effort of enzymatic phosphatase exudation and exploitation of mineralized P. Further, we found that root branching ratio had a positive relationship with SRL, despite species variation. Thus, we suggest that root branching might be part of the "do it yourself" strategy for the collaboration gradient, and root mycorrhizal colonization and root PME occupy the "outsourcing" strategy. Although our opposite trend of PME and root branching ratio was confirmed for pre-hurricanes samples, we do consider important to consider more species and greater sample size to confirm the patterns. Finally, we found that only root PME decreased with the hurricane disturbance (not including mycorrhizal percent colonization in the analysis), which could have been related to the changes in soil nutrient concentrations, plant demand, low C allocation to enzyme production due to low foliar cover, or microbial composition and abundance in the rhizosphere.

The complexity and multidimensionality of root traits can be driven by different selection pressures and be constrained by multiple environmental drivers that might not necessarily be related to P uptake. Thus, finding general trade-offs between root traits that can inform P acquisition strategies between species is a challenging task, and it is likely that mycorrhizal symbiosis represents another dimension in addition to the two already suggested in the literature. Further studies on these trade-offs are necessary, as is accounting for interspecific and intraspecific root trait variations and environmental drivers, all of which are important factors to consider when interpreting trade-offs.

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Appendix

*Table 8. P-value results from one-way ANOVA comparing root traits: root diameter (RD), root branching ratio (RBR), root branching intensity (RBI), mycorrhizal colonization percentage (AM col.), root phosphatase activity (PME), root phosphorus percentage (RP), specific root length (SRL), A) between sites for each species that are repeated in sites, and B) between species within each site. *Significant values.*

A. Root trait differences between sites for each species

Species	RD	RBR	RBI	AM col.	PME	RP	SRL
Prestoea montana	0.092	0.633	0.071	0.625	0.028*	0.404	0.627
Cecropia scheberiana	0.481	0.295	0.350	0.323	0.317	0.097	0.333
Calophyllum calaba	0.051	0.016*	0.221	0.097	0.276	0.115	0.632

B. Root trait differences between species for each site

Site	RD	RBR	RBI	AM col.	PME	RP	SRL
EVS	<0.001*	0.007*	0.016*	0.002*	<0.001*	0.220	0.343
EV1	<0.001*	0.024*	0.250	0.004*	0.284	0.079	0.01*
SB2	0.310	0.043*	0.006*	0.001*	0.095	0.282	0.180

Table 9. Mixed effect models; dependent variables; fixed effects; random effects; P values; R^2 marginal and R^2 conditional; coefficient estimates.

Model (lmer)	Dependent variable	Fixed effects	Random effect	P value	R^2m/R^2c	Estimate
Root PME~SRL+ (1|Site)	Root PME	SRL	(1|Site)	0.89	0.00/0.19	-0.65
Root PME~SRL+ (1 | Site/Species)	Root PME	SRL	(1 | Site/Species)	0.84	0.00/0.95	-0.16
Root PME ~ Mycorrhizal col +(1|Site)	Root PME	Mycorrhizal colonization	(1|Site)	0.01	0.2/0.38	25.90
Root PME ~ Mycorrhizal col +(1|Site/Species)	Root PME	Mycorrhizal colonization	(1 | Site/Species)	0.22	0.08/0.55	16.60
Root PME ~ Root brancing ratio +(1|Site)	Root PME	Root branching ratio	(1|Site)	0.09	0.09/0.36	-10.80
Root PME ~ Root brancing ratio +(1|Site/Species)	Root PME	Root branching ratio	(1 | Site/Species)	0.11	0.07/0.72	-10.00
Root PME ~ Soil available P +(1|Site)	Root PME	Soil available P	(1 | Site)	0.75	0.01/0.26	-18.73
Root PME ~ Soil available P +(1|Site/Species)	Root PME	Soil available P	(1 | Site/Species)	0.23	0.02/0.74	-52.11
Root PME ~ Soil organic P +(1|Site)	Root PME	Soil organic P	(1 | Site)	0.94	0.00/0.38	<0.00
Root PME ~ Soil organic P +(1|Site/Species)	Root PME	Soil organic P	(1 | Site/Species)	0.94	0.00/0.38	<0.00
Root P ~ Root PME +(1|Site)	Root P	Root PME	(1 | Site)	0.30	0.05/0.21	<0.00
Root P ~Root PME +(1|Site/Species)	Root P	Root PME	(1 | Site/Species)	0.30	0.05/0.21	<0.00

Table 9 (continued)

Model (lmer)	Dependent variable	Fixed effects	Random effect	P value	R^2m/R^2c	Estimate
Root P ~ Mycorrhizal col +(1|Site)	Root P	Mycorrhizal colonization	(1 | Site)	0.05	0.14/0.26	0.03
Root P ~ Mycorrhizal col +(1|Site/Species)	Root P	Mycorrhizal colonization	(1 | Site/Species)	0.05	0.14/0.26	0.03
Root P ~ Root branching ratio +(1|Site)	Root P	Root branching ratio	(1 | Site)	0.94	0.00/0.08	<-0.00
Root P ~Root branching ratio +(1|Site/Species)	Root P	Root branching ratio	(1 | Site/Species)	0.80	0.00/0.16	<0.00
Mycorrhizal col~ Root branching ratio +(1|Site/Species)	Mycorrhizal colonization	Root branching ratio	(1 | Site)	0.04	0.25/0.32	-0.06
Mycorrhizal col~ Root branching ratio +(1|Site)	Mycorrhizal colonization	Root branching ratio	(1 | Site/Species)	0.77	0.00/0.84	<0.00
Root branching ratio~ SRL+(1|Site/Species)	Root branching ratio	SRL	(1 | Site)	0.65	0.00/0.00	0.05
Root branching ratio~ SRL +(1|Site)	Root branching ratio	SRL	(1 | Site/Species)	0.04	0.11/0.72	0.24
Mycorrhizal col~ SRL+(1|Site/Species)	Mycorrhizal colonization	SRL	(1 | Site)	0.93	0.00/0.00	<-0.01
Mycorrhizal col~ SRL +(1|Site)	Mycorrhizal colonization	SRL	(1 | Site/Species)	0.57	0.00/0.82	0.01

Table 10. Mixed effect model ANOVA; dependent variables; fixed effects; random effects (ID refers to the specific tree ID); P values; Emmeans are the estimated marginal means for time 1 (6 months before the hurricanes), and time 2 (6 months after the hurricanes).

Model (lmer)	Dependent variable	Fixed effects	Random effect	P value	Emmeans time 1	Emmeans time 2
Root PME ~ Time + (1|Site/Species/ID)	Root PME	Time	(1|Site/Species/ID)	<0.01	222.80	79.60
Root ratio ~ Time+ (1|Site/Species/ID)	Root branching ratio	Time	(1|Site/Species/ID)	0.38	3.43	3.99
Root intensity ~ Time+ (1|Site/Species/ID)	Root branching intensity	Time	(1|Site/Species/ID)	0.20	1.81	2.28
Root diameter ~ Time+ (1|Site/Species/ID)	Root diameter	Time	(1|Site/Species/ID)	0.24	0.37	0.32
SRL ~ Time+ (1|Site/Species/ID)	SRL	Time	(1|Site/Species/ID)	0.98	4.72	4.78
Root P ~ Time+ (1|Site/Species/ID)	Root P	Time	(1|Site/Species/ID)	0.21	0.15	0.13
Soil available P ~ Time+ (1|Site/Species/ID)	Soil available P	Time	(1|Site/Species/ID)	0.07	0.66	0.21

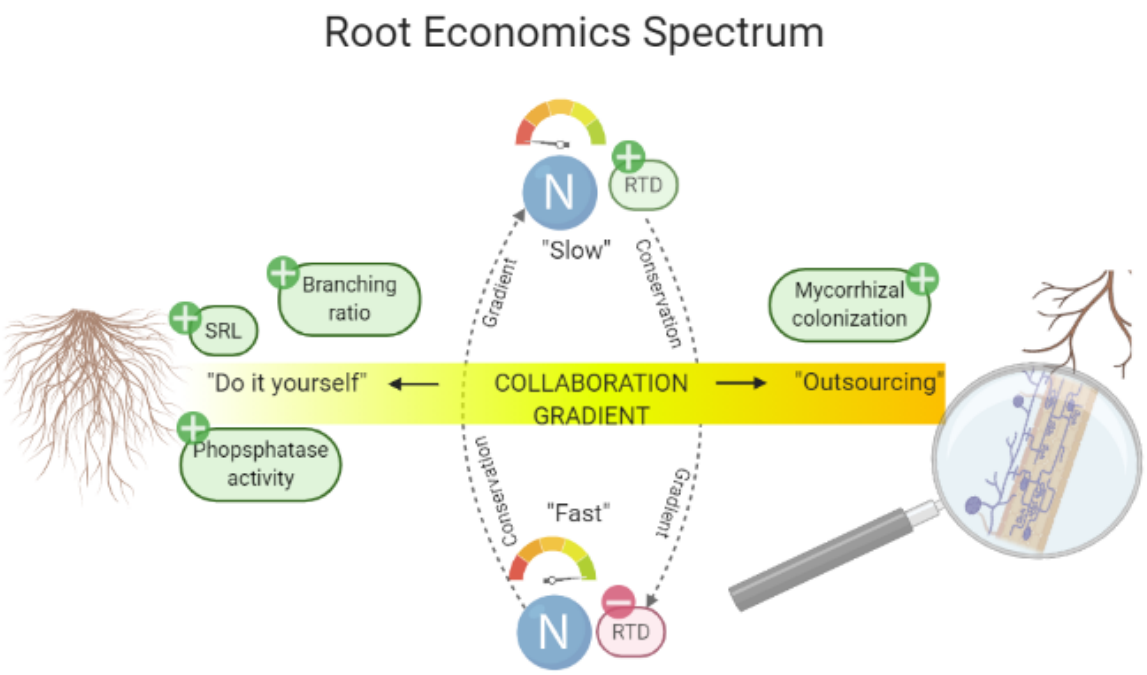

Figure 17. Conceptual framework of the Root Economic Spectrum adapted from Bergmann et al. (2020) and Kramer-Walter et al. (2016). The collaboration gradient (horizontal line) ranges from "do-it-yourself" soil resource acquisition strategy to "outsourcing" strategy. The conservation gradient (dotted lines) represents a "fast-slow" resource return on investment.

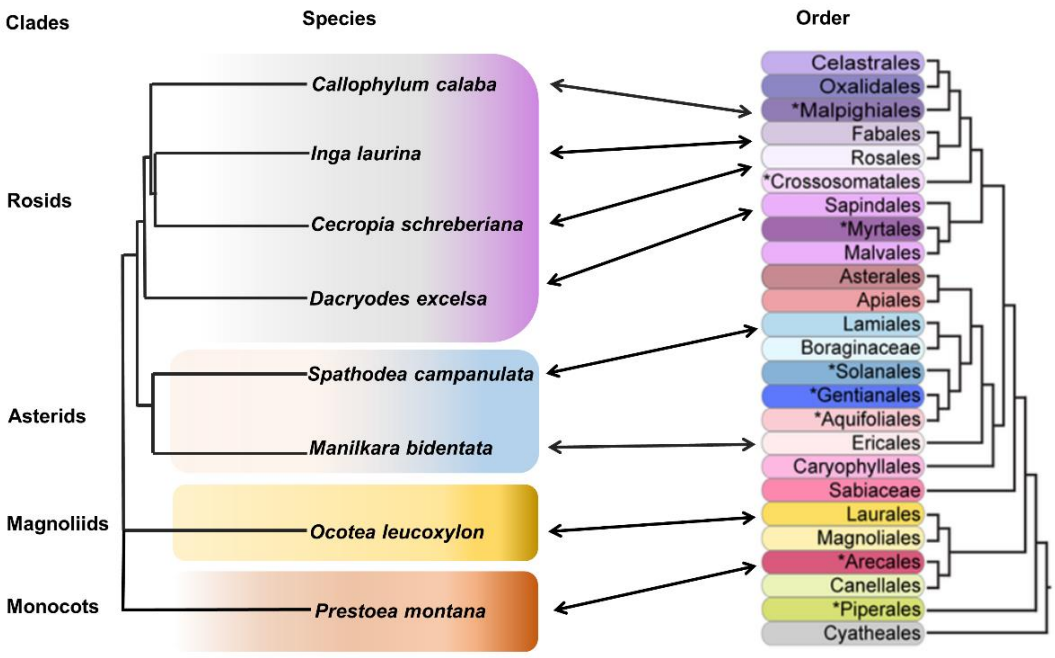

Figure 18. Phylogenetic tree representing all the species considered in the study grouped by the clades and matching with their respective order from a phylogenetic tree of Puerto Rican tree species in the Dynamics Plots from the Luquillo Experimental Forest (Kress et al., 2010).

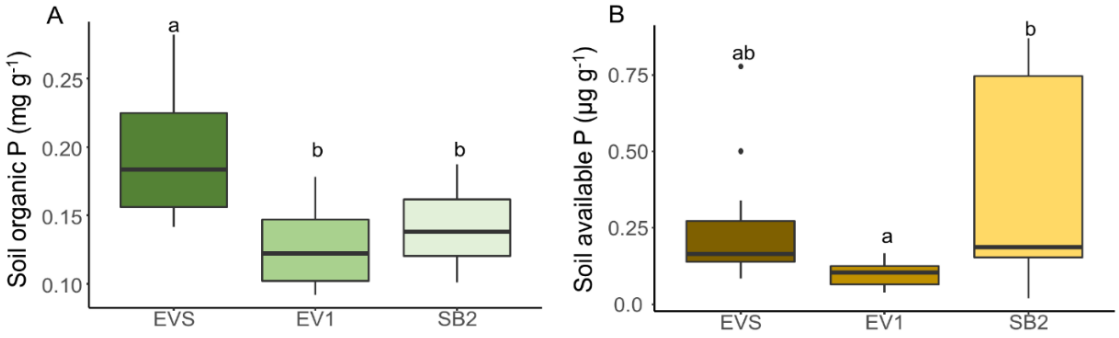

Figure 19. Soil organic P (A), and available P (B), from the three sites: El Verde Station (EVS), El Verde 1 (EV1), and Sabana 2 (SB2). Significant Tukey posthoc differences are designated with different letters.

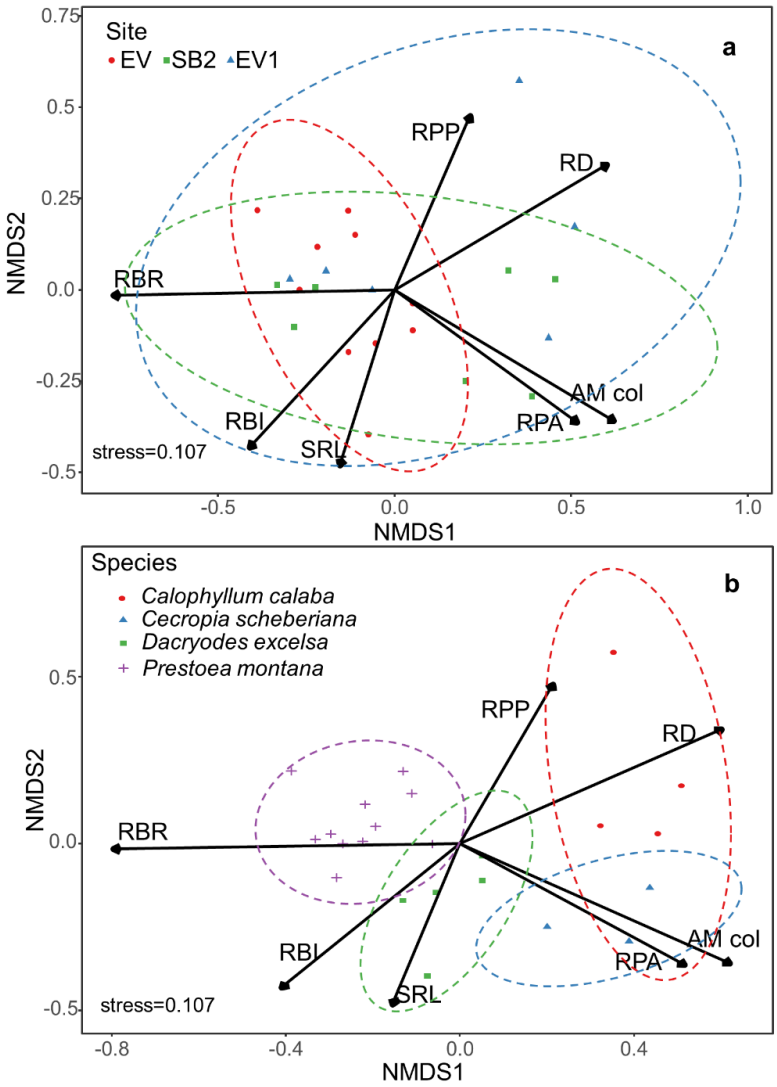

Figure 20. Non-metric multi-dimensional scaling of root traits: root branching ratio (RBR), root branching intensity (RBI), specific root length (SRL), root phosphatase activity (PME), root diameter (RD), root P percent (RPP), and arbuscular mycorrhizal colonization (AM col). The traits are grouped by a) site, and by b) species.

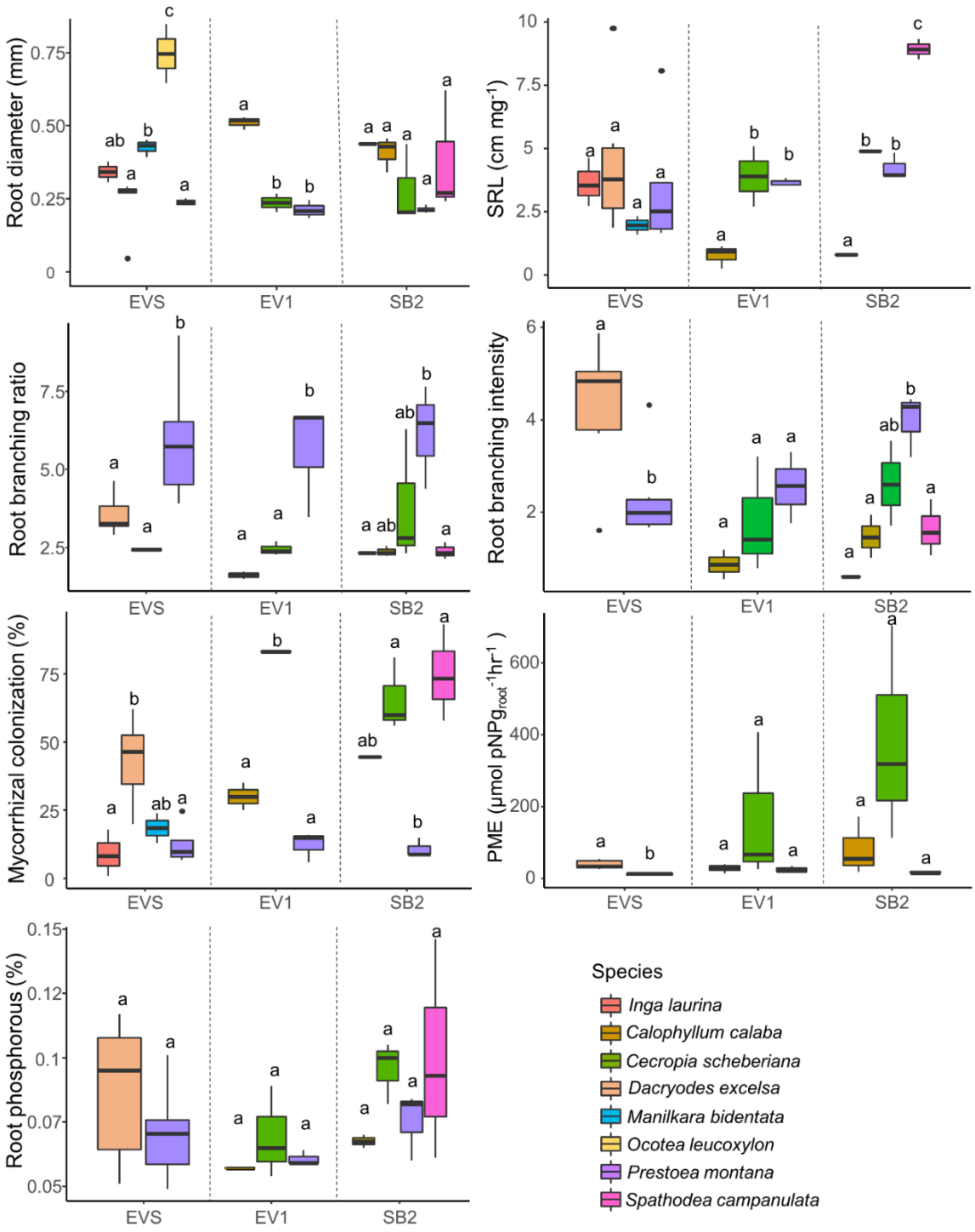

Figure 21. Root trait values represented by species (colors) and divided by sites (dotted vertical lines). Significant Tukey Posthoc differences within each site are designated with different letters.

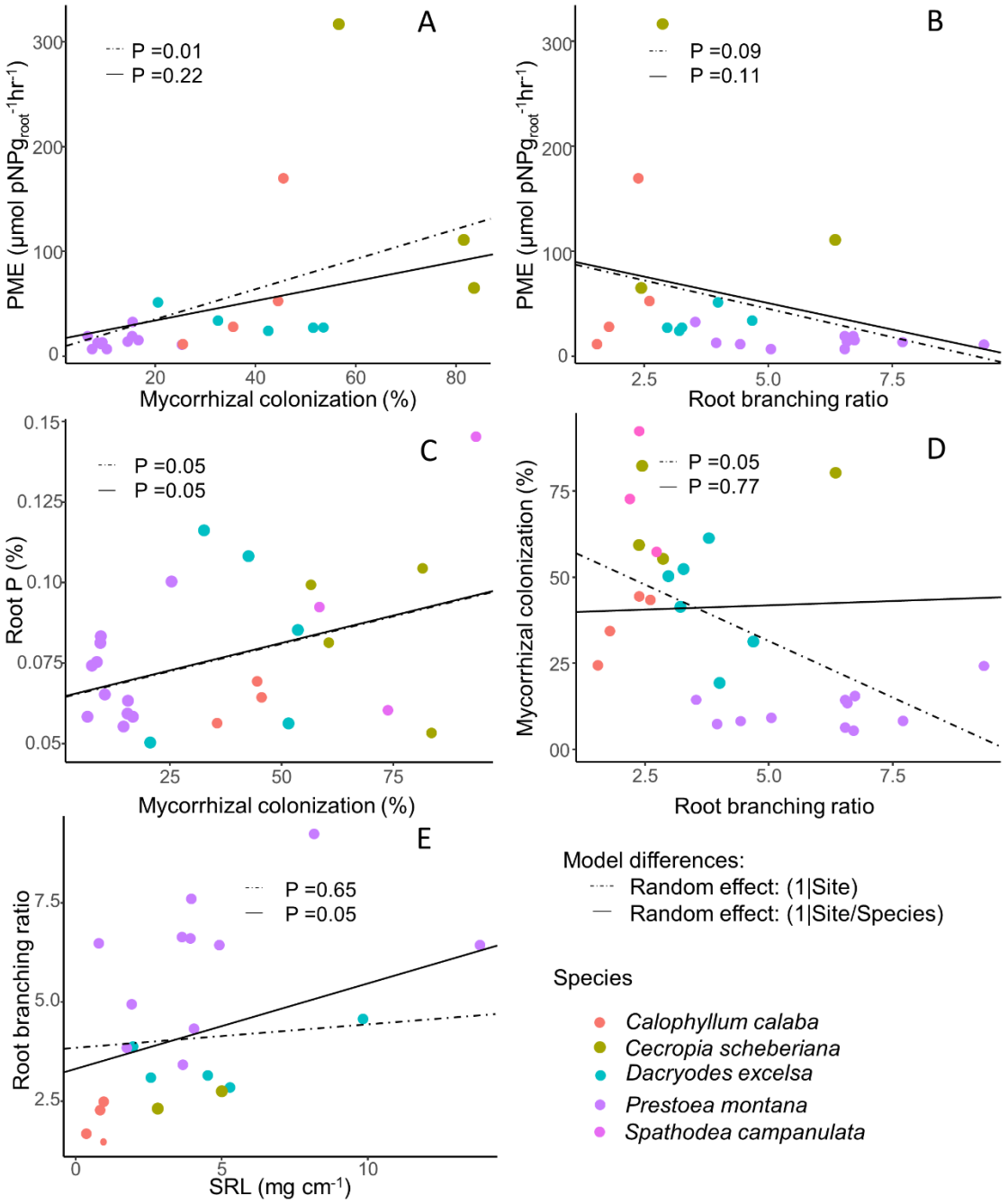

Figure 22. Significant regressions between root mycorrhizal colonization, root branching ratio, root PME, and root P percent. The patterns were evaluated under two different model regression lines. P-values are reported in the graphs for each model.

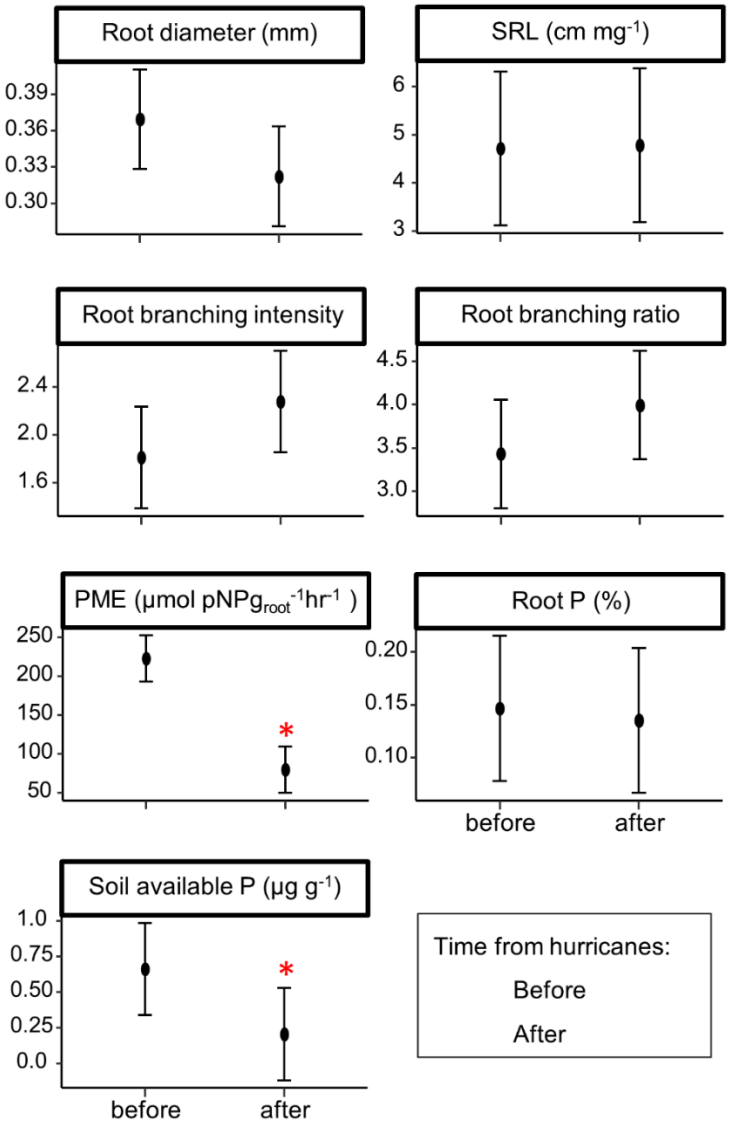

Figure 23. Estimated marginal means and standard error for each root traits before and after the hurricanes taken from the mixed model. Differences of root traits before and after the hurricanes with a P-values <0.1 are represented with a red asterisk.

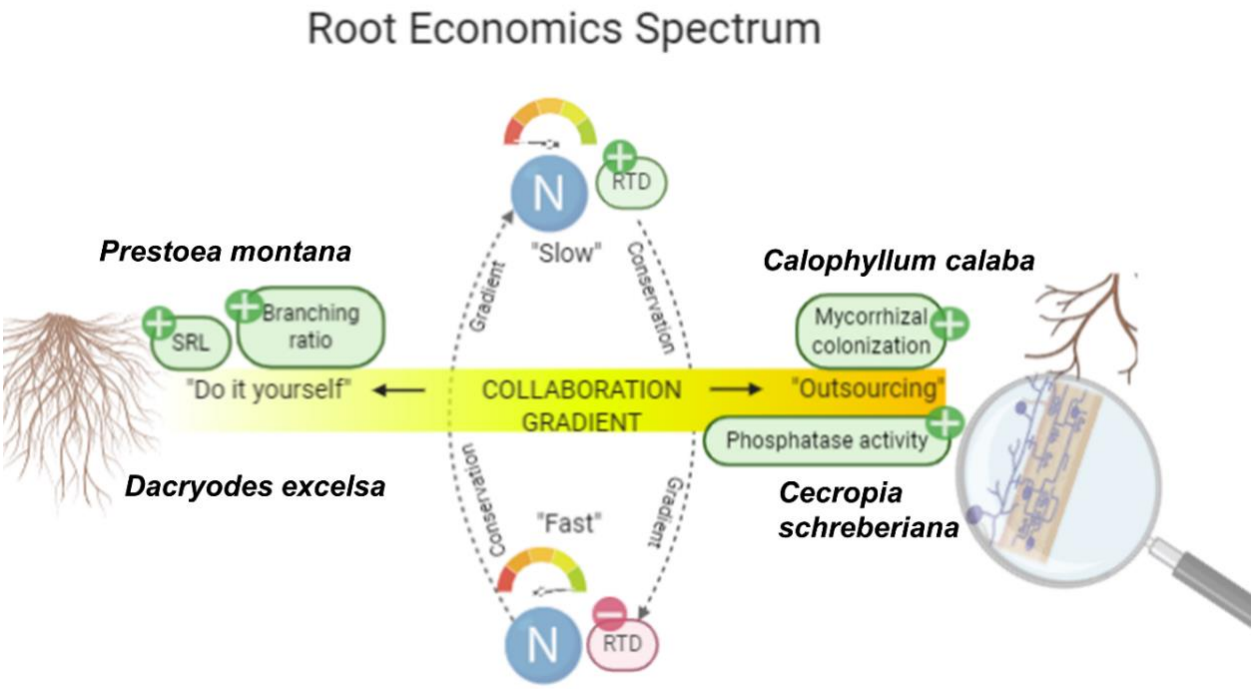

*Figure 24. Conceptual framework of the Root Economic Spectrum space adapted from Bergmann et al. (2020) and Kramer-Walter et al. (2016), with the correspondent location of the tree species based on their root traits over the "collaboration" gradient (*horizontal line*), and the location of the root traits based on this study results. Root tissue density is RTD, and root nitrogen is N.*

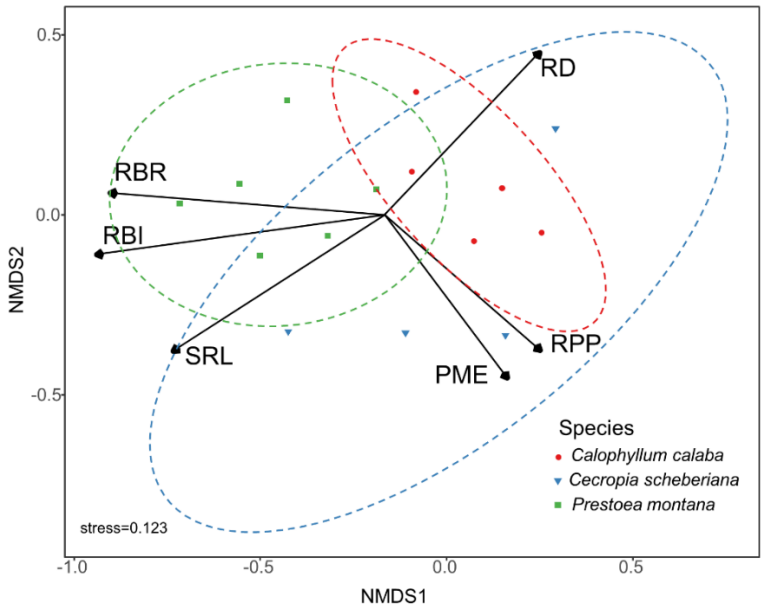

Figure 25. Non-metric multi-dimensional scaling of root traits: root branching ratio (RBR), root branching intensity (RBI), specific root length (SRL), root phosphatase activity (PME), root diameter (RD), root P percent (RPP). The traits are grouped by a) site, and by b) species before the hurricane events.

Chapter 5

Conclusions

The contribution of plant roots to C storage, and C and nutrient cycling is variable across ecosystems (Hungate *et al.*, 1997; Norby, 1997). Roots can store up to 80 % of the C from the vegetation, and account for up to 30% of the plant production (Jackson *et al.*, 1997). However, the relationships between root structure (e.g., morphology) and function (e.g., production, nutrient uptake), is still under debate. Further, the extent to which we can predict root response to disturbances and their effect on the ecosystem function in a changing world is also understudied. Therefore, roots are often considered as part of the "black box" in the C budget (Norby, 1997) and the "hidden half" of the ecosystem (Waisel *et al.*, 1991). Broaden our understanding on the participation of roots to the C cycle, and how the diversity of root structure relates to its function is essential for predicting C balance in a changing world. Further, despite their great contribution on C cycling, tropical ecosystems have been less represented in root studies than temperate and boreal ones.

Root traits are mostly underrepresented in Terrestrial Biosphere Models (TBM). For example, the Energy Exascale Earth System Model (E3SM) Land Model (ELM) represents roots as a pool of carbon by depth in the belowground and considers the nutrient uptake based on the plant demand (Zhu *et al.*, 2019). However, root traits such as mycorrhizal colonization, root branching ratio, and root phosphatase activity can regulate the amount of nutrient and carbon that are being cycled from and to the plant, but are usually not considered. In turn, these root traits are also correlated to some morphological traits such as specific root length (SRL) and root diameter. Currently, there are some hypothesis on how root traits relate to the plant performance, growth, and nutrient acquisition strategies, but there are still conflicting conclusions (Kong *et al.*, 2014; Weemstra *et al.*, 2016; Bergmann *et al.*, 2020). My study resulted in: 1) A synthesis on root traits measured from tropical forests in Puerto Rico for the past 50 years in order to inform public databases and therefore improve tropical root representation in the models. 2) An experimental research on the effect of warming, and hurricane disturbance on root production and biomass. And, 3) a research study on the root trait strategies related to phosphorus (P) acquisition in common species at the Luquilllo Experimental Forest, Puerto Rico.

Root traits at the Island scale

Root studies in Puerto Rico have been very representative for tropical ecosystems considering that its land surface only represents 0.03% of the tropics. From all tropical root data in the Fine-Root Ecology Database (FRED) (Iversen *et al.*, 2017), 4% is from Puerto Rico (Iversen *et al.*, 2017). Most of the data was collected from wet, rain, and cloud forests in the Luquillo Experimental Forest (LEF). Thus, other forests of the Island were underrepresented. Further, from the studies considered in my review, only 18% focused primarily on root measurements, and the rest included roots as a secondary measurement. The most studied root traits were root chemistry and root dynamics. The least studied root traits were root architecture, morphology, and anatomy.

Despite the shallow root distribution in the wet forest (above 20 cm) that I found in the literature, only three studies considered rooting distribution deeper than 30 cm. Although the dry forest has slightly deeper root distribution than the wet forest, only one study measured root vertical distribution below 30 cm of soil depth. Rooting depth distribution from other forests (e.g., Southwest and Northwest) would increase the accuracy of root vertical distribution representation over the island. I also found that root biomass is greater in the wet forest than in the dry forest; and plantations followed by urban sites in the dry area of the island have the lowest root biomass from all studies. Root:shoot ratio is species-specific, but it varies depending on the tree bole diameter (smaller trees have lower root:shoot ratio than larger trees), and it is higher in Puerto Rico compared to other tropical sites, mainly due the great representation of larger trees measured by whole tree excavation. Root N and P concentration is also species-specific and varies significantly with root diameter classification. Thus, a functional classification or an order-based classification are greatly recommended for future root studies in the island. Finally, studies show that tree roots from Puerto Rico present some adaptations to hurricanes, such as the root grafting. Yet, the multiple functions (e.g., nutrient exchange) of root grafting are still underdeveloped, and the responsiveness of different root traits to hurricanes or other disturbances have not been investigated in depth.

This synthesis can be used in global databases to better represent tropical roots, better inform modules of TBM, and open questions for future studies of the underground in Puerto Rico and the tropics in general. As a result of this review, I was able to investigate some of the gaps presented in belowground studies in the island such as the response of root traits to warming and hurricane disturbances, considering a rooting depth up to one meter. Further, I was able to extend root trait measurements, including those that were least studied before such as root morphology, architecture, and enzymatic activity from some common tree species at the LEF.

Root-dynamics traits under warming on a forest scale

Increasing temperatures can affect directly root production by alterations to metabolic processes, or indirectly by affecting nutrient mineralization, water input, and carbon allocation (Fitter *et al.*, 1999; Pregitzer *et al.*, 2000; Burton & Pregitzer, 2003; Tierney *et al.*, 2003; Piatek & Allen, 2010; Zhou *et al.*, 2011). However, the effect of temperature increase in root systems, is not consistent across tree species or forest type (Forbes *et al.*, 1997; Wan *et al.*, 2004; Dukes *et al.*, 2005). Most studies found either a positive response of root production to the increase of temperature, or an increase in mortality due to the secondary negative effect of water shortage (Forbes *et al.*, 1997; Fitter *et al.*, 1999; Majdi & Öhrvik, 2004; Wan *et al.*, 2004; Pugnaire *et al.*, 2019). However, most warming experiments took place on temperate and boreal biomes (Norby *et al.*, 2004; Zhou *et al.*, 2012), where initial environmental condition are very different than in the tropics. I studied how root dynamics responded to an experimental warming (4^{o}C greater than ambient) in a wet tropical forest of Puerto Rico. Contrary to results from higher latitude ecosystems, I found that higher temperature decreased root production over time, declining root biomass. This shows the negative effect of warming on root dynamics in a tropical forest.

Root dynamics traits under hurricane disturbance on a forest scale

Hurricanes are considered one of the most intense weather disturbances affecting forest ecosystems in the Caribbean (McDowell, 2011). Forest carbon budgets and fluxes recovery,

including from plant roots, take from 10 up to 60 years following a Category 4 hurricane disturbance (Parrotta & Lodge, 1991; Lugo *et al.*, 2000; Teh *et al.*, 2009). I studied how two consecutive hurricanes affected roots from plots that were previously exposed to soil warming (4^{o}C greater than ambient) for a year. I found that after 10 months from the hurricanes, root production and biomass increased compared to values before the hurricanes. This rapid change in root dynamics and stock mirrored the aboveground change in plant composition during the forest recovery. More grass and herbaceous plants occupied the plots compared to woody plants post-hurricanes. However, we found that the increase in root stock was greater in the control plots, compared to the previously warmed plots, even when separating exclusively woody roots from the others. This shows the strong effect of hurricanes on root stock and root dynamics, as well as the legacy effect of the previous warming on the recovery of roots from the hurricanes.

Root traits related to P acquisition on a species scale

In tropical forest where soil available P is low, trees will present adapted functional traits, including in their roots, to enhance P acquisition (Vitoussek & Sanford, 1986; Kitayama *et al.*, 2000; Turner *et al.*, 2018). According to the Root Economics Spectrum (RES), due to the cost required to invest in some of the root traits related to P acquisition, trees will usually enhance some traits over the others (Weemstra *et al.*, 2016; Bergmann *et al.*, 2020). In this study I measured seven root traits from eight common species of the LEF in Puerto Rico, to identify the strategies these species have to optimize their soil P exploration and uptake. I considered root branching, root mycorrhizal colonization, and root phosphatase activity to be the root traits closely related to P uptake. I found that species that presented higher root branching ratio, presented lower mycorrhizal colonization and phosphatase activity (e.g., *Prestoea montana*), than those using the opposite strategies (e.g. *Cecropia schreberiana*). Species considered in this study that had more mycorrhizal colonization also had high root phosphatase activity, suggesting a possible combination of phosphatase enzymes from the root and the fungal partner, which is still under debate in the literature (Turner, 2008; Nasto *et al.*, 2017; Lugli *et al.*, 2019). My results also suggest that whereas pioneer species such as *C. schreberiana* and *Spathodea campanulata* will likely rely more on root mycorrhizal colonization and root enzymatic exudation to enhance P uptake, non-pioneers like *Dacryodes excelsa* and *P. montana* will rely on

their root branching for soil P exploration and acquisition. Finally, this study showed that although root traits vary by species, there are certain trade-offs between the cost involved in optimizing any of these traits related to enhance their P uptake, and that species cannot invest in all of them.

Representation of the belowground system in this study

From my findings, I developed an ecosystem diagram (Figure 26) adapted from Odum's energy flow system diagram (Odum, 2007). In this model I separated "warming (+4^{o}C)" as a source of temperature provided exclusively from the warming experiment that interact with "weather" which includes ambient temperature, wind, precipitation, and air conditions (e.g., O_2 concentration). I represented the ecosystem storages I studied throughout this dissertation (C and P in plants and soil biomass), the consumers (microbes), and the basic sources involved (sun, warming, weather, CO_2). I represented the paths of C and P fluxes by arrows between different storage pools and to outside the system. I represented "root traits" as a "modifier" (black box) of these fluxes in and out of the roots to and from the microbial symbionts (AMF).

The diagram shows that the experimental increase of temperature "warming (+4^{o}C)" interact with weather sources that affect plant photosynthesis (Figure 26). Depending on the initial ambient conditions, the interaction of warming could either increase or decrease photosynthetic rates (Reich *et al.*, 2018). In tropical forests such as the ones in the LEF, canopy temperature is already exceeding their photosynthetic temperature optima (Topt; Mau *et al.*, 2018). The understory, on the other hand, is usually under its Topt (Carter *et al.*, 2020), but can reach and exceed its Topt with the +4^{o}C of the warming, especially during summer. If plants are not able to acclimate to the increase of temperature, as is the case on our plots (Carter *et al*., 2020), then the photosynthesis rate can potentially decrease and consequently affect C storage and C allocation to the roots. In turn, this could result in less root production and consequently less root biomass. The ambient conditions also can affect decomposition, by temperature changes and precipitation (which regulates soil O_2), which can influence nutrient availability (Av. P; Cornejo *et al.*, 1994).

The increase of temperature by warming could have additional effects on decomposition (Majdi & Öhrvik, 2004; Wan *et al.*, 2004; Zhou *et al.*, 2012; Pugnaire *et al.*, 2019). Nutrient pools availability can up-regulate plant growth (Wright, 2019), consequently root growth (Cuevas & Medina, 1988; Wurzburger & Wright, 2015). Another possibility is that root soil exploration and demand of nutrient is down-regulating the soil nutrient availability. From my results I can suggest that root production and biomass are decreasing possibly due to the secondary effect of warming on C allocation, and that root dynamics are affecting nutrient availability and not the other way around. However, I did not measure C allocation, root respiration, root chemistry, decomposition rate, nutrient fluxes, or photosynthesis, and the photosynthesis and dark respiration measured from the leaves by Carter *et al*. (2020) represent only two common species of the plots. Therefore, I suggest incorporating some of these different measurements and results collected from the plots by other TRACE members, to better infer causation. I also suggest considering the initial effect of weather, and seasonality in root dynamics.

The hurricane disturbance in the diagram (Figure 26) interacts with the weather as well, increasing precipitation, wind, and changes the temperature in different time scales. During the hurricane event, temperature can decrease due to the interaction of the wind, precipitation, and the lack of direct sun to the system. However, on the longer term, the canopy openness caused by the hurricane disturbance can allow temperature to increase in the system. The hurricane as an interaction of weather can affect photosynthesis, carbon allocation to the roots, and decomposition. Further, the hurricane disturbance, acting as a modifier in the diagram, can also affect the structure and composition of the forest vegetation, changing the storage and fluxes of C and P throughout the system.

In my study, I found that the canopy openness caused by the hurricanes provided gaps of light that allowed mostly grass and herbaceous plants to grow in the understory, but also woody seedlings, and this combination increased the root production and biomass greatly. Yet, this increase in biomass was greater in control plots than in the previously warmed plots for both, woody roots, and other plant type roots. We assume that the woody roots were from trees or woody seedlings that were already in the plots before the hurricanes, and that these plants had less C stored due to the warming treatment in the warmed plots, which can explain the legacy

effect of warming after the hurricanes for these plants. However, the legacy effect of warming on new herbaceous, grass, and other seedlings might have involved a more complex interaction of nutrient fluxes, microbial composition change, and potentially effects on the seed bank.

Other root traits, such as morphology, architecture, mycorrhizal colonization, and root phosphatase activity are underrepresented in land models (e.g., ELM). In my system diagram (Figure 26), I represented them as a "modifier", or they can also be considered as a regulator of nutrient (P) uptake from soils or the fungal partner to the plant, and of C allocated from the plant to the fungal partner and the rhizosphere. These fluxes from the root to and from the mycorrhizal fungi are represented in the diagram as a "transaction", which symbolizes the mutual symbiosis. Since in most TBM nutrient uptake is a function of plant demand, including root traits as a modifier/regulator of the nutrient flux to the plant in the models, we would be accounting for the soil nutrient limitations that are not only based on the soil available P pools, but also on the different strategies plants might have to buffer the limitation (e.g. more root phosphatase enzyme exudation, or more root branching). There are few models (e.g. Functionally Assembled Terrestrial Ecosystem Simulator (FATES; Koven *et al.*, 2019) that are including plant functional types (PFT), and identifying the root traits that can cover these PFTs could better model the flux of nutrients and C.

Implications of this study

This research has contributed with over 1,000 root data records from 46 previous publications that now are centralized and are being added in the Fine-Root Ecology Database that will help improve the representation of tropical roots. This research has also broadened root traits measurements from common tree species in the LEF in Puerto Rico, which not only will increase root traits representation for the island and the tropics in general, but will also provide evidence of root trait strategies used to potentially enhance P acquisition. The study of the co-occurring disturbance stresses on root dynamics showed the importance of considering the multiple interactions and forces that can shape the ecosystem. The negative effect of warming to root dynamics and the legacy of warming on root recovery after the hurricane disturbance have implications for carbon cycling since root dynamics affect the amount and residence time of

carbon in the soils (Kell, 2012). This also has relevant implications on soil stability, thus, forest community and in the longer term, human society (Gyssels *et al.*, 2005). Inferring causation and increasing tropical monitoring studies that measure ecosystem responses to multiple disturbances will help us project and anticipate forest response and trajectory heading to a warmer world.

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Appendix

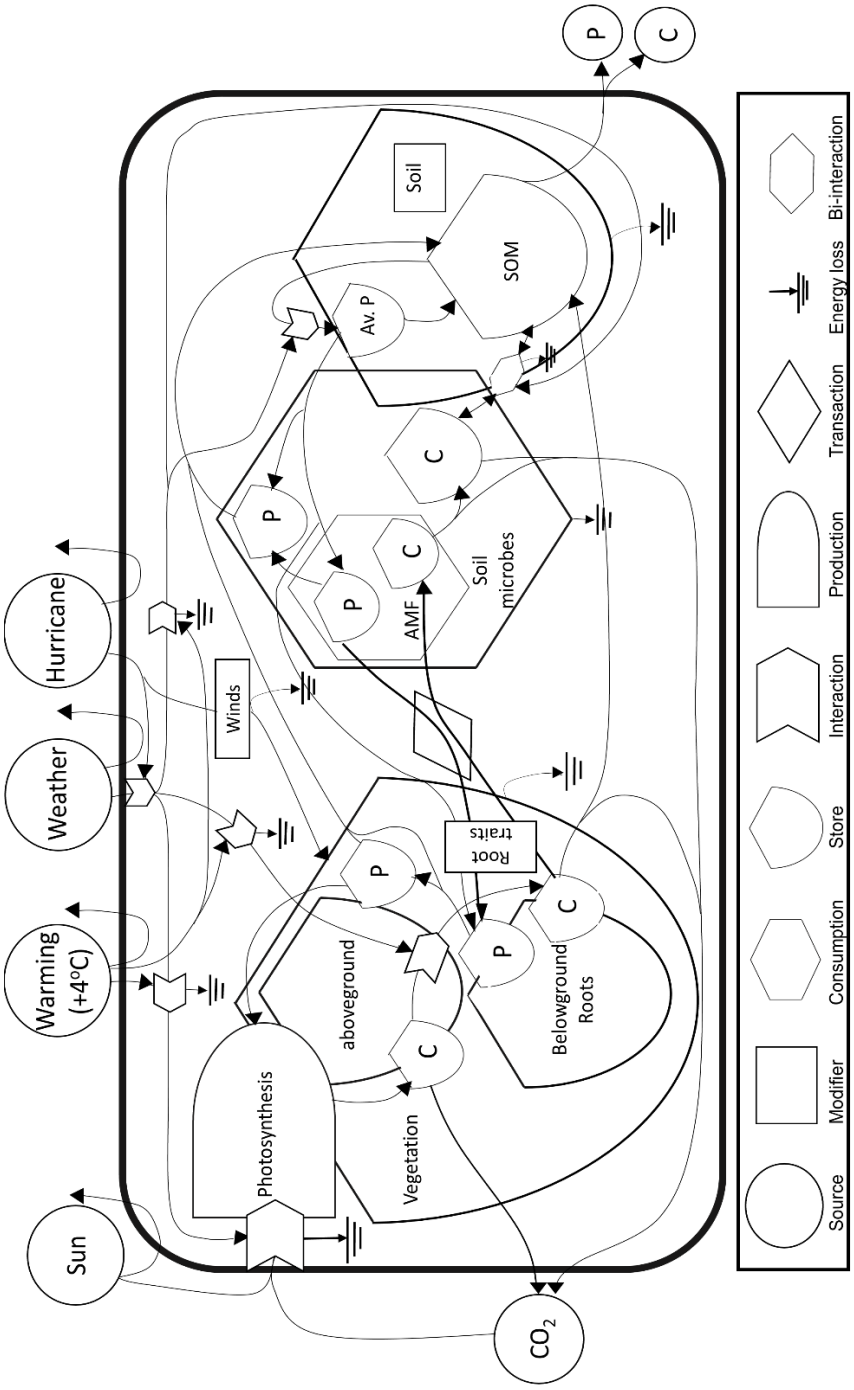

Figure 26. Ecosystem diagram representing the pools and fluxes of C and P studied in this research including root traits as a regulator from the microbial pool to the vegetation belowground and vice versa. The system is inside the main box. Warming and hurricane disturbance are represented as external sources, just like weather, sun, and CO_2. All symbols are explained in the rectangle below the System. This diagram is based on Odum System Diagrams.

Vita

Daniela Yaffar de La Fuente was born in La Paz, Bolivia where she received her Bachelor of Biology degree with a major in Ecology from the University of Saint Andrew (UMSA). She started her postgraduate studies at the Department of Natural Science, University of Puerto Rico (UPR), but transferred to the Department of Ecology and Evolutionary Biology at the University of Tennessee Knoxville (UTK) after Hurricanes Irma and María hit Puerto Rico. As a biologist with a background in tropical ecology, she is most interested in understanding the contribution of tree roots on carbon and nutrient cycling, and the response and role of plant roots facing global disturbances. With experience in forest carbon monitoring in the Bolivian Amazon, and in forest management policy, she aims to create research programs and participate in policy making that can benefit Bolivian communities applying forest sustainable management, and preserving natural ecosystems and resources of her country.