



## FINE ROOT DENSITY ACROSS SOIL PROFILES IN TROPICAL MOUNTAIN CLOUD FOREST AND ADJACENT MANAGED FIELDS IN VERACRUZ (MEXICO): INFLUENCE OF SOIL PROPERTIES †

### [DENSIDAD DE LAS RAÍCES FINAS EN EL PERFIL DE SUELO DEL BOSQUE MESÓFILO DE MONTAÑA Y DE TERRENOS MANEJADOS CIRCUNDANTES, EN VERACRUZ (MÉXICO): EFECTO DE LAS PROPIEDADES DEL SUELO]

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#### SUMMARY

**Background.** Fine roots play a crucial in ecosystem functioning as they supply nutrients and water to plants and represent one of the main pathways for carbon transfer to the soil. **Objective.** This study assessed the vertical distribution of fine root density and analyzed its relationship with soil properties from a land use change perspective in three vegetation cover types (tropical mountain cloud forest, grassland, coffee crop). **Methodology.** Soil samples of known volume per horizon were extracted from the soil profile. In laboratory, fine roots ( $\leq 2$  mm in diameter) were tweezers picked, rinsed with water and subsequently oven-dried (70 °C), and weighed. While, the soil was air-dried for chemical analysis. **Results.** In all soil profiles, average fine root density was greater in grassland ( $16.06 \text{ kg m}^{-3} \pm 3.5$ , average  $\pm$  SE) than in tropical mountain cloud forest ( $8.45 \text{ kg m}^{-3} \pm 1.3$ ), and coffee crop ( $2.73 \text{ kg m}^{-3} \pm 0.44$ ). On average, grassland, tropical mountain cloud forest, and coffee crop showed 78.4%, 73.2%, and 65%, respectively, of fine root density on the first soil horizon. **Implications.** Soil properties were strong predictors of fine root density variation. For example, there was a significant positive correlation between fine root density and soil organic carbon, total soil nitrogen, and effective cation exchange capacity (nutrient availability) in all vegetation covers. A significant negative correlation was observed between fine root density and pH in tropical mountain cloud forest but was not correlated in grassland and coffee crop. In all vegetation covers, there was a significant negative correlation between fine root density and soil bulk density and a strong positive correlation between it and soil water content at field capacity. **Conclusion.** This research contributes data that will improve our understanding of fine root density-soil interactions in a changing regional environment.

**Keywords:** fine root density; soil properties; soil profile; mountain forest; grassland; coffee crop.

#### RESUMEN

**Antecedentes.** Las raíces finas desempeñan un papel fundamental en el funcionamiento del ecosistema, ya que suministran nutrientes y agua a las plantas y representan una de las principales vías de transferencia de carbono al suelo. **Objetivo.** Este estudio evaluó la distribución vertical de la densidad de raíces finas y analizó su relación con las propiedades del suelo desde una perspectiva de cambio de uso del suelo en tres tipos de cobertura vegetal (bosque mesófilo de montaña, pastizal, cultivo de café). **Metodología.** En el perfil de suelo, se obtuvieron muestras de suelo de volumen conocido por horizonte. En el laboratorio, las raíces finas ( $\leq 2$  mm de diámetro) fueron recolectadas con pinzas, se lavaron con agua, se secaron a 70 °C, y luego se pesaron. Al mismo tiempo, el suelo se secó al aire para su análisis químico. **Resultados.** En todos los perfiles, la densidad promedio de raíces finas fue mayor en los pastizales ( $16.06 \text{ kg m}^{-3} \pm 3.5$ , promedio  $\pm$  SE) que en el bosque mesófilo de montaña ( $8.45 \text{ kg m}^{-3} \pm 1.3$ ) y que en el cultivo de café ( $2.73 \text{ kg m}^{-3} \pm 0.44$ ). En promedio, el pastizal, el bosque mesófilo de montaña y el cultivo de café mostraron 78.4%, 73.2% y 65%, respectivamente, de densidad de raíces finas en el primer horizonte del suelo. **Implicaciones.** Las propiedades del suelo fueron fuertes predictores de la variación de la densidad de raíces finas. Por ejemplo, hubo una correlación positiva significativa entre la densidad de las raíces finas y el carbono orgánico del suelo, el nitrógeno total del suelo y la capacidad de intercambio catiónica (disponibilidad de nutrientes) en todas las coberturas vegetales. Se observó una correlación negativa significativa entre la densidad de las raíces finas y el pH del suelo en el bosque mesófilo de montaña, pero no se correlacionó en el pastizal y en el cultivo de café. En todas las coberturas de vegetación, hubo una correlación negativa significativa entre la densidad de raíces finas y la densidad aparente del suelo, y una fuerte correlación positiva entre la densidad de raíces finas y el contenido de agua del suelo a capacidad

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de campo. **Conclusión.** Esta investigación aporta datos que mejoran nuestra comprensión de las interacciones entre la densidad de las raíces finas y el suelo en un entorno regional cambiante.

**Palabras clave:** densidad de raíces finas; propiedades del suelo; perfil de suelo; bosque mesófilo; pastizal; cultivo de café.

## INTRODUCTION

Fine roots ( $\leq 2$  mm in diameter) are the most dynamic and physiologically active component of below-ground biomass. They play a crucial role in the flux of energy and matter in the terrestrial biosphere, carrying out the essential functions of soil resource acquisition such as inorganic nutrient and water uptake (Norby and Jackson, 2000; Schenk and Jackson, 2002). Therefore, fine root production represents the dominant pathway by which carbon enters the soil (Kögel-Knabner, 2017). The acquisition of soil nutrients by plants and nutrient losses from the ecosystem by leaching strongly depend on the exploration of the soil by roots (Stark and Jordan, 1978). Vertical fine root distribution plays an integral role in plant survival and productivity (Ogle and Reynolds, 2004), and is related to soil characteristics including nutrient availability, pH, and bulk density (e.g., Leuschner and Hertel, 2003; Godbold *et al.*, 2003; Carvalheiro and Nepstad, 1996). To a large extent, forest ecosystems are characterized by a densely rooted surface layer due to the fact that available nutrients decrease with depth (Claus and George, 2005; Yang *et al.*, 2004). At that point, fine root density could provide valuable data on the strategies by which plants acquire and use soil resources (Jackson *et al.*, 1997). The variation in fine root mass across a landscape results from such influences as species composition, soil properties, topography, and microclimate (Day and Bassuk, 1994; Tateno *et al.*, 2004; Mattia *et al.*, 2005).

There is considerable need to quantify the fine root density held within soil profile layers and their relationship to soil properties, in order to understand soil functioning as well as to model key ecosystem processes (Jackson *et al.*, 1997; Mokany *et al.*, 2006). Root growth is a dynamic process that is highly sensitive to environmental changes, such as global warming and land use change (Gill and Jackson, 2000; Majdi and Öhrvik, 2004; Schenk and Jackson, 2005). The structure and function of forests are disrupted by increased interference from anthropogenic activities via land use pattern changes (Freschet *et al.*, 2017; Du *et al.*, 2019). Such anthropogenic effects, particularly when the new land use modifies plant community composition and soil environment, may lead to changes in vertical distribution pattern of fine roots among plant communities (Freschet *et al.*, 2017). There is increasing evidence that quantitative characterization of fine root mass and recognition of its heterogeneity holds the key to a deeper understanding of the biogeochemical processes that take place

(Jackson *et al.*, 1997; Leuschner and Hertel, 2003). For example, nutrient availability, soil acidity, water availability, and other environmental conditions are key factors that influence fine root biomass (Eissenstat *et al.*, 2000; Lauenroth and Gill, 2003; Leuschner and Hertel, 2003).

Estimating fine roots and their relationship with soil properties significantly advance our appreciation of belowground contributions to terrestrial biosphere processes (Jackson *et al.*, 1997; Chang *et al.*, 2012; McCormack *et al.*, 2015). However, the nature and significance of relationships between fine root density and the soil properties that operate at the soil profile scale are still poorly understood. Here, we analyze fine root density in conjunction with soil properties in an effort to develop a more robust quantitative understanding of belowground environments in tropical mountain cloud forest and surrounding managed fields. The specific research questions were as follows: (i) How much fine root mass enters the soil? (ii) What differences are there in the fine root densities of three contrasting vegetation cover types? (iii) Is there an association between fine root density and soil properties? It was hypothesized that land use change would produce different patterns in the vertical distribution of fine roots, that fine root density would differ among vegetation cover types, and that the density and vertical distribution of fine roots would be related to soil properties.

## MATERIALS AND METHODS

### Study site

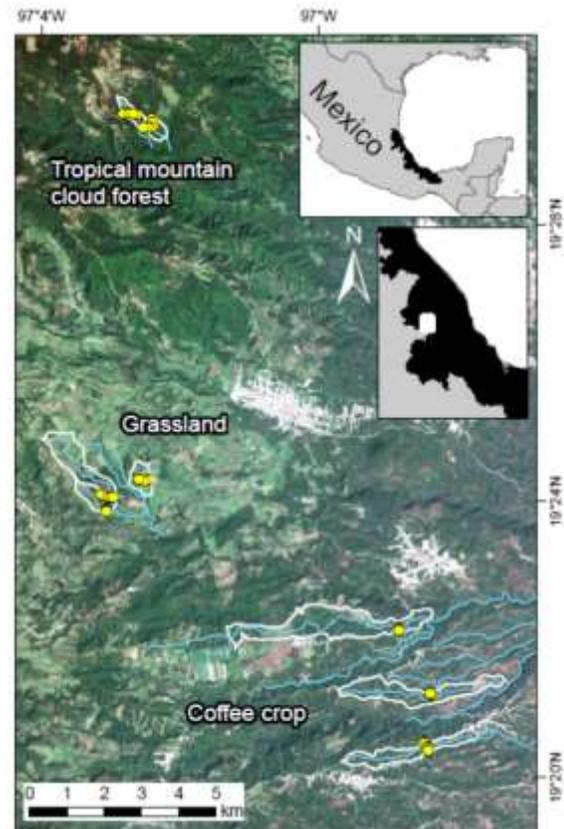
The study region is located between the latitudes 19°20'29''N and 19°29'36''N and longitudes 96°58'22''W and 97°02'41''W in the middle and slightly lower portion of Cofre de Perote Volcano's eastern slope (Figure 1). This study was conducted on typical small watershed slopes (Figure 1). Each watershed includes a small perennial stream fed by base-flow. The study covered the following three land use types: native vegetation, grassland, and coffee crop. The altitude there varies from 1089 to 2169 m above sea level. According to climate data (Garcia, 1988; Holwerda *et al.*, 2010; Muñoz-Villers and McDonnell, 2013), total average annual precipitation ranges from 1700 to 3200 mm and mean monthly temperature from 15 °C to 23 °C. Soils are derived from volcanic materials and there is altitudinal variation in soil type, changing from Histic Hydric Andosol (IUSS Working Group WRB, 2015), with high organic matter content, to Ferric Acrisol (IUSS

Working Group WRB, 2015), with low organic matter content. Two adjacent small watersheds (with size of 25.0 ha and 25.6 ha) covered with native vegetation were used for this study (Figure 1). The vegetation type in natural areas is representative of tropical mountain cloud forest, and the main tree species found in this forest are *Hedyosmum mexicanum* Cordem, *Parathesis melanosticta* (Schltdl.) Hemsl., *Clethra mexicana* DC., *Alchornea latifolia* Sw., *Quercus xalapensis* Humb. & Bonpl., *Quercus corrugata* Hook, and *Quercus laurina* Humb. & Bonpl. (García-Franco *et al.*, 2008). Three adjacent small watersheds (with size of 32 ha, 118 ha and 41 ha) covered with grassland were selected for the study (Figure 1). Data from Hoffmann (1993) indicate that livestock have been raised in this region since 1940, converting large areas of native vegetation into grassland for cattle-grazing. According to Hoffmann (1993), this grassland is a grazed mixed-grass prairie dominated by *Pennisetum clandestinum* Hochst. ex Chiov., *Paspalum notatum* Flüggé, and *Cynodon dactylon* (L.) Pers.; it maintains cattle throughout the year and has a stocking rate of 1.0 animal unit per hectare. Inorganic fertilizer is not applied to grassland. Three adjacent small watersheds (with size of 380 ha, 177 ha, and 80 ha) covered with coffee crop were selected for this study (Figure 1). Here, coffee (*Coffea arabica* L.) crop covers most of the landscape; this is the part of the study site where agricultural activity has taken place for the longest time. Based on the data from Hoffmann (1993), large areas of native vegetation were converted to coffee crop beginning in the late 19th century. For coffee crop, the following is applied three times a year (in January, June, and September): 144 kg N ha<sup>-1</sup>, 42 kg P ha<sup>-1</sup>, and 40 kg K ha<sup>-1</sup> in the form of urea, triple superphosphate, and potassium chloride, respectively.

### Soil and fine root sampling and processing

For each land use type (Figure 1), representative slopes were selected in small watersheds. In tropical mountain cloud forest, slopes have a gradient of 40°, 25°, and 20° and a length of 194 m, 254 m, and 105 m, respectively. In grassland, slope have a gradient of 20°, 28°, and 13° and a length of 70 m, 140 m, and 250 m, respectively. In coffee crop, slopes have a gradient of 20°, 8°, and 12° and a length of 110 m, 130 m, and 140 m, respectively. Slopes were divided into three segments according to their topographic elements: shoulder, backslope, and footslope. A soil profile was dug out at the center of each topographic element, and bulk soil samples were obtained from recognized pedogenetic horizons following standard protocol (Schoeneberger *et al.*, 2012). A soil volume of 150 mm x 150 mm x horizon depth (except in the litter layer of forest sites, which was 200 mm x 200 mm x O horizon depth) was dug up and placed in a plastic bag. In laboratory, fine roots ( $\leq 2$  mm in diameter) from soil volume were handpicked with tweezers and

subsequently rinsed with water to remove soil particles. Promptly, fine roots were placed into paper bags, oven-dried (70 °C for 48 h), and weighed. Meanwhile, the remaining soil was air-dried and ground to pass through a 2 mm sieve for chemical analysis. Fine root density (kg m<sup>-3</sup>) was obtained by dividing fine root dry mass by the sampled volume.



**Figure 1.** Location map of study sites and soil profiles.

Soil pH was measured in water using a 1:2.5 soil/water ratio with a glass electrode. Exchangeable Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, and Na<sup>+</sup> were estimated using 1 M ammonium acetate buffered at pH 7. Soil organic carbon was determined by the Walkley and Black dichromate oxidation method, while total N was measured following the Kjeldahl method. Effective cation exchangeable capacity (ECEC) was calculated by summing exchange bases plus exchangeable acidity (Al<sup>3+</sup> and H<sup>+</sup> from 1 M KCl by titration). Soil bulk density was determined by dividing the mass of oven-dried soil by the volume of the soil sample (cylinder of 53.5 mm diameter and 30.0 mm height). All analyses of soil samples were determined using the methods of Dane and Topp (2002) and Sparks (1996).

For determination of soil-water content at -10 kPa (field capacity, FC) and -1500 kPa (permanent wilting

point, WP), samples preserved with their natural structure and field moisture conditions were placed in plastic rings and then wetted until saturation. After saturation and equilibrating at -10 kPa and -1500 kPa water potential using membrane plates and pressure chambers, respectively (Dane and Topp, 2002), the samples were oven-dried at 105 °C for 48 h. Gravimetric soil-water content at each pressure level was determined by dividing the mass of water by that of dry soil. Available water content (AW) was calculated as the difference between the water content at FC and that at WP, with the unit g water g<sup>-1</sup> over dry soil.

### Experimental design and data analysis

The experiment followed a completely randomized design. Treatments consisted of three land use types (tropical mountain cloud forest, grassland, and coffee crop), and typical slopes chosen for each land use type were used as replicates. To analyze fine root density per soil horizon in each vegetation cover, normality of data was checked with the Shapiro-Wilk test using SigmaPlot 14.0 (2018). As fine root density data did not show a normal distribution, they were statistically analyzed with the Kruskal-Wallis non-parametric test. Dunn's multiple-rank post hoc tests with  $p < 0.05$  were used to assess whether fine root density differed significantly among soil horizons within each vegetation cover.

The vertical pattern of fine root density from the data set was modeled by the following negative exponential depth function (Thornley and Johnson, 2000; Minasny *et al.*, 2016):

$$FR(d) = FR_0 * exp^{-kd}$$

where  $FR(d)$  represents the measured fine root mass as a function of soil depth,  $FR_0$  fine root density at the soil surface, and coefficient  $k$  a descriptor of the rate of fine root mass decrease with soil depth ( $d$ ).

To assess which soil properties were most closely associated with fine root variation at the native forest and managed field levels, we used fine root density as a dependent variable and soil properties as independent factors. Correlation graphs were produced using SigmaPlot 14.0 (2018) to show Pairwise Pearson's correlations of fine root density with soil properties. To reduce the number of variables characterizing the soil environment and their co-linearity, a PCA was performed with the soil variables measured in this study: soil nitrogen (N) and organic carbon (C), pH, sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), exchangeable aluminum (Al), effective cation exchangeable capacity (ECEC), soil water availability (WA), soil water content at field capacity

(FC) and at permanent wilting point (WP), and soil bulk density (BD).

## RESULTS

### Depth variation of fine root density

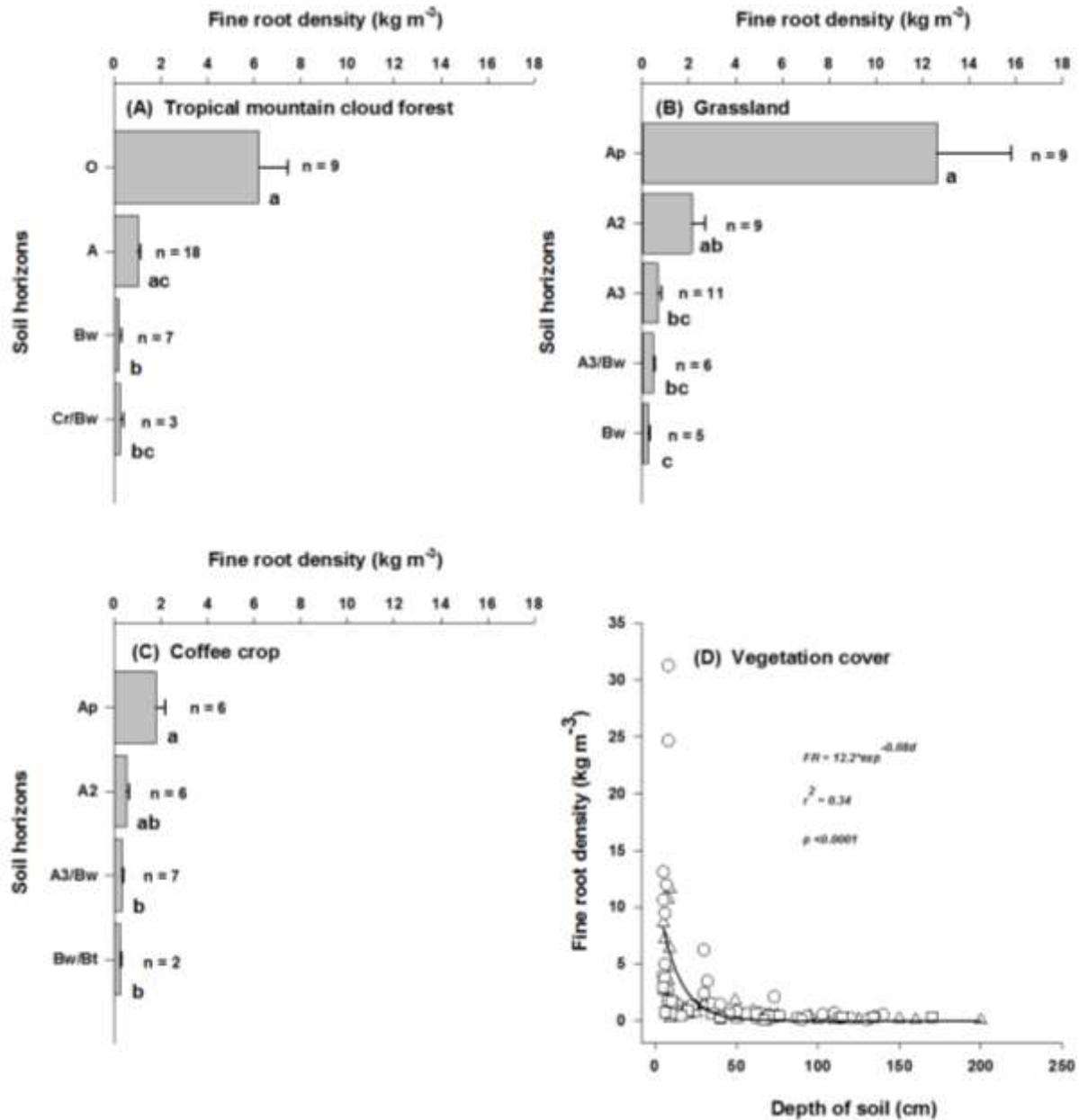
Vertical fine root density distribution under different vegetation covers is shown in Figure 2. From these results, fine root density is demonstrated to be heterogeneous among vegetation covers, revealing their varying capacity to explore the soil. Figure 2 shows that most of the fine roots were found in the first horizon (e.g., O and Ap horizons) and decreased markedly with depth. Notably, grassland had higher fine root density (especially in the upper soil horizon), followed by tropical mountain cloud forest; coffee crop registered lower values. Specifically, in grassland, mean fine root density values ranged from  $12.6 \pm 3.1$  (mean  $\pm$  SE) kg m<sup>-3</sup> near the soil surface (Ap horizon) to  $0.26 \pm 0.07$  kg m<sup>-3</sup> at deeper in the profile (Bw horizon). In this case (Figure 2B), fine root density across soil profiles was significantly higher for Ap horizon than A3, A3/Bw and Bw horizons, while A2 horizon showed no significant difference ( $P < 0.05$ ). For tropical mountain cloud forest, mean fine root density values ranged from  $6.2 \pm 1.2$  kg m<sup>-3</sup> in the upper soil layer (O horizon) to  $0.22 \pm 0.18$  kg m<sup>-3</sup> for Bw horizon. Across all soil profiles (Figure 2A), O horizon fine root density values were significantly higher than for Bw and Cr/Bw horizons, but A horizon registered no significant difference ( $P < 0.05$ ). In the case of coffee crop, mean fine root density values ranged from  $1.78 \pm 0.40$  kg m<sup>-3</sup> in the upper soil layer (Ap horizon) to  $0.26 \pm 0.03$  kg m<sup>-3</sup> in the deeper layer studied (Bw/Bt horizon). At this site (Figure 2C), fine root density was significantly greater in Ap horizon than for A3/Bw and Bw/Bt horizons, but no significant differences appeared for the A2 horizon. Additionally, no significant differences in fine root density values were observed among A2, A3/Bw, and Bw/Bt horizons. In terms of exponential depth function (Figure 2D), the results showed that the model described 34% of dataset variance.

### Dimensionality of soil environmental variation

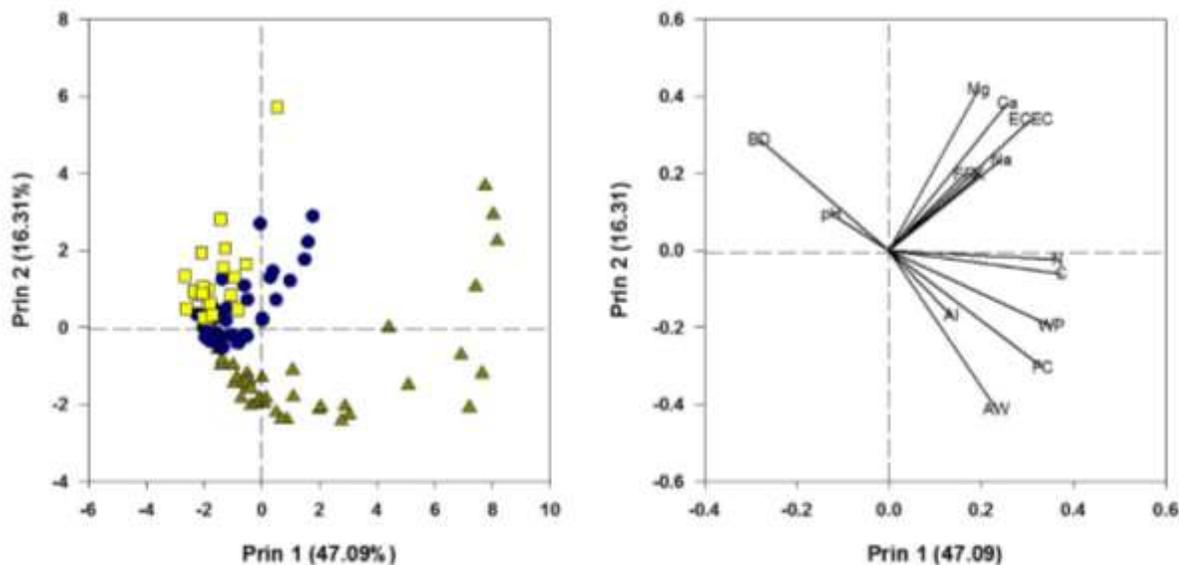
Based on PCA results, the fourteen soil parameters measured at the vegetation cover level could be summarized by two independent dimensions, which together accounted for 63.4% of total variance (Figure 3). The first principal component, with an eigenvalue of 6.59, explained approximately 47% of variance and was heavily loaded on soil water retention (FC, WP) and soil organic matter (C, N). The second principal component, with an eigenvalue of 2.28, described 16.3% of the variance and was related to soil nutrient availability (Ca, Mg, ECEC) and available water in soil (AW). A third principal component, with an

eigenvalue of 1.80, accounted for an additional 12.9% of variance and represented covariation between pH and Al. Ordination showed a clear distinction between forest and managed fields (coffee crop and grassland). For example, forest had higher soil organic matter content (C, N) and water holding capacity (FC, WP) than managed fields. Ordination also suggests that some grassland and forest sites featured high soil nutrient concentrations (e.g. ECEC, Ca, Mg). Unlike data for forest, coffee crop appears to be associated

with low-fertility soils; this means that there are less accessible nutrients in the soil solution, probably due to soil management. Given this evidence, one might expect that a larger input of litter residues (which are the main precursor of soil organic matter) on coffee plots could provide a substantial input of energy and nutrients for the coffee system, with positive effects on soil quality that, in turn, could lead to improved productivity and sustainability.



**Figure 2.** (A, B, C) Changes in fine root density across soil horizons in three vegetation covers. The error bars represent standard mean error. The lowercase letters indicate significant differences ( $p < 0.05$ ) among soil horizons per vegetation cover. (D) Model that describes variation of the data set for fine root density with soil profile depth of vegetation covers (triangle, tropical mountain cloud forest; circle, grassland; square, coffee crop).



**Figure 3.** Multivariate analysis (PCA) of soil parameters for each vegetation cover (tropical mountain cloud forest: dark yellow triangle; grassland: dark blue circle; coffee crop: yellow square).

### Bivariate relationships between fine root density and soil parameters

Correlation analysis indicated that fine root density across the soil profile responds markedly to soil conditions (Figs. 4, 5, 6). For example, this study found a significant positive correlation between fine root density ( $\text{kg m}^{-3}$ ) and soil organic carbon (%) (Figs. 4a, 4b, 4c). The latter effect was more evident in tropical mountain cloud forest, where the correlation coefficient was stronger ( $r = 0.79$ ) than in coffee crop ( $r = 0.62$ ) and grassland ( $r = 0.55$ ). This suggests that fine root density mediates soil carbon inputs and can have an important impact on soil carbon sequestration. The analysis also showed that fine root density was significantly positively correlated with total soil nitrogen (Figs. 4d, 4e, 4f). In the present context, fine root density showed a very strong correlation with soil nitrogen in tropical mountain cloud forest ( $r = 0.84$ ) and was also strong in coffee crop ( $r = 0.78$ ) and grassland ( $r = 0.60$ ). These positive correlations, across all vegetation covers, suggest that fine root density is strongly dependent on soil nitrogen concentration.

Fine root density correlated significantly and negatively with soil pH in tropical mountain cloud forest ( $r = 0.49$ ) but showed no significant correlation in grassland and coffee crop soils (Figs. 5a, 5b, 5c). In the case of tropical mountain cloud forest, the negative correlation implies higher fine root density at greater soil acidity. In this same line, fine root density (Figs. 5d, 5e, 5f) showed a strong significant positive

correlation with effective cation exchange capacity in soils under tropical mountain cloud forest ( $r = 0.65$ ) and coffee crop ( $r = 0.66$ ), but it was moderate in grassland soils ( $r = 0.54$ ). These positive correlations indicated that fine root density tended to be higher in more favorable environments (e.g., O horizon) in terms of nutrient availability.

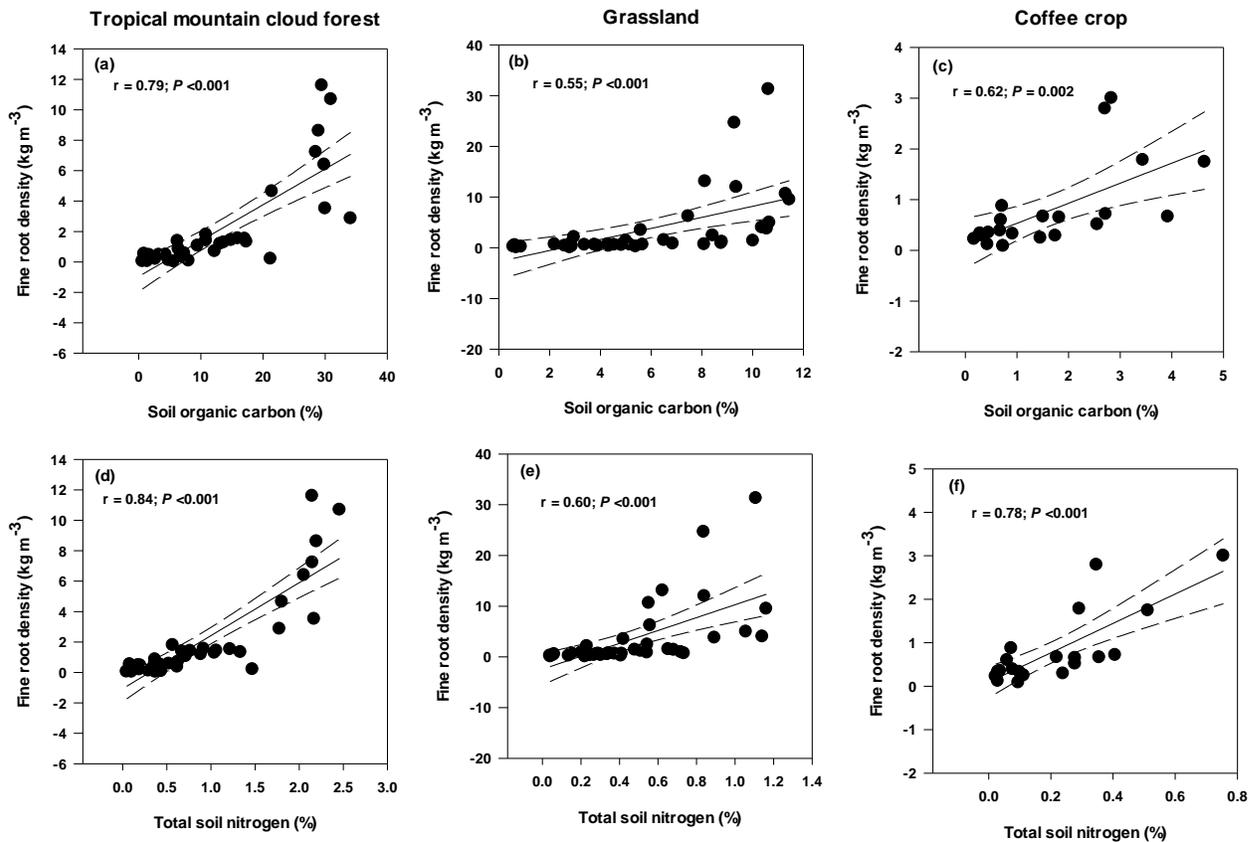
In the present study, correlation analysis revealed that fine root density was significantly negatively related to soil bulk density in all of the vegetation covers studied (Figs. 6a, 6b, 6c). Specifically, fine root density showed a moderate correlation with soil bulk density in tropical mountain cloud forest ( $r = 0.54$ ) and coffee crop ( $r = 0.55$ ) but was weak in grassland ( $r = 0.36$ ). As to soil water content (Figs. 6d, 6e, 6f), a very strong significant positive correlation was observed between fine root density and field capacity in tropical mountain cloud forest soils ( $r = 0.85$ ), but no significant correlation in grassland and coffee crop soils. Thus, these results demonstrate that an increase in soil water content promotes fine root production in tropical mountain cloud forest.

### DISCUSSION

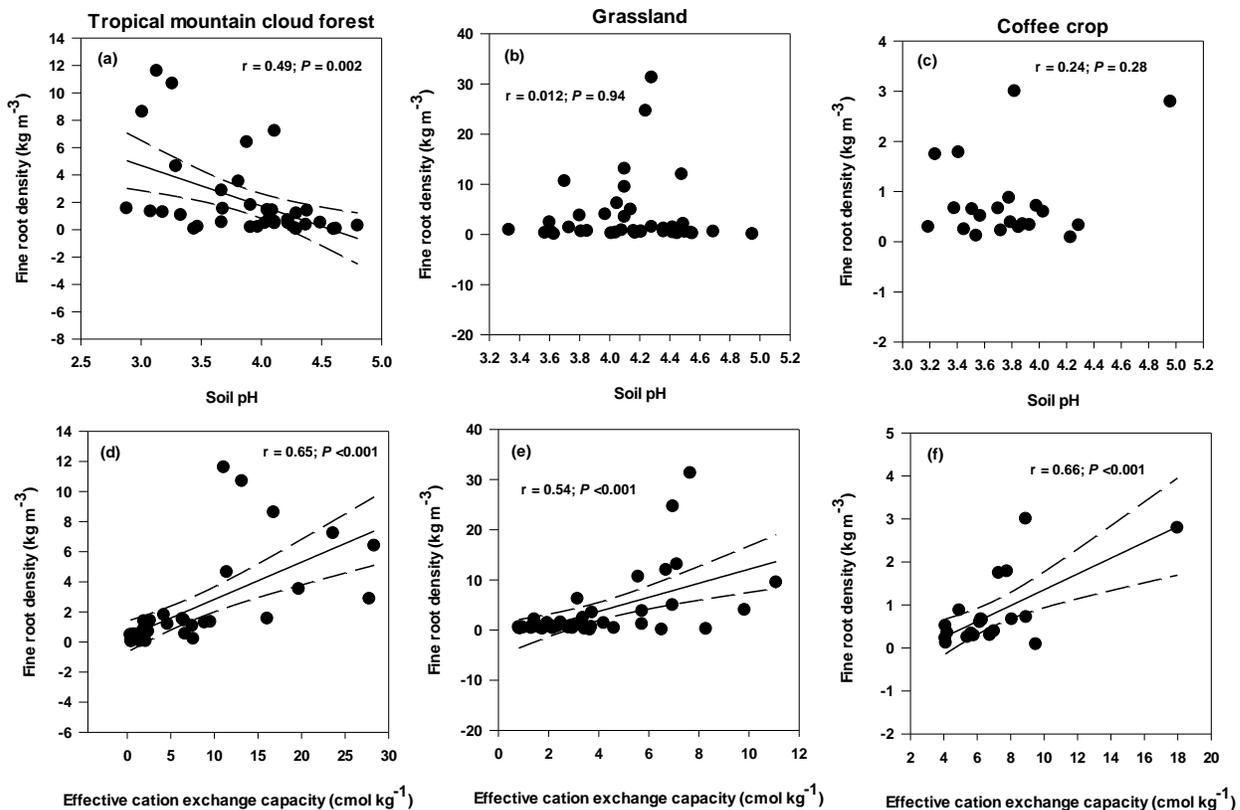
Over the course of several decades, a number of studies have focused on fine root mass through various terrestrial biomes. With regard to tropical cloud mountain forest, our results were consistent with data reported for other regions. For example, Rosado *et al.* (2011) found a fine root mass of  $2.9 \text{ kg m}^{-3}$  in the top

30 cm of a tropical mountain rain forest in Brazil; Vance and Nadkarni (1992) estimated it at  $2.7 \text{ kg m}^{-3}$  in the organic layer (0-15 cm) of a moist tropical montane forest in Monteverde Cloud Forest Reserve, Costa Rica; and Hertel *et al.* (2003) reported an average value of  $6.6 \text{ kg m}^{-3}$  in the organic layer (19 cm depth) of old-growth tropical montane forest in the Cordillera Talamanca, Costa Rica. In this same way, Leuschner *et al.* (2006) calculated an average fine root mass of  $4.9 \text{ kg m}^{-3}$  for the organic layer, and for mineral soil at 0-10 cm depth an average value of  $2.3 \text{ kg m}^{-3}$ , in a tropical montane forest in Central Sulawesi, Indonesia. With regard to the grassland system, the results presented here are in line with other global studies. For example, in mountain grassland (northern part of the Czech Republic), fine root mass varied between  $20.0$  and  $24.4 \text{ kg m}^{-3}$  in the 0-6 cm layer (Pecháčková *et al.*, 1999). Also, in some cases, Jackson *et al.* (1996) reported a fine root mass of over  $17.5 \text{ kg m}^{-3}$  in upper soil layers of temperate grasslands. Schenk and Jackson (2002) mention that most species in natural grasslands have predominantly shallow rooting. Another study (Zhou *et al.*, 2012)

carried out in a tallgrass prairie ecosystem in Oklahoma, USA, recorded fine root mass values of  $2.8$  and  $1.0 \text{ kg m}^{-3}$  at depths of 0-15 and 15-30 cm, respectively. A more recent study (Zhao *et al.*, 2018) found that the average fine root mass in grassland soils ranged from approximately  $5.1 \text{ kg m}^{-3}$  in the upper profile (0-15 cm) to  $0.3 \text{ kg m}^{-3}$  in the deeper section (80-120 cm). With regard to fine root mass in coffee crop, the results of the present study are in agreement with Defrenet *et al.* (2016), who recorded  $0.65$ ,  $0.52$ , and  $0.41 \text{ kg m}^{-3}$  at depths of 0-10, 10-20, and 20-30 cm, respectively. Furthermore, Jackson *et al.*'s review (1996) reported that cropland had the lowest fine root densities, with values never over  $2 \text{ kg m}^{-3}$ . According to Brunner and Godbold (2007), knowledge of fine root distribution with depth is essential for understanding ecosystem functioning. For example, the exponential model describes fine root functioning under many factors including different climate conditions, soil water and nutrient supplies, anchorage forces, and plant species (Tobin *et al.*, 2007; Zuo *et al.*, 2013).



**Figure 4.** Variation in the relationships between fine root density and soil parameters (soil organic carbon, soil nitrogen) for the three vegetation covers considered in this study. Solid lines represent linear correlations with 95% confidence intervals (dotted lines).



**Figure 5.** Variation in the relationships between fine root density and soil parameters (soil pH, ECEC) for the three vegetation covers considered in this study. Solid lines represent linear correlations with 95% confidence intervals (dotted lines).

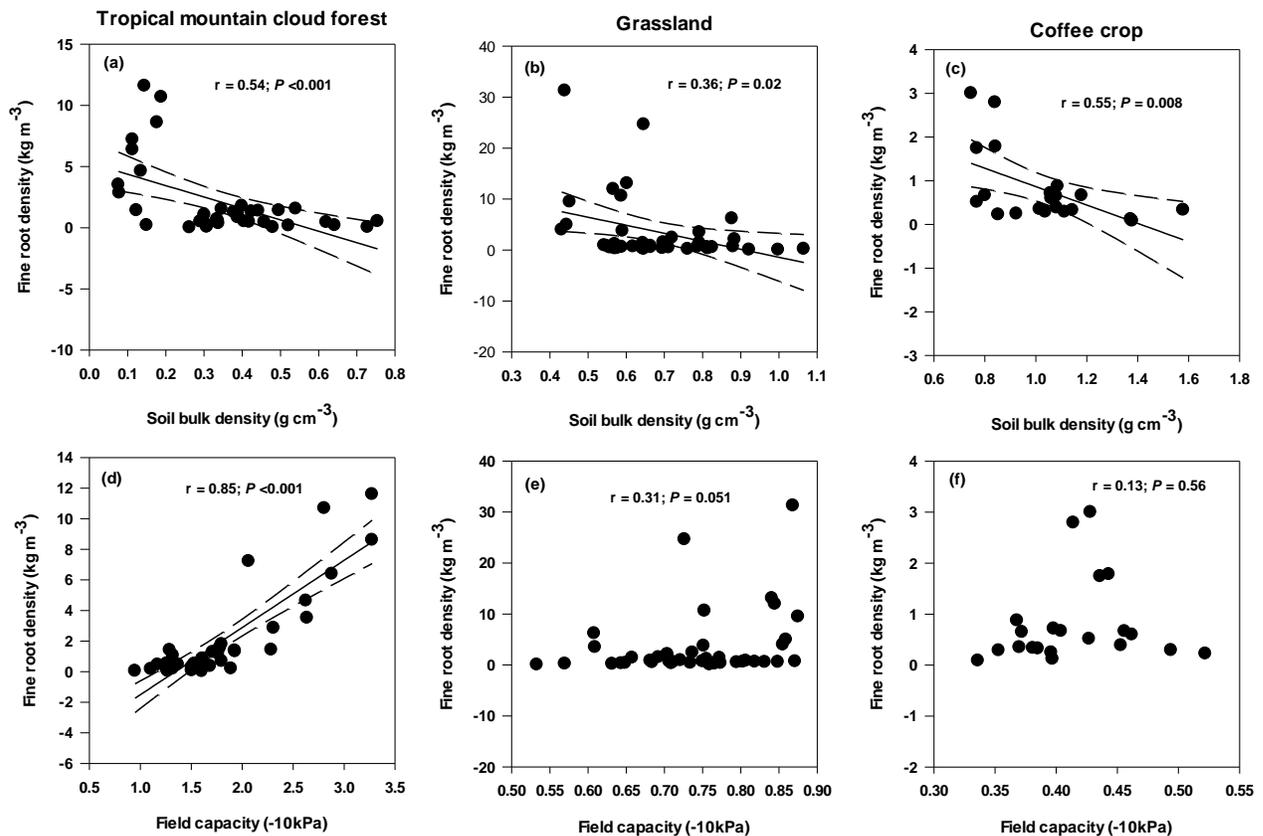
Fine roots perform a number of physiological functions (such as water and nutrient acquisition and the synthesis of certain growth hormones) that are essential to plant survival and productivity (Du *et al.*, 2019; Guo *et al.*, 2019). Our research found that fine root density in the three vegetation cover types decreased with increasing soil depth. These results suggest that soil layer and vegetation cover type have significant effects on fine root density. We observed that the vertical distribution pattern of fine root mass was affected by land use change, supporting the first hypothesis. In our study, the variation in vertical distribution pattern of fine root mass resulting from land use change could be due to replaced species and to changes in soil environment, as different species often have different optimal water and nutrient requirements for plant growth. The spatial configuration of the root system in soil is vital for plants because it determines soil exploration and therefore nutrient and water acquisition (Du *et al.*, 2019). In this sense, the differences in fine root mass observed across vegetation covers could reflect roots' ability to explore and forage resources through land use change, in relation to the nutrient variations in soil. For example, in our study area for topsoil, tropical mountain cloud forest with a high nutrient concentration had a lower fine root mass than

grassland, which had a lower nutrient concentration. The explanation might be that grass has a strong preference for investing fine roots in topsoil for nutrient foraging in a greater soil volume. Whereas the fine root mass in coffee crop topsoil could relate to the species' exploration strategies in terms of nutrient availability due to fertilizer applications and to soil environmental changes represented by a denser soil in deeper layers, which is known to increase the resistance to root penetration and restricts its growth. The alterations in the vertical distribution pattern of fine root mass due to land use change may result from modification in species composition and in the response of soil environmental conditions (Du *et al.*, 2019; Isaac and Borden, 2019). In line with our second hypothesis, in all soil profiles, average fine root density was greatest in grassland, followed by tropical mountain cloud forest and, lastly, coffee crop. Our results showed that fine root density in the first soil horizon was higher than in other soil layers. More than 65% of fine root mass was confined to the first soil horizon. These findings are consistent with previous studies (e.g., Gautam and Mandal, 2012; Zhou *et al.*, 2016) indicating that in most ecosystems, roots tend to be the most abundant near the soil surface, decreasing exponentially with increasing soil depth. The highest fine root density in upper soil layers may well be the

more efficient strategy for vegetation types to acquire nutrients (Pinheiro *et al.*, 2018). For example, high fine root density in topsoil represents a greater root capacity to explore a given soil volume, which in turn increases the contact between plants and soil, thus improving nutrient uptake rates (Gautam and Mandal, 2012; Lazarovitch *et al.*, 2018; Isaac and Borden, 2019). In forest ecosystems, high fine root density near the soil surface is important for nutrient conservation (Gautam and Mandal, 2012). Studies (Wang *et al.*, 2016; Shu *et al.*, 2018) have indicated that fine root mass varies with vegetation types and that there is significant variation among soil horizons due to different water and nutrient contents in soil layers (from Du *et al.*, 2019). In our study, the vertical distribution of fine root mass decreased more sharply in forest than grassland, which suggests that pasture has a fine root system capable of reaching deeper soil horizons, increasing the soil exploration capacity for nutrient and water acquisition. The main reason for deeper root distribution in grassland is probably increased resource demands (Arndal *et al.*, 2018). Forest ecosystems can benefit from an O horizon where nutrients are more abundant and available. Plant roots acquire nutrients that are heterogeneously distributed in a diverse and complex

soil matrix and that often selectively proliferate in soil patches (that contain high concentrations of nutrients) to optimize the efficiency of nutrient capture at so-called nutrient hotspots (Chen *et al.*, 2018; Isaac and Borden, 2019). Fine root production in terrestrial ecosystems represents about 33% of global annual net primary productivity (Jackson *et al.*, 1997; McCormack *et al.*, 2015; Arndal *et al.*, 2018). In grassland, most of the net primary production occurs belowground (Arndal *et al.*, 2018).

In this study, we found that fine root mass in soil profiles followed (in many cases) the vertical pattern in nutrient availability, as well as differences in physical properties such as moisture and bulk density. Studies have shown that fine root vertical distribution is associated with the distribution pattern of soil properties (such as nutrients, bulk density, etc.) in the soil profile (Jobbágy and Jackson, 2004; Pei *et al.*, 2018; Isaac and Borden, 2019). Really, important soil properties changes with soil depth, which can have an effect on fine roots; for example, nutrients available to plants decrease and the apparent density increases with soil depth. Since fine roots mass generally increases in response to nutrient sufficiency in topsoil, the same



**Figure 6.** Variation in the relationships between fine root density and soil parameters (soil bulk density, field capacity) for the three vegetation covers considered in this study. Solid lines represent linear correlations with 95% confidence intervals (dotted lines).

can be expected with vertical gradients in nutrient availability from topsoil to subsoil. For soil nutrients, bulk density and, to some extent, soil moisture, our third hypothesis (which stated that the vertical distribution of fine root mass would relate to soil properties) was consistently supported. For example, we observed that the spatial pattern of fine root mass across soil profiles followed at vertical pattern in soil carbon and total nitrogen concentration in all vegetation cover types. This is confirmed by highly significant ( $P \leq 0.002$ ) positive linear relationships between fine root mass and soil organic carbon or total soil nitrogen. One of the main pathways by which organic carbon inputs to subsoil occur is through fine root mass (Rasse *et al.*, 2005; Kong and Six, 2010; Tückmantel *et al.*, 2017). An important fraction of carbon assimilated by plants through photosynthesis is root transferred, to increase excavation capacity in nutrient acquisition (McCormack *et al.*, 2015; Pausch and Kuzyakov, 2018; Cai *et al.*, 2019). Furthermore, roots introduce carbon into the soil in the form of root tissue, thus controlling the vertical distribution of soil organic matter (Tückmantel *et al.*, 2017; Cai *et al.*, 2019). Belowground carbon allocation through roots may also stimulate the microbial community and enhance soil organic mineralization rates (Arndal *et al.*, 2018). Additionally, studies show that root-derived carbon is more easily retained in soil than carbon inputs from litter fall, suggesting that fine root mass is one of the main components of the terrestrial carbon budget (Schmidt *et al.*, 2011; Pierret *et al.*, 2016). Therefore, rooting patterns in the soil profile can have a strong control over C sequestration through fine root turnover (Tückmantel *et al.*, 2017; Borden *et al.*, 2019). In fact, plants allocate significant amounts of carbon belowground in order to explore deeper soil profile horizons for nutrient and water acquisition (Ven *et al.*, 2019). As illustrated by Loades *et al.* (2013), root-soil interactions are complex because of intimate functional coupling. This refers to the fact that roots have an impact on soil properties (e.g., aggregate stability), but soil also influences root properties (e.g., root architecture) (Loades *et al.*, 2013). There is ample evidence suggesting that greater fine root mass increases the plant's capacity to absorb available nitrogen from soil (Nadelhoffer, 2000; Borden *et al.*, 2019). One explanation is that fine root production is generally associated with a higher potential to proliferate in nutrient patches (Hodge, 2004), for example, in this study, the O horizon. Similarly, in our study the concentrations of soil nutrients (in particular  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$  from ECEC) decreased with the increase in soil depth, following the general vertical pattern of fine root mass in soil profiles. This is confirmed by a highly significant ( $P < 0.001$ ) positive linear relationship between fine root mass and effective cation exchange capacity. Our results were in line with the findings that higher exchangeable base (i.e.,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ) concentrations are associated

with greater fine root density (Jobbágy and Jackson, 2004; Mora and Beer, 2013; Borden *et al.*, 2019). This suggests that plants invert in fine root production in resource-rich spots in order to ensure plant growth nutrient supply under strongly nutrient-limited conditions (Reich, 2014; Kramer-Walter *et al.*, 2016; Weemstra *et al.*, 2016), for example soils with  $\text{pH} < 5.0$ . Recent studies (Freschet *et al.*, 2017) also found a strong positive correlation between fine root density and cation exchange capacity. In the present research,  $\text{pH}$  shows no remarkable differences with increase in soil depth. However, the present study revealed that  $\text{pH}$  and fine root mass were negatively correlated ( $P = 0.002$ ) in tropical mountain cloud forest, whereas they showed no significant correlation ( $P \geq 0.28$ ) in grassland and coffee crop covers. In this case, the negative effect of  $\text{pH}$  on fine root mass, clearly evident only in tropical mountain cloud forest, could be mainly attributed to proton ( $\text{H}^+$ ) production generated by plant N (ammonium) nutrition, as indicated by Wang and Tang (2018). In general, ammonium leads to acidification because of excess uptake of cations over anions by plant roots (Wang and Tang, 2018). A negative trend was also observed between soil  $\text{pH}$  and fine root density (Freschet *et al.*, 2017). In fact, soil acidity is the result of a loss of exchangeable bases cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ) and exchangeable aluminum ( $\text{Al}^{3+}$ ) activation through the soil profile (Brady and Weil, 2010). In this scenario of low nutrient availability, it has been suggested that plants develop adaptation strategies, for example greater fine root mass to explore more soil volume in order to obtain nutrients (Kong *et al.*, 2014; Postma *et al.*, 2014; Kramer-Walter *et al.*, 2016). Absorptive roots are the primary organs for plant nutrient acquisition (Robinson *et al.*, 2003; Pierret *et al.*, 2007). In our study, soil bulk density progressively increased with increasing soil depth. As expected, the fine root mass distribution in the soil profile was significantly negatively associated ( $P \leq 0.02$ ) with soil bulk density. The decrease in fine root density with an increase in soil bulk density has also been reported in some studies (e.g., Alameda and Villar, 2009) but not in others (e.g., Freschet *et al.*, 2017). In terms of physical limitations, lower fine root density under denser soils may be due to the fact that fine roots cannot easily penetrate them (Bengough *et al.*, 2011; Clark *et al.*, 2003), thus limiting soil volume exploration. In contrast, higher fine root density represents higher root capacity to explore the soil matrix for the purpose of nutrient acquisition (Robinson *et al.*, 2003; Weemstra *et al.*, 2016). There is ample evidence suggesting that high mechanical impedance lead to a decrease in fine root mass with increasing soil bulk density (Gao *et al.*, 2016). As indicated by Wang *et al.* (2020), root growth and function in subsoils are frequently limited by high bulk density (i.e.,  $1.4\text{--}1.6 \text{ g cm}^{-3}$ ). Our results revealed that soil water availability generally decreased with progressive soil depth in tropical mountain cloud forest

but a heterogeneous and similar tend along the soil profile in grassland and coffee crop covers, respectively. We found a highly significant ( $P < 0.001$ ) positive linear relationship between fine root mass and soil water availability in tropical mountain cloud forest, whereas it was not significant ( $P > 0.05$ ) in grassland and coffee crop covers. Our findings may be explained in part by the fact that land use changes (e.g., natural forest converted to managed lands) could alter soil water processes, in turn affecting fine root growth and distribution throughout the soil profile. For example, the conversion of natural forest to managed lands may lead to an increase in temperature which in turn accelerate evapotranspiration, affecting soil water availability (Li *et al.*, 2019). Fine root mass increases with soil water availability and is typically the primary factor in plant productivity and litter inputs in forests (Zhang *et al.*, 2020). This is in accordance with results obtained by Leuschner and Hertel (2003) in which fine root density showed a significant positive correlation with precipitation (water availability). Soil water content at field capacity (-10 kPa) has been assumed to represent the practical upper limit of soil water storage for plant use, and its corresponding air content could be considered the lower air capacity limit of the rhizosphere (Cooper, 2016).

## CONCLUSIONS

The data presented herein show the variability of fine root density across soil profiles under a perspective of land use change, capturing the complexity of the soil environment and permitting a better understanding of the fine root-soil relationship at the regional scale. Our results show evidence that the spatial configuration of the fine root system due to land use change is highly variable depending on adapted species and could be associated with strategies used by plant roots to optimize the efficiency of nutrient and water capture. This study revealed that fine root density is highest near the surface and declines more rapidly with increasing depth. The O horizon in tropical mountain cloud forest and the A horizon in grassland and coffee crop are the most fertile spots, which explains the higher densities of fine roots in those settings. The significant influence of soil properties on fine root density is an important finding derived from this study. The positive correlation of fine root density with soil organic carbon could be interpreted as evidence of significant contributions of fine root mass to soil carbon sequestration and could also explain soil carbon accumulation, particularly in grassland and tropical mountain cloud forest. Therefore, changes in vegetation cover could strongly affect root mass-derived carbon sequestration due to modifications in plant rooting depth. Fine root density and soil nutrients (e.g., soil nitrogen and effective cation exchange capacity) were positively correlated, which may suggest an adaptive response to gradients of nutrient

availability across the soil profile. This is supported by the fact that fine root density and pH were negatively correlated in tropical mountain cloud forest, suggesting that high fine root density could be an efficient strategy to explore more soil volume and, in turn, maximize nutrient uptake in more acid soil environments. In general, soil bulk density was negatively related to fine root mass. Thus, an increase in soil bulk density could be one of the principal causes of a decrease in fine roots' capacity to explore the soil volume, which, in turn, could lead to a decline in the absorption of nutrients and water. In the case of soil water, field capacity had a non-significant positive effect on fine root density in grassland and coffee crop, the likely result of soil structure damage due to management practices, which must modify the natural pore system. In contrast, in undisturbed soil (tropical mountain cloud forest), the water potential at field capacity was significantly positively correlated with fine root density, typifying the behavior of a stable and natural soil structure, that is, one with a natural pore system.

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