

The role of microbial fertilizers in diversified cropping systems

El papel de los fertilizantes microbianos bajo un sistema de cultivo diversificado

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ABSTRACT. Agroforestry systems combine fruit trees with crops, providing environmental and productive benefits. This study investigated the impact of a combined application of arbuscular mycorrhizal fungi (AMF), *Rhizobium*, and mineral fertilization levels on the agronomic and physiological characteristics of maize (*Zea mays* L.) and common bean (*Phaseolus vulgaris* L.) intercropped with peach (*Prunus persica*), Persian lime (*Citrus latifolia* Tan.), and litchi (*Litchi chinensis*). The study also examined changes in the physical, chemical, and microbiological properties of the soil. An agroforestry system was established during the fall-winter agricultural cycle of 2020 in the community of Campo Grande, municipality of Ixtaczoquitlan, Veracruz, Mexico. The treatment with *Rhizophagus irregularis* + *Rhizobium etli* (2) + 50% mineral fertilization produced the greatest increases in plant height: 68.5±8.6 cm (Persian lemon), 105.3±11.0 cm (peach) and 6.8±4.5 cm (lychee). It also produced the highest grain yield in corn (5 653.7 kg ha⁻¹) intercropped with beans (995.1 kg ha⁻¹). At the end of the first productive cycle, the soil pH decreased from moderately acidic (5.7) to acidic (4.9). On the other hand, the organic matter increased from 1.71 to 2.76%. There was an increase in the number of bacteria (55.0 × 10⁶ to 68.0 × 10⁶ CFU g⁻¹ soil), fungi (73.55 × 10² to 511.7 × 10² CFU g⁻¹ soil) and actinomycetes (48.61 × 10⁵ to 53.19 × 10⁵ CFU g⁻¹ soil). The establishment of diversified agricultural systems in combination with microbial consortia promotes crop productivity and improves the physical, chemical, and microbiological properties of the soil.

Key words: Milpa system, fruit trees, soil health, microbial consortia, productivity.

RESUMEN. Los sistemas agroforestales combinan árboles frutales con cultivos agrícolas, brindando beneficios ambientales y productivos. Este estudio determinó el efecto de la aplicación combinada de hongos micorrízicos arbusculares (HMA), *Rhizobium* y niveles de fertilización mineral sobre las características agronómicas y fisiológicas del maíz (*Zea mays* L.) y frijol (*Phaseolus vulgaris* L.) intercalados con durazno (*Prunus persica*), limón persa (*Citrus latifolia* Tan.) y lichi (*Litchi chinensis*), así como algunos cambios en las propiedades físicas, químicas y microbiológicas del suelo. Se estableció un sistema agroforestal durante el ciclo agrícola otoño-invierno de 2020 en la comunidad de Campo Grande, municipio de Ixtaczoquitlán, Veracruz, México. El tratamiento con *Rhizophagus irregularis* + *Rhizobium etli* (2) + 50% de fertilización mineral produjo los mayores aumentos en la altura de las plantas: 68.5±8.6 cm (limón persa), 105.3±11.0 cm (durazno) y 6.8±4.5 cm (lichi). Además, produjo el mayor rendimiento de grano en maíz (5 653.7 kg ha⁻¹) intercalado con frijol (995.1 kg ha⁻¹). Al final del primer ciclo productivo, el pH del suelo disminuyó de moderadamente ácido (5.7) a ácido (4.9). Por el contrario, la materia orgánica aumentó de 1.71 a 2.76%. Hubo un aumento en la cantidad de bacterias (55.0 × 10⁶ a 68.0 × 10⁶ UFC g⁻¹ de suelo), hongos (73.55 × 10² a 511.7 × 10² UFC g⁻¹ de suelo) y actinomycetos (48.61 × 10⁵ a 53.19 × 10⁵ UFC g⁻¹ de suelo). El establecimiento de sistemas agrícolas diversificados en combinación con consorcios microbianos promueve la productividad de los cultivos y mejora las propiedades físicas, químicas y microbiológicas del suelo.

Palabras clave: Sistema milpa, árboles frutales, salud del suelo, consorcios microbianos, productividad.

INTRODUCTION

In recent decades, crop yields have increased significantly due to crop breeding and technological advances because of the Green Revolution (Hedden 2003, Yang *et al.* 2020). However, this increase in agricultural production has been associated with the degradation of agroecosystems in terms of reduced biodiversity, soil and water pollution, loss of organic matter, increased incidence of pests and diseases with greater resistance to pesticides, and low resilience of agroecosystems (Jiao *et al.* 2011, Jones *et al.* 2012, Marcos-Pérez *et al.* 2023). It is therefore clear that a combination of productivity and sustainability is required through innovative approaches (Naudin *et al.* 2015). This can be achieved through the design and management of cropping systems that consider the principles for greater resilience to climate change and that simultaneously contribute to soil recovery and biodiversity conservation (Fargione *et al.* 2018, Jahan *et al.* 2022).

Agroforestry is highlighted for its positive effects on soil quality (Guillot *et al.* 2021). It consists of establishing rows of trees on at least 10% of agricultural land to obtain wood, firewood, or other products, in association with agricultural species or livestock (Montagnini and Nair 2004). This agronomic strategy allows farmers to increase their income and land productivity in space and time through the production of basic crops (Tsonkova *et al.* 2012, Wolz and DeLucia 2018). The importance of this form of vegetation management has added new dimensions to food security and poverty alleviation, by creating additional income, reducing inequality, and social inclusion.

Another production form of used in traditional agricultural systems is intercropping or companion cropping, which involves planting two or more species in space and time (Brooker *et al.* 2015). Different types of intercropping systems have been proposed (Burgess *et al.* 2022, Almagro *et al.* 2023) report improvement in soil structure, water and nutrient availability for plants, land productivity, among others. The most common combination in this practice is cereal-legume (Layek *et al.* 2018), however, several authors have reported other interactions such as maize-tomato (Chen *et al.* 2023), broccoli-broad bean (Marcos-Pérez *et al.* 2023). In Mexico and some South American countries, agroforestry systems and the design of intercropped maize in fruit trees, hereinafter referred to as MIAF, have been implemented to meet their basic needs with the production of maize and beans as strategic elements for the food security of rural families (Cadena *et al.* 2018, Regalado *et al.* 2020). The MIAF agricultural system has represented an excellent productive option on sloping soils. Particularly under this system, crops are used that do not compete for physical spaces, nutrients, water, and radiation, among others (Dou *et al.* 2022).

Agroforestry systems are important for their economic and environmental benefits (Schroth *et al.* 2004). However, the stable development of these complex agricultural

systems is limited by the availability of water and nutrients in the soil (Gao *et al.* 2016, Dou *et al.* 2022). Additionally, it has been reported that, under nitrogen-limiting conditions, soil microorganisms decompose more organic matter, resulting in higher greenhouse gas emissions (GHG) (Moorhead and Sinsabaugh 2006, Wang *et al.* 2014). In this sense, it is still necessary to add knowledge on sustainable forms of fertilization in the discipline of diversified crops.

Furthermore, the complementary use of microorganisms and nutrients in intercropping systems, such as the MIAF system, can be an economically viable alternative for soil fertilization and regeneration (Lai *et al.* 2022). When applied to soil in the MIAF system, microbial consortia can fix nitrogen from the air, activate soil nutrients, improve plant nutritional status, and produce active substances to stimulate plant growth as growth promoters (Jia *et al.* 2020). Several studies have revealed the changes in soil microbiological characteristics caused by the association or intercropping between different crops (Guillot *et al.* 2019, Yang *et al.* 2020, Deng *et al.* 2022, Judson *et al.* 2023). However, changes in the soil microbial community and their effect on intercropped maize in fruit trees are scarce. Our hypothesis is that the MIAF system can improve crop yield and promote fruit tree growth by increasing microbial associations in the soil and improving nutrient uptake. In general, the MIAF system is expected to improve the physical, chemical, and biological properties of the soil. Therefore, the objective of this study was to determine the effect of arbuscular mycorrhizal fungi (AMF), *Rhizobium* and fertilization levels on the agronomic characteristics of maize and beans intercropped in fruit trees, as well as the effect of the MIAF agricultural system on the physical, chemical, and biological properties of the soil.

MATERIALS AND METHODS

The research was conducted under seasonal conditions in the community of Campo Grande, municipality of Ixtaczoquitlán, Veracruz, Mexico (Figure 1), located at 18° 49' 16" N, 97° 01' 15" W, and 823 m above sea level. The soil where the trial was established is of the luvisol type, with a slope of 27%. The climate is warm and humid *Am b(i)g* (García 2004), with an annual total precipitation of 2035.5 mm. During the research period, the average daily temperature was 23.2 °C and the average daily relative humidity was 88.1%. The average daily maximum temperature was over 28 °C, in July. The coldest month of the period was November.

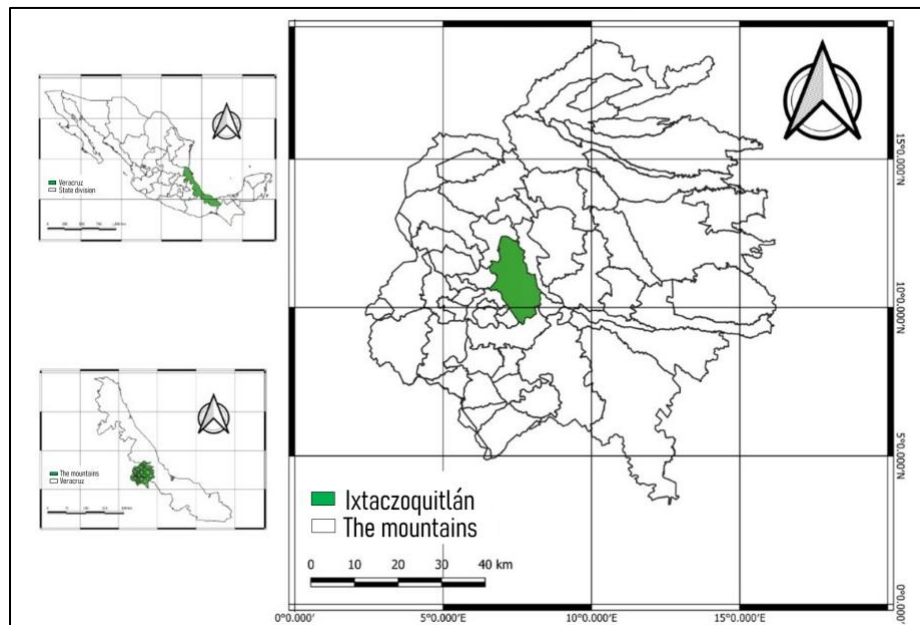


Figure 1. Location of the study area.

Establishment of the MIAF plot and plant material

A maize-intercropped-with-fruit-trees agricultural system was established in December 2020. The initial design involved tracing level curves perpendicular to the slope of the land for planting the fruit trees, which were planted in a layout of 1.0 m spacing between trees and 7.8 m between rows. Thirty fruit trees were planted on each level curve, 10 of each species: peach (*Prunus persica* L. Batsch), Persian lime (*Citrus latifolia* Tan.) and lychee (*Litchi chinensis* Sonn), with a spacing of 2.0 m between species (Figure 2). On each side strip of the fruit trees, two furrows of maize (*Zea mays* L.) were sown alternating with two furrows of beans (*Phaseolus vulgaris* L.), with a spring-summer 2021 cycle. In both crops, the spacing between furrows was 0.80 m. The spacing between plants was 0.6 m for maize and 0.3 m for beans. The first furrow of maize or beans is 2.3 m from the trunk of the fruit tree on both sides of the row. The plant density for maize cultivation was 10 416 plants ha⁻¹. The plant density for bean cultivation was 20 833 plants ha⁻¹. The total area of the land where the system was established was 0.09 hectares.

Microbiological Inoculants

The inoculants used were: *Rhizophagus irregularis* and a mycorrhizal consortium (composed of 13 AMF genera, including *Glomus dimorphicum*, *Rhizophagus fasciculatus*, and *Funneliformis mosseae*) [250 propagules g⁻¹] and two strains of *Rhizobium etli* [9 × 10⁷ CFU g⁻¹ soil], from the company BioFábrica Siglo XXI. Inoculation was carried out directly to the seed with 50 g of inoculant per kilogram of seed. Carboxymethylcellulose was used as an adhesive.

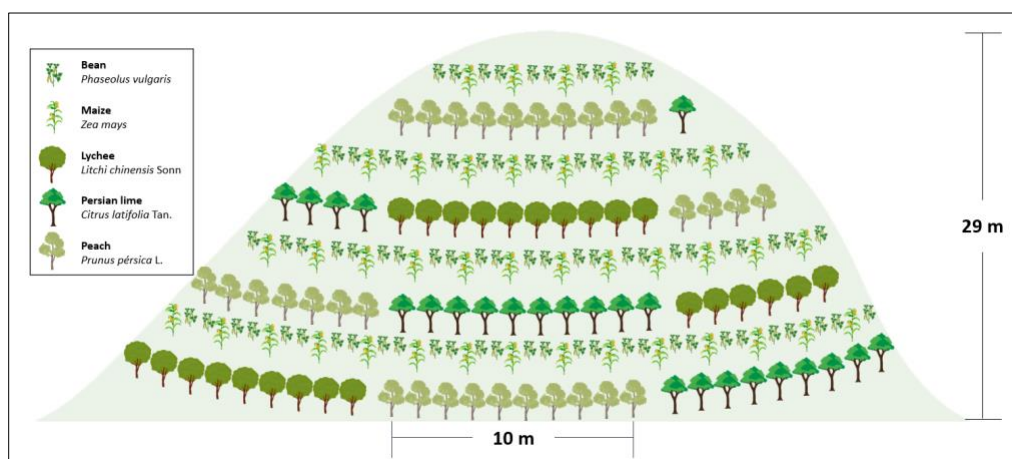


Figure 2. Distribution of crops in the experimental plot.

Experimental design

To investigate the effect of the combination of the studied microorganisms and chemical fertilization on seasonal crops, a completely randomized block experimental design with three replications was used (Table 1). In total, four combinations were involved in this experiment. The blocking factor was applied based on the slope of the land to prevent the transfer of microorganisms between the experimental plots. The slope of the land can generate water and soil movement, which could accidentally transport microorganisms from one treatment to another, affecting the integrity of the experiment's results.

Table 1. Description of the treatments applied to the maize and bean crop intercropped with fruit trees.

Treatment applied	Inoculant
T1	Consortium AMF + <i>Rhizobium etli</i> (1) + 50% de MF
T2	Consortium AMF + <i>Rhizobium etli</i> (2) + 50% de MF
T3	<i>Rhizophagus irregularis</i> + <i>Rhizobium etli</i> (1) + 50% de MF
T4	<i>Rhizophagus irregularis</i> + <i>Rhizobium etli</i> (2) + 50% de MF
T5	100% de MF (negative control)
T6	0% de MF (absolute control)

AMF: arbuscular mycorrhizal fungi, MF: mineral fertilization.

Agronomic management

Mineral fertilization for maize and bean was carried out with the formula 70 N - 60 P - 00 K and 35 N - 35 P - 00 K, respectively. At 15 days after planting (DAP) of maize, one-third of N and all the P₂O₅ were applied, subsequently, 30 DAP, the remaining two-thirds of N were applied, while for bean, all the fertilizer was applied at 15 DAP. Fertilization in the fruit trees was carried out with the formula 20 N - 10 P - 20 K.

Data recording

Maize Cultivation

Leaf area index (LAI m² m⁻²) was determined by measuring the length and width of all leaves on 5 randomly selected plants. The LAI was calculated using equation (Wang *et al.* 2023):

$$\text{LAI} = A * PD / 10000$$

Where: LAI = leaf area index (m² m⁻²), A=total leaf area (m²), PD= planting density (plants ha⁻¹).

Fresh biomass (g plant⁻¹) was determined by weighing the different plant organs separately on a digital balance with a precision of 0.01 g. Subsequently, dry biomass (g plant⁻¹) was calculated by placing the fresh organs in a drying oven at 65 °C, with forced air circulation, until a constant drying weight was reached.

The corn grain yield was recorded in kg ha⁻¹, using equation:

$$\text{Corn grain yield (kg h}^{-1}\text{)} = (TFW * DM * PG * CF) / 8600$$

Where: TFW= total field weight harvested in the experimental unit, DM= percentage of dry matter of a sample of grain from five ears, EG= estimated grain percentage of five ears, CF= conversion factor to obtain grain yield per hectare, 8600 = is a constant to estimate grain yield at a commercial moisture of 14% (Tadeo *et al.* 2014).

To measure the symbiosis between the microorganisms and the corn crop, the percentage of root colonization was determined using the triphenyltetrazolium chloride (TTC) staining technique (Phillips and Hayman 1970). The AMF spores from the samples were extracted from the soil using the wet decanting and sieving method proposed by Gerdemann and Nicolson (1963). The quantification of spores was performed by direct counting (low density) according to INVAM (2019).

Bean Cultivation

Leaf area index (LAI m² m⁻²) was calculated by measuring the length (from the petiole to the apex of the leaf) and width (widest point of the leaf) of all leaflets of randomly selected plants. LAI was calculated using equation 1 (Wang *et al.* 2023). Fresh and dry biomass (g plant⁻¹) was obtained using the same procedure employed for maize cultivation.

The bean grain yield was recorded in kg ha⁻¹, using equation:

$$\text{Bean grain yield (kg h}^{-1}\text{)} = ((NV * NG) / 1,000 g) * PD$$

Where: NV= number of pods, NG= number of grains, PD= planting density (plants ha⁻¹).

To assess the symbiosis between the microorganisms and the bean crop, the number of nodules present on the roots of 5 bean plants was counted visually. Additionally, the length and diameter of the taproot were measured using a graduated ruler. Root volume (mL) was determined by inserting the root into a graduated cylinder filled with water and recording the volume of water displaced.

Fruit trees

In the fruit trees, the initial height (cm) at the beginning of the experiment and the final height (cm) at the end of the annual crop production cycle were recorded. The height increment was calculated as the difference between the final height (cm) and the initial height (cm).

Soil quality

Soil samples were collected before the experiment was established (agricultural cycle 2020) and after the first production cycle (agricultural cycle 2021). Sampling was carried out according to the topography of the land at a depth of 0-40 cm. The sampled soil was homogenized to avoid edge effects and a composite sample was obtained. The physical, chemical, and microbiological parameters (Table 2) were determined according to the NOM-021-RECNAT-2000 standard, which establishes the specifications for soil fertility, salinity, and classification.

Table 2. Physical, chemical, and microbiological parameters of the study area.

Indicators	Units	Methods
Texture	-	Bouyoucos hydrometer
Bulk density	g cm ⁻³	Waxed lump
pH	-	Electrometric in water
Electrical conductivity	Sdm ⁻¹	Conductivity meter
Carbonates	%	Volumetric titration
CEC	cmol Kg ⁻¹	Ammonium acetate
Total nitrogen	%	Micro Kjeldahl
Assimilable P	mg kg ⁻¹	Bray and Kurtz 1
K	mg kg ⁻¹	Ammonium acetate
Ca	mg kg ⁻¹	Ammonium acetate
Na	mg kg ⁻¹	Ammonium acetate
Mg	mg kg ⁻¹	Ammonium acetate
Fe	mg kg ⁻¹	DTPA and EDTA
Cu	mg kg ⁻¹	DTPA and EDTA
Zn	mg kg ⁻¹	DTPA and EDTA
Mn	mg kg ⁻¹	DTPA and EDTA
B	mg kg ⁻¹	Azometina-h
Organic matter	%	Walkley and Black
Bateria	CFU g ⁻¹ soil	Plate count
Fungi	CFU g ⁻¹ soil	Plate count
Actinomycetes	CFU g ⁻¹ soil	Plate count

CFU: colony formin units, CEC : cation exchange capacity, DTPA : diethylenetriaminepentaacetic acid, EDTA: ethylenediaminetetraacetic acid.

Statistical analysis

All variables were subjected to a test of normality and homoscedasticity of variances using the Shapiro-Wilk and Bartlett tests (Castillo 2007). The data were analyzed using an analysis of variance (ANOVA) and a means comparison test using Tukey's HSD ($P \leq 0.05$). The data were analysed using the STATGRAPHICS® Plus 5.0 statistical software.

RESULTS

Effect of microorganisms and mineral fertilization on corn and bean crops

Treatment T3 exhibited the highest yield ($5\,653.6 \pm 310.2 \text{ kg ha}^{-1}$), followed by T4 ($4\,698.6 \pm 470.3 \text{ kg ha}^{-1}$) and T5 ($5\,230.7 \pm 807.7 \text{ kg ha}^{-1}$). Treatments T1 and T2 with HMA consortium had lower yields ($3\,865.6 \pm 651.2 \text{ kg ha}^{-1}$ and $4\,000.4 \pm 106.6 \text{ kg ha}^{-1}$, respectively). The absolute control, which received no treatment, had the lowest yield ($2\,779.6 \pm 149.1 \text{ kg ha}^{-1}$) (Figure 3a).

The highest IAF was observed in treatments T4 ($5.4 \text{ m}^2 \text{ m}^{-2}$) and T5 ($5.8 \text{ m}^2 \text{ m}^{-2}$) (Figure 3b). Treatment T5 (505.7 ± 42.1) produced the highest fresh root biomass. Dry root biomass did not show significant differences among treatments. However, treatments T3, T4, and T5 had the highest dry root biomass values (Figure 3c). Treatments T4 and T5 generated higher fresh and dry aerial biomass, with statistical significance (Figure 3d).

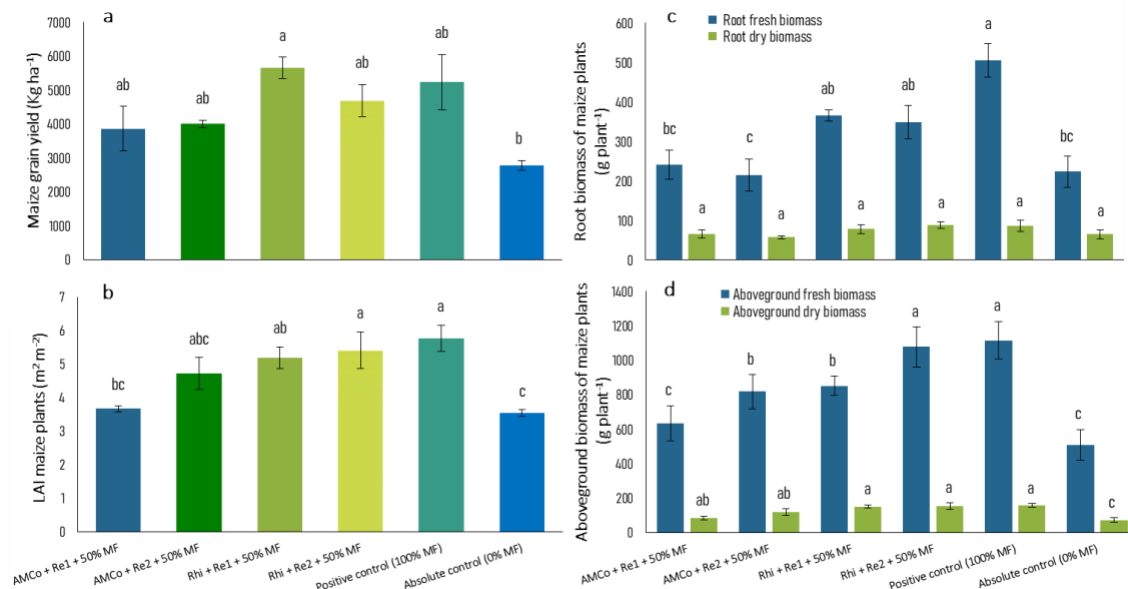


Figure 3. Effect of microorganisms and mineral fertilization on maize cultivation: a) grain yield, b) leaf area index, c) fresh and dry root biomass, d) fresh and dry aerial biomass, AMCo: arbuscular mycorrhizal consortium, Rhi: *Rhizophagus irregularis*, Re1: *Rhizobium etli* strain 1, Re2: *Rhizobium etli* strain 2, and MF: mineral fertilization. The vertical lines in the bars represent standard errors. Different letters indicate significant differences between the different treatments in the Tukey's multiple comparisons test.

Regarding the number of HMA spores, these were detected in all treatments. Treatments T3 and T4 had the highest quantities, with 377 ± 21.2 and 322 ± 39.2 spores per 50 g^{-1} soil,

respectively ($p \leq 0.05$). Treatments T1, T2, T5, and T6 did not show significant differences between each other in the number of spores (Figure 4a). The colonization percentage was also higher in treatments T3 (55%) and T4 (60%) (Figure 4b).

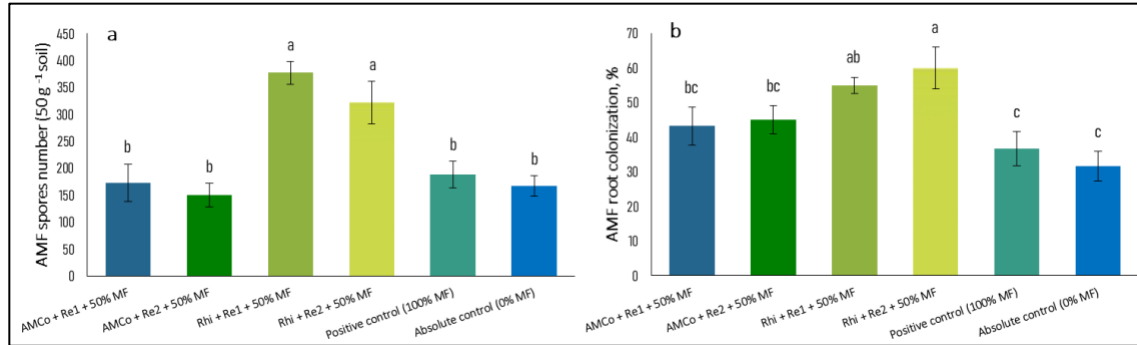


Figure 4. Effect of microorganisms and mineral fertilization on maize cultivation: a) number of spores in soil, b) percentage of colonization, AMCo: arbuscular mycorrhizal consortium, Rhi: *Rhizophagus irregularis*, Re1: *Rhizobium etli* strain 1, Re2: *Rhizobium etli* strain 2, and MF: mineral fertilization. The vertical lines in the bars represent standard errors. Different letters indicate significant differences between the different treatments in the Tukey's multiple comparisons test.

In the bean crop, treatment T4 ($1\ 123.8 \pm 129.8\ \text{kg ha}^{-1}$) achieved the highest grain yield (Figure 5a), slightly higher than the overall experiment average ($918.9\ \text{kg ha}^{-1}$). Regarding the leaf area index (LAI) values at 75 days after planting (DAP), no statistically significant differences were observed. However, treatments T3 ($1.27\ \text{m}^2\ \text{m}^{-2}$) and T5 ($1.19\ \text{m}^2\ \text{m}^{-2}$) presented the highest LAI values (Figure 5b).

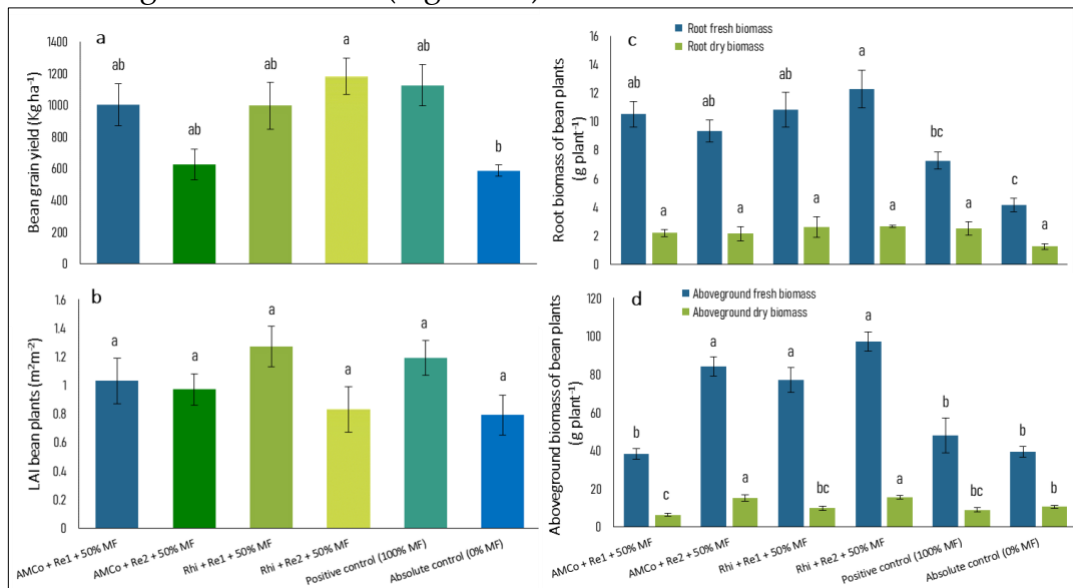


Figure 5. Effect of microorganisms and mineral fertilization on the bean crop: a) bean grain yield, b) leaf area index, c) fresh and dry root biomass, and d) fresh and dry aerial biomass, AMCo: arbuscular mycorrhizal consortium, Rhi: *Rhizophagus irregularis*, Re1: *Rhizobium etli* strain 1, Re2: *Rhizobium etli* strain 2, and MF: mineral fertilization. The vertical lines in the bars represent standard errors. Different letters indicate significant differences between the different treatments in the Tukey's multiple comparisons test.

In the bean crop, the generation of fresh root biomass was higher in treatment T4 (12.1 g plant⁻¹). No significant differences were observed in dry root biomass among the evaluated treatments; however, treatment T4 obtained the highest value, like fresh root biomass (Figure 5c). Fresh aerial biomass in treatments T3, T4, and T5 was statistically superior to that of the other treatments (Figure 5d).

In the cultivation of native variety beans, root characterization did not show significant differences in root length (Figure 6a). Treatments T3 and T4 had the plants with the highest LAI (Figure 6b). Root volume was higher in treatment T1, followed by treatments T2, T3, and T4 (Figure 6c). Similarly, the number of nodules in bean plants was statistically higher in treatments with beneficial microorganisms (T1, T2, T3, and T4) (Figure 6d).

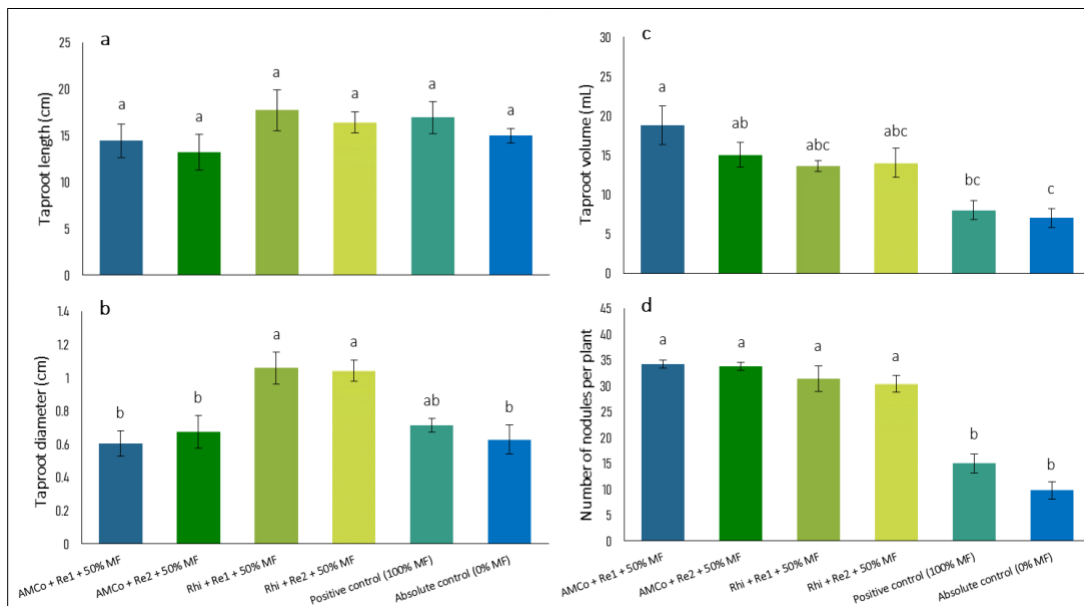


Figure 6. Effect of microorganisms and mineral fertilization on bean cultivation: a) length of the taproot, b) diameter of the taproot, c) volume of the taproot, d) number of nodules per plant, AMCo: arbuscular mycorrhizal consortium, Rhi: *Rhizophagus irregularis*, Re1: *Rhizobium etli* strain 1, Re2: *Rhizobium etli* strain 2, and MF: mineral fertilization. The vertical lines in the bars represent standard errors. Different letters indicate significant differences between the different treatments in the Tukey's multiple comparisons test.

Effect of intercropping on fruit trees

Figure 7 shows the average height increments (m) during the research period. The addition of different combinations of microorganisms and mineral fertilization in annual crops promoted significant changes in tree development, with statistically superior results observed in treatments T3, T4, and T5. Treatment T4 had a positive effect on lemon tree growth, with height increments of 68.5 ± 8.6 cm. Compared to other fruit trees, lychee tree showed low adaptability to the soil and climate conditions of the area.

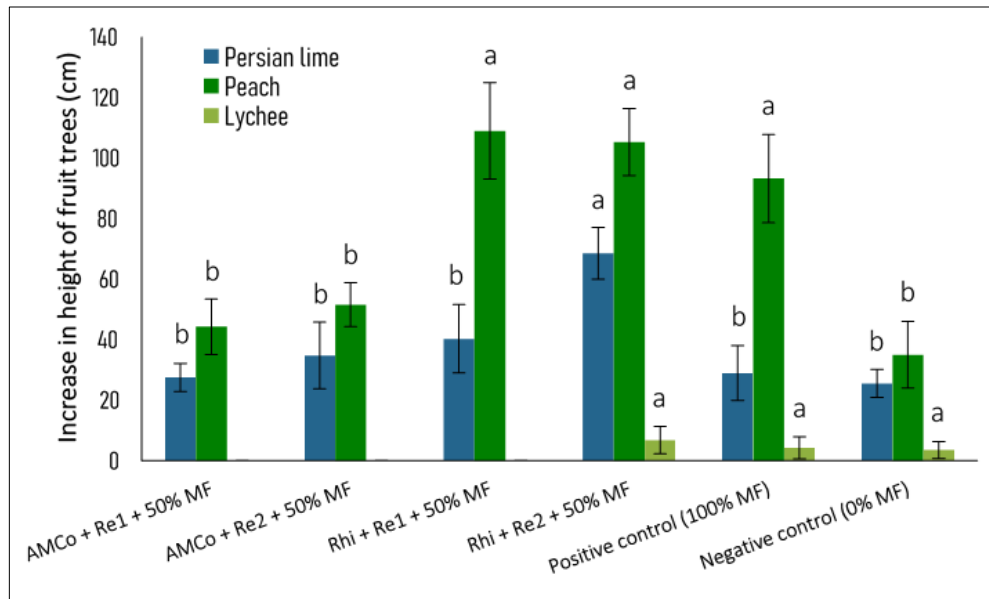


Figure 7. Effect of treatments on height increase of fruit trees (Lychee, Persian Lemon, Peach). AMCo: arbuscular mycorrhizal consortium, Rhi: *Rhizophagus irregularis*, Re1: *Rhizobium etli* strain 1, Re2: *Rhizobium etli* strain 2, and MF: mineral fertilization. The vertical lines in the bars represent standard errors. Different letters indicate significant differences between the different treatments in the Tukey's multiple comparisons test.

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Evaluation of the MIAF agroforestry system on soil properties

To assess the impact of the MIAF agroforestry system on soil properties, various soil parameters were measured. The soil in the study area is a luvisol with a clayey texture (Table 3). The bulk density of the soil decreased slightly from 1.78 g cm^{-3} at the beginning of the experiment to 1.58 g cm^{-3} at the end of the production cycle. The soil was found to be free of carbonates (0.13%) and salts (0.023 Sdm^{-1}). Electrical conductivity (EC) values increased from 0.02 to 0.04 dSm^{-1} . The Cation Exchange Capacity (CEC) also showed an increase from 13.70 to $15.38 \text{ cmol kg}^{-1}$. Organic matter (OM) increased from 1.71 to 2.76%; similarly, total nitrogen (Nt) increased from 0.08 to 0.13%. In terms of macro and micronutrients, the MIAF agroforestry system promoted increases in the content of N, P, K, B, Cu, Fe, Mn, and Zn at the study site (Table 3).

Table 3. Results of the physical, chemical, and biological characterization of the soil, before establishing the MIAF plot and at the end of the first production cycle.

Indicators	Pre-production cycle	Production cycle	Units
Texture	Arcilla	Arcilla	-
Bulk density	1.78	1.58	g cm ⁻³
pH	5.70	4.90	-
Electrical conductivity	0.02	0.04	Sdm ⁻¹
Carbonates	0.13	0.13	%
CEC	13.70	15.38	cmol Kg ⁻¹
Total nitrogen	0.08	0.13	%
Assimilable P	0.78	1.08	mg kg ⁻¹
K	37.05	56.94	mg kg ⁻¹
Ca	1 998.40	2 003.40	mg kg ⁻¹
Na	119.93	147.74	mg kg ⁻¹
Mg	9.89	10.35	mg kg ⁻¹
Fe	19.10	66.60	mg kg ⁻¹
Cu	0.30	1.22	mg kg ⁻¹
Zn	0.15	0.53	mg kg ⁻¹
Mn	5.50	17.29	mg kg ⁻¹
B	0.29	0.73	mg kg ⁻¹
Organic matter	1.71	2.76	%
Bateria	55.0 x 10 ⁶	68.0 x 10 ⁶	CFU g ⁻¹ soil
Fungi	73.55 x 10 ²	511.7 x 10 ²	CFU g ⁻¹ soil
Actinomycetes	48.61 x 10 ⁵	53.19 x 10 ⁵	CFU g ⁻¹ soil

CFU: colony forming units, CEC: Cation exchange capacity.

At the end of the first production cycle, changes were observed in the quantity of microorganisms present in the soil of the MIAF agricultural system (Table 3). The fungal population increased from 73.55×10^2 to 511.7×10^2 colony-forming units per gram of soil (CFU g⁻¹ soil), while bacteria increased from 55.0×10^6 to 68.0×10^6 g⁻¹ soil. Actinomycetes experienced an increase from 48.61×10^5 to 53.19×10^5 g⁻¹ soil. While bacteria are the most abundant microorganisms in the soil in terms of the number of individuals, fungi, due to their larger size, represent around 70% of the total microbial biomass, playing a fundamental role in the functioning of the ecosystem.

DISCUSSION

The association between mycorrhizal fungi and rhizobacteria is likely the most widespread beneficial relationship between plants and microorganisms. This ecological interaction is crucial for the ecosystem. Its potential to improve agricultural productivity and reduce

environmental impact makes it a valuable tool for sustainable agriculture (Hidalgo and Ramos 2019, García *et al.* 2012).

In staple crops, such as cereals and legumes, the use of biofertilizers has yielded satisfactory results in many tropical regions (Reyes *et al.* 2018). Inoculating seeds with beneficial microorganisms promote the speed of nutrient uptake by plants through a direct effect on the roots (Aguirre-Medina and Velasco-Zebadúa 1994). This allows for improved soil fertility, promotes biodiversity, and reduces dependence on synthetic fertilizers.

The use of HMA and *Rhizobium etli* inoculants supplemented with 50% mineral fertilization in maize and bean crops on hillslope luvisol soils resulted in yields comparable to those obtained with 100% mineral fertilization. This effect can be attributed to the characteristics of arbuscular mycorrhizal fungi (AMF) species such as *Rhizophagus irregularis*, which are characterized by being intraradical colonizers with long extraradical hyphae (Jansa *et al.* 2007, Thonar *et al.* 2011). These features allow AMF to effectively absorb nutrients from the soil and deliver them to plants. On the other hand, the symbiotic association between *Rhizobium* bacteria and legumes involves the formation of nodules, where the concentration of O₂ is regulated by leghemoglobin. The supply of carbon compounds occurs inside the nodules, which allows the bacteria to avoid competition with other microorganisms (Osorio-Vega 2009). This mutually beneficial relationship helps legumes fix nitrogen from the atmosphere, making nitrogen available to the plants.

The relationship between the number of spores in the soil and crop yield is complex, being affected by the type of crop, climatic conditions, and management practices. In the case of cultivated soils, the average number of spores is 1 000 spores 100 g⁻¹ soil (Aguilar *et al.* 2016). This study found particularly low numbers of spores. However, Cavigelli *et al.* (2005) found a lower number of spores in soils with a heavier texture, such as the soil in this study. It is important to note that organic farming has not been shown to necessarily result in many numbers of arbuscular mycorrhizal fungal spores (Gosling *et al.* 2010). However, Morton *et al.* (1993) found a positive correlation between the number of spores and the percentage of mycorrhizal colonization in a study with different plant species.

Treatments containing beneficial microorganisms and reduced mineral fertilizer had a positive effect on leaf area index (LAI) and biomass production. While LAI and biomass are influenced by a range of factors, beneficial microorganisms represent a valuable tool that can be used to improve crop management and increase productivity. This suggests that even with reduced fertilizer application, beneficial microbes can enhance plant growth and yield.

In general, Persian lime and peach showed greater adaptability to the soil and climatic conditions. Rivera *et al.* (2020) demonstrated that intercropping inoculated with mycorrhizal fungi had positive effects on the main crops (fruit trees). These

microorganisms are highly efficient in promoting crop growth, and this promotion may depend on the fungal species and soil characteristics. The correct choice of tree species can contribute to limiting competition and promoting complementarity due to the temporal and spatial separation of nutrient uptake with the crops (O'Connor *et al.* 2024).

Concerning soil quality, the results obtained in this study reinforce what has been described by various authors (Schroth *et al.* 2004, Wolz and DeLucia 2018, Guillot *et al.* 2019, Morugán-Coronado *et al.* 2020, Yang *et al.* 2020, Barros-Rodríguez *et al.* 2021, Almagro *et al.* 2023, Quandt *et al.* 2023). These authors argue that the combination of intercropping in fruit trees, perennials, and conservation tillage could have beneficial effects on the physical, chemical, and biological attributes of the soil. These attributes are indicators of soil structure recovery and improvements in water and nutrient availability for plants. This is important because in mountainous areas, agroforestry can be a more sustainable way to produce food, products, and services than monoculture agriculture (Duchene *et al.* 2017). In general, the establishment of the MIAF agricultural system allowed for increases in soil quality indicators.

According to Dominati *et al.* (2010), physical indicators are related to "inherent quality" which is influenced by soil age and past climates. In contrast, most chemical and all biological indicators can be associated with "dynamic quality" which is highly sensitive to land use and soil management (Gianfreda and Ruggiero 2006).

The use of living wall technology constructed with fruit trees and zero soil tillage practices led to a decrease in bulk density. Stine and Weil (2002) suggest that bulk density is influenced by the balance between solid particles and pore space, which is itself largely determined by soil organic matter content. High bulk density can restrict plant root growth, potentially leading to aeration stress (Stepniewski *et al.* 1994), lower soil temperature, altered biological processes (Brussaard and Van Faassen 1994), increased denitrification (Linn and Doran 1984), and loss of mycorrhizal fungi (Ellis 1998). Conversely, the increase in the CEC value suggests a greater capacity for storing nutrient cations. Soil CEC is primarily influenced by organic matter and clay minerals (Bourg and Sposito 2011).

This study observed an increase in both macro and micronutrients within the soil. Morugán-Coronado *et al.* (2020) similarly reported positive effects on nitrogen and other soil quality indicators in the presence of alley cropping and conservation tillage practices. This synergy is attributed to improved soil physical properties, enhanced water conservation, and reduced CO₂ emissions (Simoes *et al.* 2014, Almagro *et al.* 2016). These findings can explain the observed increase in soil organic matter and microbiota in this experiment. Organic matter originates from microbial decomposition and transformation of plant residues and root exudates (Guo *et al.* 2020). Several studies have shown that the

composition of dissolved organic matter in soil is primarily derived from plant litter leachates and rhizodeposits released through root exudates, secretions, and the shedding of fine roots during growth, alongside organic compounds from mycorrhizal turnover (Canarini *et al.* 2019, Shen *et al.* 2023, Zheng *et al.* 2024). Microbial communities play a crucial role in improving soil structure and chemical properties, ultimately supporting plant growth. They therefore serve as strong indicators of soil health (Sahu *et al.* 2019). Furthermore, soil organisms exemplify the cause-and-effect chain linking land management decisions with productivity and the overall health of plants and animals (Doran and Zeiss 2000).

One noteworthy indicator from the analysis is the acidic soil pH. In Mexico, acidic soils are typically found in tropical and temperate forest regions. These regions cover an area close to 14 million hectares, with the state of Veracruz having the largest expanse of such soils (Castellanos 2014). However, the acidic pH could also be attributed to the mineralization of organic matter, where NH_4 (ammonium) is produced as a final product of decomposition. This NH_4 contributes to soil acidification (Molina 1999). Another potential mechanism within agricultural systems involves the production of CO_2 through respiration processes. This CO_2 forms carbonic acid (H_2CO_3), which can combine with cations in the soil. The resulting reaction products are then leached from the soil profile, promoting acidic conditions (Valencia 1998).

CONCLUSIONS

This study underlines the potential of fostering mutually beneficial interactions between plants and microorganisms to achieve sustainable and productive agriculture. Intercropping with fruit trees demonstrably improves soil quality. This practice has been shown to enhance the physical, chemical, and biological properties, leading to improved soil structure, water availability, and nutrient retention. Further research is necessary to optimise management practices for specific crops and soil conditions, while also addressing challenges such as acidic pH.

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CONFLICTO DE INTERÉS

The authors have no financial or non-financial interests that could influence the research presented in this manuscript.

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