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Beneficial Microorganisms Associated with an Agroforestry System Enhance the Soil Quality of Cuñumbuque Pastures in San Martín, Peru

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Microbial activity plays an essential role in preserving soil quality and increasing crop production. Agroforestry systems, in turn, emerge as a promising alternative for managing soil in an agroecological and sustainable manner. This study aimed to evaluate the microbiological indicators of soil quality in an agroforestry system in the Mayo River basin, Cuñumbuque, San Martin, Peru. The experimental design was carried out using randomized blocks with 5 treatments (T) and 4 replications. The evaluated systems included: T1, Recovering pasture + chemical fertilization + agroforestry component 1; T2, Recovering pasture + beneficial microorganisms + agroforestry component 1; T3, Recovering pasture + agroforestry component 1; T4, Recovering pasture + beneficial microorganisms + agroforestry component 2; and T5, the control containing only recovering pasture. Soil sampling was conducted three times every 60 days after the experiment installation, assessing the physicochemical characteristics of the soil, microbial biomass, basal respiration, and urease and phosphatase enzyme activity. Overall, treatments T2 and T4, which included beneficial microorganisms and agroforestry components, showed the best results in terms of microbial biomass, basal respiration, urease, and phosphatase enzyme activity, standing out as suitable indicators of soil quality in pasture areas.

1. Introduction

Pasture management poses a challenge, particularly in tropical regions. An effective strategy to address this challenge is through agroforestry systems. These systems offer various environmental services, such as productive diversification, soil conservation, shade for livestock, water saving, and income generation through timber (Álvarez et al., 2023). Additionally, they ensure pasture productivity and animal yield similar to or greater than monoculture pastures (Domiciano et al., 2020). The San Martín region has 112,958 ha of pastures (SINIA-MINAM-DRASAM, 2016), most of which are under monoculture systems, leading to soil degradation.

Moreover, soil biology plays a fundamental role in its structure and composition, with organic matter decomposed by soil microorganisms that release nutrients for plants (Valdez-Nuñez et al., 2019). Biological nitrogen fixation and phosphate solubilization by beneficial microorganisms are essential for soil aggregate stabilization and plant growth (Ríos-Ruiz et al., 2023).

Overgrazing and poor agricultural management have contributed to the degradation of many pastures in the region, exacerbated by rainfall scarcity during the summer, leading to soil compaction and loss of vegetative cover. This results in nutrient loss during the rainy season and a decrease in soil microbial community, negatively affecting biogeochemical processes and pasture productivity (Suescún et al., 2023). This research presents an important novelty by integrating the use of beneficial microorganisms with agroforestry systems to improve soil quality in degraded pastures. The inclusion of microbiological indicators, such as microbial biomass and soil enzyme activity, offers a new perspective on enhancing soil quality in grazing areas. Therefore, this study aims to determine how the use of beneficial microorganisms in an agroforestry system can improve the quality of degraded soils in pasture areas in Cuñumbuque, San Martin.

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2. Methodology

2.1. Location and experiment setup

The field experiment was conducted on a livestock farm with *Brachiaria brizantha* pasture, located in Cuñumbuque (-6°18´46´´S and -76°40´17´´W), in the San Martin region, Peru. An experimental plot was established using a completely randomized block design, consisting of 5 treatments with 4 replications each, totaling 20 experimental units. The treatments (T) were defined as follows: T1 = RP (Recovering pasture) + NPK (Inorganic fertilization with nitrogen, phosphorus, and potassium) + AFC1 (Agroforestry component 1 = (Amasisa (*Erythrina poeppigiana*) + *Centrocema macrocarpum* + *Brachiaria brizantha* + Bolaina (*Guazuma crinita*) + Capirona (*Calycophyllum spruceanum*)); T2 = (RP + BM (Beneficial microorganisms = (arbuscular mycorrhizal fungi + rhizobia + phosphate-solubilizing bacteria)) + AFC1; T3 = RP + AFC1; T4 = RP + BM + AFC2 (Agroforestry component 2 = (*B. brizantha* + Bolaina + Capirona)) and T5 = RP.
In the designated area, tree and shrub species were planted according to the established experimental design. The treatment that included chemical fertilizers, especially nitrogen, was applied at a rate of 20 kg N/ha every two weeks during the trial, starting at two weeks after planting. Beneficial microorganisms were reactivated from strains existing in the culture collection of the Agricultural Microbiology Laboratory "Raúl Ríos Reátegui" of the Faculty of Agricultural Sciences of the Universidad Nacional de San Martín and multiplied following the methodologies described in Ríos-Ruiz et al. (2019) and Valdez-Nuñez et al. (2019). For arbuscular mycorrhizal fungi, the species *Acaulospora rugosa* and *Ambispora appendicula* obtained from soils in the Cuñumbuque area were used. Several variables were evaluated over three 60-day periods after the experiment installation, including physicochemical and microbiological soil analyses, such as soil microbial biomass, soil basal respiration, urease activity, and phosphatase activity.

2.2. Evaluation of the influence of beneficial microorganism use

2.2.1 Physicochemical analysis of soils

For this analysis, soil collection was carried out in each treatment by zigzagging through the area, obtaining 3 subsamples per experimental unit at a depth of 0 to 20 cm. These subsamples were homogenized to obtain a representative sample of 1 kg per experimental unit. Subsequently, the representative samples were transferred to the soil laboratory for physicochemical analysis and to the agricultural microbiology laboratory for microbiological analysis. In the laboratory, soil samples were dried, crushed, and sieved through a 2 mm mesh sieve (No. 10). The sieved samples were stored in first-use polypropylene bags and kept at 4°C until microbiological processing.

2.2.2 Microbiological analysis of soils

2.2.2.1 Soil microbial biomass (SMB)

The SMB analysis was carried out following the methodology of Anderson and Domsch (1978). Ten grams of wet soil were dried to constant weight, and the moisture content was adjusted to 60 % of the water holding capacity with distilled water. Then, 20 grams of dry soil were taken and placed in glass jars, with three replicates. Sixty milligrams of anhydrous glucose were added, and they were incubated at 22 °C for 2 h. Subsequently, 0.1 N NaOH was added, and they were incubated at 22 °C for 4 h. Titration was performed with 0.025 N HCl, recording the amount of acid consumed. The results were expressed as µg C/g of soil.

2.2.2.2 Soil basal respiration (SBR)

To determine the SBR, the methodology of Alef and Nannipieri (1995) was followed. Initially, 10 g of wet soil were dried at 105 °C to constant weight, and the moisture content was adjusted to 60 % of the water holding capacity with distilled water. Then, 100 g of sieved wet soil were weighed and transferred to 250 mL glass flasks with 0.5 N NaOH to capture the produced CO_2 and distilled water to maintain humidity. After one week of incubation at 25 °C, the excess NaOH was titrated with 0.5 N HCl. The results were expressed as C-CO_2 mg/kg.h.

2.2.2.3 Urease enzyme activity

Urease activity was evaluated following the method of Kandeler and Gerber (1988), measuring the production of NH_4^+ in sediments incubated with urea as a substrate. One gram of soil was taken in specific glass tubes, with 4 replicates per sample, and incubated for 2 h at 37 °C with horizontal agitation. After incubation, the suspension was centrifuged and filtered to determine the NH_4^+ content, using a spectrophotometer, and the results were expressed in µmol NH_4^+/g.h.

2.2.2.4 Phosphatase enzyme activity

Phosphatase enzyme activity was evaluated using the method described by Tabatabai (1982), by measuring the production of p-nitrophenol (PNP) in sediments incubated with PNP phosphate as substrate. 0.5 g of soil was taken in specific glass tubes, with 4 replicates per sample, and incubated for 2 h at 37 °C with horizontal

agitation. After incubation, additional reagents were added, and the suspension was centrifuged and filtered. The PNP content was determined using a spectrophotometer and expressed in µmol PNP/g.h.

2.3. Statistical analysis

For data analysis, the statistical software InfoStat 2019 was used. Analysis of Variance (ANOVA) and the Duncan test were applied to compare means, with a significance level of $p < 0.05$, in order to identify differences between treatments. Additionally, the Coefficient of Variation (CV) was calculated to assess data dispersion.

3. Results and discussion

3.1. Physicochemical soil analysis

The Table 1 shows the physicochemical characteristics of the soil in the experimental area plots at the beginning of the experiment.

Table 1. Physicochemical characteristics of the soil in the experimental area at the beginning of the experiment.

Sample	pH	EC µS/cm	OM %	N %	P ppm	K ppm	Sand %	Clay %	Silt %	Textural class
1	7.94	419.23	3.56	0.178	10.32	279.45	29.5	52	18.5	Clay

EC = Electrical conductivity (conductivity, soil: water ratio 1:2.5, SI Analytics Lab 960, Germany), N = Nitrogen (Micro Kjeldahl), OM = Organic matter (Walkley and Black, 1934), P = Extractable phosphorus (Modified Olsen), K = Potassium, extracted with ammonium acetate and quantified using atomic absorption spectrophotometer GBC, SavantAA, Australia.

The Table 2 shows the physicochemical characteristics of the soil from the different treatments in the experimental area, after 180 days post installation of the experiment.

Table 2. Physicochemical characteristics of the soil from the different treatments in the experimental area, after 180 days post installation of the experiment

Treatments	OM (%)	N (%)	P (ppm)	K (ppm)
1 RP + NPK + AFC1	3.76 b	0.19 a	14.31 a	270.12 a
2 RP + BM + AFC1	4.17 a	0.21 a	14.45 a	271.66 a
3 RP + AFC1	3.87 b	0.19 a	12.94 b	268.96 a
4 RP + BM + AFC2	3.99 ab	0.20 a	13.21 b	269.63 a
5 RP	3.48 c	0.16 b	10.32 c	226.20 b
CV (%)	4.58	6.94	2.98	3.57

OM = Organic matter, N = Nitrogen, P = Phosphorus, K = Potassium, RP = Recovering pasture, AFC = Agroforestry component, BM = Beneficial microorganisms. The data correspond to the average of four replicates. Different letters in each column denote statistically significant differences based on the Duncan test ($p < 0.05$).

The data from Tables 1 and 2 reveal variations in the physicochemical characteristics of organic matter (OM), nitrogen (N), and phosphorus (P). At the beginning of the experiment (Table 1), the levels of OM, N, and P were 3.56 %, 0.17%, and 10.32 %, respectively. However, after 180 days from the start of the experiment (Table 2), an increase in these three parameters was observed, with treatment T2 standing out, showing levels of OM at 4.17 %, N at 0.21 %, and P at 14.45 %. This increase suggests that the presence of beneficial microorganisms and the silvopastoral composition in this treatment positively influenced the improvement of soil nutrient composition. In a study related to the long-term implementation of a silvopastoral system, Moreno-Galván et al. (2023) evaluated the implementation of this system and observed an improvement in phosphorus availability in the soil, as well as greater bacterial diversity.

The Duncan test ($p < 0.05$) revealed significant differences among treatments for OM and soil Nitrogen. Treatments T2 and T4, which included microorganisms and agroforestry components, showed a higher increase in OM, with percentages of 4.17 % and 3.99 %, respectively, compared to treatment T5, which reached 3.48 %. Regarding nitrogen, a similar pattern was observed, where treatments T1, T2, T3, and T4 showed significant differences compared to the control treatment T5, with percentages between 0.19 % and 0.21 %, while T5 only reached 0.16 % (Table 2). The increase in average OM levels is primarily attributed to the contribution of leaf litter from the trees and shrubs of the established agroforestry components according to the different treatments of the experiment. Figure 1 illustrates the experimental area after 2 years of implementing the study, highlighting the remarkable development of the incorporated plant species (amasisa, bolaina, and capirona). The dense root systems present under the trees, shrubs, and grasses improve water infiltration and reduce runoff, thereby

minimizing nutrient loss and maximizing absorption by plants (Rolo et al., 2023). Additionally, according to Sierra-Alarcón et al. (2019), the application of increasing levels of nitrogen can increase both pasture production and quality.

Figure 1. Experimental area of the agroforestry system established in Cuñumbuque, showing considerable development of the plant species, two years after planting.

Regarding available phosphorus (Table 2), according to the Duncan test ($p < 0.05$), treatment T2 (RP + BM + AFC1) shows the highest content of available phosphorus (14.45 ppm), although it does not differ significantly from treatment T1 (RP + NPK + AFC1) (14.31 ppm). Treatments T4 and T3 also present significant levels. All of these differ notably from treatment T5, which lacks agroforestry components and inoculation of microorganisms. The high values of available phosphorus are attributed to the direct application of NPK fertilizer in treatment T1, and in T2, to the incorporation of the agroforestry component and the inoculated phosphate-solubilizing microorganisms (PSM) in the plots of this treatment. Wang et al. (2023) state that among PSM, phosphate-solubilizing bacteria can convert insoluble phosphorus into soluble forms, making it available to plants.

Regarding available potassium, Table 2 shows that treatments T1, T2, T3, and T4, which include agroforestry components, have significantly higher potassium ppm values, with no statistical differences among them but compared to treatment T5, which lacks these components. Potassium availability is influenced by factors such as clay content and soil mineralogy (Volf et al., 2023). The study area, characterized by clay soils, has elevated levels of potassium, favored by the cation retention capacity of clay soils. However, soil compaction is a significant problem in the region, limiting proper nutrient uptake by plants.

3.2. Microbiological Analysis

The SMB showed higher levels in treatments T2 and T4, which involved the inoculation of microorganisms and the integration of agroforestry components (Table 3). A significant increase in SMB was observed in the second evaluation (after 120 days post installation of the experiment) and in the third evaluation (after 180 days post installation of the experiment), with values of 1,434.64 µg C/g and 1,534.03 µg C/g, respectively, for treatment T2, and 1,436.84 µg C/g and 1,408.61 µg C/g, respectively, for treatment T4. In the third evaluation, significant differences were also evidenced between treatment T2 and control treatment T5, highlighting the positive influence of inoculated microorganisms and the integration of agroforestry components, including leguminous and non-leguminous species. Similar SMB results were found by Valdez-Nuñez et al. (2019) in other sectors of Cuñumbuque, where SMB levels ranged from 1,296.04 to 1,721.50 µg C/g. In agroforestry systems, the increase in SMB and microbial diversity is attributed to the increase in organic matter, which benefits cover crop productivity (Gupta et al., 2023).

Regarding SBR, the inoculated treatments (T2 and T4) showed moderate increases compared to other treatments, with significant differences in the first evaluation (Table 3). SBR decreases with soil depth and is significantly correlated with soil organic matter content, concentrating biological activity in the upper layer of 10 to 20 cm of soil (Angulo et al., 2023). It is important to highlight that tree legumes, such as *Erythrina poeppigiana* and *Centrocema macrocarpum*, used in this study, contributed to the incorporation of nutrients into the system, especially nitrogen, ensuring soil health and quality.

Table 3. Microbiological analysis of the soil from the different treatments in the experimental area, in the various evaluations conducted

Treatments	SMB (µg C/g)	SBR (C-CO_2 mg/kg.h)	Urease activity (µmol NH_4^+/g.h)	Phosphatase activity (µmol PNP/g.h)
First evaluation (after 60 days post installation of the experiment)				
1 RP + NPK + AFC1	1,114.19 a	4.98 ab	561.93 a	396.57 a
2 RP + BM + AFC1	1,109.30 a	4.98 ab	680.98 a	553.36 a
3 RP + AFC1	758.44 b	4.61 b	610.38 a	412.65 a
4 RP + BM + AFC2	1,130.93 a	5.16 a	638.83 a	489.31 a
5 RP	955.71 ab	4.53 b	509.31 a	525.62 a
C.V. (%)	18.75	6.77	38.26	29.43
Second evaluation (after 120 days post installation of the experiment)				
1 RP + NPK + AFC1	1,359.73 a	4.99 a	349.43 b	349.31 a
2 RP + BM + AFC1	1,434.64 a	5.40 a	436.46 a	493.83 a
3 RP + AFC1	1,428.23 a	5.01 a	327.64 b	327.29 a
4 RP + BM + AFC2	1,436.84 a	5.34 a	335.86 b	430.02 a
5 RP	1,253.28 a	3.68 b	306.09 b	365.14 a
C.V. (%)	8.91	25.23	10.57	26.92
Third evaluation (after 180 days post installation of the experiment)				
1 RP + NPK + AFC1	1,363.55 ab	4.73 a	561.10 a	268.72 b
2 RP + BM + AFC1	1,534.03 a	5.01 a	701.57 a	386.10 a
3 RP + AFC1	1,377.36 ab	4.86 a	527.41 a	315.14 ab
4 RP + BM + AFC2	1,408.61 ab	5.13 a	692.40 a	315.14 ab
5 RP	1,294.91 b	4.48 a	505.74 a	260.38 b
CV (%)	9.82	9.82	51.19	51.19

SMB = Soil microbial biomass, SBR = Soil basal respiration, RP = Recovering pasture, AFC = Agroforestry component, BM = Beneficial microorganisms. The data correspond to the average of four replicates. Different letters in each column denote statistically significant differences based on the Duncan test ($p < 0.05$).

Regarding urease activity, higher activity was observed in treatments that incorporated both microorganisms and agroforestry components, although significant differences were only observed in the second evaluation (Table 3). In this evaluation, treatment T2 reached 436.46 µmol NH_4^+/g.h and T4 reached 335.86 µmol NH_4^+/g.h, compared to treatment T5 which obtained 306.09 µmol NH_4^+/g.h. Urease activity is closely related to the nitrogen cycle and can be easily detected in soils with different coverings. As soil depth increases, urease values are likely to decrease, as mentioned by Silva et al. (2018). This decrease could be attributed to microbial growth limitation in the absence of nitrogen and oxygen sources.

Regarding phosphatase activity, treatments T2 and T4, which involved the inoculation of microorganisms and the incorporation of agroforestry components, exhibited higher phosphatase activity, with significant differences detected in the third evaluation (Table 3). Specifically, treatment T2 showed an activity of 386.10 µmol PNP/g.h, contrasting with treatment T5 which recorded 260.38 µmol PNP/g.h. According to Moreno-Galván et al., 2023, the mineralization of organic phosphorus in soil is crucial in the agricultural cycle. Enzymes, such as phosphatases, release phosphorus from soil organic compounds.

4. Conclusions

The implementation of an agroforestry system that included five treatments revealed that those inoculated with microorganisms and agroforestry components stood out for presenting better results. Additionally, the treatment that included chemical fertilizers also yielded promising results by providing nutrients available for plants. The incorporation of species from the agroforestry components into the treatments was successfully carried out under field conditions. Likewise, it was observed that soil microbial biomass and soil basal respiration parameters proved to be good indicators of soil quality. The evaluated enzymatic activities, such as urease and phosphatase, also proved to be effective indicators of soil quality. Furthermore, the results obtained in this study reveal how treatments that include beneficial microorganisms and agroforestry components can optimize microbial activity and improve soil fertility, representing a significant advancement for the sustainable management of pastures in the San Martin region.

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