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The hidden symbiont:
Exploring arbuscular mycorrhizae in the Ecuadorian Amazon

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RESUMEN

Las micorrizas arbusculares (Abr. AMF, filo *Glomeromycota*) forman una de las simbiosis terrestres más importantes, asociándose con más del 85% de plantas vasculares. Este grupo realiza una variedad de servicios ecosistémicos fundamentales que determinan la estructura de comunidades vegetales y productividad ecosistémica. En ecosistemas neotropicales, en donde se encuentra una mayor diversidad de AMF, existen pocos estudios que evalúan los impactos ecológicos, distribución y estructura de poblaciones de este grupo. Más aun, el conocimiento sobre esta simbiosis en ecosistemas naturales de Ecuador es mínimo.

La intención del presente estudio es generar información base sobre la biodiversidad y composición de comunidades de AMF asociadas a raíces de plántulas de *Inga* sp. (*Fabaceae*) en la amazonia ecuatoriana. En la Estación de Biodiversidad Tiputini de la Universidad San Francisco de Quito, ubicada en la Reserva de Biósfera Yasuní, los ecosistemas de Terra Firme y Várzea fueron muestreados para investigar si inundaciones estacionales ejercen un efecto sobre la abundancia y establecimiento de micorrizas.

Se conoce que factores abióticos del suelo son determinantes de la estructura poblacional de especies de AMF. Por lo tanto, el presente estudio evaluó el estado nutricional de suelos mediante un análisis fisicoquímico, para poder medir concentraciones de varios macro- y micronutrientes, y pH. Analizando las muestras de suelo colectadas en Terra firme y Várzea. se encontró una mayor proporción de nitrógeno total en bosques de Terra firme, en comparación con los suelos de Várzea, lo cual refleja procesos de lixiviación de este mineral., Los resultados del análisis de suelos para el resto de macro- y micronutrientes muestran que, con excepción de Mo, N y V, los suelos de Várzea presentan concentraciones más altas de macro y micronutrientes, y por lo tanto presentan suelos que son más ricos en la mayoría de nutrientes. Estos resultados se explican debido a la deposición de nutrientes durante las inundaciones estacionales por ríos de aguas blancas. Se evaluaron condiciones climáticas, como temperatura, humedad y precipitación, para el periodo de estudio 2018 a 2020, para evaluar su efecto sobre la colonización de AMF.

Abundancia de especies de *Fabaceae* subadultas y adultas, y abundancia de individuos subadultos y adultos del género *Inga* fue altos en ambos tipos de ecosistemas dentro de parcelas en EBT. Además, se evaluó la relación de brote a raíz de plántulas del genus *Inga* sp. (*Fabaceae*), recolectadas en bosques de Terra Firme y Várzea. Se obtuvieron diferencias significativas indicando que plántulas de *Inga* en bosques de Terra Firme presentaron una relación de brote a raíz más baja en comparación con plántulas que fueron recolectadas en suelos de Várzea.

Para evaluar la abundancia de AMF de forma cuantitativa, se realizó una evaluación microscópica de colonización de hifas en raíces de *Inga* sp. Adicionalmente, para la exploración del microbioma asociado a raíces de *Inga*, se utilizó secuenciación de última generación. Los resultados se analizarán con herramientas bioinformáticas para determinar los grupos taxonómicos de AMF, con la finalidad de evidenciar la presencia, abundancia y diversidad de AMF en suelos tropicales de Ecuador.

Palabras clave: Selva Amazónica, micorrizas arbusculares, simbiosis, rizosfera, Terra Firme, Várzea, micología, Ecuador, Inga, Estación Biodiversidad Tiputini.

ABSTRACT

Arbuscular mycorrhizae (Abr. AMF, Phylum *Glomeromycota*) form one of the most important terrestrial symbiotic relationships, associating with more than 85% of vascular plants. This group performs a variety of ecosystem services that are fundamental for the structure of plant communities and ecosystem productivity. In neotropical ecosystems, where a greater diversity of AMF is found, there are few studies that evaluate the ecological impacts, distribution, and population structure of this group. Furthermore, knowledge about this symbiosis in Ecuador's natural ecosystems is minimal.

The intention of the present study is to generate baseline information about the biodiversity and composition of AMF communities associated with roots of *Inga* sp. seedlings (*Fabaceae*) in the Ecuadorian Amazon. At the Tiputini Biodiversity Station from Universidad San Francisco de Quito, situated in the Yasuní Biosphere Reserve, Terra Firme and Várzea ecosystems were sampled to evaluate if seasonal floods influence the abundance and establishment of mycorrhizae.

Edaphic soil factors are known to determine the population structure of AMF species. Therefore, the present study evaluated the nutritional status of soils by means of physical and chemical soil property analyses, to measure the concentrations of various macro- and micronutrients, and pH. The analysis of soil samples collected in Terra Firme and Várzea showed a higher proportion of total nitrogen in Terra Firme forests, compared to Várzea soils, which reflects lixiviation processes for this mineral. Soil analysis results show that, except for Mo, N and V, Várzea soils present higher concentrations of macro- and micronutrients, and therefore present soils that are generally richer in nutrients. These results are explained by the deposition of nutrients during seasonal flooding by white water rivers. Climatic conditions, such as temperature, humidity, and rainfall, were assessed for the study period between 2018 and 2020, to evaluate their effect on AMF colonization.

Abundances of subadult and adult *Fabaceae* species, and abundance of subadult and adult *Inga* individuals were found to be high in both types of forest ecosystems within the TBS plots. Furthermore, the correlation between shoot and root of seedlings of the genus *Inga* sp. (*Fabaceae*) collected in Terra Firme and Várzea was evaluated. Significant differences were obtained which indicated that seedlings from Terra Firme forests resulted a lower shoot to root ratio compared to seedlings that were collected in Várzea soils.

To quantitatively assess AMF abundance, a microscopic evaluation of hyphal colonization in roots of *Inga* sp. was performed. Additionally, last generation sequencing was used to explore the microbiome associated with *Inga* roots. Results will be analyzed with bioinformatic tools to determine the taxonomic groups of AMF, to assess the presence, abundance, and diversity of AMF in tropical soils of Ecuador.

Key words: Amazon Rainforest, arbuscular mycorrhizae, symbiosis, rhizosphere, Terra Firme, Várzea, mycology, Ecuador, *Inga*, Tiputini Biodiversity Station.

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1. INTRODUCTION

1.1. Evolutionary importance of symbiotic plant-fungus associations.

One of the most important terrestrial symbiotic relationships are associations between mycorrhizal fungi and approximately 85% of all vascular plants (Brundrett, 2004; Brundrett and Tedersoo, 2018; Field & Pressel 2018; Smith and Read, 2008; Van der Heijden et al., 2015). Mycorrhizal fungi belong to a group of specialized microorganisms that colonize and inhabit the rhizosphere, which is the zone directly surrounding the surface of the plant root system (Smith and Read, 2008). This symbiosis is an ancient mutualism which is thought to have facilitated the earliest plant colonization of terrestrial ecosystems (Field and Pressel, 2018; Smith and Smith, 2011; Tedersoo et al., 2018). For more than 450 million years, this coevolution provided an important selective advantage for both symbiotic partners and explains why this relationship is so widespread in natural ecosystems (Smith and Smith, 2011).

1.2. Arbuscular mycorrhizal fungi (AMF).

Arbuscular mycorrhizal fungi (AMF) are the most diverse and widespread fungal symbionts of plants. Its members can be found in all terrestrial ecosystems (Strullu-Derrien, et al., 2018). Because of this wide distribution, they are described to perform a crucial role in the maintenance and structure of global plant communities (Marinho et al., 2018; Parniske, 2008; Walder et al., 2012). AMF are aseptate filamentous fungi that penetrate the host plant cell wall and form complex intracellular fungal structures in the apoplastic space in between plant cells, which serves as an interface for the exchange and storage of nutrients (Field and Pressel, 2018; Strullu-Derrien et al., 2018). Arbuscular mycorrhizal fungi can be identified because of their emblematic fungal structures, which on the one hand include miniature tree-like, branched structures or “arbuscules” in an Arum-type colonization of root cells, or on the other hand consist of tightly coiled hyphal structures in a Paris-type colonization (Field and Pressel, 2018;

Tang et al., 2016). Thus, both structures are involved in nutrient exchange between the plant and the fungus. Other characteristics of the establishment of AM structures are vesicles, which are enlarged intraradical hyphae which serve as nutrient reservoirs, and intercellular hyphae structures (Strullu-Derrien et al., 2018; Spatafora et al., 2016). The bidirectional exchange of fungal-acquired nutrients like phosphorus and nitrogen for plant-fixed carbon, is an integral part of the AMF symbiosis (Field and Pressel, 2018; Schappe et al., 2017). As obligate biotrophs, a part of the hyphal network of AM fungi lives inside the soil, from where they absorb nutrients. Another part of the same network resides inside the host plant root cortex, where the fungus receives organic carbon (C) in exchange for macro- and micronutrients important for plant development (Smith and Smith, 2011). Up to 20% of plant photosynthates are transferred from the host plants to AM fungi, which the fungus uses to complete its biological cycle (Tang et al., 2016). In return for these carbohydrates, AM fungi transfer the equivalent of up to 90% of the phosphorus and 80 % of the nitrogen requirements for the plant's development (Marinho et al., 2018; Smith and Read, 2008). Although this exchange is considered a costly investment for the host plant, it is through this reciprocal exchange that both partners can meet important physiological needs. In fact, it maintains a "fair trade" association that is believed to be the oldest form of plant symbiosis on planet Earth (Humphreys et al., 2010; Tang et al., 2016).

1.3. Taxonomy and phylogenetic classification of AM fungi.

Arbuscular mycorrhizal fungi belong to a monophyletic but taxonomically diverse clade within the *Glomeromycota*, a phylum distinct from the well-known phyla *Ascomycota* or *Basidiomycota*. Until the end of the last century, the taxonomy of the phylum *Glomeromycota* was based on morphological characteristics. AM fungi received their name because of the emblematic arbuscules, which have been observed for all but one genus within the

Glomeromycota (Oehl et al., 2011). Besides arbuscules, AMF identification includes spore morphology, spore formation, and specific spore cell wall structures (Oehl et al., 2011). However, due to the technological advances and easier access to molecular tools, the AMF phylogeny had to be revised. As a result, Spatafora (et al., 2016) proposed to redistribute the AM fungi in three distinct classes (*Archaeosporomycetes, Glomeromycetes* and *Paraglomeromycetes*), five orders (*Archaeosporales, Diversisporales, Gigasporales, Glomerales* and *Paraglomerales*), 16 families, 40 genera, and about 300 species known to date (Oehl et al., 2011; Öpik and Davison, 2016; Marinho et al., 2018).

1.4. AM fungi and ecosystem functioning.

Soil microorganisms, such as AMF, play an indispensable role in ecosystem functioning at multiple levels. Their functions not only orchestrate micro-interactions which happen between fungi, plant roots and soil nutrients, but also influence macro-environmental processes like plant distributions and ecosystem nutrient cycling (Field and Pressel, 2018). AMF perform a variety of ecosystem services, as their hyphal networks physically extend the range of the rhizosphere of plants. The fungus allows plants to access a larger soil volume, thereby promoting the absorption of water and nutrients (Marinho et al., 2018). Thus, AMF symbionts are believed to increase plant fitness, including boosting the plant reproductive capacity (Asghari and Cavagnaro, 2011). Mycorrhizal partnerships play an important role in the global carbon cycle by affecting soil C sequestration processes (Averill et al. 2014; Field and Pressel, 2018; Van der Heijden et al., 2015). At a local level, AMF occurrence and composition links the biotic and geochemical components of an ecosystem, with important consequences for plant dispersal, competition, and coexistence. These processes shape community composition and overall plant productivity (Cofré et al., 2019; Lugo and Pagano, 2019; Tedersoo et al., 2020). It is through these series of events, starting from the soil and ending at an ecosystem

scale, that AMF diversity and abundance, directly or indirectly, regulate important ecosystem services such as soil erosion, soil fertility, vegetation cover, invasion resistance, and pathogen and pest regulation (Lugo and Pagano, 2019). According to a study published by Rillig and Munney (2006), there are three important levels where AMF communities exert a selective pressure: Firstly, at the plant-community level, where they influence plant community structure, net primary productivity, litter quality and nutrient cycling. Secondly, at the level of the host plant, where they influence the soil water regimen, rhizodeposition and root penetration. Finally, at the fungal mycelium level, where physical, biological, and biochemical mechanisms are affected. In conclusion, AMF symbiosis is comprised of a mosaic of interactions with multiple implications, from the miniature world of microorganism, to how ecosystems are shaped and function.

1.5. Factors influencing the establishment of arbuscular mycorrhizal associations.

Edaphic factors like soil pH, water content, acidity, aeration, and the availability of nutrients can determine AMF species richness and diversity (Lugo and Pagano, 2019). Furthermore, external biotic and abiotic factors affect the soil and host plant properties (Cofré et al., 2019). Functional diversity for AMF communities depends on both plant and fungal life histories, as well as the micro and macro components of the environment (Field and Pressel, 2018). One of the most important selective biotic factors for AM fungi is host plant genetics, as root structure and root morphology can play a decisive role on which AMF species are allowed to colonize roots, and how they physically can colonize roots (Goltapeh et al., 2008). In this way, host plant preferences can determine rhizospheric AMF community dynamics (Cofré et al., 2019). The microflora in the rhizosphere of the host plant can influence the formation of AMF mycotic structures, diversity and abundance (Goltapeh et al., 2008). Abiotic factors that influence the establishment of mycorrhiza are on the one hand the prevailing

climatic conditions, and on the other hand the physical and chemical features of the soil where both host plant and fungus reside (Goltapeh et al., 2008). For example, access to light is an important factor which regulates the photosynthetic capacity of the host plant, but indirectly affects the AM fungi's access to an energy source in the form of the exchange of photosynthates. Light has been shown to stimulate development of AMF, and lack of light has been shown to reduce AMF spore production and root colonization (Goltapeh et al., 2008). Other abiotic factors like temperature and precipitation regimes can constrain AMF abundances within specific geographical boundaries, while biotic conditions such as host plant preferences can determine AMF community structures and their dynamics (Cofré et al., 2019). Temperature has been reported to influence mycorrhiza development as well, as it affected fungal spore germination, hyphal penetration of host plant roots, and hyphal proliferation within the cortical cells of the host's roots (Goltapeh et al., 2008). Finally, soil pH can also be considered a selective force for AMF community structure (Wang et al., 1993).

1.6. Arbuscular mycorrhizal fungi in Neotropical ecosystems.

The biggest effort of the scientific community working on AM fungi, has been directed towards their abundance, diversity, and functions in natural and agricultural ecosystems in temperate climatic regions. For a long time, it has been a generally accepted dogma that plants in tropical rainforests, with their exceptionally high turnover of nutrients and optimal climatic conditions around the equator, simply did not need to associate themselves with symbiotic fungi. Although at a global scale, arbuscular mycorrhiza is the most studied plant-fungus association, there is considerably less research being conducted in the tropics (Peña-Venegas and Vasco-Palacios, 2019; Cofré et al., 2019). However, in recent years, there is growing evidence that the AMF diversity in fact is higher in tropical ecosystems (Peña-Venegas and Vasco-Palacios, 2019). In general, mycologists estimate that there could be 1.5–5 million

species of mushrooms worldwide. As the number of native plants in South America is +- 144k species, and if approximately 80% of plant species worldwide have the ability to associate with AMF, in theory there might be an estimated 100,000 potential host plants (Lugo and Pagano, 2019). Moreover, 1–60 different AMF species can be associated to a single host plant. This could lead to the hypothesis that the range of AMF species associated to native plants in South America could surpass the worldwide estimate for fungal (Lugo and Pagano, 2019). Furthermore, it has been proposed that the highest number of undescribed fungal species could be found in tropical biodiversity hot spots (Lugo and Pagano, 2019). Given this extraordinary prediction about the AMF diversity in South America, there is a great potential to explore AMF within the diverse and unique biomes and geographical extension present in this region. In South America, megadiverse environments with unique ecosystems, within vast National Parks and Reserves, are areas where AMF research is still underdeveloped (Lugo and Pagano, 2019). Ecosystems that are classified as biodiversity hotspots, not only are important areas for conservation and research of the macrobiodiversity, but should be considered interesting as well for the study of microorganisms such as AM fungi. Understanding and assessing micro-ecology and -diversity in pristine ecosystems could be crucial for conservation efforts and ecosystem functioning understanding. The Amazon rainforest is thought to have existed long before the rise of the Andes mountain range, with its origin in the Late Miocene (Mörner, 2016). Due to the combination of high temperatures and humidity, Amazon soils have been leaching and show nutrient deficiencies or depletions. In fact, most of the Amazon's soil does not contain the optimal nutrient balance that is required to support tropical agriculture. Although high amounts of rainfall on the one hand can promote the decomposition of leaves and therefore favor the return of nutrients to the soil, but on the other hand, over time, this leads to lixiviation, which leaches nutrients to deeper parts or can inhibit the rate of bioavailability

of certain nutrients (Ensley-Field, 2016). Leaching due to high rainfall also has the tendency to create more acidic soils. Low net primary productivity and low species richness are often directly related to nutrient poor soils, but it is clear that this is not the case for the Amazon rainforest. In the Amazon rainforest the nutrient cycle is incredibly fast. However, the nutrient availability, such as the availability of phosphorus and nitrogen, is low. Yet, the net primary productivity and species richness are unprecedented high. Although many tropical ecosystems feature soils with low phosphorous concentrations, they still harbor the highest rates of plant diversity on the planet (Ensley-Field, 2016). Phosphorus as a limiting resource in tropical forests soils could provide an increased selective pressure on plants in these ecosystems to form AMF associations. Interestingly, tropical forests are nowadays considered hotspots for AMF diversity, as they harbor 75% of currently known members belonging to the *Glomeromycota* (Marinho et al., 2018). Therefore, it is thought that plant-associated soil fungi play a key role in promoting and maintaining high plant diversity in tropical ecosystems (Schappe et al., 2017; Bennett et al., 2017; Sheldrake et al., 2017). To fully understand the concept of nutrient availability in tropical ecosystems, a thorough understanding of soil characteristics and biogeochemical processes, and the role of microorganisms within these processes, which influence the nutrient availability, is required (Ensley-Field, 2016). Tropical forests constitute the largest terrestrial sink for anthropogenic CO_2. Therefore, these forests contribute to the regulation of the regional and global climate patterns. Anticipating the future effects of anthropogenic change in tropical forests, requires a clearer understanding of how nutrient availability limits productivity in tropical forests and to understand the specific roles of AM fungi in these complex tropical ecosystems (Alexander and Selosse, 2009; Sheldrake et al., 2017). Mycorrhizal species in tropical forests are poorly described and therefore patterns of occurrence and diversity remain largely unknown. To generate a better understanding of these

aspects at an ecological level, it is essential to generate greater knowledge about this group at a taxonomic level and the specific ecological importance of this group (de Oliveira Freitas et al., 2014). Symbiotic fungal communities in primary tropical forest ecosystems can be complex, and so are the factors that drive the establishment of the symbiotic communities. It is essential to evaluate the different spatial-temporal factors that affect the distribution of AM fungal species in order to understand ecological and evolutionary processes, both for AM fungal communities and for host plants.

1.7. Aims of this study.

The present study aims to evaluate AMF community structure within the Tiputini Biodiversity Station (TBS), located in the Napo Province, Ecuador. Situated in the Yasuní Biosphere Reserve, TBS provides the unique opportunity to study flora, fauna and fungi in pristine, natural and undisturbed tropical ecosystems. As previously mentioned, little is known in respect to AMF community composition in tropical ecosystems in South America, specifically for the Ecuadorian Amazon. Within the research station, soils of two different ecosystems, Várzea and Terra Firme, will be investigated. Várzea and Terra Firme systems have a number of differences and similarities: Várzea forests, which are periodically flooded by white water rivers, rich in sediments that originate in the Andes, have more fertile soils compared to Terra Firme forests, which are located in higher parts of the forest, out of reach of floods. On the one hand, Terra Firme forests have relatively well drained soils, and their vegetation is commonly divided by strata. Várzea forests, on the other hand, present a deeper layer of organic matter, with less clay, and a higher capacity to capture soil moisture, in comparison to Terra Firme sites (Ensley-Field, 2016). Despite these differences, being both situated in the Amazon rainforest, temperature, humidity, and rainfall are high in both ecosystems. In general, many of the same species occur in both ecosystems, however each

ecosystem can have a different frequency of these species. For example, Várzea forests tend to have higher rates of *Heliconia* sp. and alms, while Terra Firme forests have generally taller and older trees, and a greater number of for instance Kapok trees (*Ceiba pentandra*), or trees belonging to the *Meliaceae* family (Ensley-Field, 2016). One of the aims of the current study is to evaluate how changes in soil chemistry, such as differences in macro- and micronutrients, and pH, drive AMF abundance and diversity in Terra Firme and Várzea ecosystems. This study selected the *Fabaceae* family, as one of the most abundant plant families in Neotropical ecosystems. Furthermore, its members are known to form associations with both nitrogen-fixing bacteria and arbuscular mycorrhizal fungi (de Oliveira Freitas et al., 2014). For its presence in both types of ecosystems at the TBS, the genus *Inga* was selected as the host plant of interest. As part of the objectives of this study, two important research questions will be addressed. Firstly, what is the abundance and diversity of AM fungi associated to *Inga* sp. seedlings at the TBS, and secondly, what factors influence AM fungi abundance and diversity in the tropical conditions at TBS. Considering the differences in forest structure and abiotic factors within each ecosystem, we expect the AMF community structure to differ in response to these conditions. Establishing and maintaining the AMF symbiosis by plants requires a high photosynthate cost. Therefore, in Terra Firme forests, where soils tend to be poorer in comparison to Várzea soils, a higher degree of AMF colonization is expected, and possibly a higher abundance and diversity of AM fungi associated with the roots of *Inga* sp, as the host species of interest.

2. MATERIALS AND METHODS

2.1. Study area description.

The current study took place at the Tiputini Biodiversity Station (TBS), situated within a pristine area of primary Amazon rainforest with minimum human impacts in the province of Orellana, Ecuador (Figure 1). This biological research station, which is located ca. 280 km ESE from Quito (00°37'05" S, 76°10'19" W, 270 m. above sea level), was established in 1994 by Universidad San Francisco de Quito (USFQ) in collaboration with Boston University (USA). With its fluvial source high up the flanks of several volcanoes in the Eastern part of the Andes, the Tiputini river, which downstream joins the Napo river and becomes one of the tributaries of the Amazon, separates the TBS on its northern bank inside the UNESCO Yasuní Biosphere Reserve, from the border of the Yasuní National Park, which starts on its Southern river bank (Cisneros-Heredia, 2006; Espinosa and Salvador, 2017). The Yasuní Biosphere Reserve, with an extension of 9,820 km^2, occupies a unique location at the intersection of the Andes and the Amazon, and is practically situated on the equator. Moreover, Yasuní National Park is known as one of the world's last high-biodiversity wilderness areas and maintains an extraordinary biodiversity (Bass et al., 2010). Yasuní's climate is characterized by relatively high temperatures (24–27°C, year-round), and a high relative humidity of 80–94% throughout the year (Bass et al., 2010). The Yasuní Biosphere Reserve is considered part of the Core Amazon region, has a year-round high annual rainfall, which leads to the absence of a severe dry season. The soils of this area are geologically young, created by fluvial sediments derived from the weathering and erosion of the sources upstream in the Andes (Bass et al., 2010). The TBS has a total extension of 638 hectares, of forests that can be divided in Lowland Evergreen Forest or Terra Firme (~90%), and approximately 13% of its territory consists of periodically inundated Várzea (Lowland Evergreen Forest flooded by white waters), which are situated

along the riparian zone (Figure 8). Furthermore, The TBS has some small patches of inundated Igapo forest within its limits (Lowland Evergreen Forest flooded by black waters) in the lowest areas (Tiputini Biodiversity Station, n.d.).

2.2. Experimental design.

During four TBS fieldwork expeditions between June 2018 and February 2020, both Terra Firme and Várzea forests were sampled (Table 1; Figure 1).

2.3. Abiotic parameters: Meteorological data assessment.

To monitor the prevailing weather conditions, TBS has a weather station facility which logs meteorological data, located on-site near the main laboratories at the centre of the camp. This Vantage Pro 2 weather station has integrated sensors to measure hourly environmental conditions like rain, temperature, humidity, evapotranspiration, and barometric pressure (Davis Instruments Corporation, California, USA). Meteorological data were downloaded using Weatherlink 5.9 software and were analysed.

2.4. Abiotic parameters: Analysis of physical and chemical soil properties.

Soil samples were collected from the rhizosphere of Inga *sp.* seedlings in Várzea and Terra Firme forests. During sampling, Inga *sp.* seedlings where excavated, and soil clumps attached to seedling root structure where then used for physical and chemical soil sample analysis. To take a more representative sample soil, it was collected from varying depths, between 5 cm to 30 cm from the soil surface. 250g of soil was stored in 50 ml Falcon tubes. Soil samples were transported to the USFQ campus and stored at 4 ºC. Samples where then dried and sieved. An in-depth soil analysis was performed at the laboratory facilities of the USFQ Environmental Engineering Department. Soil samples were characterized based on physical and chemical parameters, using the EPA 3050B method for initial sample mineralization and EPA 6010B for instrumental measurement. Soil analysis was conducted

using a plasma spectrophotometer (Thermo Scientific brand, iCap 7000 model) with inductive coupling and optical detector (ICP-OES). Certipur® standards were used for instrument calibration, with an ICP multi-element standard solution VIII (Merck-Millipore, Switzerland). For quality control in recoveries, estuarine sediment as the Standard Reference Material (SRM®) from NIST® number 1646 was used (SigmaAldrich/Merck, Switzerland).

2.5. Biotic parameters: *Inga* sp.

For the present study, exclusively seedlings of the genus Inga of the *Fabaceae* family were sampled as possible host plants of mycorrhizal associations. This genus is characterized by the presence of winged rachis, a morphological characteristic that permits easy identification (Heywood et al., 1993). This genus can be found in both ecosystems since it presents a high abundance in tropical ecosystems. At 16 Várzea sites and 18 Terra Firme sites alongside a multitude of TBS trails, Inga *sp.* seedlings were spotted, photographed, GPS referenced, and for each seedling, root and stem length were measured using ImageJ software (Schneider et al., 2012).

2.6 Biotic parameters: AMF colonization in roots of Inga *sp.*

2.6.1. Baseline Study.

During the first field expedition in June 2018, a base line study was carried out to get acquainted with the research station and the field conditions. An initial evaluation of the two ecosystems was conducted, in which seedlings of *Inga* sp. (*Fabaceae*) were found to be present in high abundances in both ecosystems. Samples from roots of *Inga* seedlings where collected and stained using methylene blue (Walker, 2005) at the TBS laboratory facilities, to determine the presence of AMF and assess the viability of the staining process.

2.6.2. Evaluation of AMF colonization in roots of Inga *sp.*

A total of 15 *Inga* seedlings were sampled and for each seedling, three sections of root tissue were cut (2 mm). Root samples where then stored in distilled water in 2 ml eppendorf tubes and transported to the USFQ campus and stored at 4ºC until further processing. At the laboratory facilities of the USFQ Agrobusiness Department, root samples where cleaned and stained using methylene blue following the method as proposed by Walker (2005). Stained root samples where used to evaluate the mycorrhizal colonization as suggested by Trouvelot (et al., 1986), by following the proposed method in the Mycorrhiza Manual (Dodd et al., 2001). Stained root fragments where observed using an AmScope T120B-5M digital Microscope (at 40x magnification). Samples were divided into classes depending on the total level of mycorrhizal colonization. Several parameters were assessed: Frequency of mycorrhiza (F%), intensity of the mycorrhizal colonization in the root system (M%), intensity of the mycorrhizal colonization (m%), arbuscule abundance in mycorrhizal parts of root fragments (a%) and arbuscule abundance in the root system (A%). The computer program Mycocalc was used for further data processing.

2.6.3. Molecular analysis.

The soil rhizosphere of 19 Inga seedlings was sampled in the field. Soil aggregates which were still attached to the roots system, were collected, and stored in 1.5 ml eppendorf tubes with LifeGuard Soil Preservation solution. Rhizosphere samples were transported to the USFQ campus in Cumbaya and stored at -20°C to prevent denaturation of genetic material. The total DNA of the rhizosphere samples (< 2g of soil) was extracted by lysis with mechanical spheres. DNA purification was performed using the DNeasy PowerSoil kit following the manufacturer's instructions (Qiagen, USA). This method ensures that the purified nucleic acids are of the highest quality and quantity possible. Finally, concentrations of all total DNA

extractions were measured using a Nanovue Spectrophotometer. Extracted rhizosphere DNA samples were shipped to Baseclear, a laboratory specialized in sequencing of microbiome DNA (Leiden, The Netherlands). Sequencing was performed through an Illumina MiSeq platform and the construction of single-end reads was done in-house by Baseclear. For rhizospheric DNA sequencing, ITS genes were used to identify and characterize the fungal communities associated to roots of *Inga* sp. seedlings collected at the TBS. Reads were generated into FASTQ read sequence files and the final quality assessment processed by Baseclear. Sequence results will be analysed with the bioinformatic tools DaDa2 (version 1.12) and the Phyloseq package for R (version 1.30.0) to determine the taxonomic fungal diversity. Sequences matching previously submitted sequences in public databases, belonging to the phylum *Glomeromycota,* will be filtered to investigate the abundance and species distribution of AMF.

2.6.4. AMF spore identification.

A total of 15 soil samples from the rhizosphere of *Inga* seedlings where collected for AMF spore identification and stored in 50 ml Falcon tubes. Soil samples were transported to the Universidad de las Fuerzas Armadas (ESPE) located in Sangolquí, dried in an incubator at 65°C for 24 hours, and stored in a dry environment at 4ºC until further processing. Spores will be extracted from the soil samples, using the wet sieving and decanting method as proposed by Gerdemann and Nicolson (1963). Material collected after sieving process will be washed for 2 minutes with tap water and transferred to a glass Petri dish. Spores will be collected manually using an extruded 9-inch glass pipette to separate from the organic material. Fungal spores will be investigated using an optical microscope in the laboratory facilities at ESPE. AMF spores will be identified based on spore morphology, considering specific characteristics such as spore size, colour and spore wall structure.

3. RESULTS

3.1. Abiotic parameters: Meteorological data assessment.

Meteorological data logged by the TBS weather station were used to calculate the average annual temperature, humidity, and rainfall for the three years in which our research took place (Table 2a). Furthermore, for each month of a TBS sampling expedition, Table 2b provides monthly averages. Figure 2 visualizes the monthly changes in humidity, temperature and rainfall for the period January 2018 to March 2020. Data for August 2018 for all three parameters are not displayed due to maintenance of the weather station. Average temperatures as measured by the on-site TBS weather station during the specific sampling expeditions (Table 2) and the monthly differences recorded from January 2018 to March 2020, are consistent with normal seasonal changes in temperature and normal average annual temperature (25.1 °C) at the Tiputini Biodiversity Station (Personal communication TBS STAFF). However, through data analysis of the monthly rainfall averages, January, February and March 2020 were considerably drier compared to the same period in 2018 and 2019 (Figure 2). Furthermore, May and June 2018 show a specific anomaly, as the average humidity for these months is slightly higher.

3.2. Abiotic parameters: Physical and chemical properties of Várzea and Terra Firme forest plots.

During each field expedition, sample site descriptions were conducted to evaluate visible soil characteristics, and to assess possible differences between sample sites. In general, on the one hand it was observed that in the Terra Firme plots, leaf litter and humus accumulation was visibly higher, less compacted, and less degraded. On the other hand, in Várzea forest sites, leaf litter and humus accumulation in these soils had a darker colour, waterlogged, and compressed into a thinner layer. Macro- and micronutrients with important

roles in plant development where evaluated for soil samples collected at Várzea and Terra Firme sites at the TBS. The results for these parameters presented a normal distribution, which was confirmed using a Kolmogorov-Smirnov test: All samples were found to be below the critical K-S value of 0.41 (Table 3). A Student's T test (α=0.05) compared the obtained results of the physical and chemical properties between each ecosystem (Table 4). Potassium (K), zinc (Zn), Iron (Fe), Aluminum (Al), Lead (Pb), Nickel (Ni), Chromium (Cr), Cadmium (Cd), pH values, and soil conductivity did not present significant differences between the ecosystems (Table 3, labelled "ns"). All other analysed physical and chemical soil parameters showed significant differences between soil samples from Várzea and Terra Firme sites (Table 3). Figure 3 shows that soil samples collected in forest plots of Terra Firme (M=0.6) resulted in a higher Nitrogen (N) proportion ($t(8) = 0.024$, $p = 0.05$) compared to soils collected in Várzea forest sites (M=0.4). Although Phosphorous (P) was found to differ slight between ecosystems ($t(8)=0.043$, $p=0.05$), a higher concentration of this macronutrient was found Várzea forests (M=678.3) than in Terra Firme (M=518.5). Magnesium (Mg) and Calcium (Ca) showed a higher statistical significance, with p values equal or below 0.01, and both were found in higher concentrations in Várzea forests. Results of the chemical analysis of soil samples collected in Várzea and Terra Firme sites showed that Molybdenum (Mo) ($t(8)=0.0003$, $p=0.05$) and Manganese (Mn) ($t(8)=0.003$, $p=0.05$) had the significantly highest differences of all elements measured (Table 3). Figure 4 visualises that Mn was found in much higher concentrations in Terra Firme forests (M=4.06) than in Várzea (M=1.45). Similar trends could be observed for all other micronutrients, with higher average concentrations in soil samples for Várzea forest plots (Figure 4). Results of physical property soil measurements (Figure 5) showed that Várzea soils where found to be slightly less acidic (M=4.9) than in Terra Firme (M=4.6). Moreover, Várzea soils resulted in significantly higher Sodium (Na) concentrations, which might indicate

a higher concentration of sodium salts, compared to Terra Firme soils (Figure 5). Concentrations of the element Barium (Ba) ($t(8)=0.026$, $p=0.05$), was also found to be statistically different between ecosystems, with a greater average concentration found in Várzea ($M=496.24$), than in Terra Firme forests ($M=293.03$). Cobalt (Co) ($t(8)=6.897E^{-06}$, $p=0.05$), presented the highest statistical difference for all elements measured and was found at considerably higher concentrations for soil samples collected in Várzea ($M=22.36$), than in Terra Firme ($M=10.31$). In contrast, Vanadium (V) ($t(8)=0.026$, $p=0.05$), presented higher concentration in Terra Firme Forests ($M=204.05$) than in Várzea forests ($M=158.29$). In conclusion, soil analysis results show that, except for Mo, N and V, Várzea soils present higher concentrations of macro- and micronutrients, and therefore present soils that are richer.

3.3. Biotic parameters: Root and shoot measurements of sampled *Inga* seedlings.

During each field expedition, specific observations during *Inga* sp. seedling sampling indicated that the overall forest architecture differed within each ecosystem (Figure 9). In Várzea forests, less ground cover as well as less abundance of seedlings of the genus *Inga* were observed, particularly in areas with visual evidence that they had recently been flooded. These areas, which were particularly close to the Tiputini river, where observed to present distinct physical and biological structures related to the influence of seasonal floods, such as the presence of aerial roots, suppression of plant growth and the formation of erosion slides. Information regarding abundances of subadult and adult *Fabaceae* species, and abundance of subadult and adult *Inga* individuals present in both types of forest ecosystems within TBS plots and in other parts of the Ecuadorian Amazon rainforest, were kindly provided by Gonzalo Rivas-Torres. Within the TBS plots, the total number of subadult and adult tree individuals has been assessed for over 20 years, from 1998 onwards. These numbers were used to calculate local relative abundances on the family (*Fabaceae*) and genus (*Inga*) level within Terra Firme

and Várzea forest plots (Table 4). The total abundance of *Fabaceae* trees within Terra Firme plots of the TBS was 13.2%, with almost half of these trees belonging to the genus *Inga* (5.7%). Within Várzea forests, 25.4% of all subadult and adult trees encountered in the vegetation belonged to the *Fabaceae* family, of which more of half of the individuals (14.2%) could be identified as *Inga* sp. 14.2%. Although the distribution of seedlings of *Inga* sp. was not homogenous in patches along the station trails, in general it was not difficult to encounter them. For each of the 34 sampled seedlings of *Inga* sp., both root and shoot lengths were measured using ImageJ software. To determine possible statistical differences between root and shoot lengths of seedlings sampled in the different ecosystems, a Students T-test was performed. Root and shoot lengths proved not to be statistically different, but there were certain biological trends which were observed within each ecosystem: roots of *Inga* seedlings collected in Várzea sites (M=15.9 cm) where slightly longer ($t(32)=0.08$, $p=0.05$), than those collected in Terra Firme sites (M=13.7 cm). Contrasting results for the shoots indicated that *Inga* seedlings collected in Terra Firme (M=15.9 cm) were slightly longer ($t(32)=0.23$, $p=0.05$), than those of Várzea (M=14.2 cm). To further explore the relationship between shoot and root lengths between ecosystems, a shoot and root ratio was calculated. This ratio was obtained by dividing shoot length by root length for each *Inga* seedling sampled. Average shoot to root ratio for Terra Firme forest was 0.9, and for Várzea 1.26 (Figure 6). A Students T-test was performed to compare shoot to root ratios for each ecosystem, which proved to be significantly different ($t(32)=0.025$, $p=0.05$). In Figure 7, a visual representation of shoot to root ratios demonstrates that *Inga* seedlings sampled in Terra Firme forests tend to have a negative shoot to root ratio, that is, root length is often longer than shoot length. Contrastingly, in Várzea forests, seedlings tend to have a positive shoot to root ratio, where shoot length is larger than root length.

3.4. Biotic parameters: AMF assessment.

3.4.1. Microscopic evaluation of mycorrhizal structures in roots of *Inga* sp.

During the first field expedition, a baseline study was conducted to evaluate the presence of specific structures of AMF present in the roots of *Inga* sp. seedlings. Furthermore, this first expedition served to practice the staining method to evaluate how successful it was under tropical field conditions. Although the detection of AMF mycotic structures was initially difficult to assess, due to excessive amounts of staining in the roots, AMF differential staining was observed to function under tropical field conditions. Through trial and error, the staining method was refined and modified to remove excessive amounts of the alkali-soluble pigments in roots, by extending the root distaining period using KOH from 30 minutes to 24 hours. Even though *Inga* sp roots have naturally high concentrations of pigments and lignin, Figure 8a and 8b show a collection of microscopic pictures of various mycotic structures, emblematic to arbuscular mycorrhizal fungi, which were observed in the roots of seedlings of the genus *Inga.* These results effectively confirmed the presence of this group of symbiotic fungi within roots of their host plant *Inga* sp. Arbuscules, intraradical hyphae and extraradical hyphae, as well as spore-like structures were found to be abundant in the microscopy sample slides. Moreover, vesicles, which are reported to be mycotic structures that function as fungal nutrient storage organs, where observed, though not as abundant as arbuscules.

3.4.2. Evaluation of AMF colonization.

Due to the COVID-19 pandemic and the isolation measurements, a part of the planned sample processing at USFQ laboratory facilities could not be completed before the submission of this technical report. To construct an overview of the possible results in respect to the evaluation and quantification of AMF colonization in roots of seedlings of *Inga* sp., results in this current section will be based on previously published by Garcés-Ruiz et al. (2017). Their

study used a similar methodology and sample collection, and was also situated in the Ecuadorian Amazon rainforest. Their results indicated observation of root colonization of AMF for all plant species sampled in their study. The results obtained by this study are coherent with observations of our baseline study, which confirmed AM fungi to be present in root samples of *Inga* sp. seedling. Furthermore, Garcés-Ruiz et al. (2017) quantified the AMF total colonization at 56%, whereas the total vesicle colonization was estimated to be between 4% and 20%. These results also seem coherent with our observations. However, Garcés-Ruiz et al. (2017), found a low presence of arbuscules, which is in contrast with the much qualitative presence of arbuscular structures observed in our baseline study. The moment the COVID-19 confinement will end, the quantification of the various arbuscular mycorrhizal structures in the remaining roots samples of *Inga* seedlings collected during the last TBS expedition in February 2020 will be continued, which are stored at the laboratory facilities until further processing.

3.4.3. AMF spore identification and molecular analysis.

Due to the COVID-19 pandemic and enforced isolation, sample processing could not be completed and spore identification as well as molecular analysis of the ITS region 1 and 2 could not be assessed in time. To hypothesize possible expected results about the AMF community structure for the Ecuadorian amazon, a study conducted by Garcés-Ruiz (et al., 2019) was consulted. In this study, the genus *Acaulospora* was found to dominate, with an abundance of 73.4%. *Archaeospora* was the second most abundant genus, at 19.6%. In an earlier study by Garcés-Ruiz et al. (2017), *Glomus* (31%) and *Acaulospora* (25%) where found to be the most abundant genera, followed by *Rhizophagus* (23%) and *Archaeospora* (22%). These results are coherent with studies conducted in Brazil, Colombia, Venezuela, and Peru, where *Acaulospora* and *Glomus* were identified as the most abundant and diverse taxons (Cofré et al., 2019; Peña-Venegas et al., 2006).

4. DISCUSSION

4.1. Influence of climate variables on AMF.

Temperature is an environmental factor that can have major effects on both plant growth and root exudation, thereby directly influencing AMF vesicle formation and colonization (Graham et al., 1982; Fitter et al., 2004). Furthermore, studies have shown that AM fungi are also directly affected by temperature (Hawkes et al., 2008). In response to higher temperatures (24–30 °C), AMF showed a higher level of root colonization, higher abundance of extraradical mycelium, and a higher glucose uptake (Gavito et al., 2005; Hawkes et al., 2008). A possible cause of the observed higher presence of AM fungal colonization of host plant roots at higher temperatures, is the increased leakage of root metabolites from root cells into the rhizosphere, which results in a higher number of entry-points for AMF root colonization (Graham et al., 1982). This stimulating effect of elevated temperature on AMF growth and inoculation could explain why in tropical ecosystems, characterized by high temperatures, there is a greater abundance and diversity of AM fungi. It is therefore expected that these climatic conditions provide a selective force on AMF populations (Hawkes et al., 2008). Information about the effects of high humidity and high soil moisture on AM fungi growth, inoculation and community composition are limited for neotropical ecosystems. However, previous studies in arid ecosystems indicated that AM fungi promote the water uptake and offer a higher drought resistance to their host plants, by extending the host plant rhizosphere range through their extensive hyphal networks (Deepika & Kothamasi, 2015). Soil moisture levels have been reported to be positively correlated with AMF colonization (Oliveira and Oliveira, 2010). Moreover, soil moisture levels can regulate the presence or absence, and relative abundance of AMF phylotypes in host roots, as it can function as an abiotic filter and selective force affecting AM fungi community assembly associated to host plant roots (Deepika

and Kothamasi, 2015). In fact, abiotic soil properties, such as soil moisture, may determine whether AMF communities are dominated by a few species, or allow a more complex diversity of AMF (Deepika and Kothamasi, 2015). It has been reported that roots of plants in flooded soils present a low AM fungi diversity (Deepika and Kothamasi, 2015). But in general, it has been proposed that AM fungi diversity is high in ecosystems with a soil moisture regime of 15%–20%. However, in tropical ecosystems like the current study sites at the TBS, the overall soil moisture budget is much higher than 20%. This parameter could therefore function as an environmental filter, which might result in a reduced number of AMF species which can tolerate these prevailing habitat conditions. An important threshold might be their high oxygen requirement due to their aerobic lifestyle (Deepika and Kothamasi, 2015). On the one hand, this prediction might be relevant for Várzea ecosystems with their periodically inundated soils, which could result in a lower AMF diversity in these systems. On the other hand, these periodic floods might increase abundance of tolerant AMF phylotypes, specifically evolved to survive extended periods with low oxygen contents in the soils during such a flood. Interestingly, it has been documented that certain AMF species in response to floods, can display a parasitic behavior, as they are unable to continue absorbing P under these submerged conditions. Instead of contributing P to their host plants, they might dependent on P from the host plant to survive the duration of these floods (Deepika and Kothamasi, 2015). These results indicate that soil moisture, and therefore, environmental humidity, can shape local AMF communities associated with host plants in tropical habitats. It is important to evaluate local climate conditions when investigating AMF population dynamics. Like anywhere in the world, AMF populations at the TBS have adapted to the specific environmental and climatic conditions of the Ecuadorian Amazon rainforest. To understand what factors are driving the establishment of these beneficial fungal communities, is particularly important considering possible future

effects created by climate change and anthropomorphic changes in the Amazon region. Although it is outside the scope of the current study, the long-term impact of climate conditions on AMF community structures could not only generate information on evolutionary dynamics of AMF populations, but could also provide valuable information on current dispersal limitations and environmental filtering (Kivlin et al., 2011). As previously mentioned, during January to March of 2020, the Yasuni Biosphere Reserve experienced an unusual period of drought (Table 2b and 2c). Considering the effects of climate change and anthropocentric changes on land use, the extent and frequency of droughts in the Amazon basin is expected to increase. Although the Amazon rainforest does not have well defined seasons as compared to the extremes of both hemispheres, as it has a stable overall climate year-round, it does have a wetter and a drier season. Flora, fauna, but also fungi and other microorganisms inhabiting the Amazon Rainforest, have evolved under these relatively stable climatic conditions. Changes in these patterns and increases in the intensities, could have a devastating effect on population dynamics of both tropical macro- and microbiodiversity. Some microorganisms, such as AM fungi, exhibit species-area relationships and co-occurrence patterns as observed for macro-organisms (Kivlin et al., 2011). Yet, for the Amazon, little is known about species-area relationships in connection to climate conditions, and whether they can serve as selective factors for the soil's microbiome and all its populations of microorganisms. This type of research is complex and is no easy task, but a multi-disciplinary approach including the analysis and influence of beforementioned variables is encouraged.

4.2. Abiotic parameters: How edaphic factors affect AMF.

The physical and chemical analysis of soils samples collected at the TBS revealed that half of the assessed soil parameters did not present significant statistical differences between Várzea and Terra Firme ecosystems (Table 4). It is important to consider that within the area

of the Tiputini Biodiversity Station, as well as many other parts in the Amazon, river geography can change rapidly (Wittmann et al., 2006). Thus, areas that are considered drier parts, could become inundated in a short geological timeframe. Furthermore, Várzea forests are located directly next to Terra Firme forests, making these ecosystems difficult to accurately separate (Ensley-Field, 2016). The sampling areas in the current study could have been subject to recent geographical changes in river dynamics, and although they were divided in either Terra Firme or Várzea, they could have been situated in ecosystem transition zones. Furthermore, the total number of soil samples collected could have been higher. The sample number could also be a factor why some elements did not present statistical differences, and a larger sample pool would be recommended for future studies. Furthermore, it is important to clarify that small changes in soil composition could have strong impacts on soil microorganisms and could exert effects not only on AMF populations as well as on the plant community structure. It has been previously recorded that a strong influence of edaphic factors exists on AMF formation and development in acid and nutrient-poor soils in Amazonia ecosystems (Oliveira and Oliveira, 2010). Therefore, in this study, even though statistical differences between results of physical and chemical properties of Terra Firme and Várzea soils are important, because of the beforementioned points, also biological trends are important.

Mean values show that Várzea forests present a higher concentration of all chemical elements except N, Pb, Mo and V. Based on these trends, these samples confirmed that periodic inundation of white-water rivers like the Tiputini river lead to more fertile soils in Várzea forests flanking these rivers. Furthermore, the relatively lower N levels in Várzea forests could be a result of elevated processes like lixiviation. Nitrogen is a mobile compound, and it may easily be drained from the forest top soil layers in Várzea forest. Molybdenum is required for the synthesis and activity of the enzyme nitrate reductase in plants and is vital for the process

of symbiotic nitrogen fixation by *Rhizobia* bacteria in legume root modules (Kaiser et al., 2005). This element, also found in lower amounts in Várzea forests, may not affect AM fungi, but considering that the host plant chosen for this study belongs to the *Fabaceae*, it could have an effect on the nitrogen fixing bacteria associated to members of this family (Kaiser et al., 2005). It is possible that higher concentrations of Mo in Várzea forests could be positively correlated to an increase in *Rhizobia* bacteria abundance and activity, resulting in a higher dependence on bacterial nitrogen fixation over AMF formation with regards to how *Fabaceae* obtain N. Nitrogen is a major nutrient for plant development that frequently limits primary productivity in terrestrial ecosystems. (Veresoglou et al., 2012). In highly productive systems which sustain high levels of plant biomass and are limited by nitrogen, such as those observed at TBS, even small amounts of N gained by trees may give them a competitive advantage. Under these conditions, investing in natural symbiotic associations proved beneficial and has resulted in the recruitment of AM fungi by plants. AMF colonization, temporal and spatial abundance and AMF spore density are positively correlated with soil organic matter and total soluble N (He et al., 2002). AMF have the capacity to acquire N from both inorganic and organic sources, and partially transfer this N to their host plant (Hodge and Storer, 2015). However, it has been documented that competition for N between symbionts can occur (Hodge and Storer, 2015). Resource competition for N under limiting or deficient conditions could also lead to negative interactions between AMF and soil microorganisms (Hodge and Storer, 2015). As mentioned previously, plants belonging to the *Fabaceae* family, such as members of the genus *Inga* are known to form important associations with nitrogen fixing bacteria, and therefore, under low nitrogen conditions, to alleviate competition, nitrogen fixing bacteria might be chosen over AMF symbiosis, particularly in Várzea ecosystems where N concentrations are lower. Phosphorus is another important nutrient for plant growth and plays

a significant role in many physiological and biochemical plant activities like photosynthesis, transformation of sugar to starch, and transporting of genetic traits (Gad et al., 2012). Although P is crucial to plant development, high quantities of this element in the soil could have adverse effects for AMF colonization (Gad et al., 2012). On the one hand, under low P conditions, intersymbiont competition is reduced which results in an increase in AMF colonization and functionality. On the other hand, because of low levels of P quantities, the necessity to form AMF symbiosis for plants results in a higher fitness level for AMF plant symbionts. This prediction could be an important factor to explain why tropical forests present the highest levels of AMF biodiversity (Marinho et al., 2018). Furthermore, Terra Firme forests might represent an even higher need for AMF symbiosis formation, as their P levels were the lowest values in this study. Mycorrhizal benefits are dependent on the plant-fungus combinations. In general, higher biodiversity of AMF species could be the result of a higher dependence of host plant in P-limiting soils. It has been suggested that AMF species, with higher hyphal densities inside host roots, provide higher P uptake benefits to the host plant (Deepika and Kothamasi, 2015). Certain *Glomaceae* species have been recorded to present higher root hyphal densities compared to for example *Acaulosporaceae* species, and because of this feature provided profound P benefits to their host plants (Deepika and Kothamasi, 2015). Based on the microscopical analysis of the roots of *Inga* seedlings collected in this study, high diversity of AMF species is expected to be found, which will include high root density phylotypes like species belonging to the *Glomaceae* (Deepika and Kothamasi, 2015). Cobalt (Co) is an essential element for legumes because it is involved in the atmospheric nitrogen fixing processes by Rhizobia bacteria (Gad et al., 2012). Furthermore, Co has a positive effect in promoting both shoot and root biomass, particularly under low phosphorus levels. Moreover, it has been shown to have a significant beneficial effect on P, N, Mn, Zn, Cu and K content in

plant tissue after mycorrhizal associations (Gad et al., 2012). This is of importance considering that this element was found to show the greatest statistical difference of all elements evaluated in our soil samples. It is possible that high concentrations of this element in Várzea forests could lead to a higher shoot to root ratio as observed in Figure 10, thereby increasing the dependence of nitrogen fixating bacteria over AM fungi associations by host plant. In Neotropical forests, other elements like Al, Ca, Mg, K, Fe and Mn have been reported to affect colonization and spore numbers of AM fungi (Oliveira and Oliveira, 2010). The beneficial effects of the AMF symbiosis for host plants are directly related to the fungus' ability to supply nutrients by extending the effective root absorption surface areas by incorporating its hyphal network. By doing this, the fungus can explore larger soil volumes and overcome nutrient and water depletion zones near the plant root (Clark and Zeto, 2000). Finally, pH levels in both ecosystems where observed to be acidic (pH range from 3.8 to 5), which could act as a selective force for AMF community structure. On the one hand, acidic soils decrease overall AMF colonization, possibly due to an increased microbiological activity of many microorganisms living in the soil microbiome. But on the other hand, soil pH has favored the evolutionary selection and establishment of AMF species, tolerant to acidic soil conditions (Wang, 1993). In conclusion, edaphic soil parameters, like the low levels of N and P observed in the soils at TBS, may act as an important selective driver for AMF abundance and diversity. But these effects are not easy to measure, due to complex interactions between soil nutrients, AM fungi, other soil microorganisms and the diversity and specificity of host plants. Moreover, interspecies competition, seasonal fluctuations within ecosystems, and separate effects caused by climatic conditions add another layer of complexity. In the Amazon rainforest, understanding and evaluating how AMF influence nutrient cycling and benefit host plant nutrient uptake could be a crucial component to establish sustainable tropical agricultural

practices and for mitigating adverse anthropogenic industries, like in the field of bioremediation of polluted ecosystems after petrochemical oil extractions or mining activities.

4.3. Influence of host seedlings of *Inga* sp. on AMF colonization

As observed in Table 4, members of the *Fabaceae* and its genus *Inga* predominate the TBS territory, demonstrating a high abundance in both study ecosystems, in particular in Várzea habitats. A factor that could explain the high abundance of this group might be their ability to form rhizospheric associations with both nitrogen fixing bacteria and AM fungi, in ecosystems which show some limitations for specific soil nutrients. It has been recorded that AMF can both increase overall plant biomass and can influence the N-partitioning between the shoot and the root system, particularly in mycorrhizal legume plants (Gad et al., 2012). As a result, plants with a higher level of AMF colonization and diversity of associated symbionts, have been recorded to present a lower shoot to root ratio (Gad et al., 2012). Furthermore, the shoot to root ratio in presence of AMF symbiotic formation in ecosystems with low rates of P can also results in a low shoot to ratio (Gad et al., 2012). Shoot to root ratio obtained in this study show that Terra Firme plots show a tendency to present lower ratios than in Várzea (Figures 6 and 7). These results are consistent with our hypothesis that in Terra Firme forests, there is higher diversity and abundance of AMF, due to its lower P levels.

4.4. AMF colonization, abundance, and diversity in *Inga* sp. seedling at the TBS.

During the base line study, microscopic observations of roots of *Inga* sp. seedlings showed a high abundance of extraradical and intraradical mycelium and arbuscules. However, these root samples did not show many vesicles, as vesicles could only be observed in one single root sample. Although these results were generated at the TBS, through a general microscopic observation and therefore not quantifiable, it indicated that vesicles seemed to be scarce in roots of *Inga* sp. in the ecosystems of the TBS. Three possible hypotheses might explain these

observations: First, it could be possible that the low nutrient availability results in a reduction of the capacity to form theses nutrient storing structures by AMF. Secondly, there could be a genetic tendency for AMF phylotypes that do not produce vesicles at TBS. Finally, vesicles are nutrient reservoirs located outside plant root cells, therefore they are exposed to conditions outside the protection of host plants and could be subject to competition or attack by pathogenic components of the soil microbiome that want to take advantage of these nutrient reservoirs. This last hypothesis is particularly interesting as it revolves around a trade-off between on the one hand the low abundance of P and the high turn-over and high level of lixiviation of N in Amazon rainforest soils which would indicate the need of a reservoir for these essential elements for the fungus. But on the other hand, based on the low number of observations of vesicles compared to arbuscules and AMF mycelium, the risk of losing their vesicles in a biodiversity hotspot, with undoubtedly high levels of competition above and belowground, could then act as an evolutionary selective force upon AMF to not invest energy and resources into these reservoir structures. These specific environment and pressure might have resulted in AM fungal species to have lost the ability to produce these vesicles. In conclusion, considering edaphic, climatic, and biological factors, it is expected that AMF abundance and diversity will be high at TBS. As mentioned previously, low nutrients levels result in higher plant and fungal diversity because there are a greater variety of biological niches that can be occupied, particularly in Terra Firme ecosystems. Considering this correlation, it is previously observed that a many AM fungal species can associate themselves with a single host species. Investing energy to establish symbiotic associations and inviting a higher variety of fungal phylotypes could be the difference between survival, the possibility to successfully reproduce, or to face death for plants in ecosystems governed by high levels of competition for resources.

5. CONCLUSIONS

This identified that AMF development, root inoculation, abundance and diversity are directly and indirectly influenced by edaphic conditions specific to an ecosystem, such as macro- and micronutrient availability. AMF community structure and performance has also been reported to depend on climatic conditions, such as temperature, rainfall, and humidity. Furthermore, AMF populations are important in regulating and maintaining important ecosystem functions, as well as AMF influence on host plant fitness. Arbuscular mycorrhizal fungi associate with the majority of vascular plants, including most major agricultural crops. This emphasizes that understanding factors driving AMF abundance, diversity, and functionality, might benefit tropical agricultural practices. Moreover, mycorrhizal fungi are an important part of the belowground response of terrestrial ecosystems to environmental changes, as they possess the potential to affect the carbon balance, with important implications for mitigating climate change (Hawkes et al., 2008). Understanding AMF populations in natural ecosystems, and the effect that climate change could have on population dynamics, could be crucial to use its potential for ecosystem remediation and sustainable agricultural practices (Parniske, 2008). To date, although there is much interest in the AMF symbiosis, comprehensive and thorough information regarding the biogeography of mycorrhizal fungi remains relatively unknown. Understanding these processes as well as assessing endemic AMF populations in global ecosystems will enable us to improve future understanding about the impacts of global climate change (Tedersoo et al., 2020). Understanding AMF community structure and host plant fungal dynamics at a global and local scale, considering particularities in an ecosystem's climate and abiotic conditions, could become an indispensable tool to improve yields in agriculture and forestry (Tedersoo, et al., 2020), particularly in the Amazon rainforest.

6. BIBLIOGRAPHY

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7. TABLES

Table 1: Sample collection date and sample type.

Field Expedition	Date	Sampling type
1	June 29th - 2nd of July, 2018	Baseline study Staining method evaluation
2	November 23rd - 26th, 2018	Sample collection for Soil physicochemical analysis, root microscopy and DNA extraction
3	August 12th - 16th, 2019	Sample collection for DNA extraction
4	February 21st - 24th, 2020	Sample collection for root microscopy and spore identification

Table 2: Yearly and monthly average meteorological climate assessment for TBS. Average meteorological conditions calculated from raw data taken hourly at the TBS weather station. Yearly averages are shown for 2018 through 2020 as well as monthly averages for meteorological conditions during the three months where data sampling was conducted.

Date	Average Temperature (C°)	Average Humidity (%)	Average Barometric pressure (in)	Average Rainfall (mm)	Average in Air Evapotranspiration (mm)
Yearly measurements					
2018	24.70	72.55	18.71	1.71	0.10
2019	24.50	71.81	17.06	1.58	0.11
2020	25.50	68.53	16.36	0.45	0.11
Monthly measurements					
Nov-18	25.58	72.10	29.79	2.61	0.12
Aug-2019	23.89	74.34	29.95	2.40	0.10
Feb-20	25.16	67.47	29.84	0.41	0.11

Table 3: Physicochemical soil analysis for Várzea and Terra Firme. Samples were found to present a normal distribution using a Kolmogórov-Smirnov test. Critical K-S test value for all simples was 0.41. A Student's T test was used to calculate significance between physicochemical soil samples for booth ecosystems, using α=0.5. Critical P value was 0.05. Significance is shown according to APA regulations: ns= no significance, * = P ≤ 0.05, ** = P ≤ 0.01, *** = P ≤ 0.001.

Macronutrients	Várzea	Terra Firme	K-S test Norm.	T test P Val.	Significance
Nitrogen (N)	0.4	0.6	0.15	0.024	*
Phosphorus (P)	678.3	518.5	0.11	0.043	*
Potassium (K)	7649.3	6732.4	0.8	0.063	ns
Magnesium (Mg)	4499.8	2901.0	0.4	0.010	**
Calcium (Ca)	3418.7	1336.1	0.22	0.004	**
Micronutrients	Várzea	Terra Firme	K-S test Norm.	T test P Val.	
Copper (Cu)	31.18	23.18	0.11	0.007	**
Zinc (Zn)	120.22	108.98	0.18	0.250	ns
Iron (Fe)	34710.06	28798.75	0.16	0.073	ns
Manganese (Mn)	1000.81	217.15	0.16	0.003	**
Molybdenum (Mo)	1.45	4.06	0.14	0.0003	***
Others	Várzea	Terra Firme	K-S test Norm.	T test P Val.	
pH	4.65	4.92	0.16	0.174	ns
Na	4522.95	1464.13	0.18	0.006	**
Conductivity	163.12	107.74	0.28	0.313	ns
Aluminum (Al)	75499.2	61283.8	0.11	0.062	ns
Lead (Pb)	9.78	9.92	0.10	0.400	ns
Vanadium (V)	158.29	204.05	0.09	0.026	*
Nickel (Ni)	26.99	25.15	0.13	0.353	ns
Chromium (Cr)	77.15	74.31	0.13	0.376	ns
Cobalt (Co)	22.36	10.31	0.22	6.897 E^{-06}	***
Cadmium (Cd)	4.04	3.42	0.11	0.080	ns
Barium (Ba)	496.24	293.03	0.16	0.001	***

Table 4: *Fabaceae* and *Inga* abundance in Terra Firme and Várzea. Local abundance of the family *Fabaceae* and the genus Inga depending on ecosystem type. Forrest plots labeled as outside Tiputini correspond to plots near the research station but outside its limits. Relative species abundance is calculated for each plot. Information regarding abundances of subadult and adult *Fabaceae* species, and abundance of subadult and adult Inga individuals within TBS plots and in other parts of the Ecuadorian Amazon rainforest were kindly provided by Gonzalo Rivas-Torres.

Forrest type	Total in plot	Total *Fabaceae*	Total *Inga*
Terra Firme	737	97	42
Percentage	100%	13.2%	5.7%
Várzea	714	182	102
Percentage	100%	25.4%	14.2%
Terra Firme outside Tiputini	1022	103	40
Percentage	100%	10.2%	3.9%
Várzea outside Tiputini	830	111	53
Percentage	100%	13.3%	6.3%

8. FIGURES

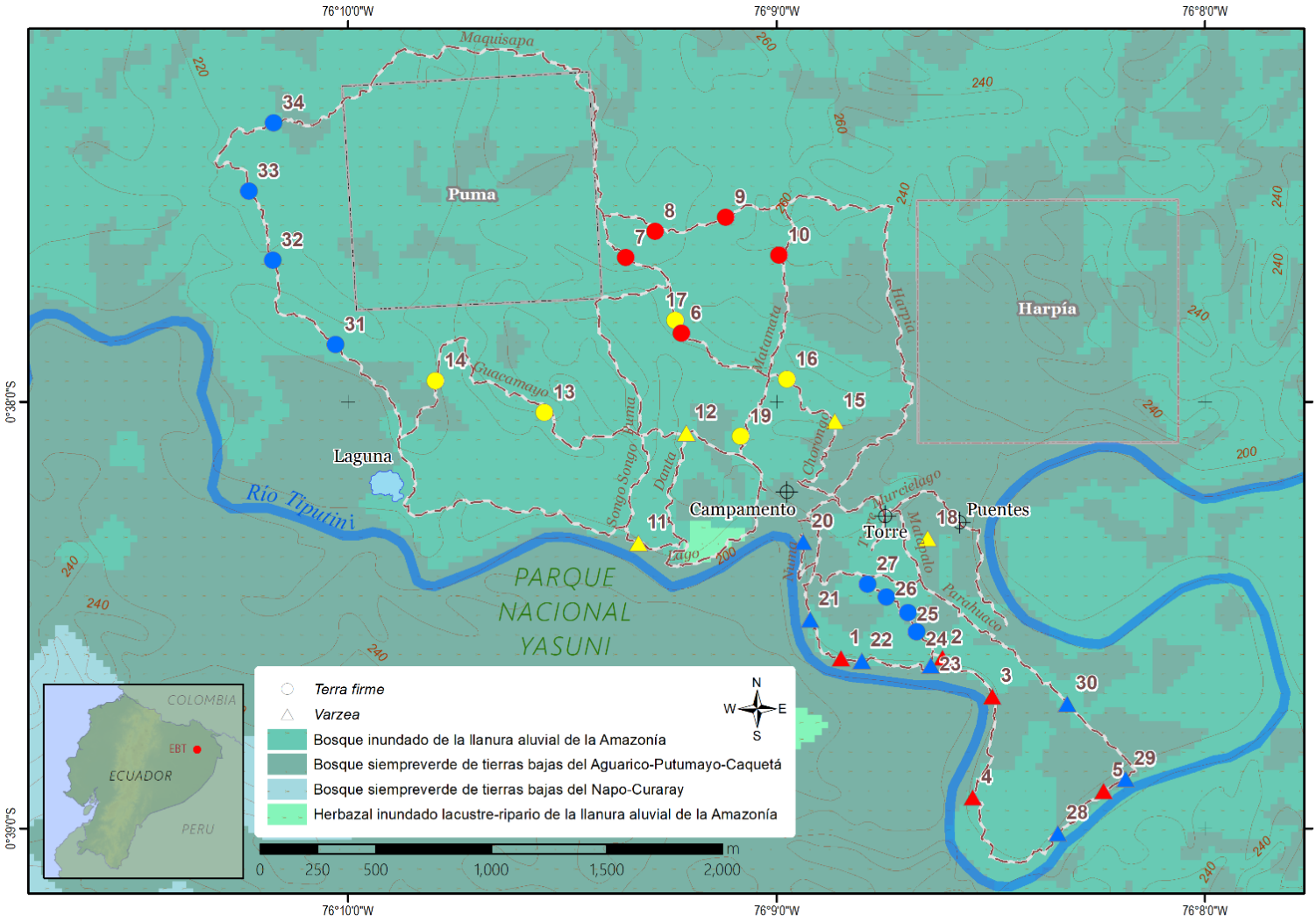
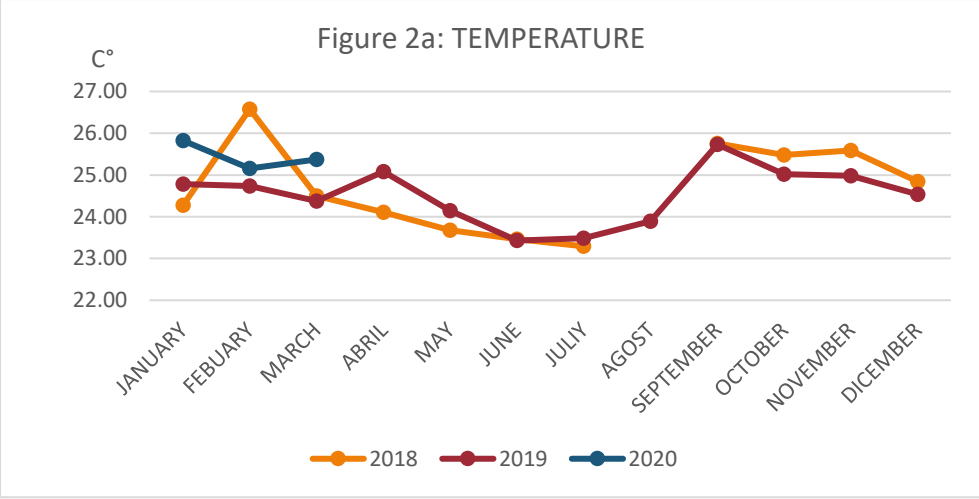
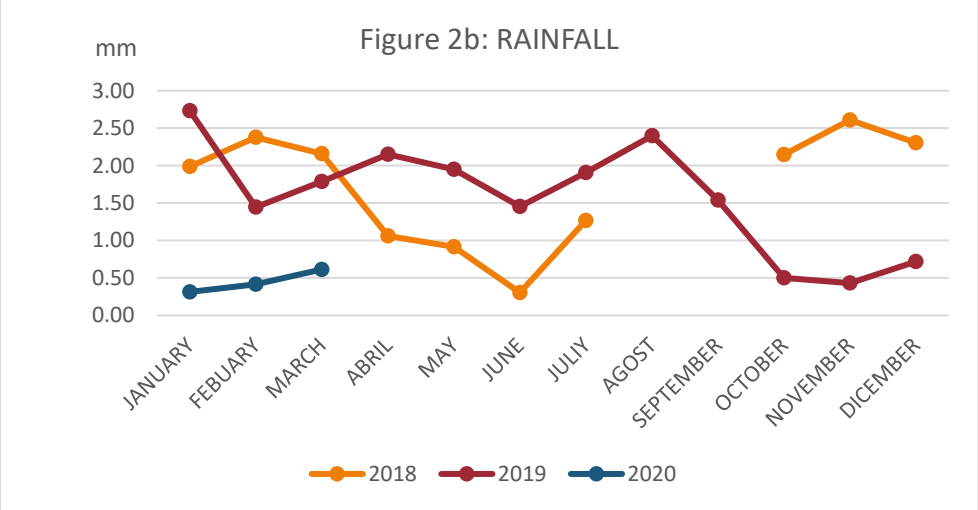


Figure 1: Sampling location for Inga seedling withing TBS trails. Map of the research station, trails and ecosystem types. Triangles show *Inga* seedlings sampled in Várzea forests. Circles show *Inga* seedling sampled for Terra Firme forests. Sampling location was taken using GPS coordinates. Colors indicate date of each field expedition where sampling took place: Red, November 2018; Yellow, August 2019; Blue, February 2020. GPS coordinates where processed and this map was created by Leo Zurita using ARCGIS.





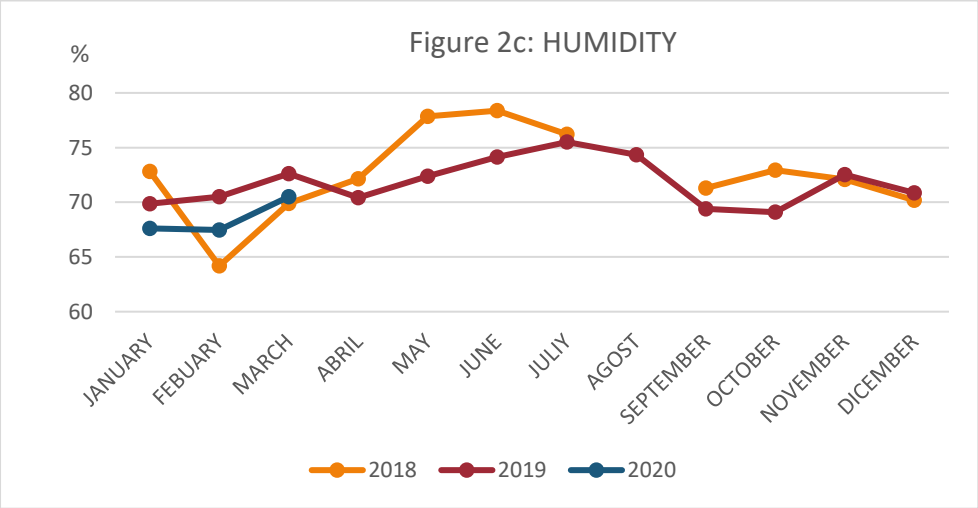


Figure 2: Average yearly temperature, rainfall, and humidity by month. Figure 2a shows the differences in average monthly temperatures for three consecutive years: 2018, 2019 and 2020. Figure 2b shows the differences in average monthly rainfall for three consecutive years: 2018, 2019 and 2020. Figure 2c shows the differences in average monthly rainfall for three consecutive years: 2018, 2019 and 2020.

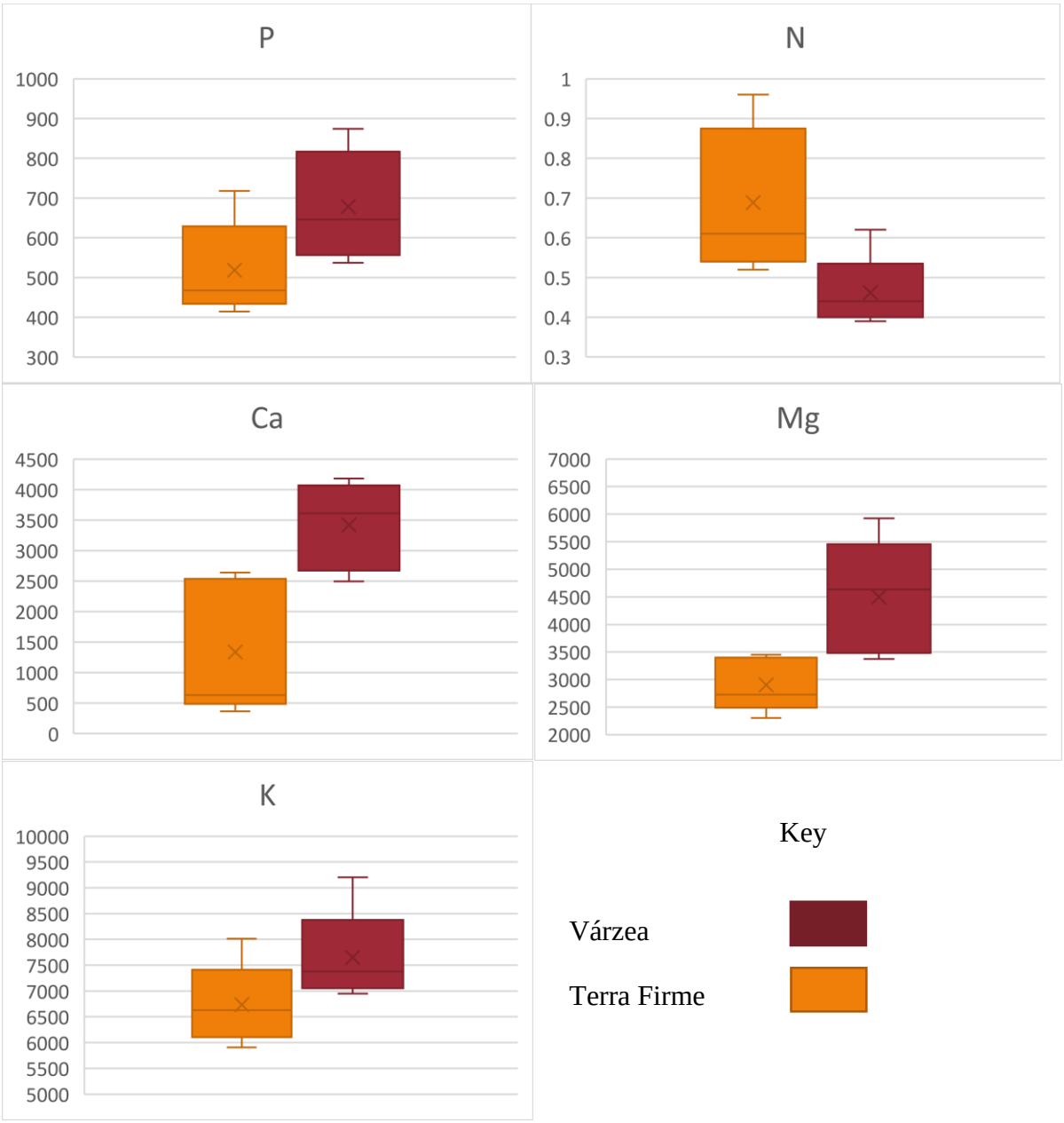


Figure 3: Macronutrient comparison between Terra Firme and Várzea. Differences in macronutrient from soils collected in Terra Firme forests and Várzea forests. Potassium (K), magnesium (Mg), phosphorus (P) and calcium (Ca) are found in greater un Várzea forests. Total nitrogen (N) is found in higher concentrations in Terra Firme soils.

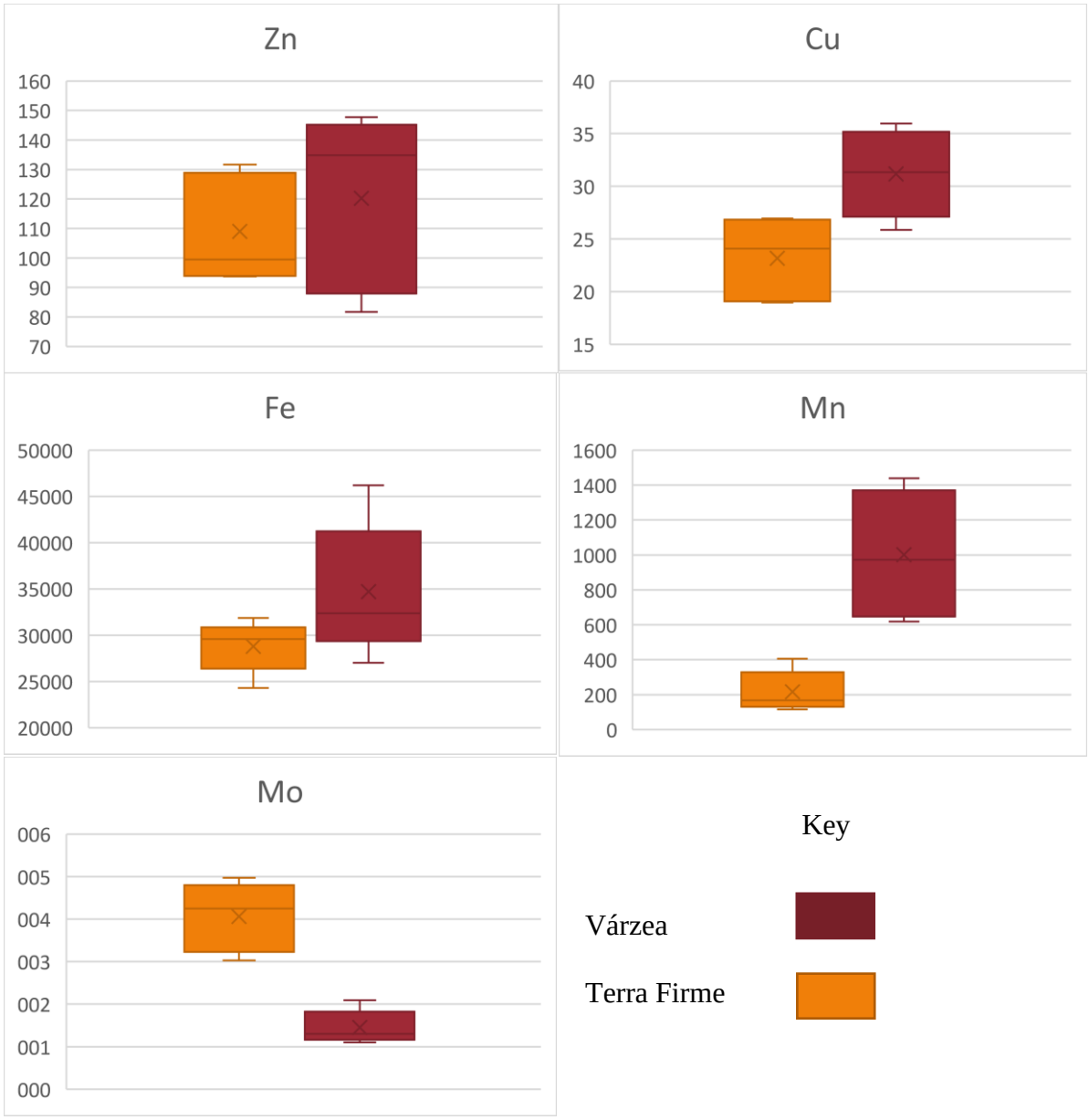
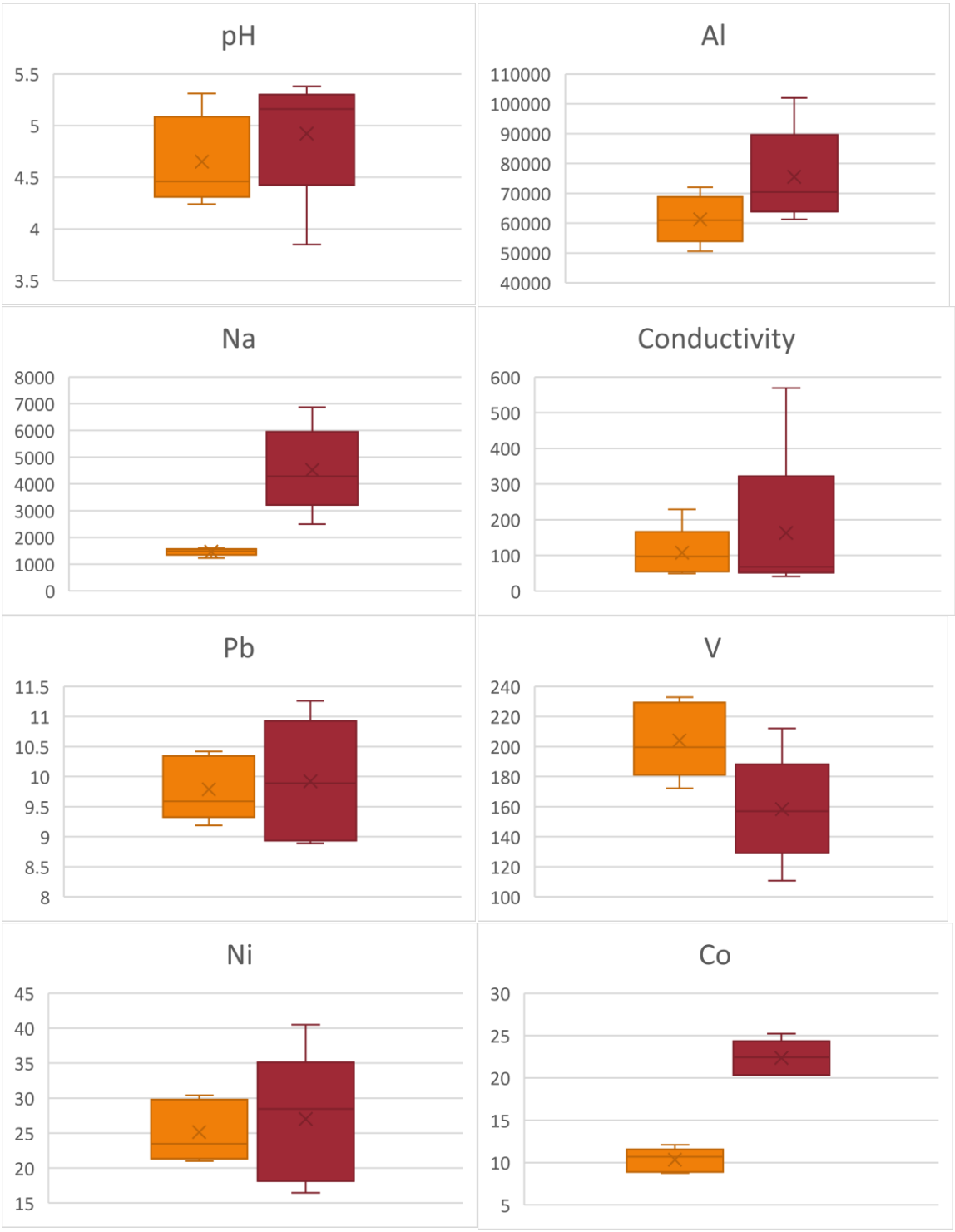


Figure 4: Micronutrient comparison between Terra Firme and Várzea. Differences in micronutrient concentrations for soils collected in Terra Firme forests and Várzea forests. Zink (Zn) does not show significant differences between both ecosystems. Copper (Cu), Iron (Fe), and manganese (Mn) are found in higher proportions in Várzea forests. Molybdenum (Mo) showed higher concentrations in Terra Firme soils.



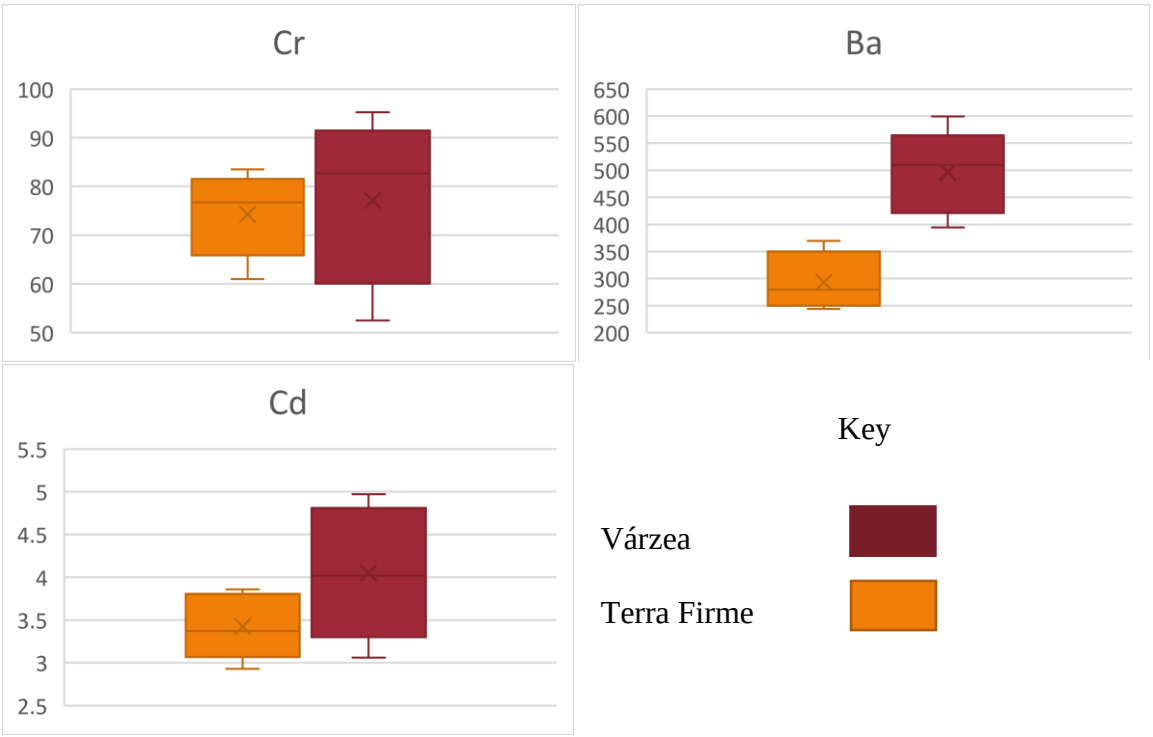


Figure 5: Other chemical soil elements and physical soil characteristics evaluated for Terra Firme and Várzea. Differences in concentrations of other chemical elements like heavy metals, pH, sodium (Na) and soil conductivity for soils collected in Terra Firme forests and Várzea forests. Sodium showed higher concentrations in Várzea forests. Soils in Terra Firme are found to be less acidic compared to Várzea soils.

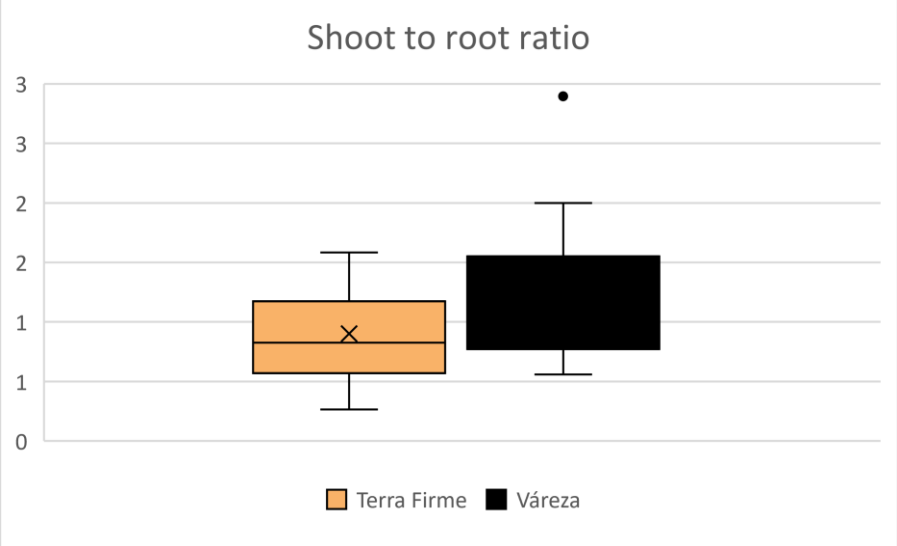


Figure 6: Shoot to root ratio for *Inga* sp. seedlings collected at Terra Firme and Várzea sites.

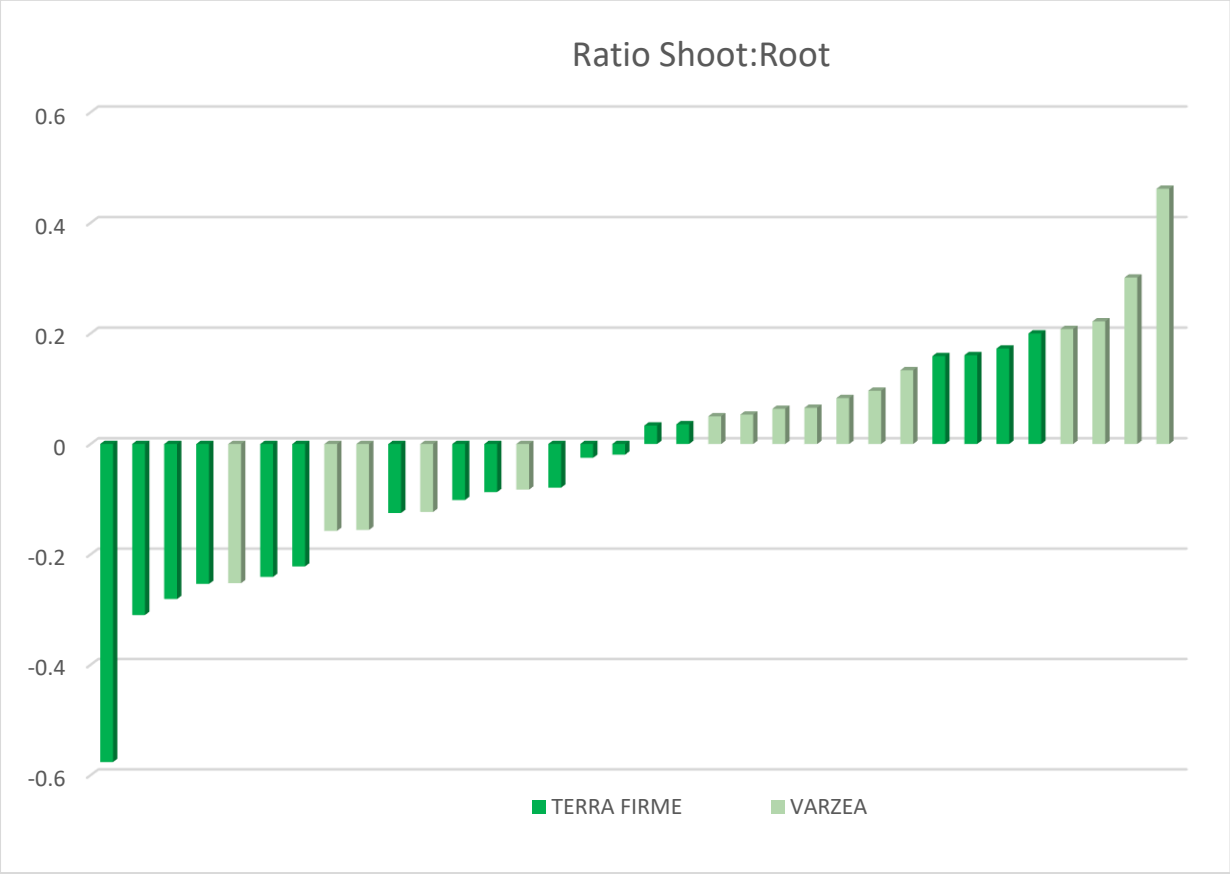
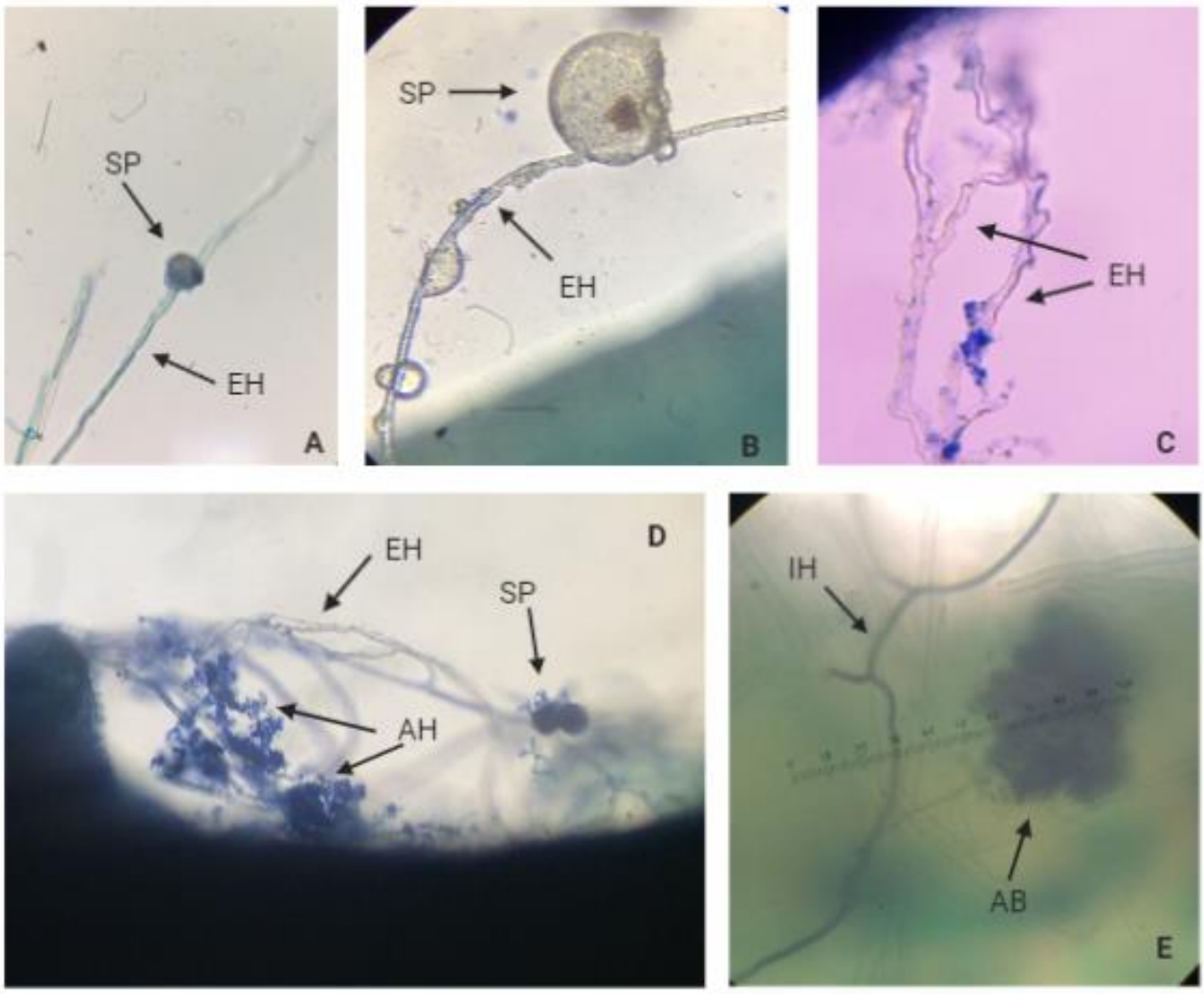


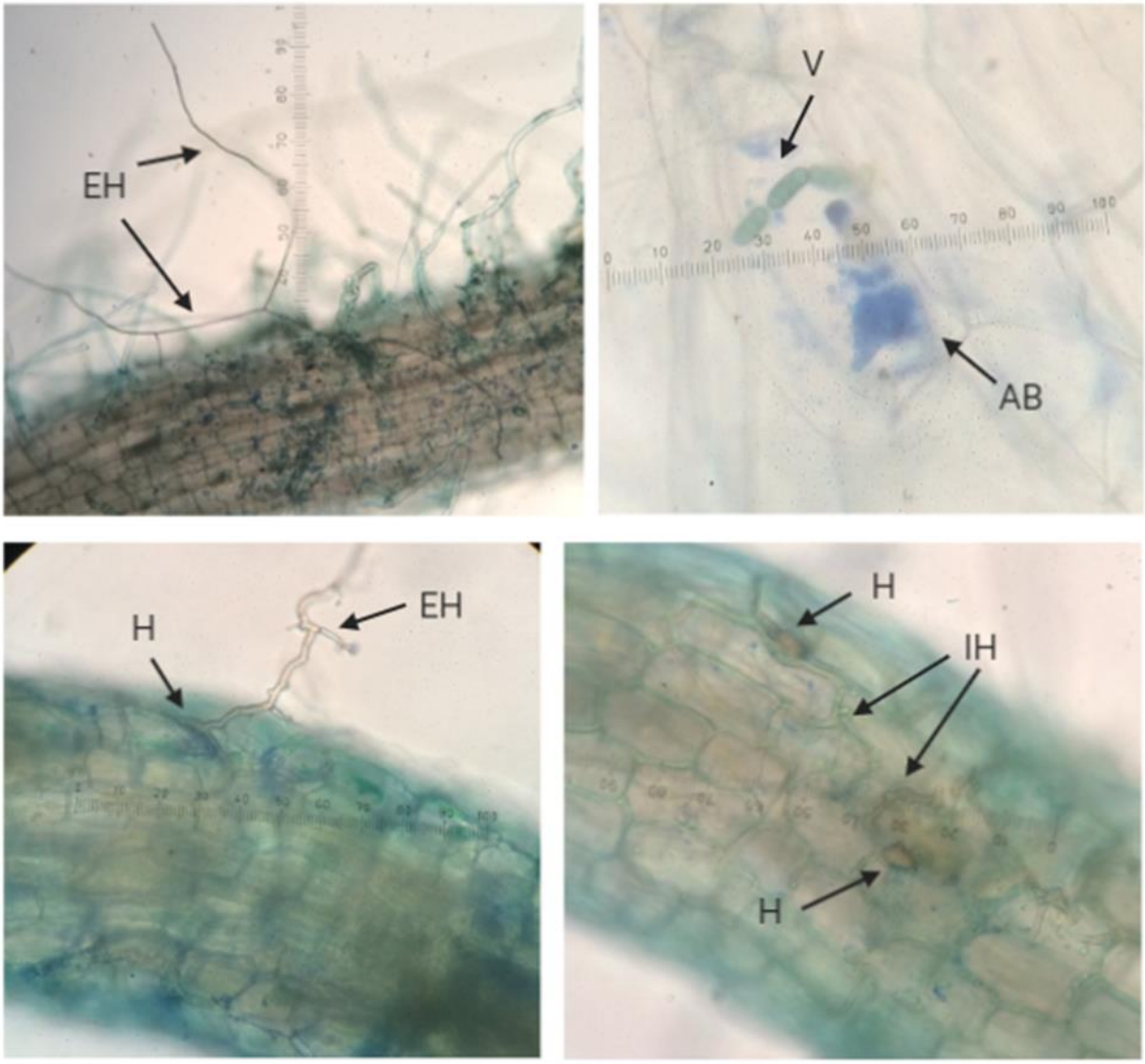
Figure 7: Ratio shoot to root. Distribution of shoot to root ratio for Terra Firme and Várzea forests.

Figure 8a: Base study microscopy of AMF mycotic structures of root samples of *Inga* sp.



Staining showing AM fungal structures proximal to roots (A-D) and withing roots (E) of Inga species. The presence of AM structures (arbuscules (AB), intraradical hyphae (IH), extraradical hyphae (EH), absorptive hyphae (AH) and spore (SP)) was assessed by means of an AmScope T120B-5M Digital Siedentopf Compound Microscope using an objective of 40×.

Figure 8b: Base study microscopy of AMF mycotic structures of root samples of *Inga* sp.



Staining showing AM fungal structures present in root samples of Inga species. The presence of AM structures (arbuscules (AB), vesicles (V), intraradical hyphae (IH), extraradical hyphae (EH), and hyphopodia (H)) was assessed by means of an AmScope T120B-5M Digital Siedentopf Compound Microscope using an objective of 40×.

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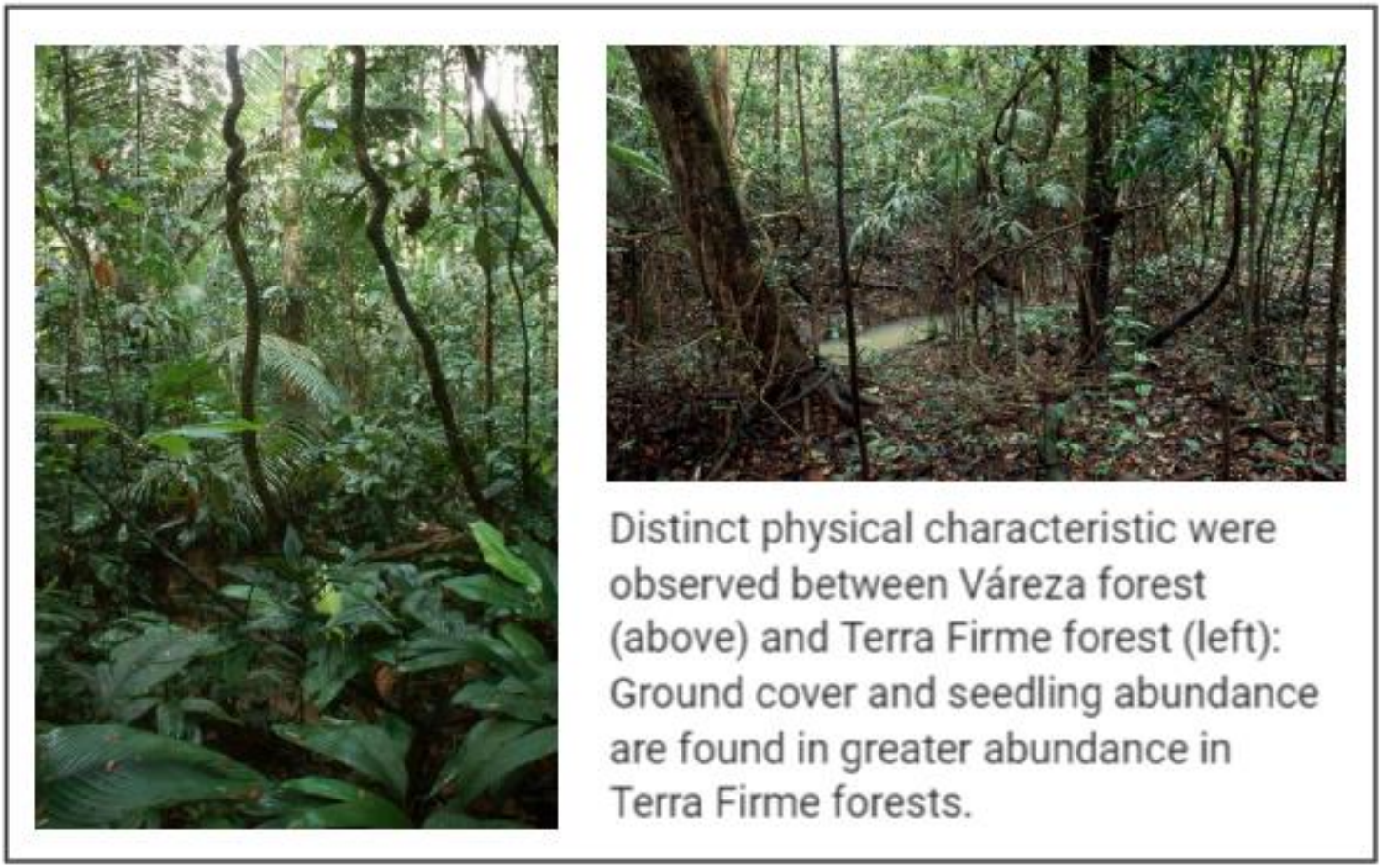

Figure 9: Visual comparison between Terra Firme and Várzea ecosystems. Visual differences in forest architecture between Várzea and Terra Firme forests. The photo for Várzea was taken near Chichico train at TBS. Here you can observe the proximity to a stream that flows into the Tiputini river. Photo for Terra Firme forest was taken near the Maquisapa trail.