Fallow improves the growth and yield of green beans and changes the rhizosphere microbial communities

Suelos en barbecho mejoran el crecimiento y rendimiento de habichuela y modifican las comunidades microbianas de la rizosfera

Nicolás Rodríguez-Romero[1], Vanessa Otero-Jiménez[1,2], and Daniel Uribe-Vélez[1]*

ABSTRACT

Soil microorganisms support key ecosystem services for agriculture, and some agricultural practices can increase soil microbial activity and improve crop productivity. Fallow periods have been considered a strategy for restoring biological activity. However, our understanding of the link between fallow periods and the biological activity restored remains limited. The present study evaluated soil microbial communities under two different management strategies: continuous agriculture and fallow. Soil physicochemical parameters and microbial communities were determined through microbiological, biochemical, and molecular techniques. The results showed that fallow soil had significantly higher values (P<0.05) of organic matter, pH, counts of soil microorganisms, and soil enzymatic activities than agricultural soil. Finally, the evaluation of plant growth showed that plants in fallow soil grew significantly better (P<0.05) than those in agricultural soil. However, after sterilization, the differences between the two soils disappeared. Leaving the soil in fallow periods allows the accumulation of organic matter, the growth of key microbial functional groups, the enhancement of soil enzymatic activities, and a significant improvement in plant growth and yield.

Key words: enzymatic activity, microbiome, *Phaseolus vulgaris*, soil health, soil fertility.

RESUMEN

Los microorganismos del suelo soportan servicios ecosistémicos clave para la agricultura, y algunas prácticas agrícolas pueden aumentar la actividad microbiana del suelo y mejorar la productividad de los cultivos. Los períodos de barbecho se han considerado una estrategia para la restauración de la actividad biológica. Sin embargo, nuestra comprensión de la relación entre los períodos de barbecho y la actividad biológica restaurada sigue siendo limitada. El presente estudio evaluó las comunidades microbianas del suelo bajo dos estrategias de manejo diferentes: agricultura continua y barbecho. Se determinaron parámetros fisicoquímicos del suelo y comunidades microbianas mediante técnicas microbiológicas, bioquímicas y moleculares. Los resultados mostraron que el suelo en barbecho presentó valores significativamente más altos (P<0.05) de materia orgánica, pH, conteo de microorganismos del suelo y actividades enzimáticas en comparación con el suelo agrícola. Además, la evaluación del crecimiento vegetal reveló que las plantas en suelo en barbecho crecieron significativamente mejor (P<0.05) que en suelo agrícola. Sin embargo, tras someter los suelos a un proceso de esterilización, las diferencias entre ellos desaparecieron. Dejar el suelo en barbecho permite la acumulación de materia orgánica, el crecimiento de grupos funcionales microbianos clave, la mejora de las actividades enzimáticas del suelo y un aumento significativo en el crecimiento y rendimiento de las plantas.

Palabras clave: actividad enzimática, microbioma, *Phaseolus vulgaris*, salud del suelo, fertilidad del suelo.

Introduction

Green bean (*Phaseolus vulgaris* L.) is one of the world's most important food legumes for direct use, particularly in least-developed countries, due to its contribution as a rich source of protein, vitamins, minerals, and fiber (Chaurasia, 2020). The demand for this crop is expected to increase based on current trends in population growth and green bean consumption, with a worldwide production of 28.3 million t by 2022, with an estimated value of US$ 31 billion (Chaurasia, 2020; FAOSTAT, 2024). Currently, this legume is widely distributed in almost all areas of the tropics and subtropics (Tridge, 2021).

Green bean production is the primary source of income for most farmers in the eastern province of Cundinamarca, Colombia. However, crop yields have considerably decreased in recent years due to inadequate practices like overfertilization, high input of herbicides, insecticides, and fungicides, and over-mechanization. These practices are

Received for publication: August 8, 2024. Accepted for publication: January 20, 2025.

Doi: 10.15446/agron.colomb.v43n1.116146

[1] Universidad Nacional de Colombia, Instituto de Biotecnología, Grupo de Investigación Microbiología Agrícola, Bogotá (Colombia).
[2] Department of Soil and Water Systems, University of Idaho, Moscow, ID (USA).
* Corresponding author: duribev@unal.edu.co

common in the tropics, where the lack of seasons encourages farmers to plant year-round. As a result, they lead to severe soil deterioration due to accelerated erosion, depletion of soil organic carbon (SOC), and loss of biodiversity (Romdhane *et al*., 2022).

Soil fertility depends on physicochemical properties and microbial composition (Kandasamy *et al*., 2019). Microorganisms play a key role in soil because they conduct many biological processes crucial for agroecosystems, such as biological nitrogen fixation and mineralization of nutrients by organic matter decomposition (Banerjee & van der Heijden, 2022). Moreover, they respond to changes in abundance, diversity, and activity with differentiated reactions under various agronomic management strategies, thereby influencing crop productivity (Mann *et al*., 2019). Consequently, a continuous cropping strategy decreases soil quality and has detrimental effects on soil microbial communities (Romdhane *et al*., 2022).

Fallow is the practice of resting land for short periods, especially in subtropical or temperate countries during the non-growing season (Garba *et al*., 2022). In some cases, the land is left fallow for several years to allow soil quality regeneration through the restoration of its natural structure and the improvement of its agrophysical properties (Burdukovskii *et al*., 2020). There are different indicators to measure this biological response. The first alternative is the evaluation of microbial communities using culture-dependent and culture-independent techniques such as Denaturing Gradient Gel Electrophoresis (DGGE) or target sequencing. An alternative approach to assessing soil quality restoration is measuring functional groups through enzymatic activities, which allows characterization of microbial processes in the soil (Klein *et al*., 1985).

Soil enzymes catalyze the essential biochemical reactions for the cycling of nutrients in the soil through the degradation and transformation of organic matter (Reardon *et al*., 2019); in this way, their activity can be used to make inferences about the quality of the soil (Sinsabaugh *et al*., 2008). Particularly, phosphatases and the phosphorus they release have been used as indicators of the effects of management practices like amendment application or reduced tillage (Bandinck & Dick, 1999; Klein *et al*., 1985). On the other hand, nitrogenases are sensitive to nitrogen availability, since the application of nitrogen fertilizers inhibits their activity (Roper *et al*., 1994). Finally, cellulases are part of a larger group of enzymes known as glycosidases (Eivazi & Tabatabai, 1990). Cellulose degradation is a critical process in soil ecosystems, playing a vital role in nutrient cycling and organic matter decomposition (Datta, 2024). These characteristics allow for the assessment of soil quality in different land uses (Dotaniya *et al*., 2019).

Traditionally, research on soil microbial communities has focused on economically important crops like wheat, rice, soybean, or maize (Brisson *et al*., 2019; Chen *et al*., 2019; Edwards *et al*., 2018; Otero-Jiménez *et al*., 2021). However, there are few studies on the microbial communities of other crops like common beans, which have primarily examined the effect of the crop domestication on rhizosphere microbial communities (Pérez-Jaramillo *et al*., 2017; 2019). In this study, we tested the effect of continuous agriculture and a five-year fallow period on green bean yield and the structure and activity of the rhizosphere microbiome. We hypothesized that fallow may improve crop productivity, enhance plant vigor, and increase soil microbial activity.

Materials and methods

Field location and soil conditions

The present study was carried out on La Colorada farm (4°29′42.96′′ N, 73°51′38.6′′ W) located in Fómeque, Cundinamarca, Colombia. The average temperature and annual precipitation were 18.4°C and 1256 mm, respectively. Soils were classified as clay loam (Pachic Melanudands) (Soil Survey Staff, 2022). We evaluated soils under two contrasting conditions: Soil A (eight years of continuous agriculture; crop rotations included passion fruit, gladiolus, tree tomato, and cape gooseberry; this field was planted with green beans in 2019 for this study) and Soil F (five years uncultivated and planted again with green bean crops in 2019 for this study).

Agronomic conditions

One green bean crop cycle was carried out in 2019 on La Colorada farm in a soil field with continuous agricultural practices (field A, 5709 m^2), and a soil field left fallow for five years (field F, 6012 m^2). Fertilization was carried out by applying NPK 10-30-10 at a dose of 250 kg ha^{-1}; the sowing density was 17 plants m^{-2}. Each field, A and F, was divided into three plots of equal area: soil A, 1903 m^2, and soil F, 2004 m^2 per plot. Each plot was considered one replicate.

Soil physicochemical parameters

Bulk soil samples were taken (depth 25 cm) in triplicate from soil A and F before the crop cycle, air-dried, and sieved (mesh size 2 mm in diameter). The physicochemical parameters were measured following the protocols of Pansu and Gautheyrou (2006). Organic matter (OM) was measured using the Walkley-Black method; the texture was measured by the hydrometer method; apparent density

(AD) was measured by the paraffin clod method; and pH was measured using the water method (1:1 v/v).

Soil sampling for microbial analysis

Six plants in each plot were pulled out and the roots were shaken vigorously to remove bulk soil. The six plant roots were combined and stored in Ziplock® bags to obtain a composite sample for each experimental plot. The bags were transported in cooler boxes (4°C) to the Agricultural Microbiology laboratory at the Biotechnology Institute of the Universidad Nacional de Colombia (IBUN), and rhizosphere soils were collected according to Barillot *et al.* (2013) to evaluate the microbial community function and structure as described below.

Study of culturable microorganisms

For the rhizosphere soil collected, heterotrophic bacteria and phosphate solubilizers were evaluated using the plate count technique on a Plate Count Agar (OxoidTM) and NBRIP culture medium (tricalcium phosphate as the sole source of phosphate), respectively (Nautiyal, 1999). Free-living nitrogen fixers were evaluated using the most probable number (MPN) technique (Chandrapati & Williams, 2014), with NFB culture medium (Alef & Nannipieri, 1995).

Soil enzymatic activities

Acid and alkaline phosphatase activities were evaluated according to the procedure proposed by Eivazi and Tabatabai (1977) in the rhizosphere soil collected. This evaluation is based on the determination of *p*-nitrophenol released after incubation of soil samples in a bis-*p*-nitrophenyl phosphate solution for 1 h at 37°C. Cellulase enzymatic activity was determined by the procedure proposed by Schinner and von Mersi (1990), which is based on the determination of reducing sugars released after incubation of soil samples using carboxymethylcellulose (CMC) as a substrate for 24 h at 50°C. Finally, nitrogenase activity was measured by the Acetylene Reduction Assay (ARA), based on the quantification of ethylene produced from a hermetically sealed bottle containing soil, where the gas phase was replaced with acetylene (Zuberer & Silver, 1978).

Bacterial community analysis by denaturing gradient gel electrophoresis (DGGE)

A DGGE analysis was carried out on the collected rhizosphere soil to determine the effect of soil management on the structure of the soil bacterial community. This technique was implemented following the protocol of Vanegas *et al.* (2013). Total DNA from rhizosphere soil was extracted using the DNeasy PowerSoil Pro Kit (Qiagen Hilden, Germany). DNA was quantified using a NanoDrop spectrophotometer (Thermo Scientific NanoDrop One) and visualized by agarose gel electrophoresis (1%). The concentration of all samples was above 30 ng μl^{-1}. The total DNA isolate was used as a template to amplify the V4 region of the 16S rRNA gene using the universal primers 338F and 518R (Muyzer & Smalla, 1998). The PCR mix contained 1X buffer, 2 mM $MgCl_2$, 0.1 mM dNTPs, 0.5 µM of each primer, 1U Taq polymerase, and 4 ng μl^{-1} of DNA per reaction tube (50 µl). PCR amplification was performed in a Thermal Cycler S1000 (Bio-Rad Laboratories, Hercules, CA, USA). The conditions were: 92°C for 2 min for initial denaturation, followed by 30 cycles (1 min of denaturation at 92°C, annealing at 55°C for 30 s, extension at 72°C for 1 min), with a final extension at 72°C for 6 min.

The DGGE was carried out using a Dcode Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA). The PCR products were run in duplicate on a 6% polyacrylamide gel in a 1X TAE buffer; the denaturant gradient was 30-80% (urea:formamide). Electrophoresis was run for 16 h at 60°C and 75 volts; the gel was stained with SYBR™ Gold and photographed under UV light using a Gel Doc™ XR+ (Bio-Rad Laboratories, Hercules, CA, USA).

Effect of soil management on crop productivity

For each replicate, which consisted of a plot of 1903 m^2 for soil A and 2004 m^2 for soil F, yield data were measured and the number of leaves and root nodules per plant were counted at the flowering crop stage in six plants per replicate. For yield, weekly harvests of the fruits were conducted, and the fresh weight of each harvest was recorded. The weekly records were summed at the end of the crop cycle to calculate total production and then divided by the area to calculate the yield.

Effect of soil microbial biomass on initial plant growth

A pot assay was implemented to assess the effect of a five-year fallow period on the initial growth of green bean plants in comparison to conventional agricultural management. A factorial design was adopted to evaluate the effect of the soil-living microbial biomass. The first factor, soil sterility, had two levels: sterile and non-sterile soil. The second factor, soil management strategy, had two levels, agricultural soil (soil A) and fallow soil (soil F). Soil sterilization was done by two sterilization cycles at 121°C and 15 psi for 40 min each. A total of four treatments with 14 independent plants were applied, where each plant was considered an experimental unit. The number of replications was calculated using the Harris-Hurvitz-Mood method. Green bean seeds were planted outdoors in plastic pots filled with 400 g of collected bulk soil collected from the top 25 cm of the soil profile, since this is the effective depth of green bean roots (Vallejo Cabrera *et al.*, 2004). The experiment was performed in the same location as the field experiment. The plant response variables were plant height, fresh weight, dry weight, and number of root nodules measured 30 d after planting.

Statistical analysis

The results were subjected to an analysis of variance ($P<0.05$) and a subsequent post hoc Tukey test ($P<0.05$) to evaluate differences between soil management strategies. For each case, the assumptions of normality were tested using the Shapiro-Wilk test ($P<0.05$), and the homogeneity of variances was tested using the Bartlett test ($P<0.05$). Multivariate analysis, principal component analysis, and Pearson correlation were calculated. All analyses were performed in R v4.2 (R Core Team, 2021) using agricolae (Mendiburu, 2023) and ggplot2 (Wickham, 2016) packages.

Results

Fallow period improves soil physicochemical parameters, microbial culturable counts, and soil enzyme activities

The soil organic matter (OM) and pH showed significantly higher values in fallow soil (F) compared to with agricultural soil (A) (Tab. 1). Apparent density was significantly higher in soil A compared to soil F (Tab. 1). Total soil microbial counts showed statistically significant higher values in soil F compared with soil A for total heterotrophic bacteria, phosphate solubilizers and nitrogen-fixing bacteria (Tab. 1). There were significant differences between the two soils, with soil F showing the highest activity for all enzymes (Fig. 1).

TABLE 1. Physicochemical properties and microbial counts across study areas on La Colorada farm.

	OM (%)	AD	pH	TB	PS	NF
	%*	mg m^{-3}*	*	LogCFU gds^{-1}*	LogCFU gds^{-1}*	LogMPN gds^{-1}*
Soil F	7.48 ±0.71 a	1.46±0.09 b	6.43±0.13 a	8.35±0.23 a	5.95±0.22 a	6.51±0.22 a
Soil A	5.91±0.6 b	1.71±0.04 a	4.99±0.03 b	8.14±0.36 a	5.60±0.34 b	5.69±0.30 b

*Significant at the 0.05 probability level. Values are presented as means ± standard deviation. Means with the same letter do not differ significantly at $P\leq0.05$, n = 3. Statistical test ANOVA-Tukey. OM: soil organic matter, AD: apparent density, TB: total heterotrophic bacteria, PS: phosphate solubilizers, and NF: nitrogen fixers, CFU: colony-forming units, MPN: most probable number, gds: g of dry soil.

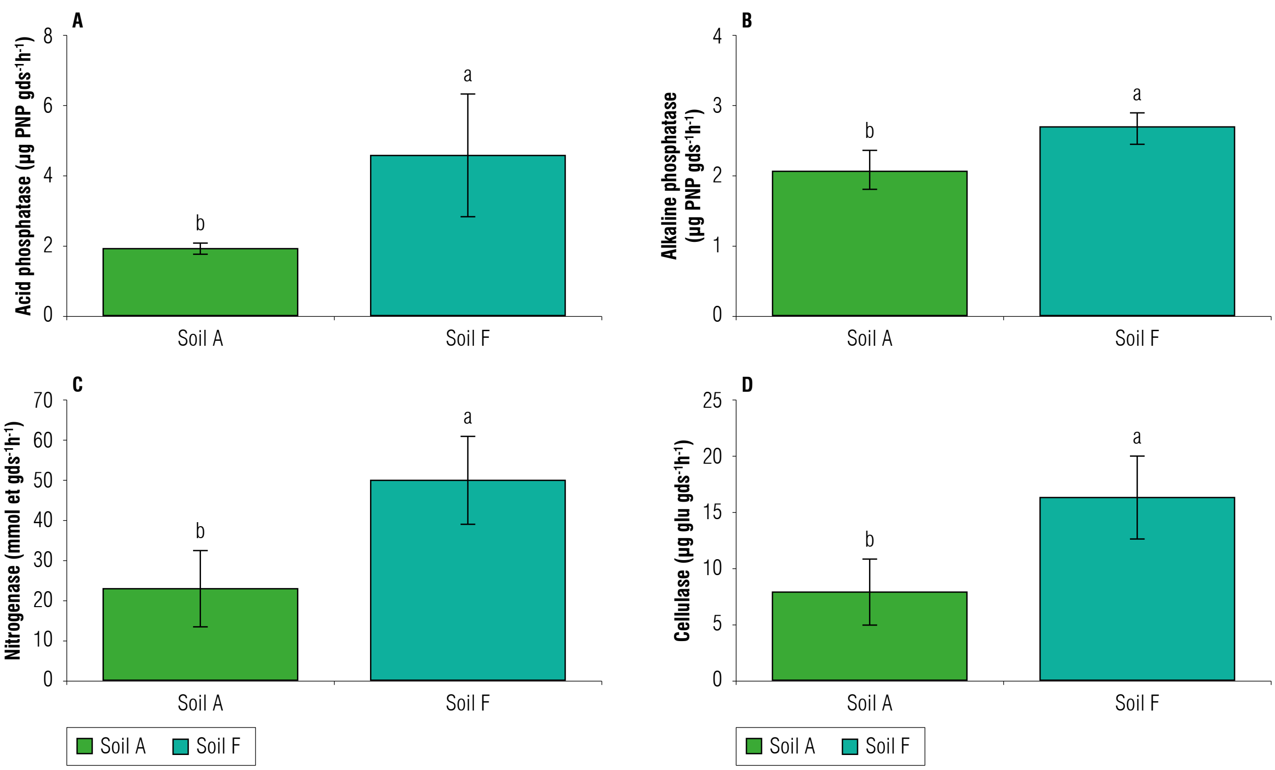

FIGURE 1. Enzymatic activities of: A) acid phosphatase, B) alkaline phosphatase, C) nitrogenase, and D) cellulase. PNP: *p*-nitrophenol; et: ethylene; glu: glucose; A: agricultural soil, F: fallow soil, gds: g of dry soil. Different letters indicate significant differences (ANOVA-Tukey, $P\leq0.05$), n=3. The bars represent the standard deviation.

Crop yield is improved in fallow soil under field conditions

The crop measurements under field conditions showed that soil F had significantly higher values for the number of nodules and leaves per plant and an increase in yield compared to soil A (Tab. 2).

TABLE 2. Effect of soil management on crop performance under field conditions at La Colorada farm.

Soil	Number of leaves per plant*	Number of nodules per plant*	Yield (t ha^{-1}) *
Soil F	38.9 ±2.8 a	33.2 ±5.06 a	14.4 ±0.14 a
Soil A	31.0 ±1.93 b	19.4 ±5.25 b	8.47±0.27 b

*Significant at the 0.05 probability level. The values are presented as means ± standard deviation. Means followed by the same letter do not differ significantly at $P\leq0.05$, n = 3. Statistical test: ANOVA followed by Tukey's test.

Bacterial communities change under different soil management strategies

Figure 2A shows a UPGMA (Unweighted Pair Group Method using Arithmetic Averages) clustering, formed from the banding patterns obtained by a DGGE approach. The analysis shows two large clades, where soil A is separated from soil F, which indicates that changes in soil management generated differences in the composition of the rhizosphere bacterial community (Fig. 2A). Figure 2B shows a correlation matrix that relates enzymatic activities, and functional group counts with physicochemical parameters and plant growth variables. Principal component analysis (Fig. 2C) shows a different clustering for samples from soil A and soil F. The response variables measured explain more than 70% of the total variance in the experiment.

Soil management and soil sterilization affect plant growth

We hypothesized that there is a differentiated effect in terms of plant growth between both soil management strategies: continuous agriculture and the fallow period. To demonstrate this effect, we took bulk soil from fields A and F and evaluated green beans growth in a pot assay. The results showed that soil management during the initial phases of green bean growth significantly influences the four variables measured, where soil F showed higher values (Fig. 3). It is important to point out that, leaving soil A and soil F with similar conditions after sterilization, these four variables did not present significant differences (Fig. 3), thus demonstrating the importance of soil microorganisms for the development and growth of the plants.

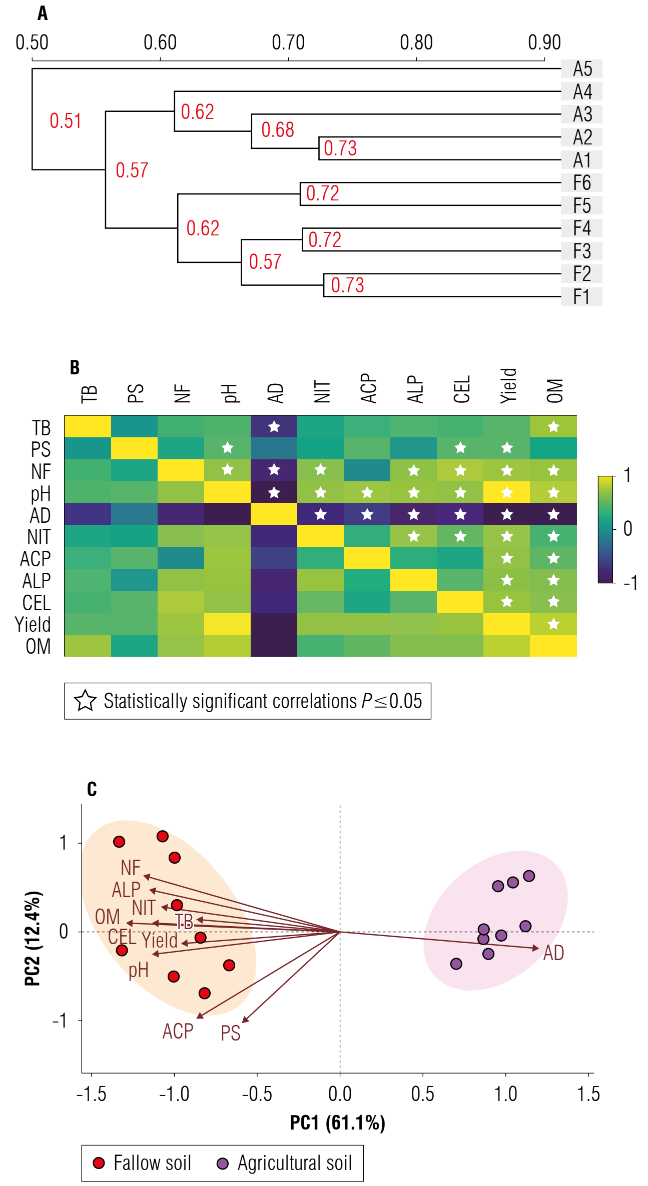

FIGURE 2. A) Analysis of relationships between non-culturable microbial communities obtained by DGGE using the UPGMA hierarchical grouping method. (B) correlation matrix between the variables measured. The result of Pearson's correlation is shown in R^2. White stars show statistically significant correlations $P<0.05$. ACP – acid phosphatase, ALP – alkaline phosphatase, CEL – cellulase, NIT – nitrogenase, OM – soil organic matter, AD – apparent density, TB – total heterotrophic bacteria, PS – phosphate solubilizers, NF – nitrogen fixers. The figure was made in R using the ggplot package. (C) Principal component analysis establishes relationships between the two soil management dynamics and the variables measured in the experiment.

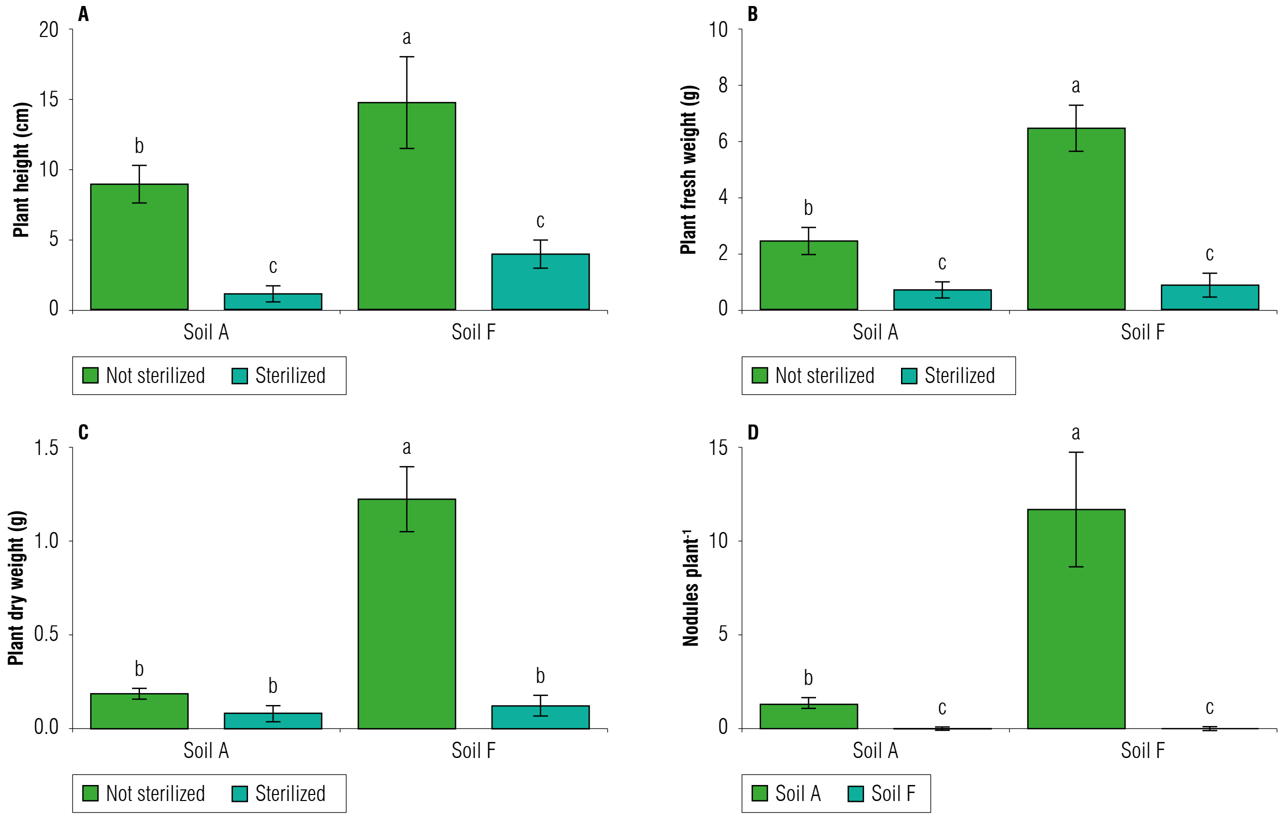

FIGURE 3. Agronomic variables measured in the green bean growth test: A) Plant height, B) Plant fresh weight, C) Plant dry weight, and D) Mean number of nodules produced per plant. Different letters indicate significant differences (ANOVA-Tukey, $P \leq 0.05$). The bars correspond to the standard deviation. Soil A: agricultural soil, Soil F: fallow soil, n=14. The figure was made in R using the ggplot package.

Discussion

This study is one of the few published reports that assesses the effect of fallow on soil microbiological activity from a functional perspective and relates it to the yield of the subsequent crop. Our results provide a reference framework, suggesting that leaving soil fallow for five years can contribute to the recovery of biological activity and, consequently, productivity. However, further research is needed to determine whether shorter fallow periods could achieve similar effects. Some meta-analyses have attempted to explore whether there is a relationship between fallow duration and crop yield (Mertz, 2002; Mertz *et al.*, 2008). Although there is empirical evidence indicating that longer fallow periods are correlated with higher yields, other factors influence yield, making it difficult to establish causal relationships between fallow duration and yield (Mertz, 2002; Mertz *et al.*, 2008).

Brennan and Acosta-Martinez (2017) showed that six years are sufficient to positively shift the microbial community in California soils under intensive agriculture when transitioning to organic cropping. Other interesting approaches demonstrated that soil amendments under different fallow periods (1, 3, and 16 years) in crops grown in continuously cultivated soils led to improved growth and resistance to pests (Howard *et al.*, 2020). However, beyond fallow periods, the long-term sustainability of organic systems also requires practices such as reduced tillage, the application of organic amendments, and the use of cover crops. Among these, cover crops are particularly effective as they provide a readily available source of labile carbon for microorganisms (Brennan & Acosta-Martinez, 2017).

We recognize that five years is a long period for a farmer to leave a crop field fallow. However, this timeframe allows us to assess the effect of fallow on the recovery of biological activity in agricultural soils. It also serves as a starting point to evaluate shorter, more economically sustainable fallow periods and management strategies. Additionally, it helps identify biological activities that indicate soil health in *P. vulgaris* crops under tropical conditions.

The soil OM content, pH, and apparent density of the soil have been reported as major determinants of the distribution of edaphic microorganisms (Fierer, 2017). These three variables in soil F showed the most favorable values for the development of microorganisms. For instance, higher OM content will provide a greater supply of carbon to soil microorganisms (Martínez-García *et al*., 2018), suggesting better microbial activity. In our study, this was indicated by the correlation between OM and total heterotrophic bacteria, nitrogen fixers, nitrogenase activity, phosphatase activity, and cellulase activity (Fig. 2B). A lower apparent density results in greater pore space, which is necessary for gas exchange and water retention; these conditions facilitate the formation of niches, which are where microorganisms live and where most biological processes of agricultural interest occur (Totsche *et al*., 2018). Our results suggest that these phenomena can be observed in the negative correlation between total heterotrophic bacteria and nitrogen fixers with apparent density (Fig. 2B).

One of the physicochemical variables that showed the greatest differences was pH, which decreased from 6.43 in the fallow soil to 4.99 in the agricultural soil (Tab.1). This pH reduction is commonly reported in soils subjected to intensive agricultural activity (Zhou *et al*., 2021). For instance, the frequent application of nitrogen fertilizers such as urea has been associated with an increase in the nitrification process (Ayiti & Babalola, 2022), contributing to soil acidification. On the other hand, a slight increase in pH has been reported in fallow soils (Fachin *et al*., 2021). Although no concrete data are available regarding the amount of fertilizers and pesticides applied to the agricultural soil, it is known that this soil had been under continuous cultivation for the past 12 years before this study, with at least three fertilizer applications per year.

A higher pH improves the survival conditions of many bacterial populations (Xiao *et al*., 2018); in this study, we identified a correlation between pH and phosphate solubilizers (Fig. 2B). From an agronomic perspective, a pH of 6.43 is optimal for the availability of most nutrients, whereas at a pH of 4.99, macronutrient availability is significantly reduced (Hartemink & Barrow, 2023). This suggests that not only are soil microorganisms affected, but plant growth is also directly limited by the low pH in agricultural soils. However, Figure 3 shows that after soil sterilization, where the only variable altered is the presence of living microorganisms, there are no differences in plant growth between fallow and agricultural soils. This suggests that the main effect of pH is exerted on soil microorganisms.

A high phosphatase activity indicates better soil quality, especially in tropical soils (Ferreira *et al*., 2016; Stone & Plante, 2014). The results obtained in our study showed a positive response to leaving agricultural soils for a five-year fallow period, since both types of phosphatases (acid and alkaline) were higher in soil F than in soil A. This suggests a more abundant fraction of organic P in this soil, as expected given the significantly higher content of OM (Tab. 1). On the other hand, it is worth mentioning that phosphatases, both acid and alkaline, are more active in the rhizosphere (Tarafdar & Jungk, 1987), especially in leguminous plants such as the green bean (Yadav & Tarafdar, 2001). We did not find a significant correlation between phosphate solubilizers and alkaline phosphatase or acid phosphatases, suggesting that the enzymatic activity observed corresponds to another type of phosphate-mineralizing microorganisms that are functionally active, or even extracellular enzymes attached to mineral and organic particles, as discussed below.

The significant correlation found between OM and alkaline phosphatase, nitrogenase, and cellulase activity (Fig. 2B) indicates that a greater amount of OM facilitates the stabilization of these enzymes in the soil matrix. These enzymes are usually excreted by microorganisms and adhere to organic particles or clays (Nannipieri *et al*., 2018). Once stabilized, enzymes remain catalytic for long periods (Dotaniya *et al*., 2019), being a potential source of enzymatic activity associated with the soil (Skujiņš & Burns, 1976). The correlation found between cellulase and nitrogenase enzymes (Fig. 2B) can be explained by considering that the energy necessary for nitrogenase activity comes mostly from carbon sources, resulting from cellulose and hemicellulose hydrolysis (Deng & Tabatabai, 1994).

Other studies analyzing the diversity and microbial structure by DGGE found differences in bacterial diversity between soils subjected to different soil management strategies. In this context, Wallis *et al*. (2010) observed lower richness in soils of intensive crop production compared to soils of natural pastures and forests. Other researchers have also shown that alternatives such as reduced tillage, cover crops, and organic amendments improve microbial diversity and abundance in soil (Kuntz *et al*., 2013; Martínez-García *et al*., 2018). Here, we demonstrated that a fallow period of five years and continuous agriculture can generate a differential configuration of the soil bacterial community, indicating that soil microbes respond to soil management practices. Principal component analysis (Fig. 2C) showed two clearly separated groups based on the type of soil management evaluated, supporting the previous discussion and the hypothesis.

Legume crops such as green beans have the inherent capacity for biological nitrogen fixation mediated by the interaction with diazotrophic bacteria (Zhong *et al*., 2024), which catalyze the conversion of nitrogen to ammonia via the nitrogenase enzyme (Chaulagain & Frugoli, 2021). Nitrogen is one of the principal macronutrients associated with plant growth and productivity. This research showed that soils under fallow for five years increased the number of leaves; in this way, more carbon can be fixed through photosynthesis. This, in turn, can be used in exchange for nitrogen produced in the nodules by nitrogen-fixing microorganisms (Chaulagain & Frugoli, 2021). Finally, this is reflected in a higher yield in fallow treatment, suggesting that plant growth on fallow plots offers a niche that allows the establishment of different microbial communities that promote greater nitrogen fixation and, consequently, increase plant growth and yield.

Our results highlight the importance of soil management, particularly its biological component, and the consequent effects on plant vigor and plant productivity, since soil fertility depends largely on biological activity, especially in the rhizosphere (Mendes *et al*., 2013).

Conclusions

The intensive use of soil leads to the continuous impoverishment of microbial communities and their functions. On the other hand, leaving fallow for five years increases organic matter and improves certain physicochemical properties, which leads to an improvement of microbial activity as well as plant vigor and productivity of the crop. All these conditions are responsible for the differences that occur in terms of plant height and weight, the number of nodules per plant, and the improvement of crop yield. Our results indicate that proper management of the biological component of the soil stimulates greater microbial activity, which leads to healthier and more productive soils. Acknowledging that leaving crop fields fallow for five years generates a greater green bean yield, the challenge is to generate similar effects using shorter periods or soil management strategies that lead to similar results.

Acknowledgments

We acknowledge Mr. Alfonso Rodriguez for providing access to his farm for the study and Ph.D. Martha Henao for facilitating the Laboratorio de Aguas y Suelos at the Facultad de Ciencias Agrarias of the Universidad Nacional de Colombia for the measurement of some physicochemical parameters. This project was partially funded by the Division of Research of the Universidad Nacional de Colombia, Bogotá, with research grant number 51274.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

All authors contributed to the study conception and design. NRR: writing the original draft, data curation, formal analysis, visualization, review, and editing. VOJ: writing, reviewing, and editing. DUV: funding acquisition, supervision, writing, reviewing, and editing. All authors have read and approved the final version of the manuscript.

Literature cited

Alef, K., & Nannipieri, P. (1995). Enzyme activities. In K. Alef, & P. Nannipieri (Eds.), *Methods in applied soil microbiology and biochemistry* (pp. 311–373). Academic Press. https://doi.org/10.1016/B978-012513840-6/50022-7

Ayiti, O. E., & Babalola, O. O. (2022). Factors influencing soil nitrification process and the effect on environment and health. *Frontiers in Sustainable Food Systems, 6*, Article 821994. https://doi.org/10.3389/fsufs.2022.821994

Bandinck, A. K., & Dick, R. P. (1999). Field management effects on soil enzyme activities. *Soil Biology and Biochemistry*, *31*(11), 1471–1479. https://doi.org/10.1016/S0038-0717(99)00051-6

Banerjee, S., & van der Heijden, M. G. A. (2022). Soil microbiomes and one health. *Nature Reviews Microbiology, 21*(1), 6–20. https://doi.org/10.1038/s41579-022-00779-w

Barillot, C. D. C., Sarde, C. O., Bert, V., Tarnaud, E., & Cochet, N. (2013). A standardized method for the sampling of rhizosphere and rhizoplan soil bacteria associated to a herbaceous root system. *Annals of Microbiology*, *63*(2), 471–476. https://doi.org/10.1007/s13213-012-0491-y

Brennan, E. B., & Acosta-Martinez, V. (2017). Cover cropping frequency is the main driver of soil microbial changes during six years of organic vegetable production. *Soil Biology and Biochemistry*, *109*, 188–204. https://doi.org/10.1016/j.soilbio.2017.01.014

Brisson, V. L., Schmidt, J. E., Northen, T. R., Vogel, J. P., & Gaudin, A. C. M. (2019). Impacts of maize domestication and breeding on rhizosphere microbial community recruitment from a nutrient depleted agricultural soil. *Scientific Reports*, *9*(1), Article 15611. https://doi.org/10.1038/s41598-019-52148-y

Burdukovskii, M., Kiseleva, I., Perepelkina, P., & Kosheleva, Y. (2020). Impact of different fallow durations on soil aggregate structure and humus status parameters. *Soil and Water Research*, *15*(1), 1–8. https://doi.org/10.17221/174/2018-SWR

Chandrapati, S., & Williams, M. G. (2014). Total viable counts: Most probable number (MPN). In C. A. Batt, & M. L. Tortorello (Eds.), *Encyclopedia of food microbiology* (2nd ed., pp. 621–624). Academic Press. https://doi.org/10.1016/B978-0-12-384730-0.00333-5

Chaulagain, D., & Frugoli, J. (2021). The regulation of nodule number in legumes is a balance of three signal transduction pathways. *International Journal of Molecular Sciences*, *22(*3), Article 1117. https://doi.org/10.3390/ijms22031117

Chaurasia, S. (2020). Green beans. In A. K. Jaiswal (Ed.), *Nutritional composition and antioxidant properties of fruits and vegetables* (pp. 289–300). Academic Press. https://doi.org/10.1016/B978-0-12-812780-3.00017-9

Chen, X., Han, X.-Z., You, M.-Y., Yan, J., Lu, X.-C., Horwath, W. R., & Zou, W.-X. (2019). Soil macroaggregates and organic-matter content regulate microbial communities and enzymatic activity in a Chinese Mollisol. *Journal of Integrative Agriculture*, *18*(11), 2605–2618. https://www.sciencedirect.com/science/article/pii/S2095311919627590?ref=pdf_download&fr=RR-2&rr=93d4a9a74c2d3ef5

Datta, R. (2024). Enzymatic degradation of cellulose in soil: A review. *Heliyon*, *10*(1), Article e24022. https://doi.org/10.1016/j.heliyon.2024.e24022

Deng, S. P., & Tabatabai, M. A. (1994). Cellulase activity of soils. *Soil Biology and Biochemistry*, *26*(10), 1347–1354. https://doi.org/10.1016/0038-0717(94)90216-X

Dotaniya, M. L., Aparna, K., Dotaniya, C. K., Singh, M., & Regar, K. L. (2019). Role of soil enzymes in sustainable crop production. In M. Kuddus (Ed.), *Enzymes in food biotechnology: Production, applications, and future prospects* (pp. 569–589). Academic Press. https://doi.org/10.1016/B978-0-12-813280-7.00033-5

Edwards, J. A., Santos-Medellín, C. M., Liechty, Z. S., Nguyen, B., Lurie, E., Eason, S., Phillips, G., & Sundaresan, V. (2018). Compositional shifts in root-associated bacterial and archaeal microbiota track the plant life cycle in field-grown rice. *PLoS Biology*, *16*(2), Article e2003862. https://doi.org/10.1371/journal.pbio.2003862

Eivazi, F., & Tabatabai, M. A. (1990). Factors affecting glucosidase and galactosidase activities in soils. *Soil Biology and Biochemistry*, *22*(7), 891–897. https://doi.org/10.1016/0038-0717(90)90126-K

Eivazi, F., & Tabatabai, M. A. (1977). Phosphatases in soils. *Soil Biology and Biochemistry*, *9*, 167–172.

Fachin, P. A., Costa, Y. T., & Thomaz, E. L. (2021). Evolution of the soil chemical properties in slash-and-burn agriculture along several years of fallow. *Science of The Total Environment*, *764*, Article 142823. https://doi.org/10.1016/j.scitotenv.2020.142823

FAOSTAT. (2024, June). *Crops and livestock products*. FAOSTAT. https://www.fao.org/faostat/en/#data/QCL

Ferreira, A. S., Espíndola, S. P., & Campos, M. R. C. (2016). Assessment and kinetics of soil phosphatase in Brazilian Savanna systems. *Anais da Academia Brasileira de Ciências*, *88*(2), 1035–1044. https://doi.org/10.1590/0001-3765201620140033

Fierer, N. (2017). Embracing the unknown: Disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology*, *15*(10), 579–590. https://doi.org/10.1038/nrmicro.2017.87

Garba, I. I., Fay, D., Apriani, R., Yusof, D. Y. P., Chu, D., & Williams, A. (2022). Fallow replacement cover crops impact soil water and nitrogen dynamics in a semi-arid sub-tropical environment. *Agriculture, Ecosystems & Environment*, *338*, Article 108052. https://doi.org/10.1016/J.AGEE.2022.108052

Hartemink, A. E., & Barrow, N. J. (2023). Soil pH – nutrient relationships: The diagram. *Plant and Soil*, *486*(1), 209–215. https://doi.org/10.1007/s11104-022-05861-z

Howard, M. M., Muñoz, C. A., Kao-Kniffin, J., & Kessler, A. (2020). Soil microbiomes from fallow fields have species-specific effects on crop growth and pest resistance. *Frontiers in Plant Science, 11*, Article 1171. https://doi.org/10.3389/fpls.2020.01171

Kandasamy, S., Liu, E. Y. R., Patterson, G., Saldias, S., Ali, S., & Lazarovits, G. (2019). Introducing key microbes from high productive soil transforms native soil microbial community of low productive soil. *Microbiology Open*, *8*(10), Article e895. https://doi.org/10.1002/mbo3.895

Klein, D. A., Sorensen, D. L., & Redente, E. F. (1985). Soil enzymes: A predictor of reclamation potential and progress. In R. L. Tate, & D. A. Klein (Eds.), *Soil reclamation processes: Microbiological analyses and applications* (1st ed., pp. 141–172). CRC Press. https://doi.org/10.1201/9781003065340

Kuntz, M., Berner, A., Gattinger, A., Scholberg, J. M., Mäder, P., & Pfiffner, L. (2013). Influence of reduced tillage on earthworm and microbial communities under organic arable farming. *Pedobiologia*, *56*(4–6), 251–260. https://doi.org/10.1016/j.pedobi.2013.08.005

Mann, C., Lynch, D., Fillmore, S., & Mills, A. (2019). Relationships between field management, soil health, and microbial community composition. *Applied Soil Ecology*, *144*, 12–21. https://doi.org/10.1016/j.apsoil.2019.06.012

Martínez-García, L. B., Korthals, G., Brussaard, L., Jørgensen, H. B., & Deyn, G. B. (2018). Organic management and cover crop species steer soil microbial community structure and functionality along with soil organic matter properties. *Agriculture, Ecosystems & Environment*, *263*, 7–17. https://doi.org/10.1016/j.agee.2018.04.018

Mendes, R., Garbeva, P., & Raaijmakers, J. M. (2013). The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiology Reviews*, *37*(5), 634–663. https://doi.org/10.1111/1574-6976.12028

Mendiburu, F. (2023). A*gricolae: Statistical procedures for agricultural research*. https://cran.r-project.org/web/packages/agricolae/agricolae.pdf

Mertz, O. (2002). The relationship between length of fallow and crop yields in shifting cultivation: A rethinking. *Agroforestry Systems*, *55*(2), 149–159. https://doi.org/10.1023/A:1020507631848

Mertz, O., Wadley, R. L., Nielsen, U., Bruun, T. B., Colfer, C. J. P., de Neergaard, A., Jepsen, M. R., Martinussen, T., Zhao, Q., Noweg, G. T., & Magid, J. (2008). A fresh look at shifting cultivation: Fallow length an uncertain indicator of productivity. *Agricultural Systems*, *96*(1), 75–84. https://doi.org/10.1016/j.agsy.2007.06.002

Muyzer, G., & Smalla, K. (1998). Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie van Leeuwenhock*, *73*, 127–141. https://doi.org/10.1023/A:1000669317571

Nannipieri, P., Trasar-Cepeda, C., & Dick, R. P. (2018). Soil enzyme activity: A brief history and biochemistry as a basis for appropriate interpretations and meta-analysis. *Biology and Fertility of Soils, 54*(1), 11–19. https://doi.org/10.1007/s00374-017-1245-6

Nautiyal, C. S. (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiology Letters*, *170*(1), 265–270. https://doi.org/10.1111/j.1574-6968.1999.tb13383.x

Otero-Jiménez, V., Carreño-Carreño, J. P., Barreto-Hernandez, E., van Elsas, J. D., & Uribe-Vélez, D. (2021). Impact of rice straw management strategies on rice rhizosphere microbiomes. *Applied Soil Ecology*, *167*, Article 104036. https://doi.org/10.1016/J.APSOIL.2021.104036

Pansu, M., & Gautheyrou, J. (2006). *Handbook of soil analysis: Mineralogical, organic and inorganic methods*. Springer. https://doi.org/10.1007/978-3-540-31211-6

Pérez-Jaramillo, J. E., Carrión, V. J., Bosse, M., Ferrão, L. F. V., de Hollander, M., Garcia, A. A. F., Ramírez, C. A., Mendes, R., & Raaijmakers, J. M. (2017). Linking rhizosphere microbiome composition of wild and domesticated *Phaseolus vulgaris* to genotypic and root phenotypic traits. *The ISME Journal*, *11*(10), 2244–2257. https://doi.org/10.1038/ismej.2017.85

Pérez-Jaramillo, J. E., de Hollander, M., Ramírez, C. A., Mendes, R., Raaijmakers, J. M., & Carrión, V. J. (2019). Deciphering rhizosphere microbiome assembly of wild and modern common bean (*Phaseolus vulgaris*) in native and agricultural soils from Colombia. *Microbiome*, *7*(1), Article 114. https://doi.org/10.1186/s40168-019-0727-1

R Core Team. (2021). *R: A language and environment for statistical computing* (4.1). R Foundation for Statistical Computing. https://cir.nii.ac.jp/crid/1370294721063650048

Reardon, C. L., Wuest, S. B., Melle, C. J., Klein, A. M., Williams, J. D., Barroso, J., & Long, D. S. (2019). Soil microbial and chemical properties of a minimum and conventionally tilled wheat–fallow system. *Soil Science Society of America Journal*, *83*(4), 1100–1110. https://doi.org/10.2136/sssaj2018.09.0344

Romdhane, S., Spor, A., Banerjee, S., Breuil, M. C., Bru, D., Chabbi, A., Hallin, S., van der Heijden, M. G. A., Saghai, A., & Philippot, L. (2022). Land-use intensification differentially affects bacterial, fungal and protist communities and decreases microbiome network complexity. *Environmental Microbiomes*, *17*, Article 1. https://doi.org/10.1186/S40793-021-00396-9

Roper, M. M., Turpin, J. E., & Thompson, J. P. (1994). Nitrogenase activity (C_2H_2 reduction) by free-living bacteria in soil in a long-term tillage and stubble management experiment on a vertisol. *Soil Biology and Biochemistry*, *26*(8), 1087–1091. https://doi.org/10.1016/0038-0717(94)90125-2

Schinner, F., & von Mersi, W. (1990). Xylanase-, CM-cellulase- and invertase activity in soil: An improved method. *Soil Biology and Biochemistry*, *22*(4), 511–515. https://doi.org/10.1016/0038-0717(90)90187-5

Sinsabaugh, R. L., Lauber, C. L., Weintraub, M. N., Ahmed, B., Allison, S. D., Crenshaw, C., Contosta, A. R., Cusack, D., Frey, S., Gallo, M. E., Gartner, T. B., Hobbie, S. E., Holland, K., Keeler, B. L., Powers, J. S., Stursova, M., Takacs-Vesbach, C., Waldrop, M. P., Wallenstein, M. D., ..., & Zeglin, L. H. (2008). Stoichiometry of soil enzyme activity at global scale. *Ecology Letters*, *11*(11), 1252–1264. https://doi.org/10.1111/j.1461-0248.2008.01245.x

Skujiņš, J., & Burns, R. G. (1976). Extracellular enzymes in soil. *Critical Reviews in Microbiology*, *4*(4), 383–421. https://doi.org/10.3109/10408417609102304

Soil Survey Staff. (2022). *Keys to soil taxonomy* (13th ed.). USDA-Natural Resources Conservation Service. US Department of Agriculture. https://www.nrcs.usda.gov/sites/default/files/2022-09/Keys-to-Soil-Taxonomy.pdf

Stone, M. M., & Plante, A. F. (2014). Changes in phosphatase kinetics with soil depth across a variable tropical landscape. *Soil Biology and Biochemistry*, *71*, 61–67. https://doi.org/10.1016/j.soilbio.2014.01.006

Tarafdar, J. C., & Jungk, A. (1987). Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. *Biology and Fertility of Soils*, *3*(4), 199–204. https://doi.org/10.1007/BF00640630

Totsche, K. U., Amelung, W., Gerzabek, M. H., Guggenberger, G., Klumpp, E., Knief, C., Lehndorff, E., Mikutta, R., Peth, S., Prechtel, A., Ray, N., & Kögel-Knabner, I. (2018). Microaggregates in soils. *Journal of Plant Nutrition and Soil Science*, *181*(1), 104–136. https://doi.org/10.1002/jpln.201600451

Tridge. (2021). *2021 Industry report: Green beans*. Market Intelligence Team. https://cdn.tridge.com/market_report_report/d5/0a/ce/d50ace8054e0dd0094fcfa891095a03e19ea08ae/Industry_Report_-_Green_Bean_-_v2.pdf

Vallejo Cabrera, F. A., Gutiérrez, A., Estrada Salazar, E. I., Cardozo Conde, C. I., García Dávila, M. A., Sánchez, M. S., & Baena García, D. (2004). *Cultivo de habichuela: variedad UNAPAL milenio* (2nd ed.). Universidad Nacional de Colombia, Palmira. https://repositorio.unal.edu.co/handle/unal/51964

Vanegas, J., Landazabal, G., Melgarejo, L. M., Beltran, M., & Uribe-Vélez, D. (2013). Structural and functional characterization of the microbial communities associated with the upland and irrigated rice rhizospheres in a neotropical Colombian savannah. *European Journal of Soil Biology*, *55*, 1–8. https://doi.org/10.1016/j.ejsobi.2012.10.008

Wallis, P. D., Haynes, R. J., Hunter, C. H., & Morris, C. D. (2010). Effect of land use and management on soil bacterial biodiversity as measured by PCR-DGGE. *Applied Soil Ecology*, *46*(1), 147–150. https://doi.org/10.1016/j.apsoil.2010.06.006

Wickham, H. (2016). *Ggplot2: Elegant graphics for data analysis* (2nd ed.). Springer International Publishing. https://ggplot2-book.org/

Xiao, D., Huang, Y., Feng, S., Ge, Y., Zhang, W., He, X., & Wang, K. (2018). Soil organic carbon mineralization with fresh organic substrate and inorganic carbon additions in a red soil is controlled by fungal diversity along a pH gradient. *Geoderma*, *321*, 79–89. https://doi.org/10.1016/j.geoderma.2018.02.003

Yadav, R., & Tarafdar, J. (2001). Influence of organic and inorganic phosphorus supply on the maximum secretion of acid phosphatase by plants. *Biology and Fertility of Soils*, *34*(3), 140–143. https://doi.org/10.1007/s003740100376

Zhong, X., Wang, J., Shi, X., Bai, M., Yuan, C., Cai, C., Wang, N., Zhu, X., Kuang, H., Wang, X., Su, J., He., X., Liu, X., Yang, W., Yang, C., Kong, F., Wang, E., & Guan, Y. (2024). Genetically optimizing soybean nodulation improves yield and protein content. *Nature Plants*, *10*, 736–742. https://doi.org/10.1038/s41477-024-01696-x

Zhou, X., u Rahman, M. K., Liu, J., & Wu, F. (2021). Soil acidification mediates changes in soil bacterial community assembly processes in response to agricultural intensification. *Environmental Microbiology*, *23*(8), 4741–4755. https://doi.org/10.1111/1462-2920.15675

Zuberer, D. A., & Silver, W. S. (1978). Biological dinitrogen fixation (acetylene reduction) associated with Florida mangroves. *Applied and Environmental Microbiology*, *35*(3), 567–575. https://doi.org/10.1128/aem.35.3.567-575.1978