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REGULAR PAPER

Mycorrhizae: a key interaction for conservation of two endangered Magnolias from Andean forests

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Background and aims – *Magnolia* species are highly endangered in neotropical forests where they are highly endemic and often very rare. However, little is known about their nutritional and soil conditions in natural forests. In this study, we focused on two endangered *Magnolia* species that cohabit in the Colombian Andean cloud forests in order to identify their conservation and nutritional status. We hypothesize that these species might exhibit mycorrhizal colonization that enhance nutrients uptake in poor and disturbed soils.
Methods – Individuals of *Magnolia jardinensis* and *M. yarumalensis* were assessed in 11 000 m^2 of Andean forests remnants from Jardín municipality (Antioquia, Colombia). Foliar and soil samples were analysed in the lab. Through a Principal Component Analysis (PCA) we identified the relationship between soil conditions and foliar nutrition. Root fragments and rhizosphere samples from seedlings and juveniles up to 3 m tall were collected to verify mycorrhizal colonization and presence of other microorganisms. Adults were excluded of the sampling due to the difficulties to differentiate their roots among the rest of the species in the forest fragments.
Key results – The surveys show that the *M. yarumalensis* population has an inverted J-shaped diametric distribution suggesting a potential recovering population while the smaller overall distribution of *M. jardinensis* in all diametric categories suggests that this species is likely to become extinct. Both species grow in acidic, infertile soils, although foliar nutrient concentrations did not correlate with soil-nutrient availability. Such a discrepancy and the high colonization levels of mycorrhizae (60–70%) and dark septate endophytes (40–45%), suggest that plant-microorganisms may facilitate nutrition and enhance survival of *Magnolia* species in stressed environments. Other fungi and bacteria were also found in their rhizosphere, but their role with respect to *Magnolia* species remains unclear.
Conclusions - Mycorrhizal colonization of endangered *Magnolia* species seems to play a key role to their performance in natural disturbed Andean forests. Aspects related to soil and rhizosphere ecology should be included in conservation projects for endangered and endemic plants.

Key words – Rhizosphere, Andean cloud forests, *Magnolia jardinensis*, *Magnolia yarumalensis*, soil fertility, tree nutrition, conservation, mycorrhizae.

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INTRODUCTION

Almost half of flowering plants are endangered, many of them in tropical countries with high diversity and unusually rapid rates of habitat depletion (Pitman & Jørgensen 2002, Vinogradov 2003). Nevertheless, not all the species are equally prone to extinction; those with restricted distributions, small populations and/or low densities are the most vulnerable (Primack et al. 2001).

Among the most vulnerable are those species in Magnoliaceae, which in Colombia are mainly found in Andean forests. At present, they are frequently recorded as isolated trees or in small forest remnants with null or recruitment limited to one or two seedlings (Yepes 2007), suggesting that fragmentation could affect reproduction and population growth (López et al. 2008). *Magnolia jardinensis* M.Serna, C.Velásquez & Cogollo (Serna et al. 2009) is considered critically endangered (CR) (IUCN 2017) because it is only recorded from a single locality in Antioquia province and its unique known population has fewer than 50 individuals. Another species, *Magnolia yarumalensis* (Lozano) Govaerts, is considered endangered (EN) (IUCN 2017) because it has experienced habitat loss and overharvesting for selective timber extraction, which has led to a greater than 50% population reduction within the last three generations (Rivers et al. 2016). Both of these Colombian endemics exhibit scarce fruit formation despite producing numerous flower buds the year round. The reasons for scarce seed production rates and low seedling recruitment in habitat are not well understood but are probably due to specialized pollinators and/or soil conditions. As well, seed germination rates in laboratory assays are in some cases relatively low (25–48%) with two pre-germinative treatments: darkness and pre-hydration for about 12 hours (CORANTIOQUIA 2011).

Andean cloud forests are characterized not only by high annual precipitation, but also by steep slopes which make them prone to erosion (Armenteras et al. 2011) and nutrient leaching, especially when the litter cover has been removed by anthropogenic disturbances, as has been recorded in other tropical forests (Chen et al. 2014). Some studies reveal that chemical (e.g. inorganic nitrogen and aluminium concentration; Andersen et al. 2010) and textural features of soils may explain species distribution (Clark 2002, Cámara-Leret et al. 2017). In turn, the spatial distribution of trees also influences quantity and type of available nutrients (Bruijnzeel 1991). In particular, availability of soil nutrients and mycorrhizal colonization might mediate plant growth and establishment according to forest fragment size (Grilli et al. 2013). In fact, almost all known plants develop some kind of symbiosis with fungi (Rodriguez & Redman 2008), and this appears to have contributed to the early evolution of land plants (Krings et al. 2013).

The rhizosphere is defined as the soil region where processes mediated by microorganisms are specifically influenced by the root system (de Souza et al. 2015). Associations with mycorrhizal and endophytic fungi inside the roots allow plants to resist drought (Rodriguez et al. 2008), enhance root development, influence sexual reproduction, and inhibit the growth of close relatives in cultivated species (Rodriguez et al. 2009). Mycorrhizae also play an important role in seedling establishment (Pellissier et al. 2013) and might contribute to the restricted distribution patterns of rare plants such as orchids (Otero et al. 2007, Swarts & Dixon 2009). To our knowledge, prior to this study nothing was known about which microorganisms are present in the roots and rhizosphere of *M. jardinensis* and *M. yarumalensis* and how their presence might influence population structure, tree nutrition, and soil fertility. We hypothesize that nutrient uptake by the *Magnolia* species in this study, which are growing in extreme environmental conditions characterized by poor soils, steep slopes, high precipitation and fragmented forests, is enhanced by mycorrhizal interactions.

The goal of this work was to identify variables that might influence the establishment of these trees in stressful soil conditions. To do so, we investigated *M. jardinensis* and *M. yarumalensis* trees in the Andean cloud forests located within the Municipality of Jardín (Antioquia province, Colombia). To evaluate the population structure, we set plots to assess trees of both species at all size categories. To assess soil fertility, we collected soil samples in each plot and analysed levels of macro- and microelements. To study tree nutrition, we collected leaves and measured foliar nutrients of adult trees for both species. We expected that leaf nutrient concentrations would correlate to soil fertility. Finally, we conducted a preliminary analysis of the microorganisms present in the roots and rhizosphere of these two endangered species. To do so we collected root fragments and rhizosphere samples of seedlings and saplings of both species to identify mycorrhizae and other endophytes inside root fragments as well as mycorrhizal spores contained in the rhizosphere. Other microorganisms were also isolated from root fragments and rhizosphere samples. As far as we know, this work is the first examination of the state of endangered *Magnolia* plants in natural forests from the perspective of soil and rhizosphere ecology.

MATERIAL AND METHODS

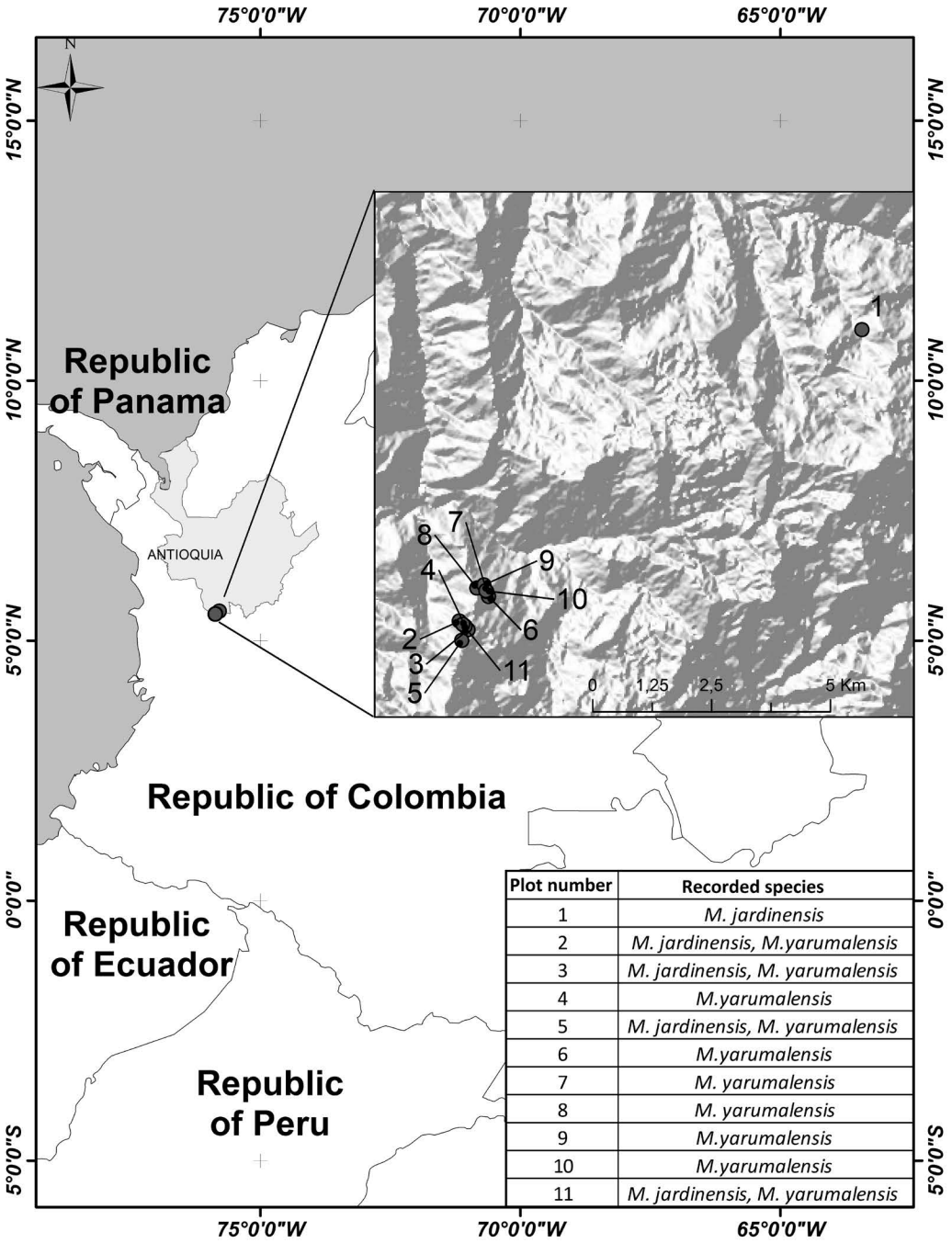
Study area

The study was performed in Jardín municipality (Antioquia, Colombia) in disturbed montane forest remnants with individuals and populations of *M. jardinensis* and *M. yarumalensis*. The forests are located between 2000 and 2550 m a.s.l. with an average air temperature from 12 to 18°C and 2000–4000 mm/year of rainfall (Holdridge 1978) and characterized by species such as *Croton magdalenensis* Müll. Arg., *Quercus humboldtii* Kotschy ex DC., *Wettinia quinaria* Burret and representatives of the genera *Nectandra*, *Blakea* and *Ladenbergia*. Scarce *Magnolia* trees were found in the study area, which is composed of grasslands and highly disturbed forests affected by selective timber extraction, including *Magnolia* trees logging (fig. 1).

Field sampling

Circular plots of 1000 m^2 (17.8 m radius) were established with a centroid tree of any of both *Magnolia* species (morphology of each species is shown in fig. 2). Within each plot, diameter at breast height (dbh) and total height of all individuals of *Magnolia* were measured and classified into ten diametric (size) classes based on dbh as follows: seed-

lings (dbh = 0–1 cm), saplings (1–2.5 cm), juveniles (2.5–10 cm), adults (> 10 cm). Tree basal area was calculated only for adult trees. Soil samples (1 kg) for physical and chemical analysis were collected around each plot and kept in plastic bags. At the laboratory, variables were calculated using the following methods: texture by *Bouyoucos* hydrometer method; pH in water 1:1, weight/volume; organic matter (OM) content (%) and organic C (%) by Walkey & Black; P ($mg.kg^{-1}$) by Bray II and colorimetric method; K, Ca, Mg ($cmol_c.kg^{-1}$) by ammonium acetate at pH 7.0 and atomic absorption; Fe, Mn, Cu, Zn ($mg.kg^{-1}$) by Olsen (0.5 M $NaHCO_3$ and EDTA) and atomic absorption; B ($mg\ kg^{-1}$) extracted by hot water; Al ($cmol_c.kg^{-1}$) by 1 M KCl and atomic absorption; S ($mg.kg^{-1}$) by 0.008 M monocalcium phosphate; NO_3^- ($mg.kg^{-1}$) by 0.025 M aluminum sulphate; NH_4 ($mg.kg^{-1}$) (1 MKCl); cation exchange capacity (CEC) ($cmol_c.kg^{-1}$) by the sum of exchangeable cations. For foliar analysis we collected 15–20 recently matured leaves (characterized by a shining light green colour, easily distinguishable from older leaves, and free from insects or fungi attack), from the centroid adult tree in each plot. These leaves were taken and kept in paper bags (Osorio & Ruiz 2013). Foliar and soil samples were



Plot number	Recorded species
1	*M. jardinensis*
2	*M. jardinensis, M.yarumalensis*
3	*M. jardinensis, M. yarumalensis*
4	*M.yarumalensis*
5	*M. jardinensis, M. yarumalensis*
6	*M.yarumalensis*
7	*M. yarumalensis*
8	*M. yarumalensis*
9	*M.yarumalensis*
10	*M.yarumalensis*
11	*M. jardinensis, M. yarumalensis*

Figure 1 – Sampling location of *M. jardinensis* and *M. yarumalensis*.

analysed in the Soil Laboratory of Universidad Nacional de Colombia, Medellín campus. To identify differences in soil nutrient availability among sites, soil variables were preliminary analysed through a principal components analysis (PCA) and then soil nutrient concentrations among the assessed plots were compared using Kruskal-Wallis tests conducted in R (R StudioTeam 2015). To evaluate the relationship of soil fertility and tree nutrition, as well as distribution of adult trees of each species, principal components analysis (PCA) was performed using Canoco v 4.56 (ter Braak & Šmilauer 2009).

For estimation of fungal colonization in roots in the laboratory, root fragments and rhizosphere samples (5 g) were taken from juveniles of both *Magnolia* species in each plot (Becker & Castillo 1990, Sánchez de Prager et al. 2010).

Mycorrhizal colonization in roots and spores isolated from the rhizosphere

Root fragments (from three to five root fragments per individual) were initially washed with water, boiled for 10 minutes in a solution of 10% KOH and subsequently washed three times with water. Fragments were submerged in a solution of 2.5% v/v vinegar-ink (Vierheilig & Piché 1998, Dalpé & Séguin 2013) and autoclaved (5 min at 121°C and 15 psi) to stain the tissues (Dalpé & Séguin 2013, Sánchez de Prager et al. 2010). The fragments were cut into 1-cm pieces and observed using an optical microscope at 40×. To estimate root colonization by fungi, we employed the gridline intercept method (Giovannetti & Mosse 1980) using slides of 75 × 25 mm gridded with 2 mm squares. For each plant, we scored the presence of any arbuscular mycorrhizal fungal (AMF) structures (hyphae, vesicles or arbuscules) at 100 random intersections. Dark septate endophytes (DSE) hyphae and microsclerotia-like structures were also recorded.

Rhizosphere samples were dried down in a stove to obtain the number of spores from 5 g of dried soil. Water was added to the soil and samples were stirred (30 min at 180 rpm) and sieved with 250 µm, 106 µm and 38 µm sieves. Ten ml of 50% sucrose and water were added until obtaining samples of 50 mL, which were stirred again (5 min at 1500 rpm). The residual solution was decanted through a 38 µm sieve, washed and placed on a marked paper with guide lines of 1 cm each to facilitate spore counting. Spores were separated by morphotypes (i.e. by morphological characters such as size, colour, shape and hyphal connection) (de Oliveira Freitas et al. 2014). After counting, some intact spores were mounted on slides for identification. This was undertaken with taxonomic keys for these groups (Oehl et al. 2011) and online references (http://invam.wvu.edu/, http://www.zor.zut.edu.pl/Glomeromycota/Taxonomy.html).

Isolation and identification of microorganisms from roots and rhizosphere

Several root fragments per plant, each c. 1 cm in length, were surface sterilized by a 2 min wash with tap water and 0.01% Tween 20 (Polyethylene glycol sorbitan monolaurate, Polyoxyethylenesorbitan monolaurate), a 1 min and 0.5 min wash with 70% ethanol and 2.5% sodium hypochlorite, and finally, a wash with sterile water. Several roots fragments of each plant were incubated in a Petri dish with potato dextrose agar (PDA) and nutritive agar (NA). Petri dishes were incubated at 25°C.

One g samples of rhizospheric soil were prepared, each sample dissolved in 9 mL of sterile distilled water (dilution 10^{-1}) and stirred in a vortex. This procedure was repeated successively until reaching a 10^{-5} dilution. One hundred µL of 10^{-2} and 10^{-4} dilutions were plated in Petri dishes with PDA to observe growth of fungi and 100 µL of 10^{-3} and 10^{-5} dilu-

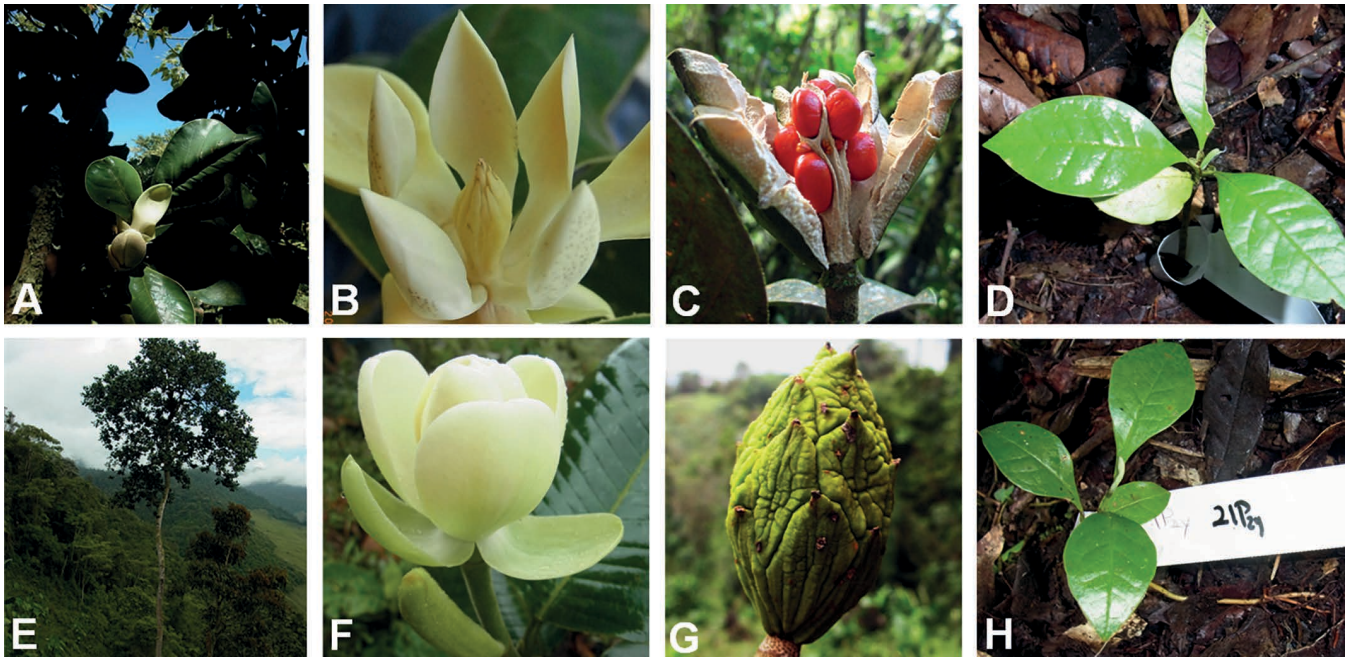


Figure 2 – Studied species. *Magnolia jardinensis*: A, adult tree; B, flower; C, fruit; D, seedling. *Magnolia yarumalensis*: E, adult tree; F, flower; G, fruit; H, seedling. Photographs by: Marcela Serna.

tions in Petri dishes with NA to observe bacteria. All samples were incubated at 25°C in the laboratory for 72 hours. This procedure was repeated in three replicates. The most abundant fungi and bacteria growing on plates were subcultured on fresh PDA or NA medium for further identification.

Fungi identification was carried out in the laboratory of Safer Agrobiológicos S.A. (Medellín, Colombia) through direct observations of shape, colour and type of edge of colonies (Girmé et al. 2014). Bacteria were identified by means of biochemical profiles with automated equipment Vitek-2 in Tecnimicro laboratory (Medellín, Colombia).

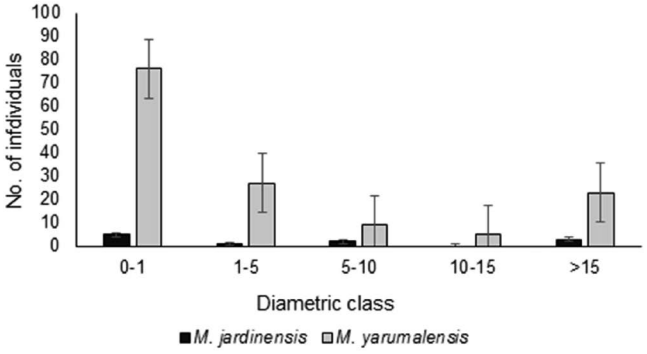


Figure 3 – Diametric distribution of *M. jardinensis* and *M. yarumalensis*. Error bars represent the standard error of the mean.

RESULTS

Diametric distribution of *Magnolia* trees

The total sampled area, with 11 circular plots, was 11 000 m^2. Adults of *M. jardinensis* were the centroid trees in two plots while trees of *M. yarumalensis* were the centroids in nine plots. 151 individuals of both *Magnolia* species were assessed in all plots: 140 (76 seedlings, 13 saplings, 31 juveniles, 20 adults) of *M. yarumalensis* and 11 individuals (5 seedlings, 3 juveniles, 3 adults) of *M. jardinensis*. That means, 10 individuals ha^{-1} of *M. jardinensis* and 127 individuals ha^{-1} of *M. yarumalensis*. While the population structure of *M. yarumalensis* has an inverse "J" shape, with a large quantity of young individuals, the graph of *M. jardinensis* showed an asymmetric shape with few individuals in the first diametric classes and none in the highest classes. Diametric distribution of both species is shown in fig. 3.

Soil fertility and tree nutrition

Soils of the studied area are classified as andisols from the Andes Cordillera, which are derived from volcanic deposits and characterized by high phosphates fixation and acidity (Jaramillo 2002). According to the chemical analysis, the

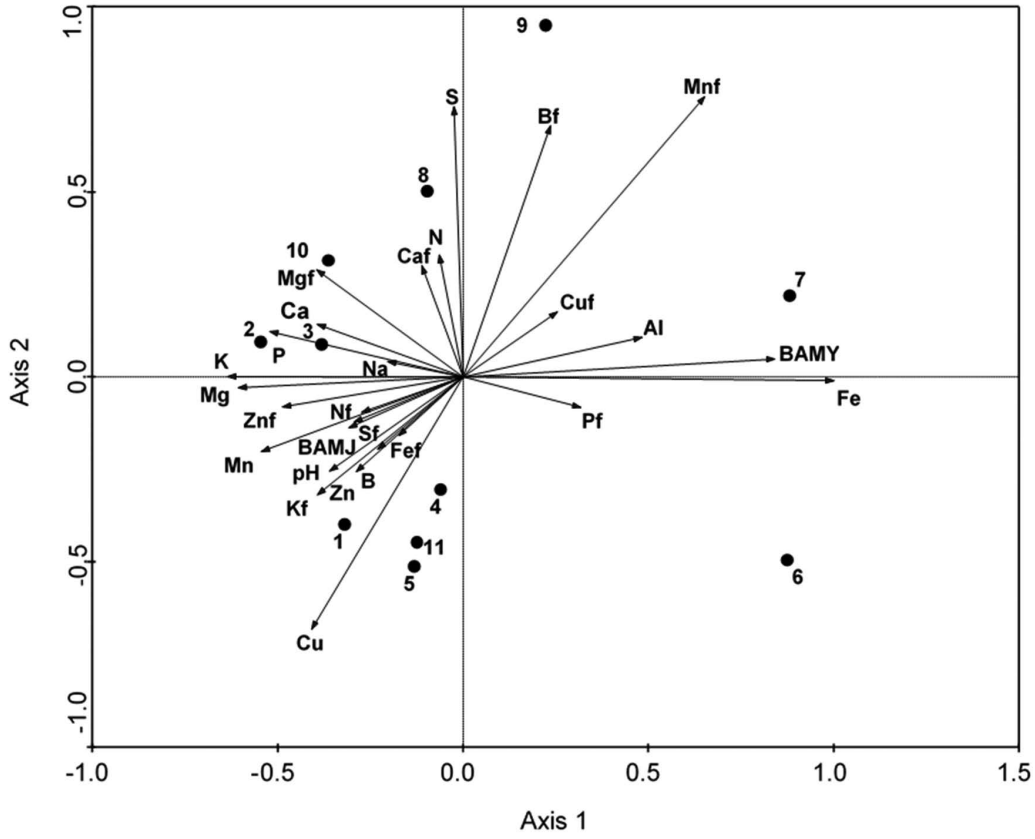


Figure 4 – PCA analysis showing the relation between nutrients in the soils and those in the leaves of trees of both species. BAMY = Basal area of *M. yarumalensis*, BAMJ = Basal area of *M. jardinensis*. Foliar nutrients are designated by "f" appended to the element name. Black circles represent the assessed plots.

samples range from very extremely acid (pH = 3.9–4.5) to strongly acid soils (pH = 5.1) according to Soil Science Division Staff (2017) and exhibit variable concentrations of P (2–17 mg.kg^{-1}), K (0.1–0.75 cmol$_c$.kg^{-1}), Ca (0.1–14.5 cmol$_c$. kg^{-1}) and Mg (0.2–8.8 cmol$_c$.kg^{-1}) (electronic appendix 1A). When analysing soil conditions of each plot through a PCA, three groups were identified (electronic appendix 1B). Differences among these groups were found for P concentrations according to Kruskall-Wallis test ($P < 0.05$).

The nutrient concentrations in the leaves of adult trees are shown in electronic appendix 2A. The percentage of essential nutrients such as N (1.17–1.73), P (0.05–0.12), Ca (0.33–1.16), Mg (0.19–0.62) and K (0.3–2.1) are considered low compared to others species of Magnoliaceae (electronic appendix 2B). Plot 8 registered the highest value of foliar B, and Plot 8 had the highest concentrations of S.

The first two axes of the principal components analysis (PCA) with eigenvalues of 0.985 and 0.012 explains 99.6 of data variance. The highest concentrations of P, Cu, Mn and B in the leaves, and Fe and Al in the soils, was related to increasing basal area values for *M. yarumalensis* (BAMY) while the highest values of pH, Mg, Mn, Cu, Zn and B in the soils and higher values of N, Zn, S, Fe and K in the leaves was correlated with increasing basal area values for *M. jardinensis* (BAMJ). In addition, while some elements in foliar tissues were positively correlated to available nutrients in the soils such as Ca, Mg, Zn and K, nutrients like P, Mn, Cu and B exhibited a negative correlation (fig. 4).

Mycorrhizal colonization, spores and other microorganisms isolated from the rhizosphere

Structures of AMF and DSE were identified after the processes of root clarification and staining (fig. 5). When comparing colonization percentage, both species exhibited similarly high levels of colonization by AMF and moderate levels of colonization by DSE (fig. 6). ANOVA analyses revealed no significant differences of colonization percentage between the species but significant differences between AMF and DSE colonization percentage in each species ($P < 0.05$).

Seedlings and juveniles were found growing on litter, which appears to be an important component of the forest rhizosphere. The number of AMF spores found in these litter-rhizosphere samples ranged from 23 to 200 spores. gr^{-1} in *M. jardinensis* and from 23 to 212 spores.gr^{-1} in *M. yarumalensis* (average of 91 and 92 spores.gr^{-1} respectively). Spores representing seven genera of AMF were identified based on descriptions in Oehl et al. (2011): *Albohypha*, *Ambispora*, *Glomus*, *Instraspora*, *Kuklospora*, *Scutellospora* and *Viscospora*.

Analysis of the rhizospheric samples in the laboratory allowed the identification through morphological descriptions of five fungi morphotypes (*Trichoderma* sp., *Aspergillus* sp., *Rhizopus* sp., *Fusarium* sp. and *Paecilomyces* sp.), that is 70% of all fungi morphotypes (7). Seven species of bacteria were identified from laboratory characterization with respect to the total (10 species, 70%): *Bacillus cereus*, *B. mycoides*, *B. sporothermodurans*, *B. thuringiensis*, *Staphylococcus lentus*, *Brevibacillus laterosporus* and *Shingomonas paucimobilis.*

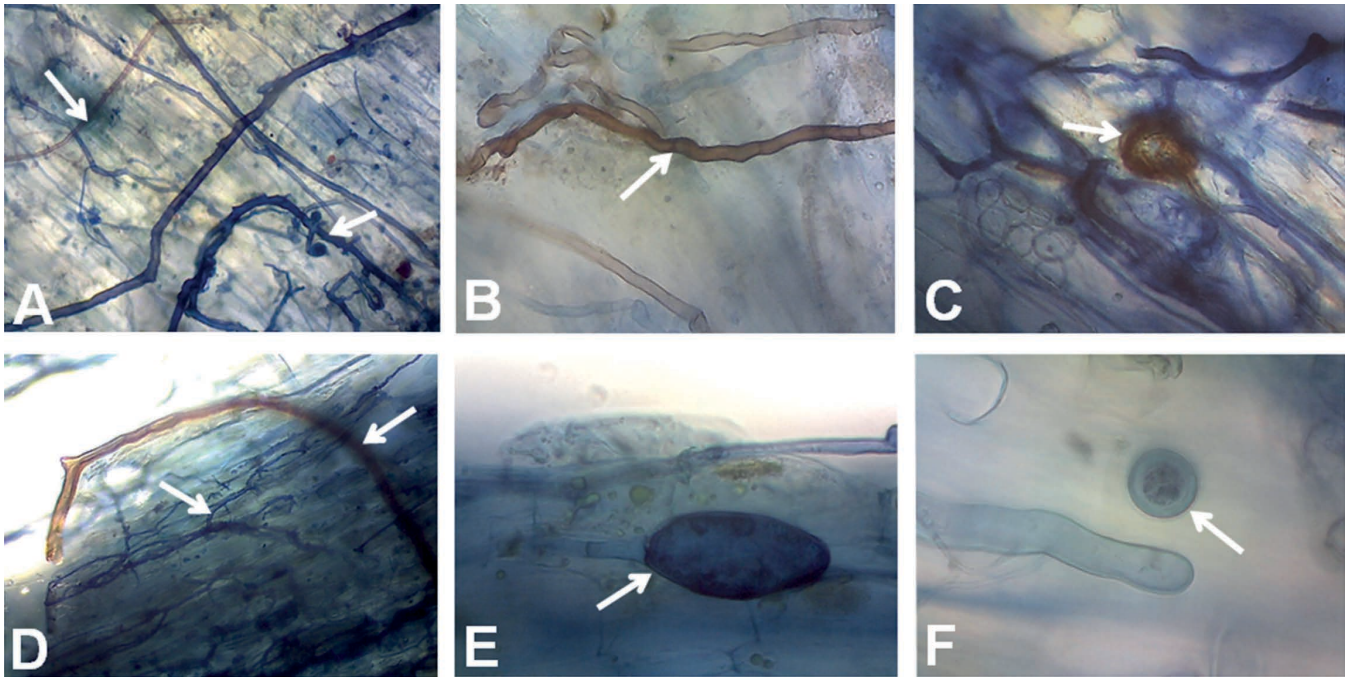


Figure 5 – Microscopic observation of AMF and DSE structures within root fragments. *Magnolia jardinensis*: A, hyphae of AMF and DSE 10×; B, visible septate hyphae of DSE; C, spore of AMF 40×. *Magonlia yarumalensis*: D, hyphae of AMF and DSE 10×; E, vesicle of AMF 40×; F, spore of AMF 40×.

DISCUSSION

Population structure of *Magnolia*

For this study, there were fewer individuals of *M. jardinensis* than of *M. yarumalensis*. *Magnolia jardinensis* was represented by only six out of the ten diametric classes. The largest tree of this species measured 45.9 cm dbh, although was not in a reproductive state by the time it was sampled. The absence of larger individuals suggests an overexploitation of this species in the past. In addition, the small number of seedlings and saplings in forest remnants also suggests poor replacement potential of old trees by young ones, reinforcing the IUCN's rating of critically endangered (CR) for this species. In fact, previous population studies of *M. jardinensis* in the same municipality exhibited the absence of seedlings and juveniles and the presence of large trees, which were attractive to wood transformation (Serna & Velásquez 2003). Current population structure of *M. jardinensis* will likely deteriorate because mature individuals are exceptionally rare, seedling recruitment is almost non-existent and size distribution is discontinuous. Besides, it is not possible to compare these results with other populations because this species is highly endemic from Colombian Andean forests and there are no reports about more populations or individuals in other localities in Colombia (Rivers et al. 2016).

The higher number of *M. yarumalensis* seedlings suggests an abundance of reproductive individuals that produce more seeds, and/or better seedling recruitment on the site. Gregarious distribution patterns are typical of *Magnolia* species, explained by barochoric seed dispersal (dispersal by gravity), finding seedlings grouped near the parent trees (Gutierrez & Vovides 1997). Cohorts of adult trees are progressively smaller due to predation, competition, harvesting and disturbance caused by the logging of large trees, causing an overall decrease in seed production. Intra- and interspecific competition for resources during growth from seedling to adult cause changes in the diametric distributions from an aggregate to an inverted J shaped distribution (Wu & Chen 2000), typical of shade-tolerant species with a decrease of large trees (Sánchez-Velásquez & Pineda-López 2009). *Magnolia yarumalensis* exhibits this pattern indicating that there are sufficient reproducing individuals to provide for seedling recruitment and suggesting a potentially recovering population. However, the decrease in the diametric classes 5 and 6 may reflect tree exploitation at sizes easier to cut and transport for further transformation, considering that these species are banned by law in local, regional and national trades. The relatively low seedling recruitment in both species could be explained by the excessive moisture and insufficient light or floor conditions as suggested in studies of other *Magnolia* species (Gutierrez & Vovides 1997, Hoshino et al. 2001), but other factors may affect population structure. Local people report that seedlings of *M. yarumalensis* have been dug up for personal collections or selling to collectors and gardeners from other places of Colombia. Another cause of scarce seedling recruitment is the forest fragmentation, which can impede litter accumulation, and diminish nutrient availability as higher temperatures and increased sun radiance may reach the forest floor. Previous studies of population structure of *M. yarumalensis* in three different localities from the Andes revealed the abundance of individuals with dbh ranging from 1 to 10 cm and the absence of seedlings and adults (Serna & Velásquez 2003), suggesting that the population assessed in this study is the least affected by forest fragmentation. In Colombia, with only 36.9% of the Andean region covered by natural forests, 5.12 million ha (62.9% of the total area) have been predicted to be cleared within the next few years (Etter et al. 2006). Further, Antioquia Province holds approximately 26% of Colombian Andean forests and deforestation rates here are currently 25 279 ha/year (Yepes-Quintero et al. 2011).

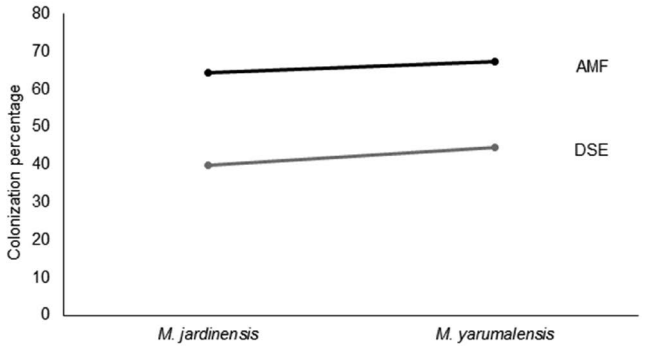


Figure 6 – Colonization percentage of AMF and DSE in roots of *Magnolia jardinensis* and *M. yarumalensis*. Error bars represent the standard error of the mean.

Soil fertility and tree nutrition

Magnolia yarumalensis and *M. jardinensis* grow in extremely poor soils. Previous studies in Colombia reveal similar soil conditions in *M. yarumalensis* (pH from 3.9 to 4.5) as recorded by Serna & Velásquez (2003). In the tropics, lower pH conditions are typical of soils with higher concentrations of Fe and Al (Jordan 1985) as seen in BAMY. Trees of *M. jardinensis* (BAMJ) appear to be associated with comparatively better soil conditions than trees of *M. yarumalensis*, showing a positive correlation with soil content of Mg, K, Mn, Cu, Zn and B. Trees of both species are influenced by soil moisture as well as availability of P and N. In this work (electronic appendix 1A), P values are considered very low (Espinoza et al. 2000). According to the soil fertility analyses, the forests in this study represent a stressed soil environment.

Regarding the nutritional status of the trees assessed in this study (electronic appendix 2A), average nutrient levels are considered low compared to data available from other *Magnolia* species (electronic appendix 2B). For example, average values of foliar P are lower than those recorded for *Magnolia sprengeri* Pamp. (Bleish & Xie 1998: 404). In addition, N values found in dry matter of *Magnolia grandiflora* L. and *Liriodendron tulipifera* L. (Perry & Hickman 1999) as well as percentages of N, P, K, Ca and Mg in dry leaf matter of *L. tulipifera* and *Magnolia macrophylla* Michx. are also higher than our recorded foliar values (Blinn & Buckner

1989). However, differences of soils and climate in natural forests from USA and Colombia must be considered.

When comparing the foliar concentrations of macro- and micronutrients found here to other species like soursop (*Annona muricata* L., Magnoliales), nutrient levels of both species but especially *M. yarumalensis* are below deficient levels of N, P and K (Castro 1996: 109). Concentrations of B in *Magnolia* leaves are also below the range of normal B levels in avocado (*Persea americana*, Magnoliidae). In seed plants, Boron promotes the assimilation of P (involved in the synthesis of RNA and DNA), mediates the transport of sugars, improves pollen grain size and pollen tube germination and contributes to resistance against diseases and climatic stresses such as low temperatures (Alarcón 2001). The low levels of nutrients, particularly B, found in the analysed trees, could be related to the low fruit production and the high floral buds abortion, particularly in *M. jardinensis*. Probably, the pollen tube germination in *M. jardinensis* is not enough to fertilize their ovules avoiding fruit formation. Studies about pollen grains germination need to be performed for Colombian *Magnolia* species in order to identify the main causes of low fruit production.

The low foliar nutrition levels found in this study could be explained by the poor fertility found in their natural forests. However, some of the foliar nutrients do not show a positive correlation with the nutrient concentration levels in the soil, suggesting that current nutrient concentrations might be mediated by mycorrhizae (Heineman et al. 2015). Under low N availability and acidic soil, plant associations with specific types of fungi seem to provide competitive advantages in favour of tree nutrition when compared to other plants, as well as general tolerance to environmental conditions (Newsham et al. 1995). However, some studies show that there are fewer mycorrhizal fungi in wet, acidic and cold habitats (Pellissier et al. 2013). Besides, the composition and structure of mycorrhizal fungi communities seem to be determined by soil factors such as pH (Fitter et al. 2011).

In stressed soil environments with surface litter, such as those in the study area, AMF could provide an increase in available nutrients (Heineman et al. 2015). Litter could thus be increasing uptake of P, N, Zn and of Cu and having an indirect effect on K uptake in P-deficient plants (Smith & Read 2008) as we have recorded. However, the correspondence between soil and foliar nutrient concentrations may not easily be detectable since slow growing shade tolerant species do not exhibit direct responses to additional nutrient supplies due to a considerable temporal uncoupling between the absorption and allocation of nutrient processes (Smith & Read 2008).

Mycorrhizal colonization and other microorganisms into the rhizosphere

Our results reveal root colonization of AMF and DSE and other microorganisms associated to the roots of both *Magnolia* species. These data are similar to other studies for *Magnolia* such as *Magnolia portoricensis* Bello, which enhances its P assimilation (Alemañy-Merly 1999) through mycorrhizae and *Magnolia cylindrica* E.H.Wilson that exhibits associations with species of the genera *Glomus*, *Acaulospora*, *Gigaspora* and *Scutellospora* (Yang et al. 2011).

Nevertheless, some studies suggest that late successional shade-tolerant species like *Magnolia ovata* (A.St.-Hil.) Spreng. are characterized by large seeds (c. 1 cm in diameter) with large reserves. Large seeds (with their enhanced food reserves), are capable of initial growth independent of mycorrhizae, and thus exhibit a low mycorrhizal dependence (Siqueira et al. 1998, Zangaro et al. 2003). According to field data, seeds of *M. yarumalensis* range from 0.7 to 1.2 cm long × 0.5 to 0.8 cm wide and are considered large, as are those of *M. ovata* (Siqueira et al. 1998). However, the high mycorrhizal colonization rates (up to 83%) found in the root fragments of seedlings and juveniles of *M. yarumalensis* suggest moderate to high mycorrhizal dependence levels which positively affect seedling establishment (Pellissier et al. 2013). In this case, both species exhibit restricted geographical distributions, which is an attribute of species with obligate mycorrhizal associations whose distribution could be conditioned by the absence of their mutualistic mycorrhizae (Pellissier et al. 2013).

Some endophytes, like the DSE found in both studied *Magnolia* species, exist in several groups of plants (Peterson et al. 2008) and are abundant in the tropics (Mandyam & Jumpponen 2005). Studies show that when inoculating with DSE, plants increase their dry weight and the concentration of P in stems and leaves. Additionally, the melanin they produce provides cell wall stiffness providing protection against herbivores and resistance to microbial attacks due to their ability to produce antibacterial and radiation protection from desiccation (Mandyam & Jumpponen 2005). *Aspergillus ustus*, considered a DSE, seems to enhance P mobilization and translocation in *Atriplex canescens* (Pursh) Nutt. (Barrow & Osuna 2002). *Aspergillus* is a known solubilizer of P compounds and other minerals that enhance soil nutrient availability for plants (Osorio 2014). In this study, we found not only a high percentage of DSE root colonization but also *Aspergillus* sp. in the rhizosphere suggesting that DSE could play a significant role related to nutrition, protection and/or resistance to stressed environmental conditions.

The nutrition of both *Magnolia* species could be also related to the presence of *Trichoderma* in the rhizosphere. These fungi are important decomposers of woody and herbaceous materials, with an ability to assimilate several substrates and produce antimicrobial compounds. Positive effects of *Trichoderma* inoculation on plants includes control of diseases, induction of systemic resistance, changes in root microflora composition, increased nutrient uptake and soil solubility and increased root development, root hair growth and deeper rooting (Cano 2011). *Paecilomyces* sp., which was found associated with both *Magnolia* species produces important antioxidants with a potential phytotherapeutic effect and may be pathogens of different insects (Whipps & Lumsden 1989).

Bacterial species recorded in the sampled rhizosphere can have a similar effect. *Brevibacillus laterosporus* inhibits the mycelial growth of pathogenic fungi and has been used as a biological control agent for insects (Reinoso et al. 2007), nematodes and mollusks (Ruiu 2013), and different strains

show broad-spectrum antimicrobial activity against other phytopathogenic bacteria and fungi (Reinoso et al. 2007, Ruiu 2013). Fungi and bacteria found on the root fragments and rhizosphere here might play a needed defence role in shade-tolerant species with slow growth such as the studied magnolias.

Some fungi are less advantageous to plants like *Rhizopus* sp., known to produce root putrescence (Kwon et al. 2001) and *Fusarium oxysporum*, a specialized pathogen (Garcés de Granada et al. 2001). In the studied *Magnolia* species, these fungi were found in low proportions, probably controlled by AMF associations, which provide defence to the host against these pathogens (Newsham et al. 1995).

The high mycorrhizal colonization found in the roots of studied *Magnolia* species suggests that both species require mycorrhizae for nutrient uptake in acid soils with low nutrient availability that is typical of high Andean forests. It is possible that nutrient deficiencies are affecting uptake of some nutrients and thus restricting the natural populations of magnolias, seedling recruitment and production of flowers and fruits. The presence of other microorganisms such as bacteria, AMF and DSE, and their interactions probably provides not only nutrients, but also greater resistance to diseases and extreme climatic conditions, as well as the production of toxins and lignified tissues, which aids in defence against predators. While there have been studies on mycorrhizae associated with litter decomposition in tropical forests, there is a need for investigations that focus on these associations as they relate more specifically to endangered plant species. Furthermore, where plant species are highly endangered, their associated AMF may also be at risk of disappearance. Further studies are necessary to identify specific mycorrhizal associations in Andean Magnolias and their implications for endangered tree species must be undertaken in order to better inform effective in situ and ex situ conservation efforts, especially as plant-soil-microbial interactions may have a profound impact on flowering and reproduction. Based on the soil results, our site 2 exhibits better levels of CEC and low Al concentrations, that could favour the establishment of juveniles and should be considered when planning reintroduction projects. However, as soil conditions are very variable even in short distances as shown in this study (plots 3, 4, 5), previous soil analyses are recommended.

SUPPLEMENTARY DATA

Supplementary data are available in pdf at *Plant Ecology and Evolution*, Supplementary Data Site (https://www.ingentaconnect.com/content/botbel/plecevo/supp-data) and consist of the following: (1) A, results of soil analysis in each assessed plot; B, PCA analysis of the soil nutrients in the assessed plots showing three groups of soil conditions; and (2) results of foliar concentration in each assessed plot.

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