The intensity of symbiotic relationships between arbuscular mycorrhizae and differentiated tree species regarding their age group and plant family in semi-arid Andine dynamical agroforestry system.

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Abstract: As research on mycorrhiza progress and scientific knowledge about organic partnerships becomes more profound, mycorrhiza symbiosis is considered an essential parameter for the vitality of ecosystems. Concerning polyculture cultivation systems, the implementation of growth-promoting and nutrient-securing symbiotic partners is a crucial step towards preserving the dynamism of involved plants and thus decisive for the yield and success of such cultivation systems. In particular, arbuscular mycorrhizal fungi (AMF) show considerable tendency in encouraging and maintaining a supply of water and nutrition for plants. Therefore, it was examined how intensive partnerships between AMF and trees in the semi-arid, dynamic agroforestry system of ‘Mollesnejita’ exist and how the species, family and age of trees are related to the respective degree of mycorrhizal intensity. This information is in turn used to decrypt relationships between nutrient provision and nutrient security in agroforestry systems and to improve them especially concerning current climate change. The results reveal that in the examined agroforestry system arbuscular mycorrhizal partnerships were found on all ten considered tree species in this study in varying intensity of the mycorrhizal structure dependent on tree species and their plant family. Nevertheless, no statistical correlation between the number of mycorrhizal elements according to primary hyphae, ramification or vesicles about the age of the trees could be proven in this study.

KeyWords: Age group, agroforestry, arbuscular mycorrhizae, semi-arid, subtropics.

Introduction

Role of Arbuscular Mycorrhiza in Ecosystems

Plants are autotrophic organisms capable of synthesizing all their components from water, carbon dioxide, and mineral elements with photosynthetic radiation. Studies of plant nutrition have shown that specific mineral elements are essential to ensure their growth and health1. To guarantee optimal nutrition sustenance, land plant evolution began to develop their nutritional mineral input at least 400 million years ago in symbiosis with mycorrhizal fungi, especially AMF of which approximately 150 - 200 species inside the family Glomeromycota exist2. The small number of AMF species might suggest their role in enriching biodiversity is limited, but the variations on their physiologic, morphologic, and genetic levels are rather high, resulting in a robust functional diversity that has a significant impact on ecology and application in plant production systems3. So far, the AMF association with terrestrial plants has been observed in 200 families of plants representing circa 1,000 genera and at least 300,000 species4. Therefore, mycorrhizal fungi, mainly AMF are ubiquitous in soil and create associations with most herbaceous angiosperms including many crops, cereals, vegetables, trees and horticultural plants4,5. The fine fungal hyphae with size of 2 – 12 µm radiating from the mycorrhiza increase the contact surface of the root with the soil7. After mycorrhizal infection roots by AMF’s fine hyphae and the following extension of the plant’s roots system facilitates the acquisition of water and mineral elements i.e. phosphorus that is relatively immobile in the soil and nutrients such as nitrogen, zinc, and copper. In return, plants provide carbohydrates8 and lipids9 to the arbuscular mycorrhizae. Likewise AMF stimulates the production of growth substances and may reduce stresses, diseases or pest attack10. AMF occurs over a global range of agro-climatic conditions in natural and agricultural ecosystems and is geographically ubiquitous11. Nevertheless, terms of the soil such as erosion, salinity, waterlogging, water holding capacity, soil types, soil porosity, fertility status, and vegetation, etc. appreciably influence AMF associations, composition, distribution, and activity12. Optimization and improvement of mycorrhizal symbiosis for different “agricultural applications can be considered as the attempt to extract the maximum plant benefit from colonization for the minimum loss of resources”13.

Improvement of Agroforestry Systems by Arbuscular Mycorrhizae

The intensification of agriculture as an inescapable consequence of the constraint to produce foodstuffs compounded with fast and uncontrolled industrialization has put an enormous burden on the natural ecosystem1. Agricultural impacts on biological, physical and chemical attributes of soils and their ecosystems, leading to biodiversity losses, decreases in soil coverage, changes in natural element cycles and the overall water balance, degradation of soil structure, erosion and contamination of groundwater and therefore resulting in unknown consequences of high complexity14,15. Considering the current cultivation methods, there is an increasing interest in agroforestry systems. Agroforestry is defined by growing trees along with various types of crops to enhance crop yields, conserve soil and recycle nutrients while producing fodder, fruit, timber, and wood for non-corporate and economic use16. Under agroforestry, the needs for ecological sustainability can be reconciled with the needs and future challenges for sustainable food production17. To ensure plant nutrition in such cultivation systems, it is crucial to understand the interactions among the

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actors and to improve plant growth by enhancing measures. Consequently, AMF has associated with improved and enhanced growth of many plant species due to production of growth-promoting substances and synergetic interactions with other beneficial and necessary microorganisms, thus affecting the whole ecosystem where AMF subsist 20. In a study 21 about subtropical agroforestry systems and the influence of mycorrhiza, 93 of the 101 tree species evaluated inside the agroforestry system were colonized by AMF. The general soil conditions prevalent in sustainable agriculture equivalent dynamic agroforestry are likely to be more beneficial to AMF than those under conventional agricultural methods corresponding pesticide application, fertilization and tillage 20. Under nutrient saturating conditions related to high-input agrarian systems, the considerable advantages are reduced while the carbohydrates costs for the plant remain, and the overall performance, including yield by AMF colonized plants can fail below that of those that are non-colonized 21. Since the mycorrhizal association may shift from a symbiotic relationship to a parasitic and damaging association because the fungus still obtains carbohydrates from the infected plant, but the host plant no longer benefits from improved nutrient uptake efficiency 20, 21. AMF may provide a more appropriate and environmentally acceptable alternative for sustainable agriculture including agroforestry, due to the fact of increasing expenses of inorganic fertilizers by agrochemical industry as well as environmental and public hazards associated with pesticides and following the pathogens resistant to chemical pesticides 21. Therefore, it was examined how intensive the potential partnerships between AMF and trees in a semi-arid, dynamic agroforestry system develop and how the tree species plus their plant-family and age of the trees are related to the degree of mycorrhizal intensity. This information is necessary to decrypt the relationships between nutrient provision and nutrient security in agroforestry systems and to improve them under adverse conditions, especially about current climate change and their resulting extreme conditions. Unfortunately, studies examining arbuscular mycorrhizal colonization not only with individual tree species but in a complete agroforestry system are scarce 21.

Materials and methods

Climate and Soil Conditions

The degree of mycorrhizal colonization was analyzed in ten different tree species on the 16 ha agroforestry area in Mollesneja at 2750 to 2840 meters above sea level beginning of May 2019. The research institute is located near the Andes border in the center of Bolivia in the proximity of Cochabamba. The study was performed during dry period southern of equator, and due to the winter season, the daylight was decreasing. Because of its altitude above sea level, the subtropical area is in the USDA winter hardness zone 10a and can, therefore, reach absolute minimum temperatures of -1.1 °C at night 22. The average annual temperature is 16.5 °C, showing respectively a minimum average temperature of 8.7 °C and a maximum average temperature of 25.4 °C. The middle yearly precipitation is 518 mm, of which only 68 mm in sum are precipitated during the seven arid months of April, May, June, July, August, September, and October. The soil profile of the agroforestry landscape was very nutrient-poor, had a high sand content and contained a high amount of unweathered Andean rocks. The phosphorus content available to the plants and salt conductivity of the soil amounted on average of only 9.339 kgP₂O₅/ha as well as an electric salt conductivity of only 0.27 mS/cm at the start of the experiment. The PH value of 5.3 proved slightly acidic. There was generally a very thin or almost nonexistent layer of humus, as large parts of the terrain were destroyed. 19 months before the start of the test due to a massive area fire. As a result, the upper humus layer was partially burnt, and therefore soil fertility was reduced. Nevertheless, the soil directly around the trunks of the trees had higher humus content and more excellent aggregate stability than the rest of the surrounding fallow land.

Biological Materials and their Preparation

To analyze the degree of mycorrhizal colonization, three trees per tree species of Inga feuerleii, Caesalpinia spinosa, Erythrina falcata, Acacia visco, Tecoma stans, Tecoma cochambensis, Jacaranda mimosaefolia, Fraxinus americanaus, Zanthoxylum coco and Schinus molle in the age classes 5, 10 and 15-20 years were examined. An exception was made for Acacia visco, as only three 10-year-old trees were available during the time of this experiment. Before sampling, the trees were surveyed for external consipicuities such as the influence of biotic and abiotic damage. All trees were externally vital at the time of sampling and showed no abnormalities except of exfoliation due to the current dry winter season. Three excavations were carried out at angles of 120° a depth of 30 cm. Several samples were taken per excavation consisting in sum of at least four light-colored fine-root strands, with a minimum length of 2 cm each. Furthermore, focus was to examine trees with the same soil samples in order to maintain comparability of the results and limit additional interrelations. Subsequently, the mycorrhiza was stained according to the method of Ungar et al. 27, which represents a slightly modified form of the established methodology of Vierheilig 21. At first, the root cells were discolored by destroying their cytoplasm in a process of heating them for 10 minutes in a solution of 10 % potassium hydroxide (KOH), then hyphae were colored with black ink (Brand: Pelikan) by heating in a 5 % vinegar solution in ratio of 1.50 for 3 minutes. The samples were then stored in a 50 % water-alcohol solution. From each pack of excavated root sample, two different fine-root strands were chosen randomly and observed under the microscope. Collectively, 180 root samples were evaluated, including 18 samples per tree species and six samples per tree and for its respective age. The absolute number of each mycorrhizal element was counted on the planar upper surface and how the tree species plus their plant-family and age of the trees were not observed in order to allow a direct comparison with other tree species and their age groups. The absolute area of each examined root sample was determined photometrically using a 12-megapixel color camera at a 90° angle to the root samples and ImageJ software Version 1.52a (Publisher: Wayne Rasband, National Institutes of Health, USA). For this purpose, the percentage background of the root sample was calculated by binary reduction (black, white) of the image and its relative proportion was subtracted from the total area of the object slice to obtain the whole root area in square millimeters and to calculate the number of mycorrhizal elements per square millimeter root area (The Mathematical calculation for the degree of mycorrhizal colonization is noted below.
The non-parametric Kruskal-Wallis test ($\alpha = 0.05$) was performed, to prove potential differences between the age groups and the proportion of hyphae elements. Since the dependent variable "age" was not normally distributed, the lowest distribution was ordinarily scaled, and the samples were independent of each other. Due to the sample size of 180 $> 30$ (critical value), the asymptotic significance value was taken into consideration. Based on the small sample size for trees 15 and 20 years old, these were grouped as category "15-20". The relationship between the relative proportion of

\[ MD = \frac{x}{\text{mm}^2} = \frac{n_e[x]}{(1 - B[N\%]) \cdot A_{\text{tot}}[\text{mm}^2]} \]

$MD$: Degree of mycorrhizal colonization [Quantity / mm²]
$n_e$: Quantity of mycorrhizal element [x]
$B_N$: Relative amount of negative binary background [%]
$A_{\text{tot}}$: Total area of object slide [\text{mm}²]

**Statistical Analysis**

The non-parametric Kruskal-Wallis test ($\alpha = 0.05$) was performed, to prove potential differences between the age groups and the proportion of hyphae elements. Since the dependent variable "age" was not normally distributed, the lowest distribution was ordinarily scaled, and the samples were independent of each other. Due to the sample size of 180 $> 30$ (critical value), the asymptotic significance value was taken into consideration. Based on the small sample size for trees 15 and 20 years old, these were grouped as category "15-20". The relationship between the relative proportion of
Results and Discussion

The results showed that AMF is omnipresent in the root structures of the studied agroforestry system and are in symbiosis with all observed tree species regardless of age (Figure 1 and 2). The findings confirm other investigations\(^3\) that mycorrhiza plays a fundamental role in balancing agrosystems and represent an essential link between soil and root. The positive effects on nutrient supply and water transfer as well as the general significance of AMF for ecosystems as already described in various literature\(^31\)-\(^35\) confirm the high occurrence of arbuscular mycorrhizae in this experiment (Figure 1 and 2) respective to the overall vital health of the trees. The thesis that acidic to neutral soils containing a large number of AM fungi\(^3\) could be confirmed by the average PH value of 5.3 on the areal and the widespread colonization by AMF (Figure 2). Because of the plural anastomosing mycorrhiza net\(^37,38\), plants of different species can exchange substances and communicate among themselves with organic carbon compounds\(^38,39,40\), which can be anticipated based on the uniformly mycorrhiza based on all examined trees, impartial with respect to their species, plant family (Figure 2) or age (Figure 1).

However, no statistical correlation between the intensity of mycorrhizal growth and age could be established by this experiment (Figure 1). Due to strong scattering of the determined values regarding primary hyphae, ramifications and vesicles, the Kruskal-Wallis test showed no correlation between the two parameters. Only the branching seems to have cumulated the parameter of the lowest test variable. All statistical analyses were performed using the open-source software PSPP Version 3.0 (Publisher: Benn Pfaff) under GNU General Public License.

Nevertheless, such assumptions must be confirmed by further investigation upon said tree samples. Predominant and extremely aversive abiotic stressors make the growth and survival of plants more difficult\(^1\), especially as the general low nutrient conditions with an electrical salt conductivity of only 0.27, the seven arid months and the resulting drought stress, as well as the high temperatures during the day in the sun in this case. Since all plants had a vital state of health, the colonization of tree roots by AMF with its water and nutrient-enriching attributes must be considered as a defense against abiotic stressors\(^35,36,37,51\).

Phosphate is an essential nutrient and limits plant growth\(^1\). Because of the low P\(_{2}O_{5}\) content of 9.339 kg/ha other relevant vectors must introduce phosphorus into the soil or make the current organic phosphorus available to the plants, otherwise sufficient growth of the trees would not be possible. Mycorrhizal fungi have been shown to mineralize organic soil phosphate through the synthesis of phosphatase\(^53,54\). Similar to phosphate, nitrogen is a major limiting nutrient to plant growth\(^1\), and most of the nitrogen in the soil is only available as ammonium (NH\(_4\)\(+) or nitrate (NO\(_3\)\(-\). Ammonium is the preferential form of nitrogen absorbed by subjected to a nitrogen deficiency\(^55,56\), but its concentration is 10 – 1000 times lower than of nitrate, especially in acidic soils like in this experiment\(^1\). Ammonium has minimal mobility in the soil and similar to phosphate, a zone of depletion is formed around the roots. AMF extraradical mycelium can absorb ammonium\(^57,58,59\) and amino acids\(^60\). It can, therefore, be assumed that the colonization of all tree species by AMF (Figure 2), regardless of the age of the trees (Figure 1), is associated with the compulsion for the plant to obtain nutrients from the soil and thus to enter into a symbiosis with the fungi.

Figure 3. Hyphal structure of arbuscular mycorrhizae on the fine root strands of (A) Tecoma stans (15 yrs. old) and (B) Schinus molle (10 yrs. old). Black bar equals 60 tm.
Conclusions

The intensity of mycorrhizal variation across all investigated parameters did not differ between the three age groups. The results indicate that in nutrient-limited, semi-arid, subtropical, dynamic agroforestry systems arbuscular mycorrhizae are present in symbiosis with all ten examined tree species in the varying intensity of fungal hyphae structure. Furthermore, it would be interesting to decipher the species of the current AMF by protein sequencing and to investigate which species are beneficial for agroforestry systems concerning growth, age of trees and climatic seasons.

Bibliographic references


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