CHLOROPHYLL FLUORESCENCE, AND ROOT TRAITS IN MYCORRHIZAL PLANTS OF *Persea americana* MILL. UNDER DIFFERENT SOIL AGRICULTURAL MANAGEMENT †

[FLUORESCENCIA DE LA CLOROFILA, Y CARACTERÍSTICAS DE LAS RAÍCES EN PLANTAS MICORRIZADAS DE *Persea americana* MILL. BAJO DIFERENTE MANEJO AGRÍCOLA]

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SUMMARY

Background. The avocado, *Persea americana*, is a fruit crop of immense importance to Mexican agriculture with increasing demand worldwide. Hence there is an increment in the cultivated area and the use of agrochemical products. Current research explores how arbuscular mycorrhizal (AM) fungi may reduce agrochemicals in avocado crops. Therefore, it is necessary to investigate the specific conditions under which the application of AM fungi is beneficial and the compatible host plants or genotypes. **Objective**. To investigate whether the origin of soil and AM inoculum from two orchards with different soil agricultural management affect the infectivity (percentage of AM colonization) and effectivity (chlorophyll fluorescence and root traits) of the avocado plants, and whether chlorophyll fluorescence and root traits correlate with the percentage of AM colonization. **Methodology**. A factorial experiment with three factors: i) the origin of seed (two seeds origin), ii) the origin of soil, and iii) the origin of AM inoculum from two orchards with different soil agricultural management was performed. **Results**. Although root-soil-AM interactions are highly complex, we found evidence that the origins of seed, soil, and AM inoculum can affect the performance of plants in terms of chlorophyll fluorescence, and root traits. Additionally, infectivity was greater when the soil and AM inoculum were agrochemical-free. We discuss our findings in the context of soil mineral nutrients and seed origin. **Implications.** This study contributes to understanding the mechanisms that underlie plant-AM interactions in plants from two seed origins and soil agricultural management in avocado plants, an important cultivated tree for humans worldwide. **Conclusion**. The combination of root traits and leaf may improve our understanding of the mechanisms that underlie plant-microbe interactions in plants from two seed origins and soil agricultural management to enhance not only avocado production but also the long-term sustainability and yield stability of avocado agroecosystems.
Key words: avocado; chlorophyll fluorescence; Nayarit; organic manures; root branching ratio; specific root length.

RESUMEN

Antecedentes. El aguacate, *Persea americana*, es un cultivo de gran importancia en la agricultura mexicana con una creciente demanda en el mundo. Lo que ha llevado a un incremento en el área cultivada y el uso de agroquímicos. Actualmente, las investigaciones exploran cómo los hongos micorrizógenos arbusculares (HMA) pueden reducir los agroquímicos en cultivos de aguacate. Por lo tanto, es necesario investigar las condiciones específicas bajo las cuales la aplicación de HMA son benéficos para el cultivo, y la compatibilidad de plantas procedentes de diferentes semillas. **Objetivo.** Investigar si el origen del suelo y el inóculo de HMA proveniente de dos parcelas con diferente manejo agrícola del suelo afectan la infectividad (porcentaje de colonización micorrícica) y la efectividad (fluorescencia y características de las raíces) en plantas de aguacate, y si la fluorescencia y las características de las raíces correlacionan con los porcentajes de colonización micorrícica. **Metodología.** Se realizó un experimento factorial con tres factores: i) origen de la semilla (dos plantas), ii) el origen del suelo, y iii) el origen del inóculo micorrícico proveniente de dos parcelas con diferente manejo agrícola del suelo. **Resultados.** Aunque las interacciones raíz-suelo-hongos son complejas, encontramos que el origen de la semilla, el suelo y el origen del inóculo micorrícico pueden afectar el desempeño de las plantas en términos de la fluorescencia de la clorofila, y las características de las raíces. Además, la infectividad fue mayor cuando el origen del suelo y el origen de los propágulos fúngicos se obtuvo de una parcela libre de agroquímicos. Discutimos los resultados en el contexto de los nutrientes minerales del suelo y el origen de las

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semillas. **Implicaciones.** Este estudio contribuye a entender los mecanismos que subyacen las interacciones planta-hongos arbusculares en plantas de aguacate procedentes de diferentes semillas y diferente manejo agrícola del suelo, un cultivo importante para los humanos en el mundo. **Conclusión.** La combinación de las características de las raíces y las hojas puede mejorar el entendimiento de los mecanismos que subyacen las interacciones planta-microbios en plantas procedentes de diferentes semillas, y diferente manejo agrícola del suelo para mejorar no solo la producción de aguacate sino también la sostenibilidad a largo plazo y la estabilidad del rendimiento en los agroecosistemas de aguacate.
Palabras clave: aguacate; fluorescencia de la clorofila; Nayarit; abonos orgánicos; proporción de la ramificación de la raíz; longitud de raíz específica.

INTRODUCTION

Avocado, *Persea americana*, is an economically important crop with a worldwide market (FAO, 2024; Cho *et al*., 2021) and valued nutritional properties. Mexico has the largest cultivated area dedicated to avocados globally, producing up to 30% of global demand, a larger share than any other country (Altendorf, 2019). From 2021 to 2023, avocado production in Mexico increased *ca*. 8.3% (SIAP, 2023), and approximately 76% of all avocados consumed in the U.S. were imported from Mexico (Cho *et al*., 2021; USDA FAS, 2021). The rising demand for avocado worldwide has increased the number and extension of avocado orchards in which the use of insecticides, herbicides, and other agrochemicals is a common practice (FAO, 2024). Expanding avocado-cultivated land jeopardizes local forests and increased green-house gas emissions due to loss of forest cover and the burning of pruning residues (Denvir, 2023; Tauro *et al*., 2023), there have even been land-use changes within protected natural sites in Mexico. Besides, soil erosion has increased considerably in avocado-producing regions of Mexico (Cho *et al*., 2021). Deforestation alters physical and chemical soil properties, and it can reduce beneficial soil microorganisms and increase soil pathogens (Martín-Robles *et al*., 2020). Sustainable agriculture alternatives are being widely explored to reduce the chemical inputs in productive systems.

Arbuscular mycorrhizal (AM) fungi (Glomeromycota, Wijayawardene *et al*., 2022) are an ancient lineage of obligate biotrophs (they need a living host plant during their life cycle) that colonize the roots of most terrestrial plant species, including cultivated plants (around 80% of plant species and 92% of plant families, Wang and Qui, 2006). In general, extraradical mycelia of AM fungi forage the soil, exploit sources of mineral nutrients inaccessible to the plant's roots, and supplement plant nutrition by exchanging mineral nutrients and water for simple carbon compounds (carbohydrates such as sugars and starch). Further, AM fungi provide bioprotection to the plants from biotic and abiotic stresses (Martin and van der Heijden, 2024). However, deforestation and agrochemicals negatively affect the capacity of AM propagules to colonize the roots of their host plants (infectivity), and also reduce mycorrhizal-derived benefits, for instance, plant growth and reproduction (effectiveness), the soil's health, and the composition of the microbiota, which directly impact crops' yields (e.g., Balderas-Alba *et al*., 2019; Vega-Frutis *et al*., 2018; Trejo *et al*., 2016).

AM fungi represent an alternative to the conventional use of agrochemical fertilizers favoring sustainable agriculture (Martin and van der Heijden, 2024; Rillig *et al*., 2019; Berruti *et al*., 2016; Rillig *et al*., 2016; but see Gupta and Abbott, 2021), and there is a fair amount of evidence of positive AM fungi effects on various crops in terms of biomass (Berruti *et al*., 2016). However, roots (e.g., thin or thick roots, and their association with AM fungi) for foraging nutrients, and leaf traits (e.g., photosynthesis, specific leaf area) should be considered in the context of acquisition *vs* conservation strategies to uptake resources, because contrasting nutrient availabilities can lead to changes in plant resource acquisition strategies and could reflect the relationships among functional traits (Zhao *et al*., 2024; Delpiano *et al*., 2020) both leaves (leaf economics spectrum) and roots (root economics spectrum). Additionally, the influence of plant genotypes within a single species (intraspecific variation) and biotic interactions also can influence above- and belowground traits (Zhang *et al*., 2024; Martin and Isaac 2021).

To our knowledge, no studies have evaluated chlorophyll fluorescence and root traits in avocado with soil and AM inoculum from contrasting soil agricultural management practices. Previous field evidence (see Balderas-Alba *et al*., 2019) showed that the use of chemical fertilizers affects AM fungi symbiosis, with lower AM colonization observed in avocado trees growing in an agrochemical-treated orchard compared to one without. Building on this field evidence, we conducted a study to determine whether the observed differences in AM colonization confer benefits to avocado plants. Specifically, we evaluated AM infectivity (percentage of AM colonization) and effectivity, assessed through chlorophyll fluorescence parameters (minimal and maximal fluorescence, and the ratio of variable to maximum fluorescence) and root traits (diameter of first-order roots, specific root length, and root

branching ratio). We used an experimental approach including, i) seeds from two avocado trees (seeds origin), ii) soil, and iii) AM fungi isolated from two orchards that qualitatively differed soil management. Specifically, one orchard used conventional agrochemicals while the second orchard was agrochemicals-free. We aimed to answer the following questions: i) does the exposure to agrochemicals affect the infectivity and effectiveness of AM fungi in avocado plants? ii) are the infectivity and effectiveness of AM fungi on avocado plants affected by the origin of the tree seeds? and iii) do chlorophyll fluorescence and root traits correlate with the percentage of AM colonization? Previous studies reported that avocado trees are highly dependent on AM fungi (e.g., Rivera *et al*., 2016), and mycorrhizal fungi improved avocado tree growth, nutrition, and photosynthesis (Lemus-Soriano, *et al*., 2021; Bañuelos *et al*., 2017; Carreón *et al*., 2014; Castro-Alvarado *et al*., 2013). Therefore, we predicted that AM fungi would colonize avocado plants, but the AM frequency will differ between seed origin. Also, the infectivity and effectivity of AM fungi would be higher in the agrochemical-free orchard (Balderas-Alba *et al*., 2019).

Additionally, it has been suggested that species with thicker roots and lower root branching ratio support greater AM colonization, suggesting greater mycorrhizal dependency and faster resource acquisition than thin and profusely branched roots (Zhao *et al*., 2024; Wen *et al*., 2019; Kong *et al.*, 2014). Therefore, we expect that AM colonization positively correlates with the diameter of roots but negatively with specific root length and root branching rate, minimizing costs for soil resource acquisition independently of seeds origin (Zhao *et al*., 2024; Kong *et al*., 2017). Also, we expect a positive correlation between the overall plant physiological status (chlorophyll fluorescence traits) and AM colonization as there is evidence showing that AM colonization increases photosynthesis (Shi *et al*., 2023; Amani *et al*., 2022).

MATERIALS AND METHODS

Study plant

Avocado (*Persea americana* Mill.) is a fruit tree native to Mesoamerica but grows worldwide (Galindo-Tovar *et al*., 2008). There are three different races or botanical varieties: Mexican (*P. americana* var. *drymifolia*), Guatemalan (*P. americana* var. *guatemalensis*) and West Indian (*P. americana* var. *americana*), which are originally from highland Mexico, highland Guatemala, and lowland Guatemala to Costa Rica, respectively (Galindo-Tovar *et al*., 2008). We set up an experiment with the Mexican variety, one of the preferred varieties used as the rootstock of commercial varieties such as Hass because of its resistance to pests and diseases (Bedoya-Ramírez *et al*., 2023; Sánchez-Pérez, 1999), particularly root diseases (Rincón-Hernández *et al*., 2011).

Seed and soil material collection

In August 2017, we collected 400 ripe fruits from two avocado trees located within the Agriculture Faculty of the Autonomous University of Nayarit (104.89085° W, 21.4288333° N). We collected seeds from two Mexican variety trees growing in the same area. Since these trees were under similar environmental conditions, the experimental responses are more likely to reflect inherent characteristics of the seeds. At the same time, using two different trees may introduce genetic diversity. We haphazardly chose 170 fruits from each tree, enumerated and weighed them. Once they reached their edible maturity, the seeds were extracted, weighed, and then stored in brown paper bags at room temperature for one week.

In September 2017, we collected soil from two avocado orchards located in the State Reserve Sierra de San Juan, Xalisco, Nayarit, Mexico, no more than 1.17 km apart. Orchard 1 (104.94894º W, 21.40142º N) was characterized as being free of pests, diseases, and chemical soil nutrition management, except for manual weeding (Luna-Esquivel, personal communication, September 2017). In orchard 2 (104.93842º W, 21.39864º N), the avocado trees are fertilized with poultry manure (30 kg plant $^{-1}$ per year, for the past three years) during the dry season, and with a chemical fertilizer mixture (N, P, K, Ca, Zn and Mg). The amount of chemical fertilizer varies according to the foliar nutrient content and soil fertility but on average is 4 kg of fertilizer mixture per plant each year, split into two applications (early summer and early fall). In orchard 2, micro-sprinkler irrigation is applied once a week (60 L per plant, Luna-Esquivel, personal communication, September 2017).

In each orchard, we marked two plots (50 m x 50 m, 2500 m^2), and in each plot, we collected approximately 150 kg soil using the five-gold method (INIFAP, 2012) at distance of 1.5 m of the tree trunks. The soil was collected with a shovel up to a depth of 20 cm. The soil samples per orchard were homogenized and divided into three parts: 1) 1 kg per orchard for physical-chemical analyses of the soil, 2) 1.5 kg per orchard to be used as inoculum, and 3) the rest of the soil was sterilized for the experiment of infectivity and effectiveness.

Germination

Avocado seeds were individually submerged in plastic cups (~200 ml volume) with 1.5% sodium hypochlorite for 5 minutes, after which they were

transferred to plastic cups (~200 ml volume) with purified water for 5 minutes (Amaya-Acosta *et al*., 2016). Later, the tip of each seed was cut and transferred into the germination trays (Hydro-Environment of 32 cavities), previously washed and disinfected. The cavities were filled with a mixture containing autoclaved commercial soil and sand (1:1). During the first 10 days, the seeds were irrigated daily with water from a well and were watered every two days.

The commercial soil and sand were heat sterilized in an autoclave (BIOBASE®) for 90 minutes at 125 °C (1.2 Mpa), after 24 hours, a second sterilization was undertaken under the same conditions mentioned above to have plants free of AM fungi before the experiment. This procedure very likely eliminates all living forms in the soil. However, microbes other than AM fungi and AM fungi can be added separately in different treatment combinations while retaining most of the abiotic properties of the soil (Johnson *et al*., 2010).

Transplant

After 45 days of germination, 60 random plants of each tree were weighed (initial fresh total biomass). At this stage, we detected significant differences in the biomass of seedlings of each tree (tree 1: 60.710 ± 1.518 g, mean ± standard error, tree 2: 50.260 ± 1.229, $F_{1,118} = 27.69$, $P < 0.001$). Afterward, the plants were transplanted into black plastic bags filled with ~2 kg of heat-sterilized (see above) soil from the orchards. Soil samples from each orchard were separated and analyzed for their physical-chemical properties. Analyses of soil were carried out in the Soil Analysis Laboratory of the Institute of Ecology A.C (INECOL) in agreement with the Official Mexican Norm PROY-NOM-021-RECNAT-2000 (SEMARNAT, 2002). The physical-chemical soil parameters are presented in Fig. 1 and Table S1.

The experiment was initiated in October 2017, and we performed a factorial experiment with three factors: i) the origin of seed (T) with two levels (T1 and T2), ii) the origin of soil (O) with two levels (O1 and O2), and iii) AM inoculum (AM) that included three levels, from orchard 1 and 2 (AMO1 and AMO2) and non-inoculated plants (NI). We had 10 replicates of each treatment combination, with 120 plants (Fig. S1).

The mycorrhizal inoculum (spores, extraradical mycelium and root fragments) consisted of 20 g of unsterilized soil per plant from each orchard (Methods in supplementary material). During the transplant, 20 g of inoculum was placed directly on the root, and for non-inoculated plants (NI), 20 g of sterilized soil was used (Johnson *et al*., 2010). Additionally, all plants received 15 ml of a soil microbial suspension. Sterile distilled water was used to suspend 20 g of soil from each orchard (unsterilized soil). Subsequently, the content was homogenized and passed through a nitrocellulose membrane Millipore filter (0.8 μm) to separate the native microbiota and partially return them to their corresponding sterile soil (Lauriano-Barajas and Vega-Frutis, 2018).

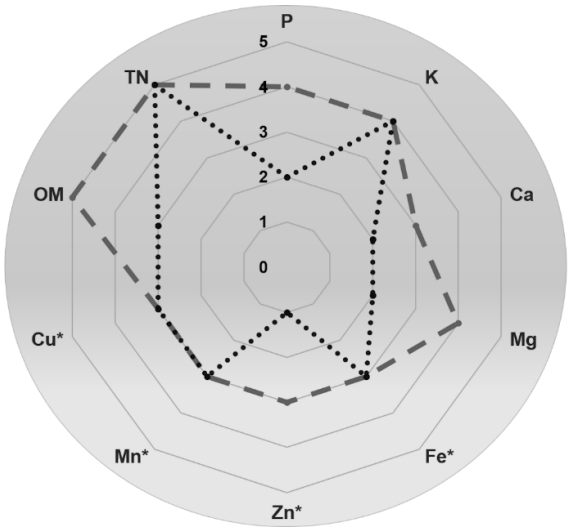

Figure 1. Summary of sterilized soil parameters (P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium, Fe = iron, Zn = zinc, Mn = manganese, Cu = copper, OM = organic matter, TN = total nitrogen) quantified from orchard 1 (O1) and orchard 2 (O2) located in the State Reserve Sierra de San Juan, Nayarit, Mexico. The numbers indicated: 1) very low or deficient, 2) low or marginal, 3) medium or adequate, 4) high, and 5) very high (following Castellanos *et al*., 2000). The dashed line (---) represent the properties of orchard 1, and the dotted line (⋯) represent the properties of orchard 2. The soil elements with an asterisk mean that its minimum scale is 1) and its maximum scale is 3).

After, the plants were assigned to the treatments, and the pots were randomly placed in a nursery garden in the Agriculture Faculty of the Autonomous University of Nayarit. To avoid potential effects due to the arrangement of the pots in the nursery garden and micro climatic-conditions, these were rotated every two weeks during the 129 days of the experiment (end of the experiment March 2018) and watered every third day with well water. In addition, in November 2017 and February 2018, we applied AgroIQC® avermectin (3 ml L^{-1} H_2O) to eliminate growing populations of the red spider mite (*Tetranychus urticae* C.L. Koch).

Chlorophyll fluorescence

After 126 days, chlorophyll fluorescence was measured using LI-COR 6800 portable photosynthesis

measuring system (LI-COR, Inc., Lincoln, Nebraska, USA). Before measuring the chlorophyll fluorescence, the leaves were put in darkness for 30 min (Goltsev *et al*., 2016). Afterward, we recorded the F_0 and F_m applying three pulses of saturating light from 1000 µmol m^{-2} s^{-1} every 5 s. These parameters were recorded in one fully expanded apical leaf per plant, and the pulses of saturating light were averaged. The variable fluorescence yield (F_v) was calculated as $F_v = F_m - F_0$, and F_v/F_m was calculated as maximum quantum efficiency of photosystem II (Goltsev *et al*., 2016; see Methods in the supplementary material for the explanation of photosynthetic parameters).

Root traits

The experiment lasted 129 days, the average time when avocado plants are transplanted to the field. During the harvest (March 2018), a representative subsample of roots was collected per plant (approximately 0.049 ± 0.002 g, dry weight per plant). This subsample, including first and second-order roots, was washed gently with running water. Clean roots were evenly laid on a tray and photographed with a high-resolution camera. Afterward, the root segments were oven-dried at 60 °C for three days. From these photographs, we measured the diameter (RD, cm) of all first and second-order roots and the total root length (cm) from each root subsample using the complementary software SmartRoot from ImageJ software (Lobet *et al*., 2011). Additionally, the specific root length (SRL, cm g^{-1}) was estimated by dividing the root length by the root dry biomass. The root branching ratio (RBR) was calculated as the number of first-order roots relative to second-order roots. These root traits are expected to influence the mycorrhizal colonization (Zhao *et al*., 2024; Lugli *et al*., 2020; Chen *et al*., 2016; Kong *et al*., 2014). Additionally, we calculated the mycorrhizal plant benefit (Methods in supplementary material).

Fungal measurements

A subsample of fine roots of all plants was collected during the harvest to estimate the percentage of roots colonized by AM fungi. The roots were processed according to the method of Koske and Gemma (1989) and stained with trypan blue (0.05%). Subsequently, the intraradical colonization by AM fungi was quantified on 15 root segments of approximately 15 mm long per plant fixed in polyvinyl-lacto-glycerol (PVLG) on permanent slides. Each root fragment was examined at three equally spaced points (at the top, middle, and bottom per each root fragment) under an optical microscope (ZEISS Primo Star) at 100x total magnification. We considered the colonization by hyphae (fungal structures that transport nutrients from the soil to roots), vesicles (fungal structures that store large amounts of lipids and can have a function either as propagules or to support the regrowth of intracellular hyphae), and arbuscules (specific fungal structures that perform the exchange of nutrients between the symbionts, Smith and Read, 2008). The presence/absence of each fungal structure across the equator from each observation field was registered. Positive counts were summed and weighted by the total number of fields of view observed and multiplied by 100 (McGonigle *et al*., 1990) to obtain the total percentage and the percentage per fungal structure.

Statistical analyses

All statistical analyses were performed using the R software environment (R Development Core Team, 2019). Before running the tests, we performed a graphical data exploration to evaluate the best potential models to analyze each type of response variable (Zuur *et al*., 2010). For all the models, we verified whether the residuals were normally distributed, the variances homogeneous, and when it was necessary, the variables were transformed (see below).

Plant measurements

To test for differences in the initial fresh biomass between the origins of the seed (tree 1 and tree 2), we used one-way ANOVA. To meet model assumptions, the response variable was square root transformed.

Before running the models, we did multi-panel scatterplots between each response variable to detect correlations between these (Fig. S2). It is important to highlight that the multi-panel scatter plots were developed to know if there is a correlation between groups of response variables (chlorophyll fluorescence, and root traits). Given that there was no correlation, we tested all variables. The effect of the origin of seed (T1 and T2), the origin of soil (O1 and O2), the origin of AM inoculum (AMO1, AMO2, and NI) and the two-way interaction between these factors on minimal fluorescence (F_0), maximal fluorescence (F_m), the ratio of variable to maximum fluorescence (F_v/F_m), specific root length (SRL), root branching ratio (RBR) and diameter (RD), were tested with analyses of covariance (ANCOVA), the initial fresh biomass (IFB) was used as a covariate. To meet the model assumptions, the response variables of F_0, F_m, and SRL were transformed with square root, F_v/F_m, RBR, and RD, were transformed with rank ("average" method). Significant differences between AM inoculum levels (AMO1, AMO2, and NI) were tested with a *posteriori* contrast based on a *t-test* using the *lsmeans* library (Lenth, 2016), which calculates the means of least squares (expected marginal means) for specific factors or combinations of factors in a linear model.

It is important to mention that we used the initial fresh biomass as covariate because it could be related to the response variables. We measured the covariate before the delivery of explanatory variables. However, we understand that the initial biomass is related to the origin of seeds. Still, they can interact with another explanatory variable that the covariate is uncorrelated. It is generally recommended to include the covariate interaction in the model, regardless of its significance. But, if the interaction between the origin of the seed and the covariate is significant, we will not discuss it.

Fungal measurements

We also used an ANCOVA to explore differences in the percentages of roots colonized by hyphae, vesicles, and arbuscules among treatments and interactions. The initial fresh biomass was used as a covariable. To meet the model's assumptions, the percentage of vesicles was transformed with range ("average" method).

Spearman correlation coefficients were employed to determine the relationship between the total colonization percentage by AM fungi and root traits (RD, SRL, and RBR) and between chlorophyll fluorescence traits (F_0, F_m, and F_v/F_m). The correlations were calculated separately for each root trait and level of the factors.

RESULTS

Chlorophyll fluorescence

None of the factors evaluated (origin of soil, seed, and AM inoculum) affected the variables F_0 and F_v/F_m (Table 1). But, F_m was affected by the origin of the soil (Table 1). The plants growing in the soil from O1 had a higher F_m than those growing from O2 (Table 2).

Root traits

The origin of the seed significantly affected the SRL (Table 1). The plants from T2 developed 26.66% more SRL (1921.405 ± 111.355 cm g^{-1}) compared with plants from T1 (1517.001 ± 94.905 cm g^{-1}). The plants growing without mycorrhizal fungi developed ~22.76% lower SRL (1467.585 ± 114.339 cm g^{-1}) than plants growing with inoculum from AMO1 and AMO2 (AMO1: 1789.987 ± 124.446 cm g^{-1}, AMO2: 1900.037 ± 143.386 cm g^{-1}), with no significant differences between them (P = 0.914), and both AM treatments differed of NI treatment (AMO1-NI, P = 0.048, AMO2-NI, P = 0.013). Nonetheless, the plants from T1 seem to benefit more when they grew in soil from O2 but inoculated with AM propagules from O1 (AMO1, Fig. S3a). In contrast, the mycorrhizal plant benefit in terms of SRL was similar regardless of the seed and orchards when the inoculum was from O2 (AMO2, Fig. S4a).

The RBR was only significantly affected by the AM inoculum (Table 1). The plants growing with fungi from AMO2 had 9.57% more RBR (0.094 ± 0.008) compared with the plants without fungi (0.085 ± 0.017, AMO2-NI, P = 0.022; Fig. 2b), and there were no statistically significant differences between AM treatments explaining the RBR (AMO1-AMO2, P = 0.402; Fig. 2b). Additionally, the plants from T1 seemed to benefit more when inoculated with AM fungi of O1 (AMO1) independently of soil origin (Fig. S3b). They were similar to the plants inoculated with fungal propagules from O2 (AMO2) but growing in soil from O1 (Fig. S4b). No statistical differences were detected for the origin of the seed, origin of soil, AM inoculum, or their interactions explaining the RD (Table 1, Fig. 2c). However, the plants from T2 showed a higher mycorrhizal benefit, independently of the origin of soil and AM inoculum (Fig. S3c and S4c).

The interaction between seed origin and AM inoculum significantly affected SRL and RBR (Table 1). Avocado plants from T1 and NI treatment developed lower SRL compared with the plants from T2 and NI treatment (Fig. 2a), and the same pattern was observed for the variable RBR (Fig. 2b) but not for RD (Fig. 2c).

Seed origin and AM inoculum with the covariable (IFB) significantly affected the SRL (Table 1). Smaller plants developed greater SRL in the AM treatments than plants in the NI treatment (Fig. S5a). The AMO1 treatment was the one that most contributed to the development of SRL in larger plants (Fig. S5a). Neither the RBR nor the RD showed significant differences among AM treatments (Fig. S5b and S5c).

Fungal measurements

The fungal parameters measured showed significant differences for the origin of seed and soil (Table 3). The plants from T2 had greater colonization by hyphae (80.064 ± 2.408%) and vesicles (67.132 ± 3.040%) compared with those from T1 (hyphae: 63.832 ± 4.345%, vesicles: 43.062 ± 4.201%), but there were no differences in the AM colonization by arbuscules between T1 and T2 (8.076 ± 1.765% and 10.710 ± 1.929%, respectively). The plants growing in soil from O1 had greater colonization by hyphae (82.765 ± 2.704%), vesicles (63.418 ± 4.058%), and arbuscules (19.836 ± 2.653%) compared with those growing with the inoculum from O2 (hyphae: 61.132 ± 3.841%, vesicles: 46.776 ± 3.781% and arbuscules: 8.343 ± 1.860%).

There were statistically significant differences in the interaction between AM inoculum and the origin of the seed, explaining the percentages of hyphae, vesicles, and arbuscules. The percentages of fungal structures were greater in the roots from T2 and growing in the AMO2 (Fig. 3a, b, and c).

Table 1. Summary of the statistical results of ANCOVA models for plant parameters analyzed in *Persea americana.*

Source of variation		Chlorophyll fluorescence						Root traits					
		F_0		F_m		F_v/F_m		SRL		RBR		RD	
	df	F	P	F	P	F	P	F	P	F	P	F	P
T - trees	1	0.054	0.815	0.019	0.888	0.024	0.876	9.059	**0.003**	1.313	0.254	0.116	0.733
O - orchard	1	0.058	0.809	5.057	**0.026**	0.774	0.380	0.855	0.357	0.016	0.897	2.009	0.159
In - inoculum	2	0.212	0.808	0.252	0.777	0.411	0.663	3.799	**0.025**	3.403	**0.036**	0.177	0.837
IFB – in biomass	1	1.333	0.250	0.201	0.654	0.700	0.404	1.335	0.250	1.028	0.312	3.598	0.060
T:O	1	0.053	0.817	0.078	0.780	0.114	0.736	0.536	0.465	1.582	0.211	0.315	0.575
T:In	2	0.514	0.599	1.410	0.248	0.426	0.653	3.585	**0.031**	3.217	**0.044**	1.870	0.159
T:IFB	1	7.229	**0.008**	0.281	0.596	4.229	**0.042**	4.637	**0.033**	2.185	0.142	0.056	0.813
O:In	2	0.068	0.933	0.110	0.895	0.007	0.992	0.012	0.987	0.196	0.821	0.300	0.741
O:IFB	1	0.172	0.679	0.943	0.333	0.0001	0.991	2.072	0.152	1.875	0.173	2.525	0.115
In: IFB	2	2.646	0.075	0.944	0.392	1.592	0.208	3.846	**0.024**	1.049	0.353	0.363	0.695
Residuals	105												

Significant values according to the statistical analyses are shown in bold. T: origin of seed (T1, T2); O: origin of soil (O1, O2); In: origin of AM inoculum (AMO1, AMO2, NI); IFB: initial fresh biomass; F_0: minimum fluorescence; F_m: maximum fluorescence; F_v/F_m: ratio of variable to maximum fluorescence; SRL: specific root length, RBR: root branching ratio; RD: root diameter.

Table 2. Means ± standard errors of chlorophyll fluorescence parameters analyzed in *Persea americana*.

	Tree		Orchard		AM inoculum		
	1	2	1	2	AMO1	AMO2	NI
F_0	671.29 ± 54.58	671.21 ± 51.93	657.41 ± 48.92	685.09 ± 57.24	681.82 ± 57.06	676.48 ± 67.73	655.45 ± 70.90
F_m	2917.12 ± 88.35	2934.81 ± 90.58	3078.50 ± 102.83a	2773.43 ± 68.19b	2970.39 ± 118.19	2861.24 ± 106.08	2946.28 ± 104.64
F_V/F_m	0.759 ± 0.021	0.759 ± 0.020	0.766 ± 0.022	0.752 ± 0.020	0.750 ± 0.027	0.756 ± 0.026	0.771 ± 0.024

Different letters indicate statistically significant differences according to *t*-test contrast ($P < 0.05$) among the origin of seed (tree), the origin of soil (orchard), and the origin of AM inoculum (AMO1, AMO2, NI). F_0: minimal fluorescence (μmol m^{-2} s^{-1}); F_m: maximal fluorescence (μmol m^{-2} s^{-1}); F_V/F_m: ratio of variable to maximum fluorescence.

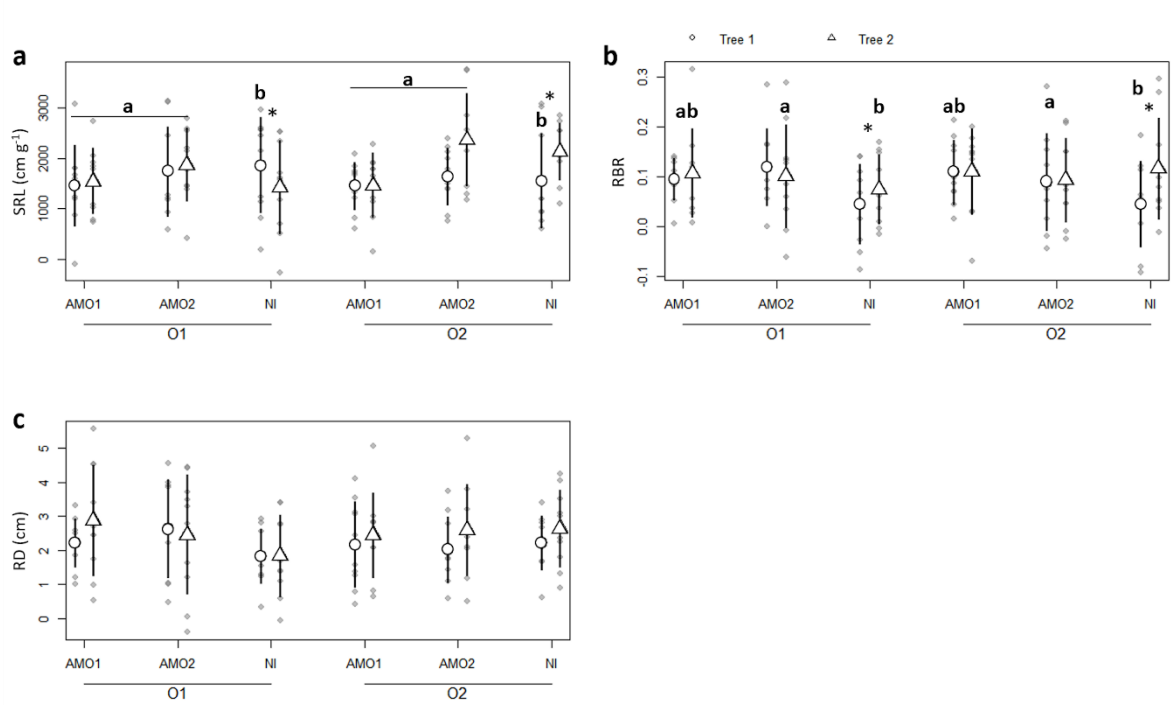

Figure 2. Interactive effects of origin of seed (circle: T1, triangle: T2), and the origin of AM inoculum (AMO1, AMO2, NI) on (a) SRL: specific root length, (b) RBR: root branching ratio, and (c) RD: root diameter in *Persea americana* plants. Different letters indicate statistically significant differences among AM treatments inside each level of origin of soil (O1, O2). The asterisks indicate statistically significant differences between origin of seed inside each level of AM inoculum according to *lsmeans* contrast *P* (<0.05). Means ± standard deviations were given without data transformation.

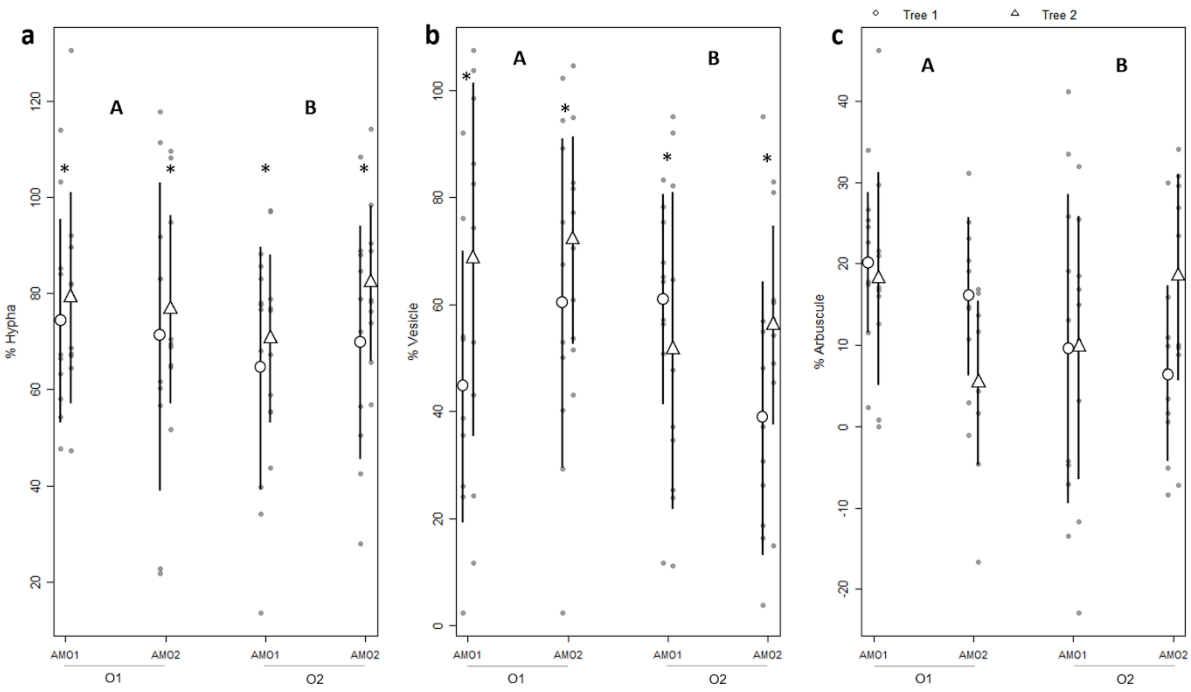

Figure 3. Interactive effects of origin of seed (circle: T1, triangle: T2), and the origin of AM inoculum (AMO1, AMO2) on the percentage of (a) hyphae, (b) vesicles, and (c) arbuscules in *Persea americana* plants. Different capital letters indicate statistically significant differences between origin of soil (O1, O2). The asterisks indicate statistically significant differences between origin of seed inside each level of AM inoculum according to *lsmeans* contrast *P* (<0.05). Means ± standard deviations were given without data transformation.

Table 3. Summary of the statistical results of ANCOVA models for the fungal structures analyzed in *Persea americana*.

Source of variation		Hypha (%)		Vesicle (%)		Arbuscule (%)	
	df	F	P	F	P	F	P
T – trees	1	15.293	**<0.001**	26.109	**<0.001**	1.757	0.189
O – orchard	1	27.166	**<0.001**	15.047	**<0.001**	14.874	**<0.001**
In – inoculum	1	1.015	0.317	0.110	0.741	1.383	0.243
IFB – in biomass	1	0.100	0.752	1.379	0.244	4.573	**0.036**
T:O	1	0.539	0.465	0.274	0.602	0.398	0.530
T:In	1	8.202	**0.005**	6.184	**0.015**	6.824	**0.011**
O:In	1	4.344	**0.041**	4.099	**0.047**	6.348	**0.014**
T:IFB	1	0.034	0.854	0.911	0.343	0.292	0.590
O:IFB	1	0.129	0.720	0.470	0.495	0.318	0.574
In:IFB	1	1.249	0.268	0.530	0.469	1.338	0.251
Residuals	69						

Significant values according to the statistical analyses are shown in bold. T: origin of seed (T1, T2); O: origin of soil (O1, O2); In: origin of AM inoculum (AMO1, AMO2); IFB: initial fresh biomass.

An important interaction occurred between AM inoculum and soil origin explaining the percentages of fungal structures (Table 3). The plants growing in the soil from O1 and with AM inoculum isolated from the same orchard (AMO1) had a greater percentage of AM colonization by hyphae, vesicles, and arbuscules. In contrast, there were no statistically significant differences for the plants inoculated with mycorrhizas from O2 (AMO2). However, the AM colonization with inoculum from O1 (AMO1) tended to decrease in the presence of soil from O2 (Fig. 3). Additionally, all NI plants remained without AM colonization at the end of the experiment.

Spearman correlation for each level of factor evaluated showed a negative correlation between the total mycorrhizal colonization (T1, 63.94 ± 4.33%, T2, 80.06 ± 2.41%, O1, 82.88 ± 2.66%, O2, 61.13 ± 3.84%, AMO1, 74.04 ± 3.40%, and AMO2, 69.97 ± 4.01%) and RBR, but only by the origin of soil from orchard 1 (Table S2). Yet, there were no correlations between the total mycorrhizal colonization and RD, SRL, F_0, F_m, and F_v/F_m for any level of factors evaluated (Table S2).

DISCUSSION

This study investigated whether the origin of soil, AM inoculum from two orchards with different soil agricultural management affect the performance of avocado plants in terms of chlorophyll fluorescence and root traits. Additionally, infectivity was greater in plants growing in soil and AM inoculum of the orchard with no agrochemicals. This observation was consistent with the negative correlation between AM colonization and RBR. An important point in our study is soil agricultural management because avocado yield is highly dependent on climatic conditions and soil nutrients such as soil porosity, pH, and availability of essential nutrient elements.

Chlorophyll fluorescence

The photosynthetic parameters are a good measurement of the physiological condition of plants, and it is used to predict crop yields under various environmental conditions (Croce *et al*., 2024; Goltsev *et al*., 2016). In this way, AM fungi lessen the damage caused to the PSII by stress conditions (Amani *et al*., 2022; Zhu *et al*., 2011; Zhu *et al*., 20l0). In avocado plants, Castro-Alvarado *et al*. (2013) showed that plants with AM inoculum had 20% higher chlorophyll concentration compared with non-inoculated plants. This finding suggests that these fungi may increase photosynthesis.

In the present study, the F_v/F_m ratios were close to 0.8, suggesting that avocado plants were not stressed as they grew under nursery garden conditions. Recently, Shezi *et al*. (2020) showed that the optimum photosynthetic efficiency of avocado plants was under low light intensity, i.e., the excess sunlight affects functions of photosystems I and II. This evidence suggests that excess energy may cause photooxidative damage to the photosynthetic membrane. Low F_m (indicator of loss of chemical energy) levels in O2 (with soil nutrition management) indicate that the reaction centers of leaves were not completely closed compared to plants growing in soil from O1. It has been suggested that the deficiency of nutrients such as P, Ca, Mg, and Zn (which were lower in soil of orchard 2 than orchard 1) have a relationship with a decrease in F_m. For instance, P deficiency can modify the structure of grana and thylakoids. The Mg and Ca are important components of chlorophyll, and the deficiency of these elements affects the function of chloroplasts. At the same time, the Zn is necessary to keep the photosystem II active. Therefore, the shortage of these elements could negatively impact the photosystem II (Kalaji *et al*., 2016; Liu *et al*., 2009; Wu *et al*., 2006). Furthermore, the cation

exchange capacity (a measure of the ability of a soil to hold and exchange cations) was lower in O2. Additionally, it has been shown that biotic stress (fungal plant pathogen) decreases the efficiency of photosystem II, but this response depends on avocado genotypes (Martínez-Ferri *et al*., 2016). Although we detected the presence of the red spider mite, this was eliminated immediately.

Root traits

Plants have evolved strategies to acquire soil nutrients and respond to nutrient availability space and time. These strategies include increasing in length and branching of absorptive fine roots and varying their reliance on AM fungi to optimize nutrient acquisition (e.g., Zhang *et al*., 2024; Lugli *et al*., 2020; Chen *et al*., 2016; Liu *et al*., 2015; Maherali, 2014). The avocado plants growing with AM inoculum had greater SRL than non-inoculated plants, and the same pattern was found for the RBR, which agrees that species with high SRL also produce a higher RBR (e.g., Kramer-Walter *et al*. 2016; Liu *et al*. 2015). However, these findings disagree with several studies suggesting that plants with higher SRL and RBR frequently have less dependence on mycorrhizas (e.g., Laliberté 2017; Liu *et al*., 2015; Maherali, 2014), i.e., we expected high SRL and BRB in non-inoculated plants. Additionally, species with high SRL tend to be highly successful competitors, as SRL reflects the root system's capacity for nutrient foraging. In our study, we found that SRL varies depending on the seed's origin. These findings along with the lack of differences in root diameter (RD), might indicate that the avocado plants would benefit by investing in both strategies at the same time (high SRL, BRB, and AM colonization), indicating a collaboration gradient as recently was pointed by Bergmann *et al*. (2020).

Some studies have shown a negative correlation between SRL and RBR with mycorrhizal colonization (Comas *et al*., 2014). Our results did not show a correlation between SRL and AM colonization, consistent with some studies (Lugli *et al*., 2020; Liu *et al*., 2015; Maherali, 2014). However, we observed high SRL in mycorrhizal plants suggesting higher nutrient acquisition rates through of mycorrhizal fungi (Zhang *et al*., 2024; Kong *et al*., 2017) as we pointed out before. There was a negative correlation between RBR and AM colonization but only in plants that grew in soil from orchard 1 (without nutritional management). Recently, Fassio *et al*. (2020) showed that avocado trees propagated by seed or clonal techniques differed in their root branching orders (first, second and third-order roots). Clonal root systems branched more extensively and developed a vascular system with a larger transport capacity than seed root systems (Fassio *et al*. 2020).

Our findings suggest that roots of avocado could have phenotypic plasticity and can respond to soil conditions, at least in young plants grown in a nursery. The analysis of root traits is a novel topic that will help reduce the amounts of fertilizers required to achieve the same yield and reduce the negative effects of agricultural practices on soil ecosystems and their functions. Furthermore, understanding root traits is essential for comprehending plant responses to climatic change, and their adaptation to heterogeneous environments. However, root traits are likely shaped by multiple evolutionary pressures.

Fungal measurements

Our results showed that the plants growing in the soil from O1 had greater root colonization by hyphae, vesicles, and arbuscules. This finding agrees with a previous study under field conditions, which showed that avocado plants from O1 had greater AM colonization compared with O2 (Balderas-Alba *et al*., 2019). Furthermore, several studies suggest that AM symbioses depend on stoichiometry, mainly of available N and P. In general, low availability of mineral nutrients is related to higher mycorrhizal colonization (Smith and Smith, 2011; Johnson, 2010; Johnson, 1993).

Our soil analyses showed that O1 had a greater concentration of P, N, organic matter, along with other nutrients. These nutrients are probably unavailable for the plants, compared with the O2, which received chemical nutrients available for the plants. In addition, the high content of organic matter favors microorganisms in the rhizosphere (Castellanos *et al*., 2000). It also has been reported that the loss of organic matter can reduce the exchange of essential plant nutrients such as N, P to plants (Lal, 2020; and references therein). However, the direct cause-effect relationship could be confounded by multiple factors as biotic and abiotic environments.

We observed that plants growing in soil from O1 (without nutrient management) and inoculated with AM propagules from the same orchard had greater percentages of all the fungal structures quantified. Johnson *et al*. (2010) did a reciprocal AM inoculation experiment to test if the soil is a key driver of local adaptation in arbuscular mycorrhizal symbioses. They showed that the plants (*Andropogon gerardii*) are adapted to their local soil and indigenous AM fungal communities. In the same way, Edwards *et al*. (2015) showed that rice seedlings growing in sterile soil recruited similar microbiomes to field rice plants under greenhouse

conditions. Therefore, the edaphic origin of AM fungi should be considered in agricultural management programs to obtain sustainable agriculture, given that AM fungi are part of most crops.

Finally, several studies in avocado plants both under field and greenhouse conditions have shown that avocado plants are mycotrophic and have observed colonization percentages above 75% (Rivera *et al*., 2016; Carreón-Abud *et al*., 2016; Castro-Alvarado *et al*., 2013; Osorio *et al*., 2012). Our findings showed that biotic (seeds origin, see Raya-Hernández *et al*., 2020; Morales-Londoño *et al*., 2019) and abiotic (mineral soil nutrients) factors are important drivers of the outcomes observed in avocado plants. When considering multiple mycorrhizal-influenced parameters, it may also become evident that mycorrhiza may influence some plant traits positively and others negatively. In this work, the diversity of AM fungi in the orchards was not analyzed, but the abundance of mycorrhizal propagules in both orchards was similar. Therefore, estimating the richness and abundance is necessary because fertilization can select AM fungi that are less competitive, as Johnson (1993) pointed out. Likely, the combination of fertilizers (chemical and organic) in O2 has been decisive so as not to ultimately affect the beneficial effects of mycorrhizas present in this orchard.

In conclusion, our study contributes to a more holistic understanding of the field of root-soil-mycorrhiza interactions. Different factors, including soil management and host genotype influence the root-associated microorganisms. However, the ecological processes are poorly understood. In our study, the origin of seed and soil were determinant factors for plant effectiveness (chlorophyll fluorescence and root traits) and infectivity (mycorrhizal colonization). The combination of root traits (e.g., architectural, morphological, physiological, and biotic traits) and leaf (e.g., photosynthesis rate, chlorophyll fluorescence) may improve our understanding of the mechanisms that underlie plant-microbe interactions in plant from different seeds, and soil agricultural management to enhance not only avocado production but also long-term sustainability and yield stability of avocado agroecosystems.

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Compliance with ethical standards. Consent was obtained from the orchard's owners.

Data availability. The data used to analyze the results are available with Dr. Rocío Vega-Frutis (rocio.vega@uan.edu.mx) upon request.

Author contributions statement (CRediT). A. Balderas-Alba. Conceptualization, field and lab work, data analysis, writing – original draft preparation. **R. Vega-Frutis.** Conceptualization, methodology, supervision, data analysis, writing – review and editing, funding acquisition. **G. Luna-Esquivel**. Field work, writing – review and editing.

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Supplementary Figures and Tables

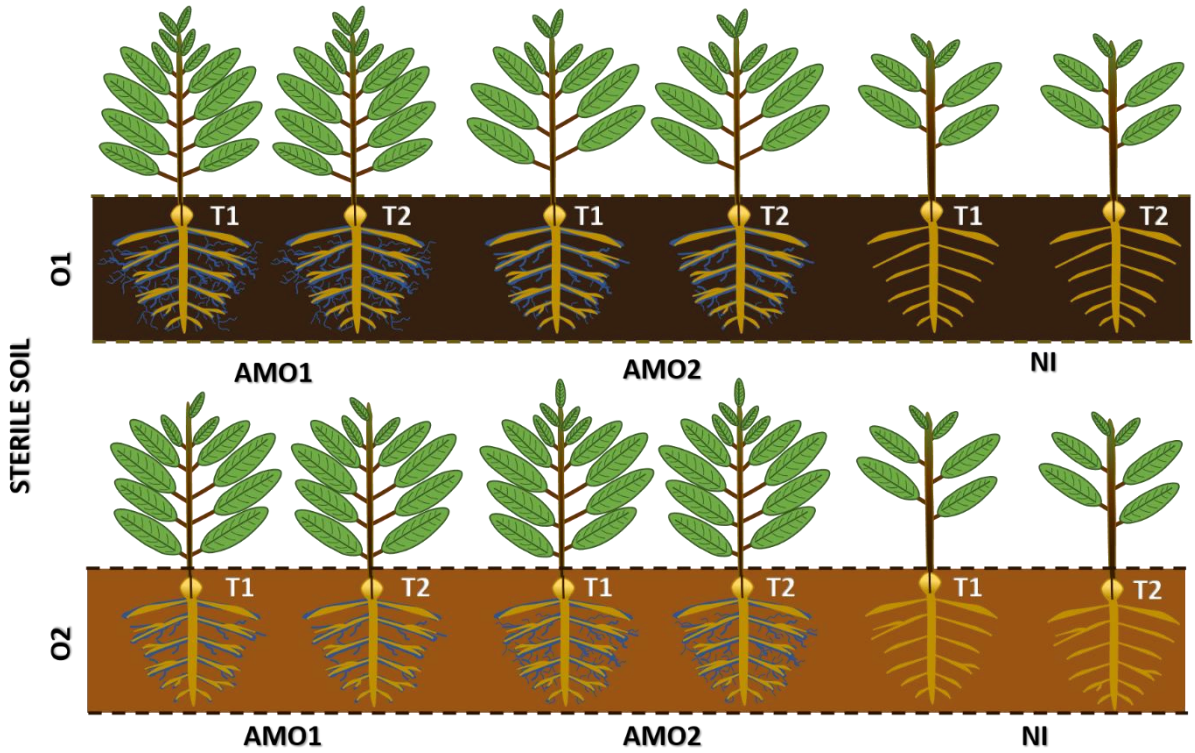

Figure S1. Schematic representation of the factorial experiment with three factors: 1) origin of the soil from orchard 1 (O1), and orchard 2 (O2) with different soil nutrition management, 2) origin of seeds from tree 1 (T1), and tree 2 (T2), and 3) origin of AM inoculum from orchard 1 (AMO1), orchard 2 (AMO2) or without inoculation (NI). We had 10 replicates of each treatment combination, with 120 plants.

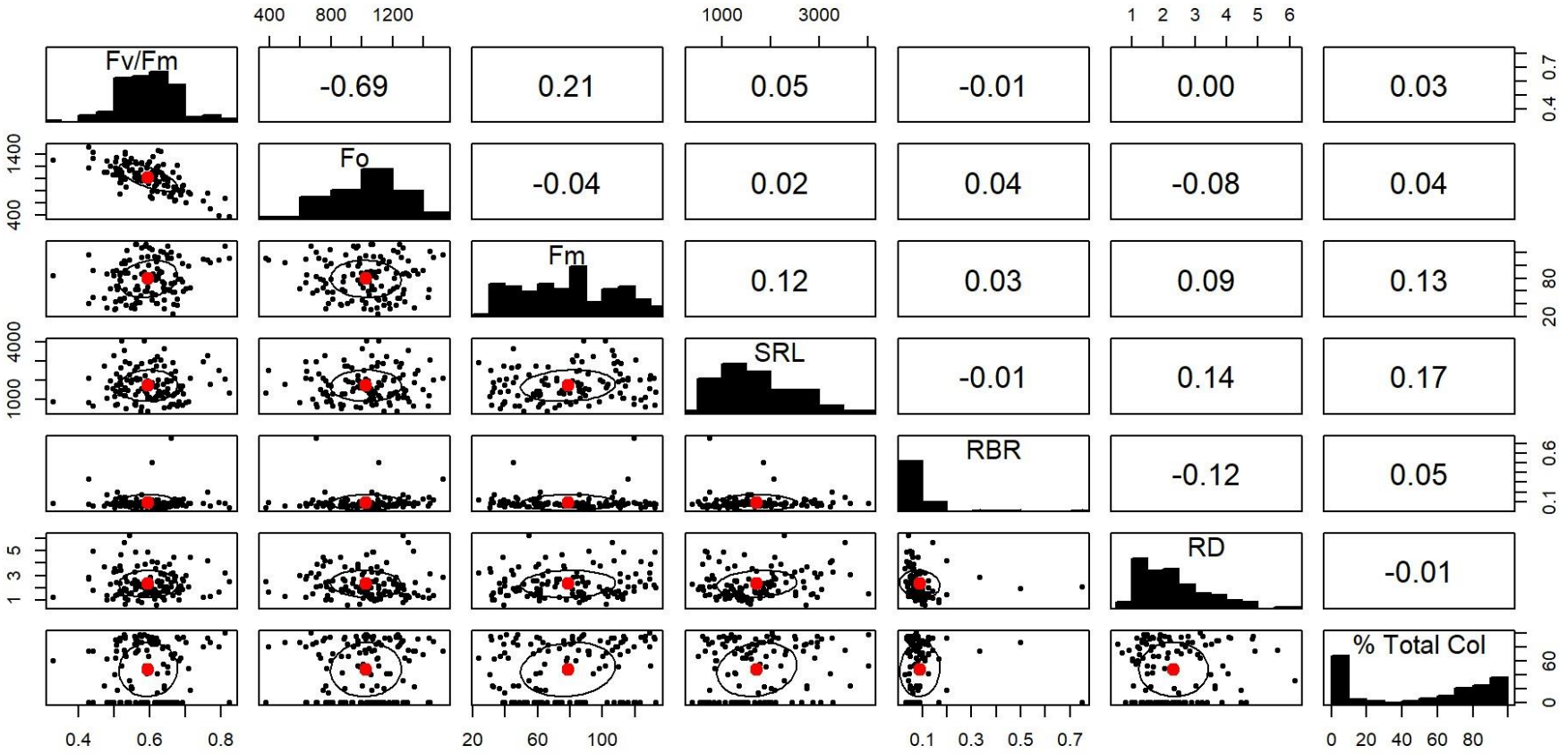

Figure S2. Multi-panel scartterplots of plant response variables. F_v/F_m: ratio of variable to maximum fluorescence, F_o: minimum fluorescence, F_m: maximum fluorescence, SRL: specific root length, RBR: root branching ratio, RD: root diameter, and % Total Col: percentage of AM colonization. The upper/right panels contain Pearson correlation coefficients, and the lower/left panels show pairwise scatterplots between the variables. The red dot enclosed in the ellipse represents the mean. The histograms of the variables are shown on the main diagonal panels.

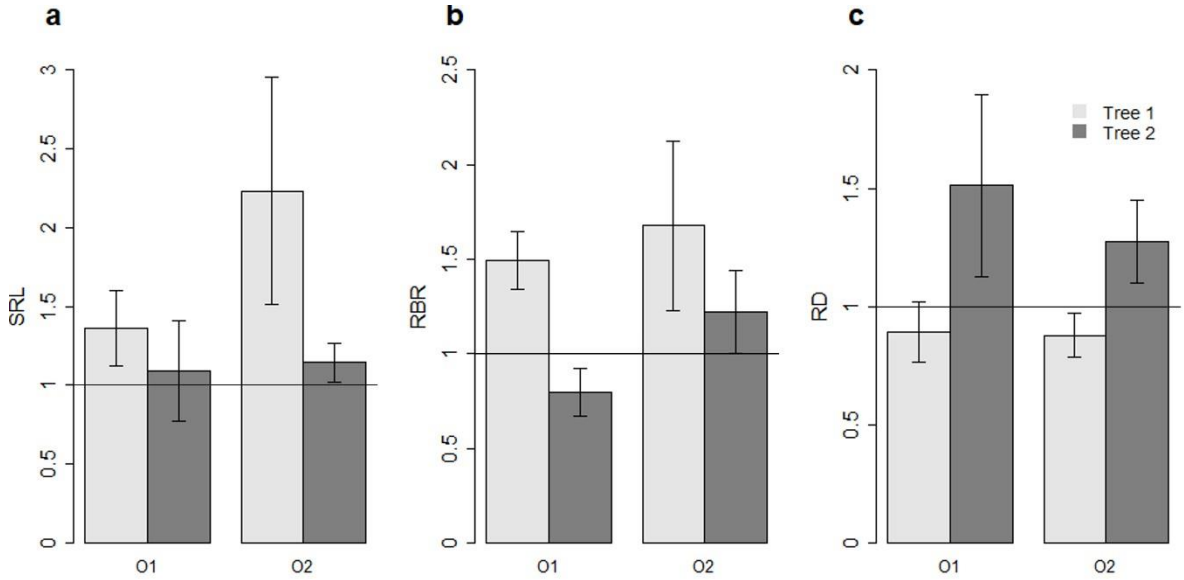

Figure S3. Mycorrhizal plant benefit, defined as the ratio between mycorrhizal and nonmycorrhizal plants in terms of (a) SRL: specific root length, (b) RBR: root branching ratio, and (c) RD: root diameter in *Persea americana* plants from tree 1 (light grey bars) or tree 2 (grey bars) that grew in soil from orchard 1 (O1) or orchard 2 (O2) and that were inoculated with AM inoculum from orchard 1 (AMO1). Values above the line denote positive mycorrhizal plant benefit.

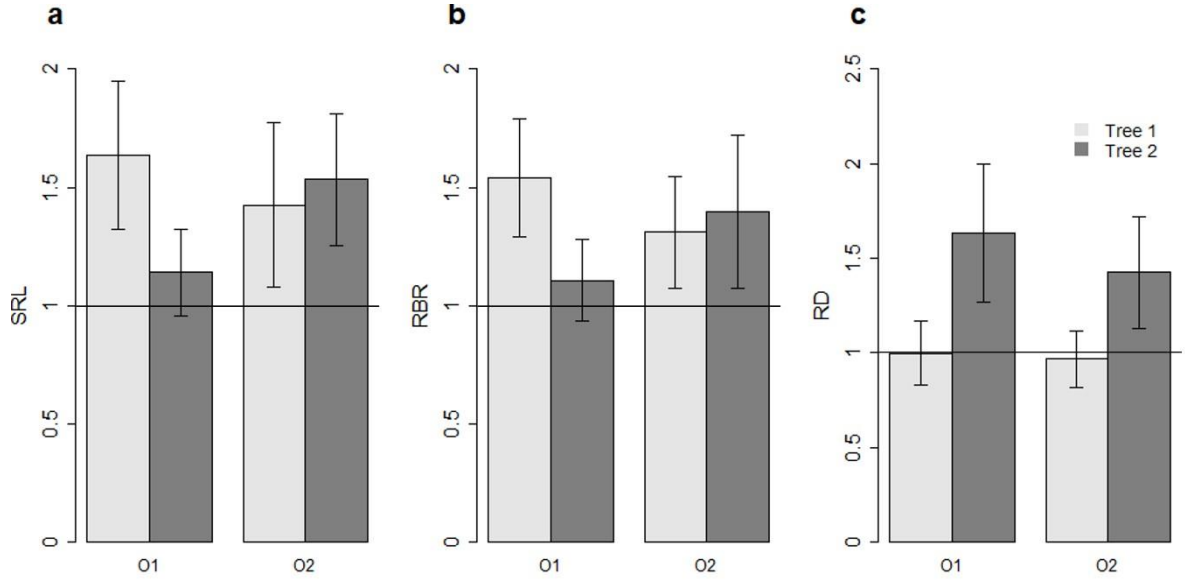

Figure S4. Mycorrhizal plant benefit, defined as the ratio between mycorrhizal and nonmycorrhizal plants in terms of (a) SRL: specific root length, (b) RBR: root branching ratio, and (c) RD: root diameter in *Persea americana* plants from tree 1 (light grey bars) or tree 2 (grey bars) that grew in soil from orchard 1 (O1) or orchard 2 (O2) and that were inoculated with AM inoculum from orchard 2 (AMO2). Values above the line denote positive mycorrhizal plant benefit.

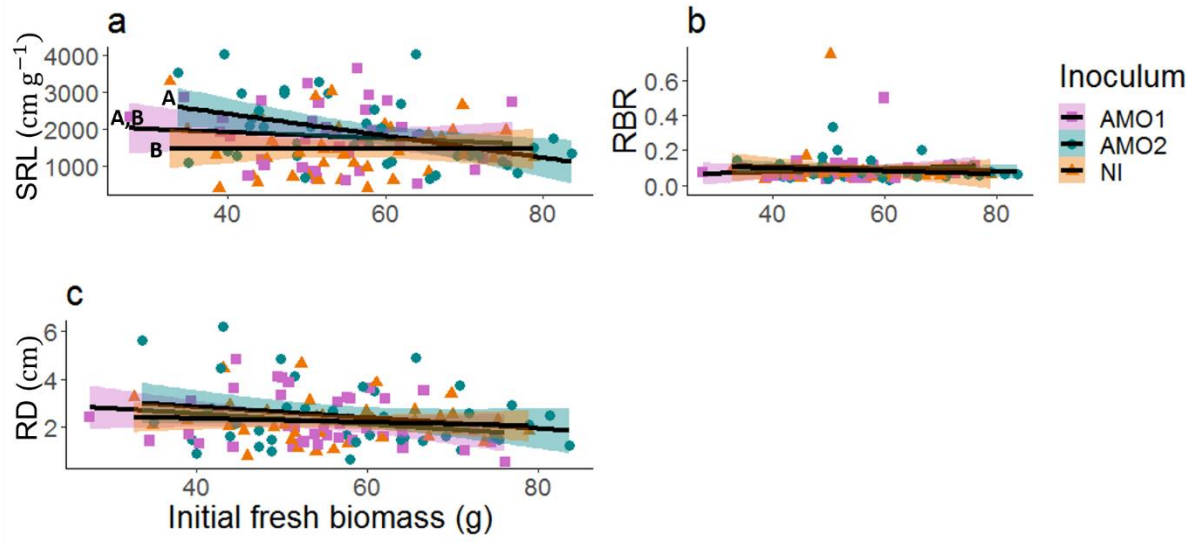

Figure S5. Interactive effects of the AM inoculation and the initial fresh biomass (covariate) on (a) SRL: specific root length, (b) RBR: root branching ratio, and (c) RD: root diameter in *Persea americana* plants inoculated with AM fungi from the O1 (AMO1, orchid square), O2 (AMO2, turquoise circle) and a control (NI, orange triangle). The respective lines represent the regression lines with their confidence intervals (shaded). Different letters in the lines indicate statistically significant differences among AM inoculum or origin of seed respectively according to *lsmeans* contrast *P* (<0.05).

Table S1. Summary of sterilized soil parameters quantified from orchard 1 (O1) and orchard 2 (O2) located in the State Reserve Sierra de San Juan, Nayarit, Mexico. P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium, Fe = iron, Zn = zinc, Mn = manganese, Cu = copper, OM = organic matter, TN = total nitrogen, CEC = cation exchange capacity.

	Determination	Properties	
		O1	O2
ppm = mg kg^{-1}	P-Bray	52	2
	K (acetate NH_4 pH 7)	503.1	390
	Ca (acetate NH_4 pH 7)	1966	544
	Mg (acetate NH_4 pH 7)	738.8	133.65
	Fe (DTPA)	82.5	77.9
	Zn (DTPA)	2.2	0.4
	Mn (DTPA)	147.4	103.2
	Cu (DTPA)	0.3	1.12
%	OM	6.19	3.25
	TN (Kjeldahl)	0.87	0.66
	pH (1:2 H_2O)	6.31 (mod. acid)	5.21 (mod. acid)
cmol kg^{-1}	CEC	16.27	9.98
%	Texture	Sandy clay crumb	Clay crumb
	Clay	24.2	34.2
	Silt	26.0	24.0
	Sand	49.8	41.8

Table S2. Spearman correlations between arbuscular mycorrhizal (AM) colonization for each level of factor evaluated [origin of seed: tree 1 and tree 2, origin of soil: orchard 1 and orchard 2, and origin of AM inoculum: from orchard 1 (AMO1) and orchard 2 (AMO2)], and RD (cm) = diameter of roots, SRL (cm g^{-1}) = specific root length, RBR= root branching ratio, F_0 (µmol m^{-2} s^{-1}) = minimal fluorescence, F_m (µmol m^{-2} s^{-1}) = maximum fluorescence, and F_v/F_m = ratio of variable to maximum fluorescence. Significant values are shown in bold.

Variables	Origin of seed		Origin of soil		Origin of AM inoculum	
	Tree 1	Tree 2	Orchard 1	Orchard 2	AMO1	AMO2
	Mycorrhizal colonization (%)					
RD	rho = -0.09 P = 0.59	rho = 0.11 P = 0.50	rho = 0.17 P = 0.31	rho = -0.20 P = 0.24	rho = -0.04 P = 0.79	rho = 0.13 P = 0.44
SRL	rho = -0-11 P = 0.51	rho = -0.14 P = 0.42	rho = -0.18 P = 0.30	rho < 0.01 P = 0.98	rho = -0.18 P = 0.27	rho = 0.08 P = 0.65
RBR	rho = 0.08 P = 0.61	rho = -0.16 P = 0.33	rho = -0.36 **P = 0.03**	rho = 0.30 *P = 0.07*	rho < 0.01 P = 0.97	rho = -0.11 P = 0.51
F_0	rho = 0.18 P = 0.26	rho = 0.02 P = 0.86	rho = 0.18 P = 0.27	rho = 0.12 P = 0.47	rho = 0.07 P = 0.65	rho = 0.12 P = 0.47
Fm	rho = 0.13 P = 0.43	rho = 0.04 P = 0.81	rho = -0.01 P = 0.95	rho = 0.20 P = 0.22	rho = 0.26 P = 0.10	rho = 0.02 P = 0.87
F_v/F_m	rho = -0.12 P = 0.46	rho = 0.02 P = 0.87	rho = -0.13 P = 0.42	rho = -0.03 P = 0.86	rho = 0.07 P = 0.66	rho = -0.09 P = 0.55

Supplementary Material and Methods

Inoculum preparation

The 20 g of fungal propagules were constituted by approximately 332 ± 42.394 (mean ± standard error) and 402 ± 25.438 spores from O1 and O2 respectively. The spores were extracted using the wet sieving and decanting method (Gerdemann and Nicolson 1963) in three replicates per orchard and counted at 35x total magnification with a stereomicroscope (LEICA EZ4). For extraradical mycelium, we used 5 g of dry soil from three samples per orchard, and the Sylvia (1992) method was used to extract the mycelium. The length of extraradical mycelium was estimated using a grid in the eyepiece of a light microscope (ZEISS Primo Star) at 400x total magnification, in agreement with the formula of Brundrett *et al*. (1994). On average, 2.895 ± 0.143 cm g^{-1} soil and 2.672 ± 0.236 cm g^{-1} soil of extraradical mycelium were estimated from O1 and O2 respectively. In addition, we separated and weighted all potentially colonized root fragments from three dry soil samples of 5 g per orchard, and 25.900 ± 4.696 mg and 28.766 ± 2.907 mg of root fragments from O1 and O2 respectively were obtained.

Chlorophyll fluorescence

Chlorophyll fluorescence assesses the photosystem II (PSII) status and surrogates the overall plant physiological status. The main fluorescence parameters are i) minimal fluorescence (F_0) is the level of fluorescence when Q_A [primary quinone electron acceptor of photosystem II (PSII)] is maximally oxidized (PSII centers open), ii) maximal fluorescence (F_m) is the level of fluorescence when Q_A is maximally reduced (PSII centers closed), and iii) the ratio of variable to maximum fluorescence (F_v/F_m) provides an estimate of the maximum efficiency of PSII photochemistry at a given PPFD (photosynthetically active photon flux density), which is PSII operating efficiency if all the PSII centers were open. Optimal values of F_v/F_m has been estimated to around 0.8 (± 0.05) in most plant species including cultivated plants. Values lower than 0.75 are considered an indicator of damage caused by abiotic stresses to photosystem II (photoinhibition, Maxwell and Johnson 2000; Baker 2008; Márquez 2017).

Mycorrhizal plant benefit

Mycorrhizal plant benefit, defined as performance ratio between inoculated plants and noninoculated plants (Kytöviita *et al*. 2003), was calculated for SRL, RBR, RD. Because our factorial experiment included 80 inoculated plants and 40 non-inoculated plants, non-inoculated plants were randomly sampled (within each orchard) with replacement and assigned to each inoculated plant to estimate mycorrhizal dependency. The above-described procedure was bootstrapped 1000 times to minimize biases. Then we worked out whether the mean mycorrhizal dependency estimates in each orchard differed significantly from the random expectation (Fig. S6 and Fig. S7). Ratios > 1 indicate that AM colonization was beneficial to the avocado plants, ratios < 1 indicate that AM colonization was detrimental to the avocado plants, and ratios close to one indicate no net mycorrhizal benefit for the plant.

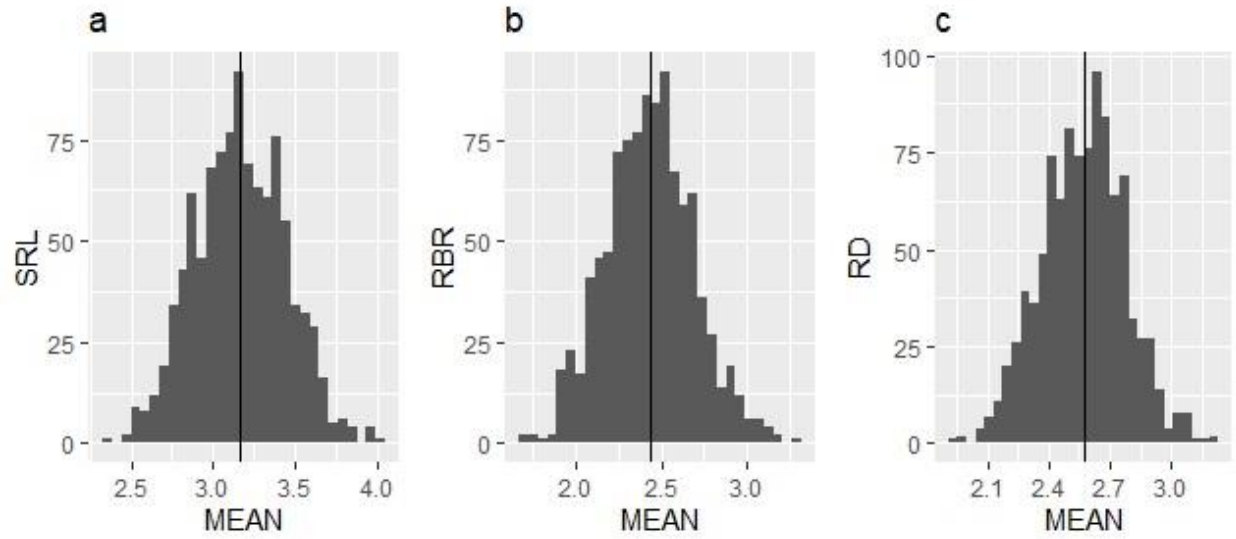

Figure S6. Mean of mycorrhizal plant benefit calculated (black line) and histograms of the means after 1000 random resamplings for plants inoculated with AM from orchard 1 (AMO1). The mycorrhizal plant benefit was calculated for the variables (a) SRL: specific root length, (b) RBR: root branching ratio, and (c) RD: root diameter.

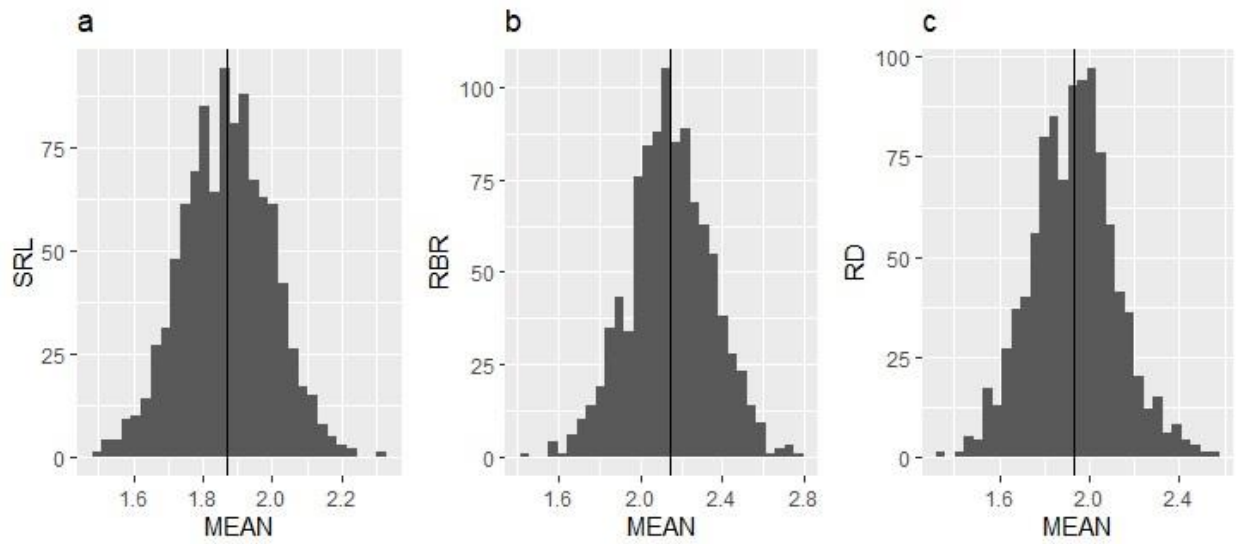

Figure S7. Mean of mycorrhizal plant benefit calculated (black line) and histograms of the means after 1000 random resamplings for plants inoculated with AM from orchard 2 (AMO2). The mycorrhizal plant benefit was calculated for the variables (a) SRL: specific root length, (b) RBR: root branching ratio, and (c) RD: root diameter.

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