



OPEN ACCESS

EDITED BY

Everlon Cid Rigobelo,
São Paulo State University, Brazil

REVIEWED BY

Bruno Bachiega Navarro,
University of São Paulo, Brazil
Rafael Barroca Silva,
São Paulo State University, Brazil

*CORRESPONDENCE

Richard Solórzano Acosta
✉ investigacion_labsaf@inia.gob.pe

RECEIVED 02 June 2026

REVISED 17 August 2026

ACCEPTED 20 August 2026

PUBLISHED 17 September 2026

CITATION

Bellido Tenorio E, Alarcón Romani S, Condori Ataupillco T, Palomino-Espinoza C, Rengifo Sanchez R, Erazo JM, García-Blásquez C and Solórzano Acosta R (2026) Co-inoculation with *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense* outperforms nitrogen fertilisation in terms of yield, crude protein content, and profitability of green pea (*Pisum sativum* L.) cultivation in the Peruvian Andes. *Front. Sustain. Food Syst.* 10:1898585. doi: 10.3389/fsufs.2026.1898585

COPYRIGHT

© 2026 Bellido Tenorio, Alarcón Romani, Condori Ataupillco, Palomino-Espinoza, Rengifo Sanchez, Erazo, García-Blásquez and Solórzano Acosta. This is an open-access article distributed under the terms of the [Creative Commons Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Co-inoculation with *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense* outperforms nitrogen fertilisation in terms of yield, crude protein content, and profitability of green pea (*Pisum sativum* L.) cultivation in the Peruvian Andes

Enrique Bellido Tenorio¹, Susan Alarcón Romani²,
Tatiana Condori Ataupillco², Claudio Palomino-Espinoza²,
Raihil Rengifo Sanchez³, José Manuel Erazo⁴,
Cayo García-Blásquez¹ and Richard Solórzano Acosta^{3*}

¹Escuela de Agronomía, Facultad de Ciencias Agrarias, Universidad Nacional San Cristóbal de Huamanga (UNSCH), Ayacucho, Peru, ²Estación Experimental Agraria Canaan, Dirección de Servicios Estratégicos Agrarios (DSEA), Instituto Nacional de Innovación Agraria (INIA), Ayacucho, Peru, ³Centro Experimental La Molina, Dirección de Servicios Estratégicos Agrarios (DSEA), Instituto Nacional de Innovación Agraria (INIA), Lima, Peru, ⁴Estación Experimental Agraria Pucallpa, Dirección de Servicios Estratégicos Agrarios, Instituto Nacional de Innovación Agraria (INIA), Pucallpa, Peru

Green pea (*Pisum sativum* L.) is a key crop for food security in communities of the Peruvian Andes; however, its productivity is constrained by low soil fertility and the high cost of nitrogen fertilisers. In this context, the effects of co-inoculation with *Rhizobium leguminosarum* bv. *viciae* (strain Ar-014) and *Azospirillum brasilense* (strain Az-007) on crop yield, crude protein content, and economic profitability were evaluated in comparison with conventional nitrogen fertilisation. The study was conducted at the Canaan Agrarian Experimental Station (Ayacucho, Peru; 2,750 m.a.s.l.) using a randomised complete block design with five treatments and four replicates: T01 (*Rhizobium leguminosarum* bv. *viciae* inoculation), T02 (*Azospirillum brasilense* inoculation), T03 (co-inoculation), T04 (mineral-N comparator, 82.5 kg N ha⁻¹), and T05 (uninoculated and unfertilised control). The results showed that T03 (co-inoculation) significantly outperformed nitrogen fertilisation (T04) ($p < 0.01$), achieving higher green pod yield (6.73 vs. 5.63 t ha⁻¹), crude protein content (24.28 vs. 23.67%), and economic profitability (262.75 vs. 176.78%). Furthermore, T03 recorded the highest values for root length (21.73 cm), number of nodules per plant (20.27), and number of seeds per pod (6.07). Individual inoculation treatments (T01 and T02) also improved specific agronomic traits, such as aboveground biomass and number of pods per plant, respectively, although neither surpassed the performance of the co-inoculation treatment (T03). The Relative Yield Index (RYI_N) in the inoculated treatments was 16.85–19.49% higher than that observed under nitrogen fertilization. Overall, these results indicate that co-inoculation with *R. leguminosarum* b. *viciae* and *A. brasilense* (T03) represents a promising alternative to conventional nitrogen fertilization for green pea production under the evaluated high-Andean conditions, improving agronomic performance and economic profitability.

KEYWORDS

Azospirillum brasilense, co-inoculation, Peruvian Andes, PGPR, *Pisum sativum* L., *Rhizobium leguminosarum* bv. *viciae*, sustainable agriculture

1 Introduction

Pea (*Pisum sativum* L.) is a legume of major value in the agriculture of the Peruvian highlands due to its capacity to establish symbiotic associations with *Rhizobium leguminosarum* bv. *viciae*, enabling biological nitrogen fixation (BNF) (FAO, 2019). Globally, pea cultivation covers approximately 7.41 million hectares for dry peas and 2.66 million hectares for green peas, underscoring its importance to food security due to its high crude protein content (FAOSTAT, 2023). In Peru, green pea production occupies approximately 55,125 hectares (Ministerio de Desarrollo Agrario y Riego, 2023), with yields ranging from 4 to 6 t ha⁻¹ depending on crop management practices, nitrogen availability, and prevailing climatic conditions (Briones Vásquez et al., 2016).

In the Peruvian Andes, agriculture is practised across a wide diversity of soil types, whose distribution is influenced by parent material, altitude, and physiography, resulting in substantial variability in their physical and chemical properties (Lama-Isminio et al., 2024). In the high-Andean agricultural areas, Inceptisols are common and are characterised by variable fertility and, in many cases, limited availability of nitrogen and phosphorus for crop development (Visscher et al., 2024). Andisols are also widespread and develop from volcanic ash; they exhibit a high water-retention capacity and strong phosphorus adsorption due to the presence of short-range-order minerals, which reduces phosphorus availability to plants (Leceta et al., 2024). Phosphorus availability directly influences nodule formation and functioning because biological nitrogen fixation has high energy requirements (Janati et al., 2021). Similarly, soil physicochemical properties regulate the establishment and effectiveness of symbiotic associations between legumes and nitrogen-fixing bacteria (Kebede, 2021).

Nitrogen is essential for the growth and development of legumes; however, its availability in agricultural soils is often limited, thereby constraining crop productivity (Backer et al., 2018). This limitation has led to a high dependence on nitrogen fertilisers, whose intensive use increases production costs and generates adverse environmental impacts (Zhang et al., 2025). In legume production systems, rhizobial inoculation is widely recommended as a strategy to enhance biological nitrogen fixation and reduce dependence on nitrogen fertilisers (Masson-Boivin and Sachs, 2018). However, the successful implementation of microbial inoculants depends on the availability of high-quality products, efficient bacterial strains, and effective technology-transfer strategies that facilitate their adoption in agricultural production systems (Santos et al., 2019).

In this context, inoculation with rhizobia and other beneficial microorganisms represents a sustainable alternative, as these microorganisms convert atmospheric nitrogen (N₂) into forms of nitrogen available to legumes, thereby enhancing crop productivity and improving soil fertility (Masson-Boivin and Sachs, 2018).

Plant growth-promoting rhizobacteria (PGPR) associated with the rhizosphere play a fundamental role in crop development (Granada et al., 2018). Among these, the genus *Azospirillum brasilense*

is particularly notable, as its diazotrophic bacteria colonise plant roots and promote growth by producing phytohormones such as auxins, gibberellins, and cytokinins (Boleta et al., 2020). In particular, indole-3-acetic acid (IAA) stimulates the formation of lateral roots and root hairs, thereby enhancing water and nutrient uptake (Spaepen et al., 2007). Furthermore, *Azospirillum brasilense* high adaptability to diverse environmental conditions enhances its potential for application across a wide range of agricultural systems (Wisniewski-Dyé et al., 2012). Another important attribute of *Azospirillum brasilense* is its capacity for biological nitrogen fixation, which can reduce nitrogen fertiliser requirements by up to 30% in crops such as maize (Condori et al., 2024).

The use of co-inoculation with *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense* has gained increasing attention as an effective strategy to enhance the benefits of microbial inoculation (Tsegaye et al., 2022). The combination of symbiotic and associative bacteria integrates complementary mechanisms of action, resulting in improved nutrient uptake, greater tolerance to biotic and abiotic stresses, enhanced plant growth and development, improved nodulation and nitrogen accumulation, and increased pea yield (Oleńska et al., 2020; Ejaz et al., 2020; Bocatti et al., 2022; Ibrahim and El-Sawah, 2022; García-Blásquez Morote and Sato Palomino, 2019). Furthermore, microbial inoculation, whether applied individually or as microbial consortia, has been shown to improve physiological, biochemical, and yield-related traits, demonstrating clear advantages over non-inoculated controls (Mahmoud et al., 2023).

However, in the high-Andean ecosystems of Peru, information on the combined effects of *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense* in pea cultivation remains limited, particularly regarding their impact on yield and economic performance, despite national studies reporting positive outcomes from co-inoculation (García-Blásquez Morote and Sato Palomino, 2019; Huanhuayo Zuasnarbar and Acero Gonzales, 2025; Palomino Espinoza, 2023; García-Blásquez-Morote et al., 2026). In this context, the present study aimed to evaluate the effects of co-inoculation with *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense* on green pea yield, yield components, crude protein content, and crop profitability, in comparison with conventional nitrogen fertilisation under high-Andean conditions in Ayacucho, Peru.

2 Materials and methods

2.1 Study area

This study was conducted at the Canaan Agrarian Experimental Station (EEA), part of the National Institute of Agrarian Innovation (INIA), during the period from January 29 to May 6, 2024. The station is located in the district of Andres Avelino Caceres Dorregaray, Huamanga Province, Ayacucho Region, Peru. The experimental plot was located at 13°10'05"S and 74°12'14"W, at an altitude of 2,750 m a.s.l. Meteorological

data were obtained from the INIA Canaan Meteorological Station (13°09'S; 74°13'W; 2,761 m a.s.l.), belonging to the network of the National Meteorology and Hydrology Service of Peru (SENAMHI). During the experimental period, the mean air temperature was 17.92 °C, calculated as the average of the weekly mean air temperatures. The maximum and minimum air temperatures were 28.6 °C and 7.0 °C, respectively, corresponding to the highest and lowest values among the weekly temperatures recorded during the experimental period. The mean relative humidity was 86.44% (see Figures 1, 2).

2.2 Physicochemical characteristics of the soil

Before sowing, five soil subsamples were collected in a zigzag pattern across the experimental area at a depth of 0–30 cm. The subsamples were homogenized to obtain a composite sample, from which 1 kg was submitted to the Soil, Water, and Foliars laboratory of the EEA Canaan–INIA for analysis. The following soil properties were determined: texture (NOM-021-RECNAT-2000, 2002), pH (USEPA Method, 9045D, 2004), electrical conductivity (ISO 11265:1994, 1994), organic matter (NOM-021-RECNAT-2000, 2002), total nitrogen (ISO 11261:1995, 1995), available phosphorus (NOM-021-RECNAT-2000, 2002), available potassium (Bazán, 2017), and exchangeable cation concentrations (Ca, Mg y K), determined

according to NOM-021-RECNAT-2000 (2002) and expressed as cmolc kg⁻¹.

2.3 Experimental design and treatments

A randomised complete block design (RCBD) was implemented to evaluate five treatments, including inoculated treatments and an uninoculated control, using the pea (*Pisum sativum* L.) cultivar 'Remate'. The experiment was established over a total area of 1,224 m² (36 × 34 m) and arranged into four blocks. The experimental area comprised 20 experimental units, with 5 units per block. Each experimental unit measured 6 × 4.8 m, corresponding to 28.8 m², resulting in a total plot area of 576 m². The remaining 648 m² of the experimental area corresponded to pathways and border areas (Figure 3A). The net plot area used for yield data collection consisted of the central rows of each experimental unit and comprised 4 m² (2 × 2 m). Border rows were excluded to minimize edge effects (Figure 3B). A description of the evaluated treatments is provided in Table 1.

2.4 Crop management

Pea seeds (*Pisum sativum* L.) of the cultivar 'Remate' were used in this study. The seeds were obtained from the Canaan Experimental Centre of the National University of San Cristóbal de Huamanga (UNSCH) and were characterised by high purity and a 98% germination rate. Soil preparation and furrow formation were carried out

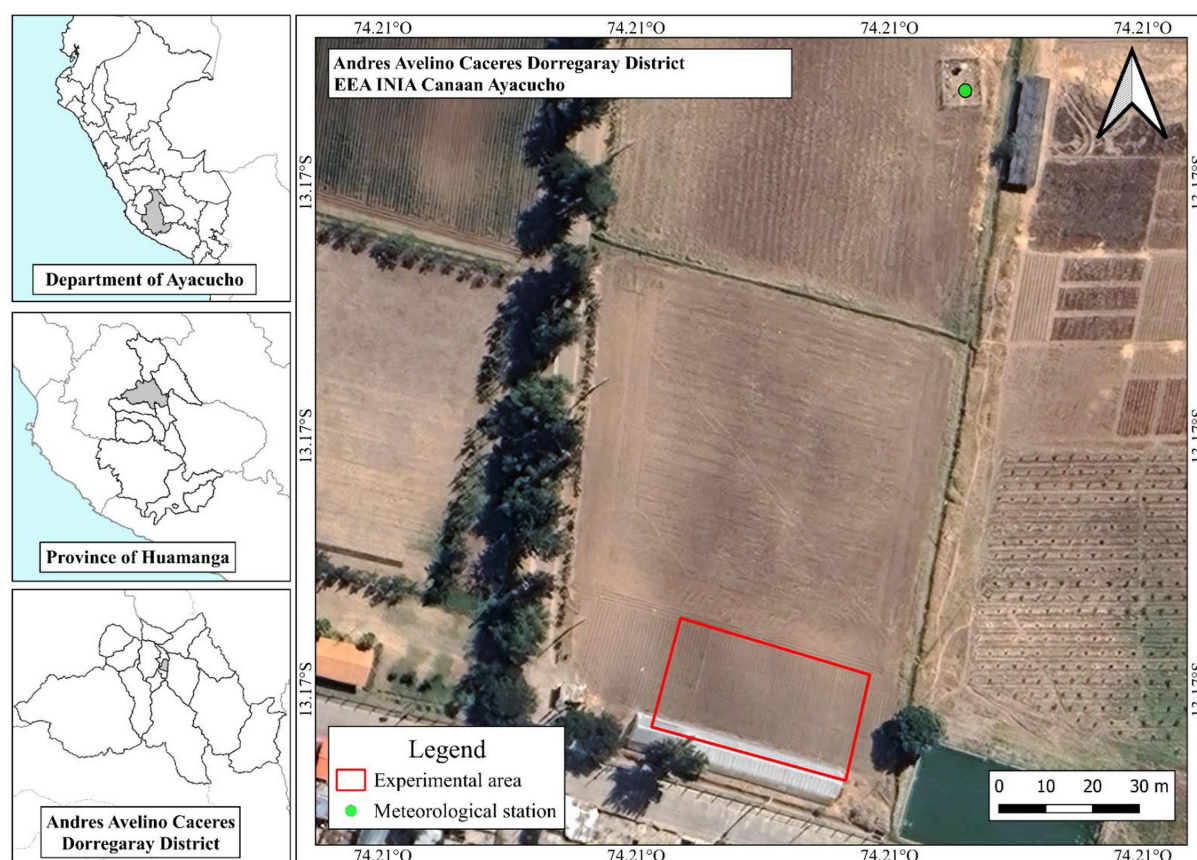


FIGURE 1

Location of the experimental area and the INIA Canaan Meteorological Station of the National Meteorology and Hydrology Service of Peru (SENAMHI), Andrés Avelino Cáceres Dorregaray District, Huamanga Province, Peru. Map prepared using QGIS 3.40.6 in February 2026. Source: Google Satellite imagery.

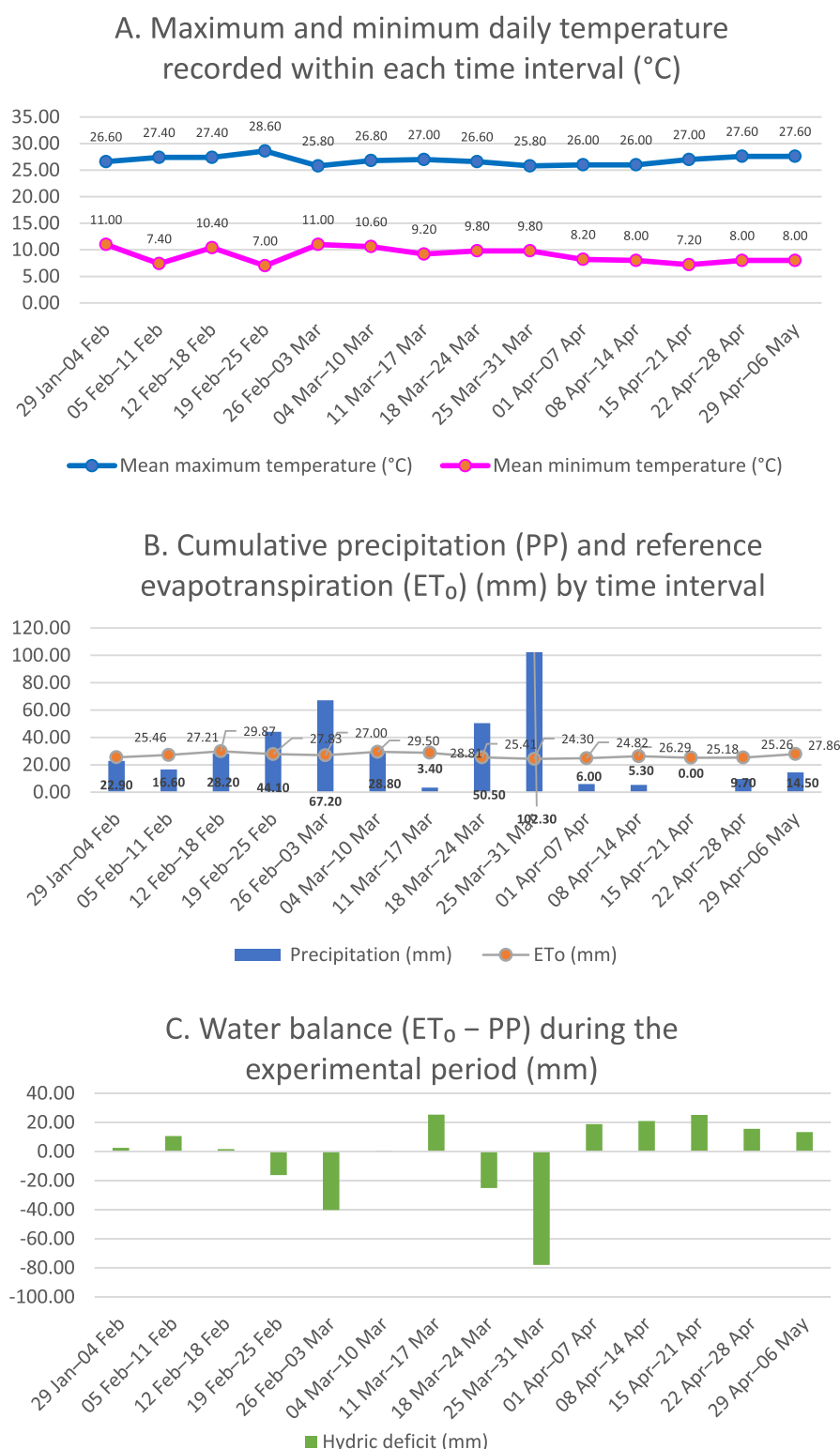


FIGURE 2

Meteorological conditions during the experimental period. (A) Maximum and minimum daily temperatures by time interval (°C). (B) Cumulative precipitation (PP) and reference evapotranspiration (ET₀) by time interval (mm). (C) Water deficit calculated as ET₀ – PP (mm). Values correspond to the date ranges shown on the x-axis; the first 13 intervals comprise 7 days each, and the final interval comprises 8 days (29 April–6 May).

mechanically. Basal fertilisation consisted of applying triple superphosphate (TSP) at 150 kg ha⁻¹ and potassium chloride (KCl) at 100 kg ha⁻¹ to all plots. In the mineral N treatment (T04), urea (46% N) was applied at a total rate of 179.3 kg urea ha⁻¹, equivalent to

82.5 kg N ha⁻¹. The urea dose was divided into two applications of 89.65 kg urea ha⁻¹ each, corresponding to 41.24 kg N ha⁻¹ per application. The first application was carried out at sowing, and the second was applied before the onset of flowering.

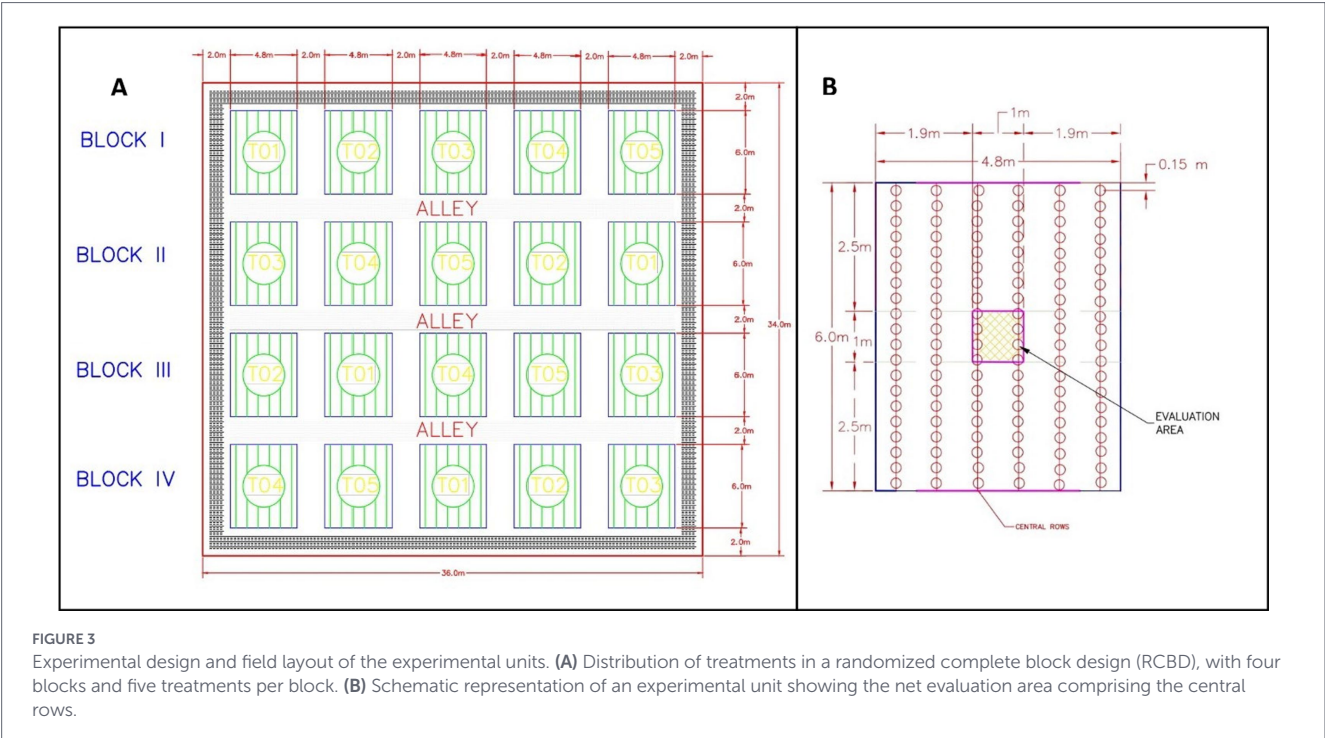


TABLE 1 Evaluated treatments.

Treatment	Description
T01	With <i>Rhizobium leguminosarum</i> bv. <i>viciae</i> inoculation
T02	With <i>Azospirillum brasilense</i> inoculation
T03	With co-inoculation of <i>Rhizobium leguminosarum</i> bv. <i>viciae</i> + <i>A. brasilense</i>
T04	With nitrogen fertilisation (N)—mineral-N comparator
T05	Without nitrogen fertilisation (N)—uninoculated and unfertilised control

Sowing was performed manually using a system of three seeds per hole, with a spacing of 0.3 m between plants and 0.8 m between furrows. Each plot measured 6.0 × 4.8 m and comprised 6 m-long rows spaced 0.8 m apart, covering an area of 28.8 m² (Figure 3B), while the total experimental plot area was 576 m². The sowing rate was 90 kg ha⁻¹. Seeds were treated 24 h prior to inoculation and sowing with the fungicide carboxin + captan (Vitavax-300) at a rate of 8 g kg⁻¹ of seed. Subsequently, seeds were inoculated with *R. leguminosarum* bv. *viciae* and *A. brasilense* according to the experimental treatments. To minimize the potential effect of the fungicide on the viability of the inoculated microorganisms, sowing was performed immediately after inoculation. This procedure followed recommendations for the combined use of fungicides and inoculants in legumes, which advocate selecting fungicides with lower toxicity to rhizobia and minimizing the interval between inoculation and sowing to preserve bacterial survival and promote symbiosis establishment (Curley and Burton, 1975; Henning et al., 1997; Hungria et al., 2001; Kunal and Sharma, 2011; Santos et al., 2021).

The crop was cultivated during the rainy season. Weed control was performed manually throughout the experimental period. Hilling was

carried out at 30 days after sowing (DAS), while staking was performed when plants reached approximately 30 cm in height to ensure adequate plant support and proper growth. During crop development, phytosanitary monitoring was conducted to detect the presence of pests and diseases. At 20 days after sowing (DAS), *Diabrotica speciosa* and aphids were detected and controlled by applying beta-cyfluthrin insecticide (Beta Baytroide 125 SC) at a rate of 0.15 L ha⁻¹. Subsequently, at 30 DAS, *Fusarium* spp. (damping-off) and powdery mildew (*Erysiphe polygoni*) were detected and controlled by applying carbendazim (Protexin) at a rate of 0.521 L ha⁻¹. Phytosanitary applications were performed only once during the crop cycle and were uniformly applied to all treatments using a Jacto XP-20 manual backpack sprayer with a 20 L capacity. The spray volume was 347 L ha⁻¹. Applications were carried out over the total experimental area of 576 m², following technical recommendations for equipment calibration and operation. Nodulation was assessed during crop development as an indirect indicator of the establishment of the symbiotic relationship between *Rhizobium leguminosarum* bv. *viciae* and the host plant, as well as of the response to inoculation (Gomes et al., 2017; Bueno et al., 2003). However, the evaluation of nodulation does not constitute a direct assessment of the compatibility between the phytosanitary program and the inoculant, nor does it allow for the determination of the survival of *Azospirillum brasilense* following exposure to pesticides. Previous studies have suggested that reducing the interval between seed inoculation and sowing may decrease the exposure of microbial inoculants to seed-applied pesticides, thereby reducing the risk of incompatibility associated with prolonged exposure (Seidenglanz et al., 2010; Pereira et al., 2020; Takahashi et al., 2021; Gorshkov et al., 2024; Kaur et al., 2025). In the present study, sowing was carried out immediately after inoculation, following this management strategy to minimize the exposure time of the inoculants to phytosanitary products. Nevertheless, this practice does not provide experimental evidence of the survival of *Azospirillum brasilense* or of the specific compatibility of the products used.

Harvesting was performed at 97 DAS, when pods reached commercial maturity.

2.5 Inoculation with *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense*

The inoculants used in this study were provided by the National University of San Cristóbal de Huamanga (UNSCH) through the FOCAM-UNSCH project entitled Biological Nitrogen Fixation and Plant Growth-Promoting Microorganisms. The strains are maintained as working material in the project's laboratory and do not have accession number or deposit in a public culture collection.

The *Rhizobium leguminosarum* bv. *viciae* strain Ar-014 was isolated from pea nodules collected in the district of Chiara, Ayacucho Region, Peru. The strain was characterized through macroscopic and microscopic evaluations, as well as nodulation assays in pea plants, following conventional methodologies for rhizobial characterization (Vincent, 1970; Somasegaran and Hoben, 1994; Hungria and Araújo, 1994; Zahran et al., 1994; Masson-Boivin and Sachs, 2018; Lindström and Mousavi, 2019). Subsequently, the strain was selected based on its agronomic efficiency in laboratory, greenhouse, and field trials.

The *Azospirillum brasilense* strain (Az-007) was isolated from white maize roots in the community of Ccerayocc, Ayacucho, Peru. Its characterization included macroscopic and microscopic assessments, growth in semisolid NFb medium, and standard tests for diazotrophic bacteria, following Döbereiner et al. (1995) and Bashan et al. (2004), supplemented with current criteria for plant growth-promoting rhizobacteria (Bashan and de-Bashan, 2010; Cassán et al., 2020; Spaepen et al., 2007). This strain was also selected based on its agronomic performance in different trials.

Before inoculant production, both strains were reactivated in their respective specific media, and their purity was verified based on the uniformity of their colony and microscopic characteristics, thereby ruling out contamination. The strains were stored at -20°C in sterile glycerol as a cryoprotectant and are maintained as working materials in the internal culture collection of the UNSCH FOCAM project, which is not publicly accessible.

For inoculant production, the preserved strains were reactivated in culture media specific to *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense* (Vincent, 1970; Döbereiner et al., 1995). The peat-based and liquid inoculant formulations were subjected to quality control by determining viable cell concentrations through serial dilution and plate counting and by verifying purity through growth on specific media and Gram staining (Somasegaran and Hoben, 1994). The inoculants were stored at temperatures below -20°C . Bacterial quantification was performed by direct enumeration using the serial dilution and plate count method for viable bacteria, resulting in measured viable concentrations of 1×10^8 CFU mL $^{-1}$ for *Azospirillum brasilense* and 1×10^9 CFU g $^{-1}$ for *Rhizobium leguminosarum* bv. *viciae* (Hungria and Araújo, 1994). The inoculation rates were established based on preliminary assessments of agronomic efficiency and nodulation conducted by the FOCAM-UNSCH project.

Pea (*Pisum sativum* L.) seeds were inoculated using the simple inoculation method, following technical recommendations for effective seed inoculation (Somasegaran and Hoben, 1994; García-Blásquez Morote and Sato Palomino, 2019; Huasasquiche et al., 2025). The procedure was carried out on the day of sowing during the coolest

morning hours. For the *Rhizobium* treatment, 7.14 g of peat-based inoculant was applied per kg of seed. The inoculant had a measured viable concentration of 1×10^9 CFU g $^{-1}$, resulting in an estimated dose of 7.14×10^9 CFU kg $^{-1}$ of seed. For the *Azospirillum brasilense* treatment, 5 mL of liquid inoculant was applied per kilogram of seed. The inoculant had a measured viable concentration of 1×10^8 CFU mL $^{-1}$, resulting in an estimated dose of 5×10^8 CFU kg $^{-1}$ of seed. In both cases, 40% (w/v) arabic gum-based adhesive was added at a rate of 10 mL kg $^{-1}$ of seed to enhance inoculant adhesion. The treated seeds were then air-dried for 30 min and sown on the same day.

Co-inoculation with *Rhizobium leguminosarum* bv. *viciae* (Ar-014) and *Azospirillum brasilense* (Az-007) was performed by jointly applying both inoculants to the seeds. This combination was based on previous studies reporting beneficial effects of co-inoculation with rhizobia and *Azospirillum brasilense* in legumes (Remans et al., 2008; Hungria et al., 2013).

2.6 Biometric parameters

2.6.1 Vegetative growth

At 60 DAS, during the flowering stage, five plants were randomly sampled from each experimental unit to evaluate the following parameters: root length (RL, cm); root fresh weight without nodules (RFW, g), root dry weight without nodules (RDW, g), and root fresh weight with nodules (RFWN, g); aboveground biomass fresh weight (ABFW, g); and aboveground biomass dry weight (ABDW, g). Plant height (PH, cm) was evaluated at 93 DAS under field conditions, without destructively sampling the plants. Ten plants were randomly selected per experimental unit, excluding border plants. Measurements were taken from the base of the plant (root collar) to the apex of the main stem using a measuring tape. Root length was determined by measuring from the stem base to the apex of the primary root. Roots with nodules were first weighed, after which nodules were removed and the roots were weighed again. Root, aboveground biomass, and nodule samples were dried in an oven (Memmert UN30) for 48 h until a constant weight was reached and subsequently weighed using an analytical balance with a precision of 0.001 g (BOECO BPS41).

2.6.2 Nodulation

The presence of nodules in pea plants was used as an indicator of rhizobial symbiotic activity. Large nodules located on the taproot and crown regions, present in high numbers and exhibiting a deep red coloration when sectioned due to the presence of leghemoglobin, were considered indicative of effective nodulation and greater nodule activity (Hungria et al., 2013). Nodulation parameters were assessed in five plants sampled at the flowering stage. Nodules were manually detached from the roots, and the number of nodules per plant (NNPP), as well as fresh and dry nodule weight per plant (NFWPP and NDWPP, g), were determined using a precision analytical balance (BOECO BPS41) and a drying oven (Memmert UN30).

Nodule internal coloration was assessed visually after sectioning the nodules using a three-category ordinal scale: 0 (plants without nodules), 1 (nodules without internal coloration), and 2 (nodules with pink internal coloration). This qualitative assessment was used to characterize the visual appearance of the nodules and was not considered a direct measure of leghemoglobin concentration, nitrogenase

activity, or biological nitrogen fixation. Pink internal coloration was interpreted as a qualitative morphological characteristic commonly associated with functional nodule tissue; however, it does not provide a direct quantification of leghemoglobin concentration or nitrogenase activity (Senthikumar et al., 2021; USDA, 2021; Jhu and Ledermann, 2025).

2.6.3 Relative yield index (RYI_N)

The Relative Yield Index (RYI_N) was calculated to compare the yields of the inoculated treatments with that of the mineral nitrogen treatment (T04), which was used as the reference treatment, according to Equation 1. The index was calculated as the ratio of the mean green pod yield (t ha^{-1}) of the treatment being evaluated to the mean green pod yield (t ha^{-1}) of T04, which received mineral nitrogen fertilization, multiplied by 100. In the equation, the numerator represents the mean green pod yield of the treatment being evaluated, whereas the denominator represents the mean green pod yield of the reference treatment T04 (Somasegaran and Hoben, 1994; López-Alcocer et al., 2020).

RYI_N was calculated using the following Equation 1:

$$(\text{RYI}_N)(\%) = \frac{\text{Yield of the treatment}}{\text{Yield under nitrogen fertilisation}} \times 100 \quad (1)$$

This index facilitates the interpretation of the relative performance of inoculated treatments compared with mineral nitrogen fertilization, particularly when microorganisms such as *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense* are used (Salvagiotti et al., 2008; Peoples et al., 2009; Congreves et al., 2021).

2.6.4 Seed yield and quality

Yield components were evaluated at 97 days after sowing (DAS), when the pods reached commercial maturity. Green pod yield was determined by harvesting all pods from a 2 m^2 , net plot located within the central rows of each experimental unit, excluding the lateral borders and row ends to minimize edge effects. The yield obtained from the net plot area was extrapolated to tonnes per hectare (t ha^{-1}). Seed yield was determined using the same plot. Subsequently, 10 plants were randomly selected from the central furrows of each experimental unit, outside the area used for yield assessment, to evaluate yield components, including the number of pods per plant (NPP), number of seeds per pod (NSP), and pod length (PL, cm). Pod length was measured using a graduated measuring tape.

To evaluate crude protein content, representative samples of 25 g were collected and submitted to the Soil, Water, and Foliar Laboratory (LABSAF) of the Canaan Agrarian Experimental Station (EEA-Canaan) for total nitrogen determination using the Kjeldahl method, following the laboratory's standardised analytical procedures. Crude protein content was estimated by multiplying total nitrogen content by the conversion factor 6.25, according to the official AOAC method 954.01. [Protein (Crude) in Animal Feed and Pet Food; AOAC Official Method 954.01 Protein (Crude) in Animal Feed and Pet Food, 2023].

2.7 Profitability

The green pod yield (t ha^{-1}) was determined when the pods reached the appropriate physiological stage for fresh consumption. For this purpose, the net weight of green pods harvested from each experimental unit was recorded using a digital balance with a precision of $\pm 1 \text{ g}$.

The economic analysis of the treatments was performed using a profitability analysis based on production cost (PC), gross production value (GPV), net production value (NPV), and profitability index (R), following the methodology proposed by (Brown, 1979; CIMMYT, 1988). The GPV was calculated based on the previously determined green pod yield and the assumed selling price of the product. The NPV was estimated as the difference between the GPV and the PC, whereas the profitability index (R) was calculated as the percentage ratio between NPV and PC, according to the following Equations 2–4:

$$\text{GPV} (\text{S/ha}^{-1}) = \text{Yield} (\text{kg ha}^{-1}) \times \text{Selling price} (\text{S/kg}^{-1}) \quad (2)$$

$$\text{NPV} (\text{S/ha}^{-1}) = \text{GPV} - \text{PC}, \text{ where PC represents the total production cost} (\text{S/ha}^{-1}) \quad (3)$$

$$R (\%) = (\text{NPV} / \text{PC}) \times 100 \quad (4)$$

Yield values obtained in kilograms per plot were extrapolated to tonnes per hectare (t ha^{-1}). For the economic analysis, yield was subsequently converted to kg ha^{-1} and multiplied by the assumed selling price of S/3.50 kg^{-1} of green peas. This average market price was recorded between April and May at the Nery García Zárate wholesale market in Ayacucho, according to records from the Ministry of Agrarian Development and Irrigation (Ministerio de Desarrollo Agrario y Riego, 2024). The resulting economic indicators were used to estimate the relative economic returns of the treatments under the specified production costs and market price assumptions.

2.8 Statistical analysis

Data were analyzed in R (version 4.5) using generalized linear models (GLMs) and generalized linear mixed models (GLMMs) (Bolker et al., 2009), implemented with the glmmTMB package (Brooks et al., 2017). In all models, treatment and block were included as fixed effects. Block was specified *a priori* as a fixed effect because the four blocks corresponded to predefined sections within a single experimental field and were used to control spatial heterogeneity, rather than representing a random sample from a broader population of blocks (Dixon, 2016). Plots and plants were assigned unique identifiers and included as random effects according to the sampling structure described in Supplementary Tables S1, S2. No random effects were included for variables evaluated at the plot level. For variables evaluated at the plant level, plot was included as a random effect, whereas for variables evaluated at the pod level, both plot and plant were included as nested random effects. Considering the data structure, candidate models with different distributions were fitted for each response variable according to its characteristics, as detailed in Supplementary File 1. For count variables, Poisson, generalized Poisson, Conway–Maxwell–Poisson, negative binomial, and

Tweedie distributions were evaluated. For continuous variables, gamma, lognormal, Tweedie, Gaussian, skew-normal, and Student's *t* distributions were assessed. Percentage variables were converted to proportions by dividing their values by 100 before model fitting. Because the resulting proportions remained strictly between 0 and 1, with no boundary values, no additional corrections were required. Therefore, models with beta and Gaussian distributions were evaluated.

The model for each variable was selected by jointly considering the Akaike information criterion (AIC) (Burnham and Anderson, 2002), the adequacy of residual diagnostics, and the nature of the response variable. When two or more models differed by less than 2 AIC units, they were considered to have comparable empirical support, and the final selection was based additionally on model diagnostics and the consistency of the assumed distribution with the characteristics of the response. The selected models were validated using simulated residuals generated with the DHARMA package (Hartig, 2024). Residual uniformity, the absence of overdispersion or underdispersion, homogeneity of variance, and the absence of outliers were assessed. For the GLMMs, the approximate normality of the random effects was also evaluated. When dispersion heterogeneity was detected, dispersion was modeled as a function of block or treatment, depending on the response variable. When the diagnostics indicated heterogeneity or dispersion-related problems, an additional dispersion structure was incorporated, specified as a function of block, treatment, treatment + block, or treatment $\times$ block interaction. With these considerations, the selected models generally showed satisfactory diagnostic performance, and are presented in Supplementary Table S3.

Estimated marginal means for each treatment were obtained from the validated models using the emmeans package (Lenth, 2024). For each response variable, the estimated means were compared pairwise among the five treatments, yielding 10 contrasts. Their *p*-values were adjusted using the Benjamini–Hochberg procedure to control the false discovery rate within this family of tests. Differences were evaluated at a significance level of $\alpha = 0.05$. Compact letter displays were generated from the adjusted contrasts. Treatments sharing at least one letter did not differ significantly, whereas treatments with no letters in common differed significantly.

Subsequently, variables recorded at the plant or pod level were aggregated by calculating their mean for each plot, with each experimental plot considered an observational unit. No missing values were present in the dataset; therefore, all 20 experimental plots were included in the analysis. Using the aggregated dataset, all variables were mean-centered and scaled by their standard deviations to remove differences associated with their measurement units and magnitudes. Principal component analysis (PCA) was then performed using the FactoMineR package (Lê et al., 2008). The results were visualized using biplots for the PC1–PC2 and PC1–PC3 planes, in which the experimental plots, identified according to their respective treatments, were projected together with the evaluated variables. Convex hulls were also included to graphically delimit the distribution of plots within each treatment for descriptive and exploratory purposes.

3 Results

Soil analysis results (Table 2) indicated that the experimental area had a clay loam texture, a characteristic that favors water and nutrient retention. The soil was slightly alkaline and had a low organic matter content, conditions that may influence the availability of some

TABLE 2 Physicochemical characterisation of the soil in the experimental plot.

Variable	Units	Result
Texture	–	Clay loam soil
pH	–	7.8
Electrical conductivity	mS/m	12.7
Organic Matter	%	1.24
N	%	0.06
P	ppm	12.41
K	ppm	610.33
Ca	cmolc kg ^{−1}	9.34
Mg	cmolc kg ^{−1}	0.80
K	cmolc kg ^{−1}	0.85
CEC	cmolc kg ^{−1}	11.14
Fe	ppm	16.93
Cu	ppm	0.13
Zn	ppm	2.41
Mn	ppm	50.31

nutrients essential for crop growth. Regarding soil fertility, nitrogen concentrations were low, phosphorus levels were intermediate, and potassium concentrations were high. Calcium, magnesium, and copper contents are reported according to the values obtained from the soil analysis; however, potential nutritional limitations were not inferred because specific interpretation criteria were unavailable.

3.1 Root morphological evaluation

Root analysis revealed significant treatment effects for all evaluated variables ($p < 0.01$), whereas block effects were significant for root fresh weight without nodules and root fresh weight with nodules (both $p < 0.01$). Plot was included as a random intercept in all models, and the estimated standard deviations associated with this effect, expressed on the linear predictor scale with a log link, are presented in Table 3. These estimates are model-specific and depend on the response variable, distribution, and dispersion structure considered.

Multiple comparison analysis showed that T03 (*Rhizobium leguminosarum* bv. *viciae* + *Azospirillum brasilense*) achieved the greatest root length (21.73 cm), as shown in Figure 4A. For root fresh weight without nodules, the highest values were recorded in T02 (*Azospirillum brasilense* inoculation), T03 (*Rhizobium leguminosarum* bv. *viciae* + *Azospirillum brasilense*), and T04 (mineral-N comparator) with values of 2.33, 2.21, and 2.22 g, respectively (Figure 4B). Regarding root dry weight without nodules, T03 exhibited the highest value (0.39 g) (Figure 4C). Similarly, nodulated root fresh weight showed significant differences among all treatments, following the descending order T03 > T02 > T01 > T04 > T05, with values of 3.23, 3.04, 2.94, 2.19, and 2.03 g, respectively (Figure 4D).

3.2 Nodulation assessment

Treatments significantly affected all nodulation variables analyzed ($p < 0.01$), whereas block effects were significant only for the number of nodules per plant (NNPP; $p < 0.01$). Plot was included as a random intercept in all models, and the estimated standard deviations

TABLE 3 Overall effects of the treatments on root morphology, nodulation, shoot biomass, yield, and seed quality of *Pisum sativum* L.

Factors	RL (cm)	RFW (g)	RDW (g)	RFWN (g)	NNPP	NFWPP (g)	NDWPP (g)	ABFW (g)	ABDW (g)	PH (cm)	NPP	PL (cm)	NSP	Yield (t ha ⁻¹)	Protein (%)
Fixed effects (p-value)															
Blocks	0.23	<0.01	0.10	<0.01	<0.01	0.04	0.05	0.57	<0.01	0.26	0.12	0.37	0.18	0.43	<0.01
Treatments	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Random effects (σ²)															
Plot	<0.01	0.07	<0.01	<0.01	0.03	<0.01	<0.01	<0.01	0.04	<0.01	0.03	<0.01	<0.01	-	-
Plant	-	-	-	-	-	-	-	-	-	-	-	-	0.08	-	-

The p-values corresponding to the fixed effects of blocks and treatments are shown, together with the estimated standard deviations (σ²) of the random effects, expressed on the linear predictor scale of each model. Plot was included as a random effect for variables evaluated at the plant level: root length (RL), root fresh weight without nodules (RFW), root dry weight without nodules (RDW), root fresh weight with nodules (RFWN), number of nodules per plant (NNPP), nodule fresh weight per plant (NFWPP), nodule dry weight per plant (NDWPP), shoot biomass fresh weight (ABFW), shoot biomass dry weight (ABDW), plant height (PH), number of pods per plant (NPP), and pod length (PL). In addition to plot, plant was included as a random effect for the number of seeds evaluated at the pod level (NSP). Both green pod yield and crude protein content were evaluated at the plot level; therefore, no random effects were included. A dash (-) indicates that the random effect was not included in the corresponding model.

associated with this effect, expressed on the linear predictor scale with a log link, are presented in Table 3. Although all models were fitted using a Tweedie distribution and a log-link function, each estimate is specific to the response variable and the dispersion structure considered. Multiple-comparison analysis showed that T03 had a significantly greater number of nodules per plant (20.27) than all other treatments (Figure 5A). For nodule fresh weight per plant (NFWPP), no significant differences were observed among T01, T02, and T03, with estimated means of 0.99, 0.90, and 0.97 g, respectively. These treatments had significantly higher values than T05 and T04 (Figure 5B). For nodule dry weight per plant (NDWPP), T03 had the numerically highest mean (0.13 g) and did not differ significantly from T01 (0.12 g), but had significantly higher values than T02, T05, and T04. Furthermore, T01 did not differ significantly from T02 (Figure 5C).

Internal nodule color was analyzed descriptively using frequencies and proportions because complete separation between treatments and response categories precluded fitting an ordinal model. In T01, T02, and T03, 100% of the observations corresponded to nodules with pink internal coloration. In T04, 80% of the observations corresponded to plants without nodules and 20% to nodules without internal coloration. In T05, 50% of the observations corresponded to nodules with pink internal coloration, 25% to nodules without internal coloration, and 25% to plants without nodules (Figure 5D).

3.3 Aboveground biomass evaluation

The analysis of shoot biomass-related variables showed significant treatment effects on shoot biomass fresh weight (ABFW), shoot biomass dry weight (ABDW), and plant height (PH) ($p < 0.01$), whereas block effects were significant for ABDW ($p < 0.01$). Plot was included as a random intercept in all models, and the estimated standard deviations associated with this effect, expressed on the scale of the linear predictor with a log link, are presented in Table 3. Although all models were fitted using a Student's t distribution, these estimates are specific to each response variable and the corresponding dispersion structure.

This overall response was reflected in the multiple comparisons shown in Figure 6, where T01 exhibited the highest values for both ABFW (70.11 g) and ABDW (11.39 g) (Figures 6A,B). In contrast, the tallest plants were recorded in T03, T02, and T01, at 120.35, 119.83, and 119.69 cm, respectively (Figure 6C).

3.4 Pea yield and quality

The analysis of yield and quality traits showed significant treatment effects on the number of pods per plant (NPP), pod length (PL), number of seeds per pod (NSP), green pod yield, and crude protein content ($p < 0.01$), whereas block effects were significant only for crude protein content ($p < 0.01$). Plot was included as a random intercept in the NPP, PL, and NSP models. For NSP, plant was additionally included as a random intercept to account for the hierarchical structure of pods nested within plants. The estimated standard deviations associated with these random effects, expressed on the linear predictor scale, are presented in Table 3. These estimates are model-specific and depend on the response variable, distribution, link function, and dispersion structure considered. Green pod yield and crude protein content were evaluated at the plot level; therefore, their models did not include random effects.

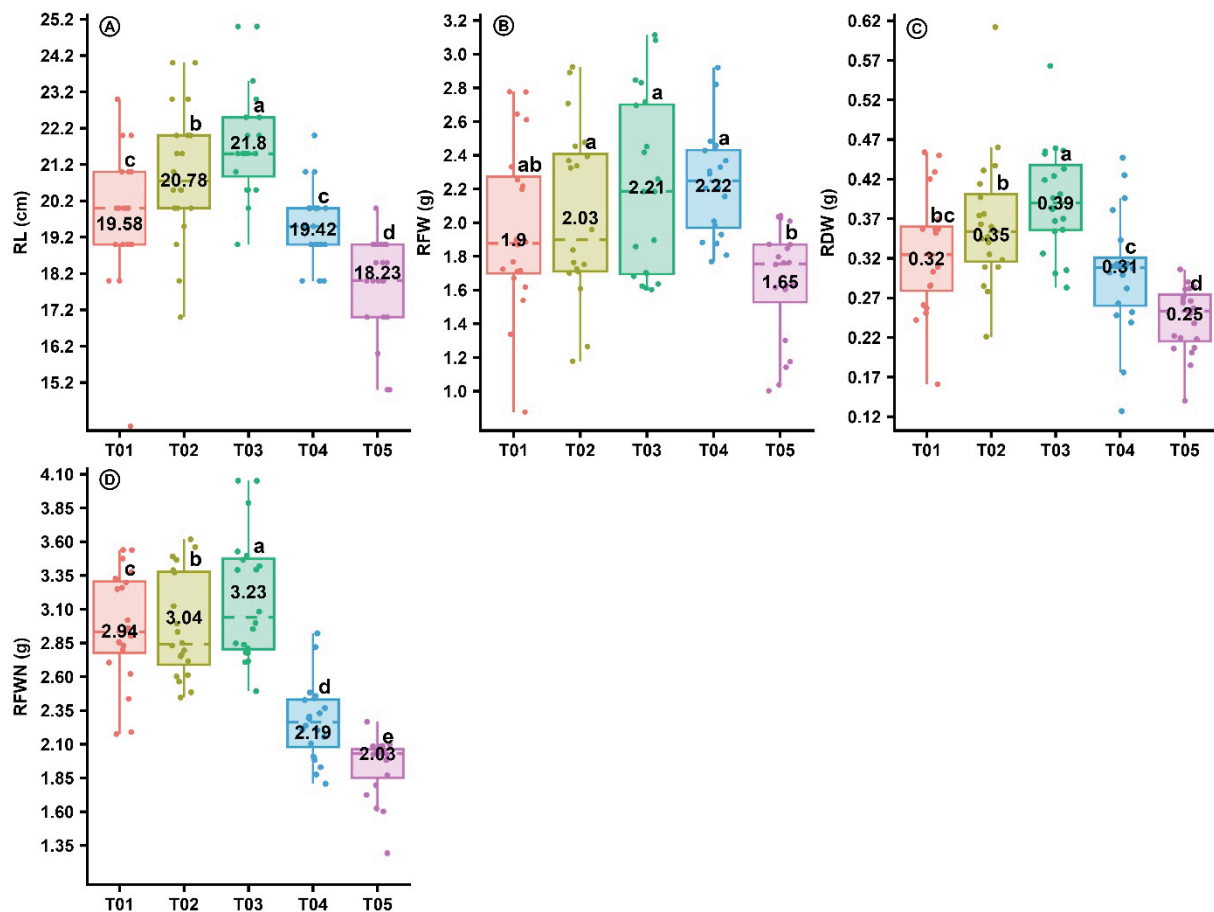


FIGURE 4

Estimated marginal means of root morphological traits across treatments. Root length [RL, cm; (A)], root fresh weight without nodules [RFW, g; (B)], root dry weight without nodules [RDW, g; (C)], and root fresh weight with nodules [RFWN, g; (D)]. In each panel, the 20 data points per treatment represent five plants sampled from each of four experimental units ($n = 4$). Boxes indicate the interquartile range, horizontal lines represent the medians, and whiskers extend to 1.5 times the interquartile range. Numerical values indicate the estimated marginal means obtained using a gamma model for RL, lognormal models for RFW and RFWN, and a Student's t model for RDW. Pairwise comparisons among treatments were adjusted using the Benjamini–Hochberg procedure to control the false discovery rate. Different letters within each panel indicate significant differences among treatments ($\alpha = 0.05$). Complete model specifications and diagnostic results are provided in [Supplementary Table S3](#).

Multiple-comparison analysis revealed differences among treatments in the evaluated yield and quality traits (Figure 7). For the number of pods per plant (NPP), T02 had the numerically highest mean (23.34) and was significantly higher than T01, T03, and T05, but did not differ from T04. In turn, T04 did not differ significantly from T01 or T03. For pod length (PL), T03 had the numerically highest mean (8.26 cm) and did not differ significantly from T02 (8.13 cm), although it was significantly higher than T01, T04, and T05. For the number of seeds per pod (NSP), T03 had the numerically highest mean (6.07) but did not differ significantly from T01 (6.01); both treatments had significantly higher values than T02, T04, and T05. Regarding green pod yield, T03 had the numerically highest mean (6.73 t ha⁻¹); however, no significant differences were observed among the inoculated treatments T01, T02, and T03. Moreover, T01 and T03 produced significantly higher yields than T04, whereas T02 did not differ significantly from T04. T05 had the significantly lowest yield (3.70 t ha⁻¹). Finally, T03 had the highest crude protein content (24.28%), which was significantly higher than those of all other treatments, whereas T01 and T04 did not differ significantly from each other.

3.5 Multivariate analysis of the effects of treatments on the evaluated variables

Four principal components with eigenvalues greater than one were retained, jointly explaining 86.71% of the total variability. PC1 explained 57.07% of the variance, followed by PC2 (11.52%), PC3 (11.00%), and PC4 (7.12%), as shown in [Supplementary Table S4](#). The variables with the greatest contributions to PC1 were RFWN (9.53%; $\cos^2 = 0.82$; loading = 0.90), Yield (9.38%; $\cos^2 = 0.80$; loading = 0.90), and RDW (9.25%; $\cos^2 = 0.79$; loading = 0.89). For PC2, the variables with the greatest contributions were RFW (33.02%; $\cos^2 = 0.57$; loading = 0.76), ABDW (22.21%; $\cos^2 = 0.38$; loading = -0.62), and NFWPP (11.41%; $\cos^2 = 0.20$; loading = -0.44). For PC3, the main contributors were NNPP (22.95%; $\cos^2 = 0.38$; loading = 0.62), NDWPP (20.08%; $\cos^2 = 0.33$; loading = 0.58), and NFWPP (13.47%; $\cos^2 = 0.22$; loading = 0.47). Finally, for PC4, the variables with the greatest contributions were Protein (39.74%; $\cos^2 = 0.42$; loading = -0.65), NSP (23.75%; $\cos^2 = 0.25$; loading = -0.50), and NPP (18.03%; $\cos^2 = 0.19$; loading = 0.44). The complete values for the contributions, $\cos^2$, and factor loadings of all variables are presented in [Supplementary Table S5](#).

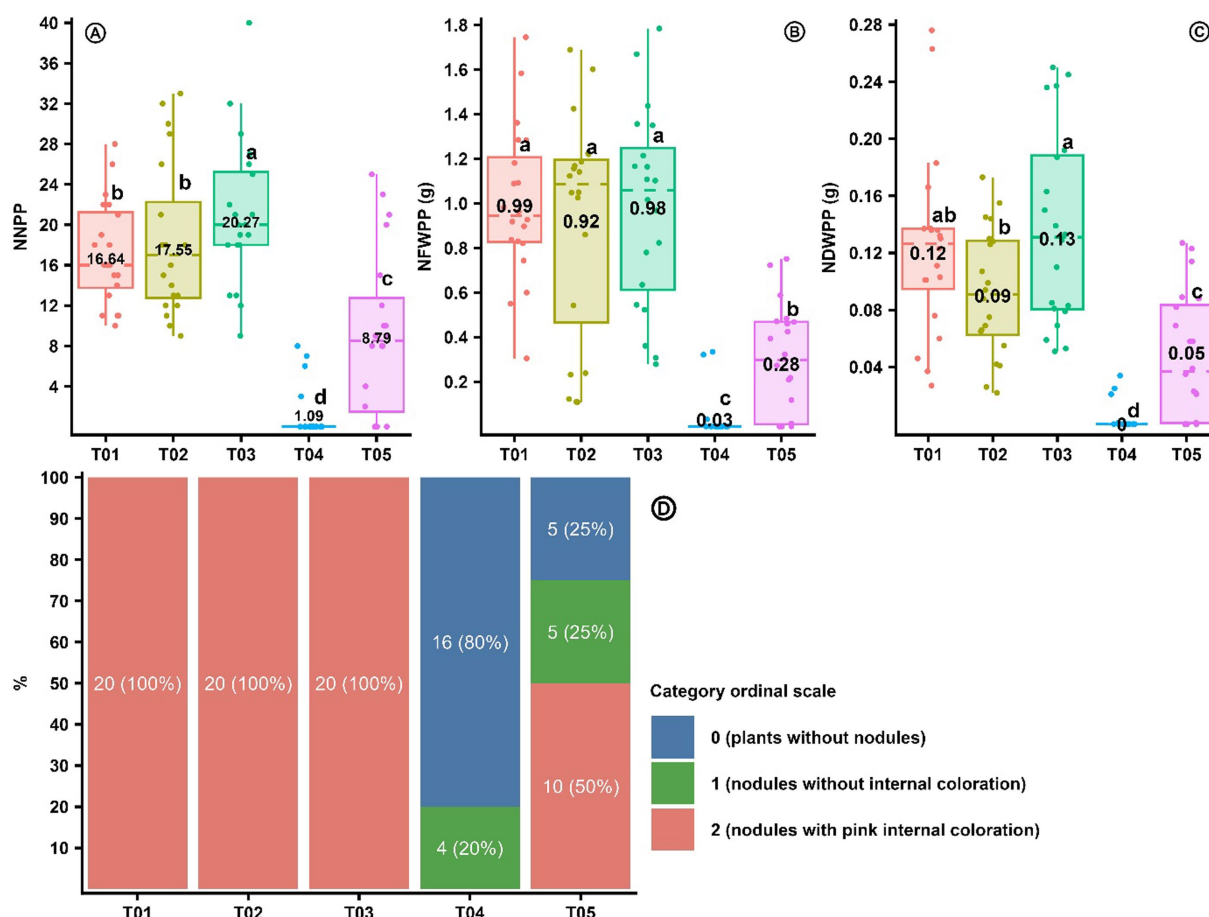


FIGURE 5

Marginal means of nodulation characteristics and internal nodule colour by treatment. The number of nodules per plant [NNPP, nodules plant⁻¹ (A)], nodule fresh weight per plant [NFWPP, g plant⁻¹ (B)], nodule dry weight per plant [NDWPP, g plant⁻¹ (C)] and internal nodule coloration (D). For NNPP, NFWPP, and NDWPP, the 20 data points per treatment represent five plants sampled from each of four experimental units ($n = 4$). Boxes indicate the interquartile range, horizontal lines represent the medians, and whiskers extend to 1.5 times the interquartile range. Numerical values indicate the estimated marginal means obtained using Tweedie models. Pairwise comparisons among treatment estimated marginal means were adjusted using the Benjamini–Hochberg procedure to control the false discovery rate. Different letters within each panel indicate significant differences among treatments ($\alpha = 0.05$). Complete model specifications and diagnostic results are provided in [Supplementary Table S3](#). In panel, the stacked bars represent the frequencies and proportions of the 20 observations per treatment according to the following categories: 0 (plants without nodules), 1 (nodules without internal coloration), and 2 (nodules with pink internal coloration). Values within the bars indicate the number and percentage of observations in each category. Internal nodule coloration is presented descriptively, and an ordinal model was not fitted because of complete separation between treatment and response categories, as all observations for T01, T02, and T03 were concentrated in a single category.

The PC1–PC2 representation explained 68.59% of the total variability. In this space, the plots corresponding to the inoculated treatments T01, T02, and T03 showed positive PC1 scores and were located in the same general direction as the variables that characterized this component, whereas the plots of T05 were located at the negative extreme of PC1 ([Figure 8A](#)). The PC1–PC3 representation explained 68.07% of the total variability; the plots corresponding to T03 showed positive scores on both components and were located in the same sector as the variables NNPP, NDWPP, and NFWPP. In contrast, the plots of T04 showed negative scores on both PC1 and PC3 and were located in the sector opposite to these variables ([Figure 8B](#)).

3.6 Relative yield index (RYI_N)

The Relative Yield Index (RYI_N), calculated from the estimated marginal means for yield and using T04 as the reference treatment (100%), varied among treatments ([Figure 7E](#)). The RYI_N was 119.49% for co-inoculation with *Rhizobium leguminosarum* bv. *viciae*

+ *Azospirillum brasilense* (T03), 116.85% for inoculation with *Rhizobium leguminosarum* bv. *viciae* (T01), and 113.57% for inoculation with *Azospirillum brasilense* (T02). These values correspond to estimated yields that were 19.49, 16.85, and 13.57% higher than that estimated for T04, respectively. In contrast, T05 had an RYI_N of 65.79%, corresponding to an estimated yield 34.21% lower than that of the reference treatment. The estimates and their 95% confidence intervals were derived from the marginal means of the fitted yield model.

3.7 Profitability

A summary of production costs for each treatment is presented in [Table 4](#), while the economic indicators (green pod yield, gross production value, net production value, and profitability) are presented in [Table 5](#).

Under the selling-price assumption (S/ 3.50 kg⁻¹) and the production costs established for this study, the highest estimated profitability

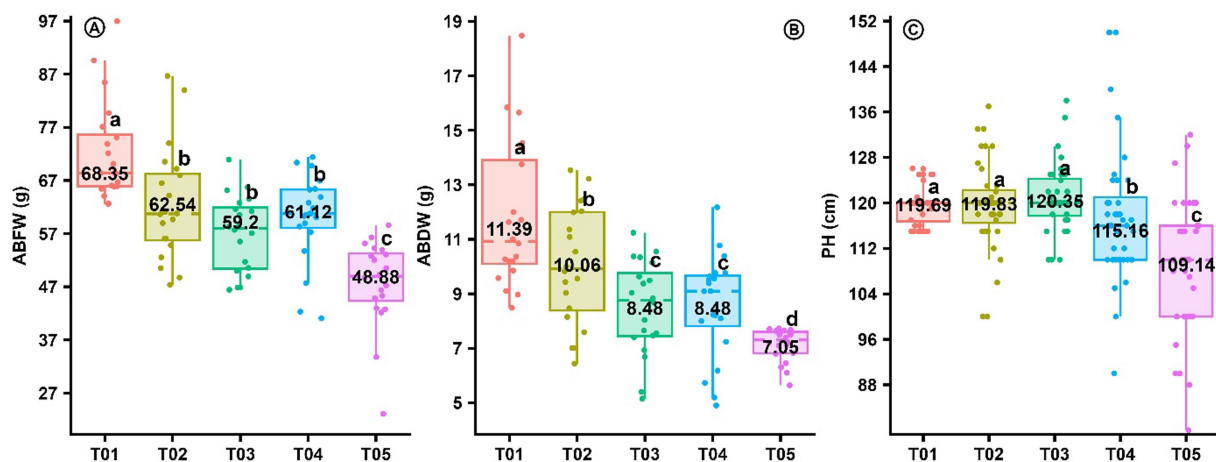


FIGURE 6

Marginal means of aboveground biomass and plant architecture according to treatments. Shoot biomass fresh weight (ABFW, g) (A), shoot biomass dry weight (ABDW, g) (B) and plant height (PH, cm) (C). For ABFW and ABDW, the 20 data points per treatment represent five plants sampled from each of four experimental units ($n = 4$), whereas for PH, the 40 data points per treatment represent 10 plants sampled from each of four experimental units ($n = 4$). Boxes indicate the interquartile range, horizontal lines represent the medians, and whiskers extend to 1.5 times the interquartile range. Numerical values indicate the estimated marginal means obtained using Student's t models. Pairwise comparisons among treatment estimated marginal means were adjusted using the Benjamini–Hochberg procedure to control the false discovery rate. Different letters within each panel indicate significant differences among treatments ($\alpha = 0.05$). Complete model specifications and diagnostic results are provided in [Supplementary Table S3](#).

for green pea production (Table 5) was obtained with treatment T03, co-inoculation with *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense*, which achieved a profitability index of 262.75% and a net production value of S/ 17,061.59 ha⁻¹. In comparison, treatment T04, the mineral nitrogen comparator, showed an estimated profitability of 176.78% and a net production value of S/ 12,585.64 ha⁻¹. Under these assumptions, T03 generated an estimated net production value that was S/ 4,475.95 ha⁻¹ higher than that of T04. Likewise, treatments individually inoculated with either *Rhizobium leguminosarum* bv. *viciae* or *Azospirillum brasilense* also showed higher estimated profitability than both T04 and the uninoculated, nitrogen-unfertilized control (T05).

4 Discussion

The co-inoculation treatment (T03) showed the highest values for most of the evaluated variables, including root length (21.73 cm) and root dry weight (0.39 g), which were significantly higher than those observed in the mineral N treatment (T04) and the control (T05). These results are consistent with the findings of Star et al. (2012), who reported that the combined application of *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense* may enhance root system development by combining the benefits of biological nitrogen fixation by *Rhizobium leguminosarum* bv. *viciae* with the production of phytohormones, particularly auxins, by *Azospirillum brasilense*. These mechanisms may contribute to improved root development, root exploration capacity, and nutrient acquisition; however, they were not directly evaluated in the present study. Similarly, Shah et al. (2024) reported that the combined application of these microorganisms may improve water and nutrient uptake, achieving effects comparable to or even greater than those obtained through nitrogen fertilisation. Their findings further support co-inoculation as a sustainable and effective strategy for reducing dependence on synthetic nitrogen fertilisers. According to Nisha et al. (2024), *Azospirillum brasilense* also enhances

the viability and effectiveness of *Rhizobium leguminosarum* bv. *viciae* root colonisation and attachment, thereby facilitating nodulation and the establishment of a functional symbiosis. Enhanced root development increases the number of potential nodulation sites, which could favour biological nitrogen fixation; however, this process was not directly quantified in the present study.

Nodulation parameters reached their highest values in T03 (co-inoculation treatment with *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense*), suggesting a greater nodulation response and development of functional nodules, consistent with previous studies (Kaziūnienė et al., 2025; Flores-Félix et al., 2020). Likewise, signals derived from *R. leguminosarum* have been shown to modify root architecture and nitrate responses; therefore, increased nodulation may be associated with more efficient root exploration and improved physiological performance (Boeglin et al., 2022). In the present study, the inoculated treatments exhibited greater nodulation, and the nodules observed in these treatments showed pink internal coloration. This characteristic is commonly associated with functional nodule tissue and may provide qualitative information on nodule physiological status. However, because leghemoglobin concentration was not biochemically quantified and neither nitrogenase activity nor biological nitrogen fixation was directly determined, the observed internal coloration should not be interpreted as evidence of differences in leghemoglobin concentration, nitrogenase activity, or biological nitrogen fixation (Ott et al., 2005). Therefore, the differences in internal nodule coloration observed among treatments are reported only as a qualitative morphological characteristic and may be consistent with differences in nodule physiological status, but they do not provide direct evidence of differences in leghemoglobin concentration, nitrogenase activity, or biological nitrogen fixation. In contrast, T04 (mineral-N comparator) and T05 (uninoculated and non-N-fertilised control treatment) showed lower leghemoglobin colouration, suggesting a lower nodulation response. The lower nodulation observed in T04 may be associated with the inhibitory effect of mineral nitrogen on symbiotic establishment. Evidence indicates that high concentrations of mineral nitrogen fertilisers can suppress

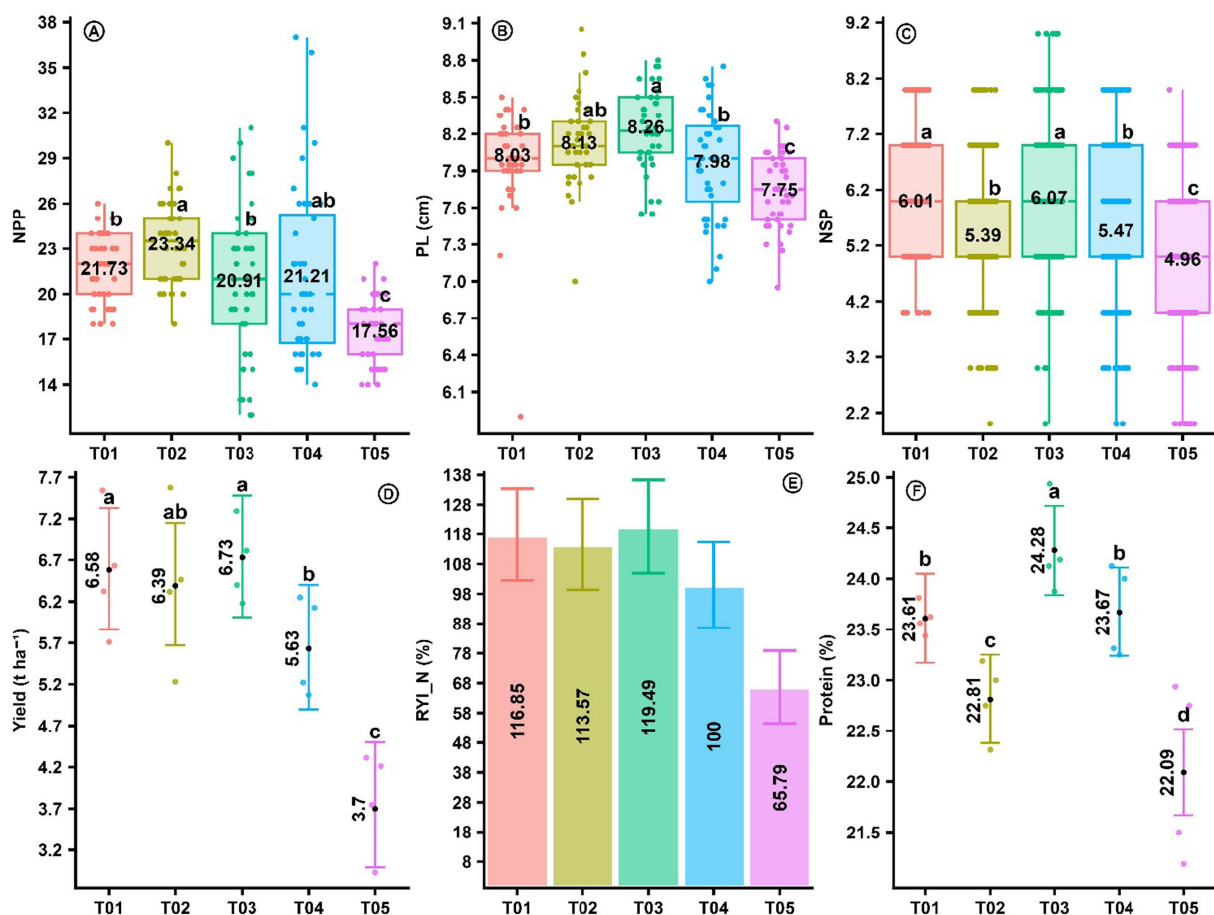
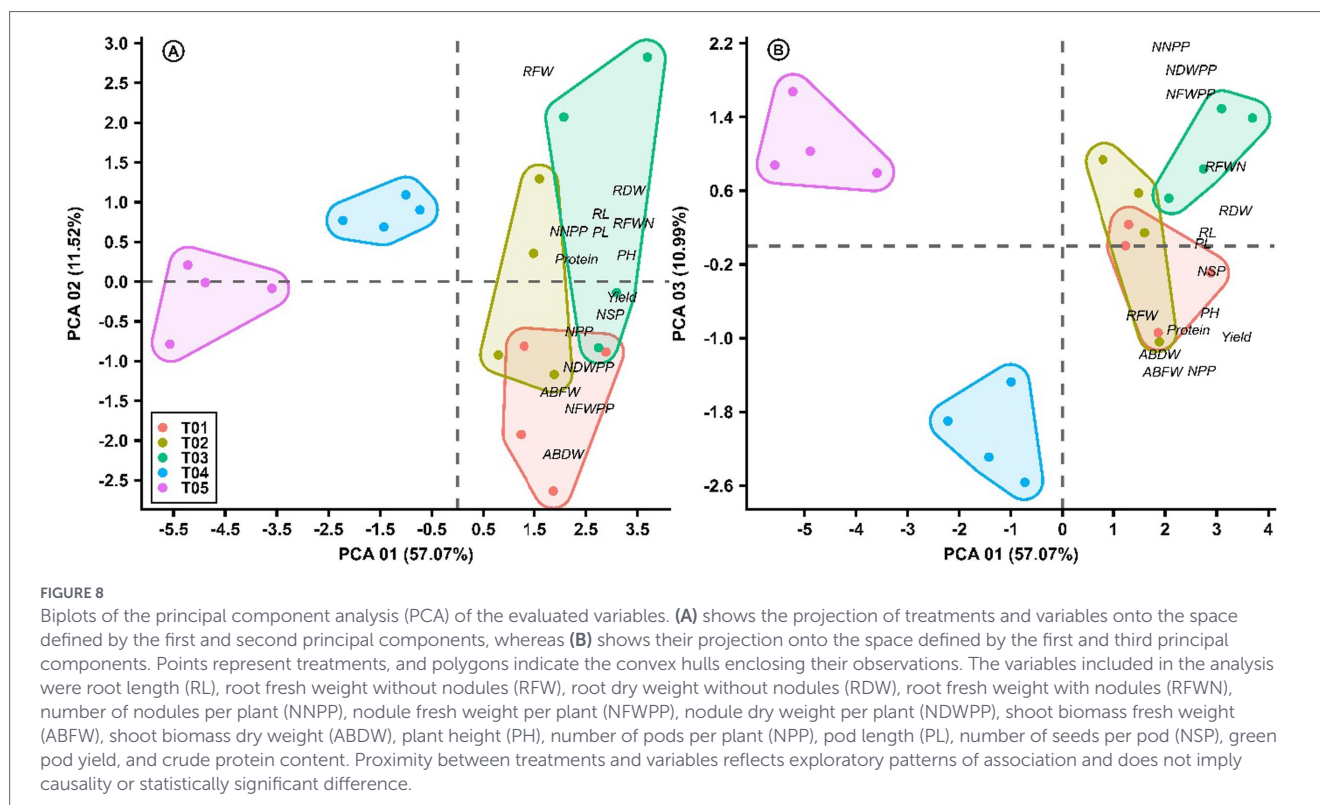


FIGURE 7

Marginal means of pea yield and quality according to treatments. The number of pods per plant [NPP, pods plant⁻¹ (A)], pod length [PL, cm (B)], number of seeds per pod [NSP, seeds pod⁻¹ (C)], green pod yield [Yield, t ha⁻¹ (D)], Relative Yield Index (RYI_N), [RYI_N, % (E)], and crude protein content [Protein, % (F)]. For NPP and PL, the 40 data points per treatment represent 10 plants sampled from each of four experimental units ($n = 4$). For NSP, the 400 data points per treatment represent 10 pods sampled from each of 10 plants in each of four experimental units ($n = 4$). Boxes indicate the interquartile range, horizontal lines represent the medians, and whiskers extend to 1.5 times the interquartile range. Numerical values indicate the estimated marginal means. In (D,F), colored points represent the experimental units within each treatment ($n = 4$), whereas black points and numerical values indicate the estimated marginal means, and error bars represent their 95% confidence intervals. In (E), bars represent the RYI_N calculated from the estimated marginal means for yield, and error bars indicate the corresponding 95% confidence intervals. Estimated marginal means were obtained using Conway–Maxwell–Poisson models for NPP and NSP, a Student's t model for PL, a lognormal model for yield, and a beta model for protein content. Pairwise comparisons among treatment estimated marginal means were adjusted using the Benjamini–Hochberg procedure to control the false discovery rate. Different letters within (A–D) and (F) indicate significant differences among treatments ($\alpha = 0.05$). Complete model specifications and diagnostic results are provided in [Supplementary Table S3](#).

nodule initiation and nitrogen fixation activity (Nishida and Suzuki, 2018). In T05, the absence of inoculation resulted in greater reliance on native rhizobial populations, whose lower abundance or symbiotic efficiency, combined with the low temperatures characteristic of high-Andean production systems, may have limited nodulation (Masson-Boivin and Sachs, 2018; Liu et al., 2018). These findings are consistent with studies demonstrating that symbiotic efficiency is closely related to the structure and functionality of the symbiotic association plant within nodules (Tsyganova et al., 2023). Treatment T02 also produced a relatively high number of nodules, likely resulting from association with native rhizobial strains, although these nodules may have exhibited lower nitrogen-fixing efficiency. This response has a physiological basis, as *Azospirillum brasilense* produces indole-3-acetic acid (IAA) and other growth-promoting compounds that stimulate lateral root formation and increase the number of potential nodulation sites available for rhizobia (Carrillo-Flores et al., 2022); nevertheless, these mechanisms were not quantified in the present study.

The evaluation of aboveground biomass revealed that treatment T01 (*Rhizobium leguminosarum* bv. *viciae* inoculation), inoculated with *Rhizobium leguminosarum* bv. *viciae* only, produced the highest fresh and dry aboveground biomass (ABFW and ABDW), demonstrating greater effectiveness in biomass accumulation. Previous studies have shown that biomass production in *Pisum sativum* is closely associated with carbon assimilation capacity and nutrient use efficiency, both of which are enhanced through improvements in physiological processes such as photosynthesis and biological nitrogen fixation (Cabeza et al., 2024; Hu et al., 2025). Although *Rhizobium leguminosarum* bv. *viciae* inoculation alone promoted greater aboveground biomass accumulation, this did not translate into the highest yield. In contrast, co-inoculation with *Rhizobium leguminosarum* bv. *viciae* + *Azospirillum brasilense* resulted in superior reproductive performance, which may be associated with physiological processes that favour resource allocation towards pod and seed development; however, these processes were not directly evaluated in the present study. This response may be related to the combined application of both



microorganisms, which has been reported to influence root development, nutrient uptake, and source–sink relationships, thereby promoting greater reproductive efficiency and yield (Hungria et al., 2013; Cassán et al., 2020). Regarding plant height (PH), treatments T03, T02, and T01 consistently exhibited the highest values, indicating superior vegetative growth in inoculated plants compared with non-inoculated treatments. However, Hu et al. (2025) reported that plant height is influenced primarily by genetic factors and genotype $\times$ environment interactions, rather than by specific agronomic management practices alone. Nevertheless, the limited variability observed in aboveground biomass dry weight (ABDW) suggests that this parameter may be more sensitive to abiotic factors, such as water availability or soil fertility, even under controlled experimental conditions, given its susceptibility to environmental fluctuations (Janusauskaite, 2025). In this regard, an adequate nutrient supply and favourable growing conditions have been shown to significantly enhance biomass accumulation and productivity in legumes (Zhang et al., 2025).

The number of pods per plant (Figure 7A) was significantly higher in the T02 (*Azospirillum brasilense* inoculation) than in the other treatments, highlighting its role as a plant growth-promoting rhizobacteria through the synthesis of phytohormones that stimulate root development and enhance nutrient uptake (Bashan and de-Bashan, 2010; Cassán et al., 2020). Similarly, pod length was greater in the inoculated treatments, suggesting that microbial inoculation may have indirectly promoted pea growth and reproductive development by improving nutrient uptake and overall plant nutritional status, consistent with previous studies (Fukami et al., 2018). Regarding the number of seeds per pod, inoculated treatments showed a slight advantage over the treatment without nitrogen application. Previous studies have demonstrated that the combined application of *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense* improves assimilate partitioning efficiency, resulting in positive effects on yield components

(Hungria et al., 2015; Vejan et al., 2016). Nevertheless, assimilate partitioning was not directly quantified in the present study.

Co-inoculation (T03) produced the best overall performance, particularly in terms of green grain yield and crude protein content, outperforming the mineral nitrogen comparator (T04). These findings indicate that co-inoculation was associated with improved crop performance under the conditions of this study. The higher crude protein content observed in the co-inoculated treatment is consistent with previous reports describing the beneficial effects of microbial inoculation on legume productivity and seed quality (Fukami et al., 2018; Hungria et al., 2015). However, this study did not determine the source of the nitrogen contributing to protein accumulation in pea grains. Therefore, it cannot be established whether this response was associated with biological nitrogen fixation, enhanced uptake of soil nitrogen, or other mechanisms related to microbial inoculation. Although the mineral nitrogen comparator (T04) showed favorable values for some traits, such as root fresh weight and crude protein content, it did not achieve the same grain yield as the co-inoculated treatment. In contrast, the unfertilized control treatment (T05) generally exhibited lower values for most productive traits. This poorer performance is consistent with a possible nitrogen limitation, although other factors that may have influenced the observed response cannot be ruled out. Similar responses have been reported in legumes, in which limited nitrogen availability is associated with reduced plant growth and grain production (Massa et al., 2022).

The principal component analysis revealed that the inoculated treatments were primarily associated with root dry weight (RDW), yield, plant height (PH), root length (RL), and pod length (PL), variables that collectively accounted for 56% of the total variability. Specifically, treatment T03 was closely associated with most of the variables evaluated in the PCA biplot, whereas treatment T04 was

TABLE 4 Production costs by treatment (\$/ ha⁻¹).

Summary of production costs					
Item	T01 (Rhizobium)	T02 (Azospirillum)	T03 (Rhizobium + Azospirillum)	T04 (with nitrogen fertilisation)	T05 (without nitrogen fertilisation)
Direct costs (\$/ ha ⁻¹)	5,923.00	5,823.00	5,863.00	6,448.00	5,233.00
a. Land preparation	930.00	930.00	930.00	930.00	930.00
Limpieza del terreno	210.00	210.00	210.00	210.00	210.00
Plowing	280.00	280.00	280.00	280.00	280.00
Harrowing or soil pulverization	140.00	140.00	140.00	140.00	140.00
Furrowing	300.00	300.00	300.00	300.00	300.00
b. Seed purchase	700.00	700.00	700.00	700.00	700.00
Pea seed	700.00	700.00	700.00	700.00	700.00
c. Biological inputs	40.00	40.00	80.00	0.00	0.00
Cost of <i>Rhizobium</i> inoculant	40.00	0.00	40.00	0.00	0.00
Cost of <i>Azospirillum</i> inoculant	0.00	40.00	40.00	0.00	0.00
d. Fertilizers	650.00	650.00	650.00	1,250.00	650.00
Urea	0.00	0.00	0.00	600.00	0.00
Triple superphosphate (TSP)	350.00	350.00	350.00	350.00	350.00
Potassium chloride (KCl)	300.00	300.00	300.00	300.00	300.00
e. Inoculation activity	50.00	50.00	50.00	0.00	0.00
Inoculation	50.00	50.00	50.00	0.00	0.00
g. Field operations	2,632.00	2,532.00	2,632.00	2,742.00	2,332.00
Sowing	280.00	280.00	280.00	280.00	280.00
Fertilizer application	280.00	280.00	280.00	490.00	280.00
Weed control and hand weeding	532.00	532.00	532.00	532.00	532.00
Tutoring	360.00	360.00	360.00	360.00	360.00
Phytosanitary control	280.00	280.00	280.00	280.00	280.00
Harvesting	900.00	900.00	900.00	800.00	600.00
h. Materials, equipment, and inputs	921.00	921.00	821.00	826.00	621.00
Materials and equipment	921.00	921.00	821.00	826.00	621.00
Supplementary activities (\$/ ha⁻¹)	634.61	627.61	630.41	671.36	479.31
Soil and other analyses	634.61	627.61	630.41	671.36	479.31
Total production cost (\$/ ha ⁻¹)	6,557.61	6,450.61	6,493.41	7,119.36	5,712.31

\$/, Peruvian sol (PEN); values are expressed on a per-hectare basis.

primarily associated with root fresh weight (RFW). Overall, the PCA revealed distinct patterns of association between treatments and agronomic variables under the experimental conditions of this study. Previous studies have reported similar positive associations between microbial inoculation and agronomic traits, in which beneficial microorganisms were associated with improved plant growth, root development, and crop yield (Amado-Rodríguez et al., 2022; Sharifi and Ryu, 2018).

TABLE 5 Economic analysis of the application of *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense* in green pea production.

Treatment	Yield (t ha ⁻¹)	Selling price (S/kg ⁻¹)	Production cost (S/ ha ⁻¹)	Gross production value (S/ ha ⁻¹)	Net production value (S/ ha ⁻¹)	Estimated Profitability (%)
T01 (<i>Rhizobium</i>)	6.58	3.50	6,557.61	23,030.00	16,472.39	251.20
T02 (<i>Azospirillum</i>)	6.39	3.50	6,450.61	22,365.00	15,914.39	246.71
T03 (<i>Rhizobium</i> + <i>Azospirillum</i>)	6.73	3.50	6,493.41	23,555.00	17,061.59	262.75
T04 (with nitrogen fertilisation)	5.63	3.50	7,119.36	19,705.00	12,585.64	176.78
T05 (without nitrogen fertilisation)	3.70	3.50	5,712.31	12,950.00	7,237.69	126.70

Reference prices were obtained from the SISAP–MIDAGRI market information system for the Ayacucho market (May 2024). Production costs included seed, land preparation, sowing, crop management practices, and harvesting. For treatments T01, T02, and T03, production costs also included the preparation of microbial inoculants, including bacterial strains, culture media, peat, adhesive, and labor.

The Relative Yield Index (RYI_N) was 19.49% higher in the co-inoculated treatment with *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense* (T03) than in the mineral-N comparator (T04). The results indicate that, under the experimental conditions of this study, the co-inoculated treatment achieved a higher green grain yield than the mineral nitrogen-fertilized treatment. However, the RYI_N alone does not allow identification of the nitrogen source that supported crop growth. Therefore, it cannot be concluded whether the observed response resulted from biological nitrogen fixation, enhanced uptake of soil nitrogen, or other mechanisms associated with microbial inoculation. Confirmation of the contribution of biological nitrogen fixation would require complementary measurements, such as isotopic techniques using N, natural abundance analysis, nitrogen balance approaches, or determination of total nitrogen accumulation in plant tissues (Herridge et al., 2008; Peoples et al., 2009). Likewise, the favorable performance observed in treatment T02 (*Azospirillum brasilense*) may be associated with the beneficial effects of microbial inoculation reported in previous studies (Giri et al., 2025; Cruz-Hernández et al., 2022). However, the mechanisms underlying these responses were not evaluated in the present study.

The economic analysis of green pea production revealed that the use of microbial inoculants generated higher profitability than conventional nitrogen fertilization. The results suggest that, under the conditions evaluated in this study, inoculation with *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense* represents a promising alternative for maintaining high productivity while reducing production costs. However, these findings should be validated across different locations, growing seasons, and economic scenarios before their widespread application. According to García-Blásquez Morote and Sato Palomino (2019), the economic profitability of pea cultivation depends not only on yield but also on the efficient use of production inputs. In this context, the present results are consistent with those reported by (Hungria et al., 2010), who highlighted that inoculants based on genera such as *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense* represent a low-cost, high-impact technology that can reduce dependence on synthetic fertilisers, whose prices have increased considerably in recent years. Consequently, microbial inoculation may represent a promising strategy for improving agronomic performance and economic efficiency under the conditions evaluated in this study.

The results of this study suggest that this technology has the potential to improve crop profitability and contribute to reducing dependence on synthetic nitrogen fertilisers under the evaluated conditions.

However, further studies across different environmental conditions, production systems, and economic scenarios are required before recommending its widespread adoption. The co-inoculation treatment was associated with higher estimated economic returns under the price and cost assumptions used. Nevertheless, the study was conducted at a single location and during one growing season. Biological nitrogen fixation was not directly quantified, and microbial colonization and phytohormonal responses were not assessed. Although nodulation provides evidence consistent with symbiotic establishment, it does not constitute a direct compatibility assay for the pesticide program or demonstrate the survival of *Azospirillum brasilense* following pesticide exposure. The economic analysis was based on a single price and cost scenario, whereas RYI_N is a comparative yield indicator and not a direct measure of biological nitrogen fixation.

5 Conclusion

The co-inoculation treatment (T03; *Rhizobium leguminosarum* bv. *viciae* + *Azospirillum brasilense*) showed the highest performance among the evaluated treatments for the main agronomic and economic variables. The co-inoculation achieved the highest values for root length (21.73 cm), number of nodules per plant (20.27), and number of seeds per pod (6.07). In terms of green pod yield, T03 produced a significantly higher yield (6.73 t ha⁻¹) than the mineral-N comparator (T04; 5.63 t ha⁻¹). Likewise, T03 recorded the highest crude protein content (24.28%), exceeding that of T04 (23.67%) and the other treatments.

Regarding the Relative Yield Index (RYI_N), all inoculated treatments showed higher values than the mineral-N comparator (T04), which was assigned a reference value of 100%. Co-inoculation (T03) exhibited the highest RYI_N (119.49%), corresponding to a yield 19.49% higher than that of T04, followed by the individual inoculation treatments with *Rhizobium leguminosarum* bv. *viciae* (T01) and *Azospirillum brasilense* (T02), with values of 116.85 and 113.57%, respectively. These results indicate that microbial inoculation was associated higher crop productivity than mineral nitrogen fertilization under the evaluated conditions. However, RYI_N is a comparative yield indicator and does not directly quantify biological nitrogen fixation or determine the source of nitrogen supporting plant growth. The economic analysis showed that co-inoculation was associated with

higher estimated net returns. The co-inoculation treatment (T03) showed the highest estimated net production value (S/ 17,061.59 ha⁻¹), exceeding that obtained with the mineral-N comparator (T04), which reached S/ 12,585.64 ha⁻¹. Consequently, T03 produced an additional net profit of S/. 4,545.95 compared with T04, indicating that co-inoculation with *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense* was associated with higher estimated net returns under the assumptions considered in the economic analysis.

Overall, co-inoculation with *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense* (T03) showed the best agronomic performance among the evaluated treatments under high-Andean conditions. Compared with mineral-N comparator (T04), co-inoculation resulted in higher agronomic performance and greater estimated economic returns. These findings indicate that microbial inoculation has the potential to enhance crop performance under the evaluated conditions; however, further studies including direct measurements of biological nitrogen fixation and plant nitrogen status are needed to determine the contribution of nitrogen fixation to the observed responses.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding author.

Author contributions

EB: Conceptualization, Investigation, Methodology, Writing – original draft. SA: Conceptualization, Investigation, Methodology, Writing – original draft. TC: Conceptualization, Investigation, Methodology, Writing – original draft. CP-E: Investigation, Writing – original draft. RR: Writing – original draft. JE: Formal analysis, Writing – original draft. CG-B: Methodology, Writing – review & editing. RS: Supervision, Writing – review & editing, Investigation.

Funding

The author(s) declared that financial support was received for this work and/or its publication. The research was funded by the Instituto

Nacional de Innovación Agraria, within the framework of the project: Mejoramiento de los servicios de investigación y transferencia tecnológica en el manejo y recuperación de suelos agrícolas degradados y aguas para riego en la pequeña y mediana agricultura en los departamentos de Lima, Áncash, San Martín, Cajamarca, Lambayeque, Junín, Ayacucho, Arequipa, Puno y Ucayali” CUI 2487112.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that Generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2026.1898585/full#supplementary-material>

References

- Amado-Rodríguez, J. L., Humeje-Pan, N. P., Blanco-Valvuen, J. O., Torres-Merchán, N. Y., and García-Parra, M. A. (2022). Effect of plant growth promoting bacteria on the phenology of the Amarilla maranganí quinoa cultivar. *Rev. Colomb. Cienc. Hortic.* 16:e13440. doi: 10.17584/rcch.2022v16i1.1344
- AOAC Official Method 954.01 Protein (Crude) in Animal Feed and Pet Food. (2023). En Oxford University Press eBooks.
- Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., et al. (2018). Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization. *Front. Plant Sci.* 9:1473. doi: 10.3389/fpls.2018.01473
- Bashan, Y., and de-Bashan, L. E. (2010). How the plant growth-promoting bacterium *Azospirillum* promotes plant growth—a critical assessment. *Adv. Agron.* 108, 77–136. doi: 10.1016/S0065-2113(10)08002-8
- Bashan, Y., Holguin, G., and de-Bashan, L. E. (2004). *Azospirillum*–plant relationships: physiological, molecular, agricultural, and environmental advances. *Can. J. Microbiol.* 50, 521–577. doi: 10.1139/w04-035
- Bazán, R. (2017). Manual de procedimientos de los análisis de suelos y agua con fines de riego. Available online at: <https://repositorio.inia.gob.pe/handle/20.500.12955/504> (Accessed May 18, 2026).
- Bocatti, C. R., Ferreira, E., Ribeiro, R. A., de Oliveira Chueire, L. M., Delamuta, J. R. M., Kobayashi, R. K. T., et al. (2022). Microbiological quality analysis of inoculants based on *Bradyrhizobium* spp. and *Azospirillum brasilense* produced “on farm” reveals high contamination with non-target microorganisms. *Braz. J. Microbiol.* 53, 267–280. doi: 10.1007/s42770-021-00649-2
- Boeglin, L., Morère Le-Paven, M. C., Clochard, T., Fustec, J., and Limami, A. M. (2022). *Pisum sativum* response to nitrate as affected by *Rhizobium leguminosarum*-derived signals. *Plants* 11:1966. doi: 10.3390/plants11151966

- Boleta, E. H. M., Shintate Galindo, F., Jalal, A., Santini, J. M. K., Rodrigues, W. L., Lima, B. H. D., et al. (2020). Inoculation with growth-promoting bacteria *Azospirillum brasilense* and its effects on productivity and nutritional accumulation of wheat cultivars. *Front. Sustain. Food Syst.* 4:607262. doi: 10.3389/fsufs.2020.607262
- Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., et al. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* 24, 127–135. doi: 10.1016/j.tree.2008.10.008
- Briones Vázquez, A., Medina Hoyos, A. E., Yoshino, M., Morita, T., and Maruyama, H. (2016). Guía de producción comercial de arveja. Available online at: <https://repositorio.inia.gob.pe/handle/20.500.12955/422> (Accessed May 20, 2026).
- Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., et al. (2017). GlmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* 9, 378–400. doi: 10.32614/RJ-2017-066
- Brown, M. L. (1979). Farm budgets: from farm income analysis to agricultural project analysis (English). World Bank staff occasional papers, No. OCP 29 Washington, DC: World Bank. Available online at: <http://documents.worldbank.org/curated/en/898231468340864373>
- Bueno, C. J., Meyer, M. C., and De Souza, N. L. (2003). Efeito de fungicidas na sobrevivência de *Bradyrhizobium japonicum* (Semia 5019 e Semia 5079) e na nodulação da soja. *Acta Sci. Agron.* 25, 231–235. doi: 10.4025/actasciagron.v25i1.2676
- Burnham, K., and Anderson, D. (2002). Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. (2nd ed.) Springer. Available online at: <https://link.springer.com/book/10.1007/b97636> (Accessed May 20, 2026).
- Cabeza, R. A., Liese, R., Lingner, A., von Stülpmagel, R., and Dittert, K. (2024). Biomass production and nitrogen fixation in *Pisum sativum* L. *Data Brief* 55:110644. doi: 10.1016/j.dib.2024.110644
- Carrillo-Flores, E., Arreola-Rivera, J., Pazos-Solis, D. M., Bocanegra-Mondragón, M., Fierros-Romero, G., Mellado-Rojas, M. E., et al. (2022). TOR participation on the root system changes of *Arabidopsis* during its interaction with *Azospirillum*. *J. Appl. Biotechnol. Bioeng.* 9, 18–23. doi: 10.15406/jabb.2022.09.00280
- Cassán, F., Coniglio, A., López, G., Molina, R., Nieves, S., de Carlan, C. L. N., et al. (2020). Everything you must know about *Azospirillum* and its impact on agriculture and beyond. *Biol. Fertil. Soils* 56, 461–479. doi: 10.1007/s00374-020-01463-y
- CIMMYT (1988). *La formulación de recomendaciones a partir de datos agronómicos: Un manual metodológico de evaluación económica*. Ed. revisada Edn. Mexico: Centro Internacional de Mejoramiento de Maíz y Trigo.
- Condori, L., Alarcón, S., Huasasquiche, L., García-Blasquez, C., Padilla, C., Velasquez, J., et al. (2024). Inoculation with *Azospirillum brasilense* as a strategy to reduce nitrogen fertilization in cultivating purple maize (*Zea mays* L.) in the inter-Andean valleys of Peru. *Microorganisms* 12:2107. doi: 10.3390/microorganisms12102107
- Congreves, K. A., Otchere, O., Ferland, D., Farzadfar, S., Williams, S., and Arcand, M. M. (2021). Nitrogen use efficiency definitions of today and tomorrow. *Front. Plant Sci.* 12:637108. doi: 10.3389/fpls.2021.637108
- Cruz-Hernández, M. A., Mendoza-Herrera, A., Bocanegra-García, V., and Rivera, G. (2022). *Azospirillum* spp. from plant growth-promoting bacteria to their use in bioremediation. *Microorganisms* 10:1057. doi: 10.3390/microorganisms10051057
- Curley, R. L., and Burton, J. C. (1975). Compatibility of *Rhizobium japonicum* with chemical seed protectants. *Agrochim. J.* 67, 807–808. doi: 10.2134/agronj1975.00021962006700060020x
- Dixon, P. M. (2016). Should Blocks be Fixed or Random? *Conf. Appl. Statist. Agric.* 23, 1–12. doi: 10.4148/2475-7772.1474
- Döbereiner, J., Baldani, V. L. D., and Baldani, J. I. (1995). Como isolar e identificar bactérias diazotróficas de plantas não leguminosas. EMBRAPA-SPI, Brazil. Available online at: <https://www.alice.cnptia.embrapa.br/alice/handle/doc/622470>
- Ejaz, S., Batool, S., Anjum, M. A., Naz, S., Qayyum, M. F., Naqqash, T., et al. (2020). Effects of inoculation of root-associated *Azospirillum* and *Agrobacterium* strains on growth, yield and quality of pea (*Pisum sativum* L.) grown under different nitrogen and phosphorus regimes. *Sci. Hortic.* 270:109401. doi: 10.1016/j.scienta.2020.109401
- FAO (2019). El reciclaje de materias orgánicas en la Agricultura de América Latina. Available online at: <https://www.fao.org/4/ar127s/ar127s.pdf> (Accessed May 22, 2026).
- FAOSTAT (2023). Estadísticas | FAO | Organización de las Naciones Unidas para la Alimentación y la Agricultura. Statistics. Available online at: <http://www.fao.org/statistics/es> (Accessed May 22, 2026).
- Flores-Félix, J. D., Carro, L., Cerda-Castillo, E., Squartini, A., Rivas, R., and Velázquez, E. (2020). Analysis of the interaction between *Pisum sativum* L. and *Rhizobium laguerreae* strains nodulating this legume in Northwest Spain. *Plants* 9:1755. doi: 10.3390/plants9121755
- Fukami, J., Cerezini, P., and Hungria, M. (2018). *Azospirillum*: benefits that go far beyond biological nitrogen fixation. *AMB Express* 8:73. doi: 10.1186/s13568-018-0608-1
- García-Blásquez Morote, C., and Sato Palomino, M. (2019). Técnicas de aislamiento, identificación, selección de cepas de *Rhizobium*, *Azospirillum* y producción de inoculantes. *Investigación* 27:175. doi: 10.51440/unsch.revistainvestigacion.2019.1.119
- García-Blásquez-Morote, J., Alarcón-Romaní, S., Rojas-Cordero, T., Quispe-Tenorio, J., and García-Blásquez-Morote, C. (2026). Co-inoculation of *Bradyrhizobium lupini* and *Azospirillum brasilense* enhances the agronomic performance of *Lupinus mutabilis* sweet in two agroecological zones of southern Peru. *Plant Prot.* 10, 169–179. doi: 10.33804/10.33804/pp.010.02.6008
- Giri, B. R., Chattaraj, S., Rath, S., Pattnaik, M. M., Mitra, D., and Thatoi, H. (2025). Unveiling the molecular mechanism of *Azospirillum* in plant growth promotion. *Bacteria* 4:36. doi: 10.3390/bacteria4030036
- Gomes, Y. C. B., Dalchiavon, F. C., and De Assis Valadão, F. C. (2017). Joint use of fungicides, insecticides and inoculants in the treatment of soybean seeds. *Revista CERES* 64, 258–265. doi: 10.1590/0034-737x201764030006
- Gorshkov, A. P., Kusakin, P. G., Vorobiev, M. G., Tsyganova, A. V., and Tsyganov, V. E. (2024). Effect of insecticides Imidacloprid and alpha-Cypermethrin on the development of pea (*Pisum sativum* L.) nodules. *Plants* 13:3439. doi: 10.3390/plants13233439
- Granada, C. E., Passaglia, L. M., De Souza, E. M., and Sperotto, R. A. (2018). Is phosphate solubilization the forgotten child of plant growth-promoting rhizobacteria? *Front. Microbiol.* 9:2054. doi: 10.3389/fmicb.2018.02054
- Hartig, F. (2024). DHARMA: Residual diagnostics for hierarchical (multi-level/mixed) regression models. R package Available online at: <https://cran.r-project.org/web/packages/DHARMA/vignettes/DHARMA.html> (Accessed May 22, 2026).
- Henning, A., Campo, R., and Sfredo, G. (1997). Tratamento com fungicidas, aplicação de micronutrientes e inoculação de sementes de soja. Available online at: <https://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/460391> (Accessed May 22, 2026).
- Herridge, D. F., Peoples, M. B., and Boddey, R. M. (2008). Global inputs of biological nitrogen fixation in agricultural systems. *Plant Soil* 311, 1–18. doi: 10.1007/s11104-008-9668-3
- Hu, C., Cun, J., Soliman, A. A., Yang, F., Ghareeb, Z. E., Yuan, X., et al. (2025). Agronomic performance and yield stability of field pea (*Pisum sativum* L.) genotypes in multi-environment trials. *BMC Plant Biol.* 25:1670. doi: 10.1186/s12870-025-07721-1
- Huanhuayo Zuasnar, D., and Acero Gonzales, J. (2025). Aislamiento y caracterización de *Rhizobium* nativo en cinco comunidades productoras de arveja (*Pisum sativum* L.) en Acobamba, Huancavelica [Tesis de grado, Universidad Nacional de San Cristóbal de Huamanga]. Available online at: <https://hdl.handle.net/20.500.14597/9425> (Accessed May 23, 2026).
- Huasasquiche, S. L., Velarde, A. L. D., Arévalo, A. Y., and Solórzano, A. R. A. (2025). *Manual para la inoculación de rizobios en leguminosas*. Instituto Nacional de Innovación Agraria (INIA). Lima, Perú: Instituto Nacional de Innovación Agraria (INIA). Available online at: <https://repositorio.inia.gob.pe/>
- Hungria, M., and Araújo, R. S. (1994). *Manual de Métodos Empregados em Estudos de Microbiologia Agrícola*. EMBRAPA. Complementar: Brasília, DF, Brasil: EMBRAPA-SPI. Available online at: <http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/199952> (Accessed May 15, 2026).
- Hungria, M., Campo, R. J., and Mendes, I. C. (2001). *Fixação biológica do nitrogênio na cultura da soja*. Londrina, PR, Brasil: Embrapa Soja. Available online at: <http://www.infoteca.cnptia.embrapa.br/handle/doc/459673>
- Hungria, M., Campo, R. J., Souza, E. M., and Pedrosa, F. O. (2010). Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. *Plant Soil* 331, 413–425. doi: 10.1007/s11104-009-0262-0
- Hungria, M., and Nogueira, M. A. y Araujo, R. S. (2013). Coinoculación de soja y frijol común con rizobios y azospirila: estrategias para mejorar la sostenibilidad. *Biol. Fertil. Soils* 49, 791–801. doi: 10.1007/s00374-012-0771-5
- Hungria, M., Nogueira, M. A., and Araujo, R. S. (2015). Soybean seed co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*: a new biotechnological tool to improve yield and sustainability. *Am. J. Plant Sci.* 6, 811–817. doi: 10.4236/ajps.2015.66087
- Ibrahim, H. M., and El-Sawah, A. M. (2022). The mode of integration between *Azotobacter* and *Rhizobium* affect plant growth, yield, and physiological responses of pea (*Pisum sativum* L.). *J. Soil Sci. Plant Nutr.* 22, 1238–1251. doi: 10.1007/s42729-021-00727-2
- ISO 11261:1995 (1995). Soil Quality—Determination of Total Nitrogen—Modified Kjeldahl Method (11261). Available online at: <https://www.iso.org/standard/19239.html> (Accessed May 15, 2026).
- ISO 11265:1994 (1994) Soil Quality—Determination of the Specific Electrical Conductivity. ISO (International Organization for Standardization). Available online at: <https://www.iso.org/standard/19243.html> (Accessed May 17, 2026).
- Janati, W., Benmrid, B., Elhaissofi, W., Zeroual, Y., Nasielski, J., and Bargaz, A. (2021). Will phosphate bio-solubilization stimulate biological nitrogen fixation in grain legumes? *Front. Agron.* 3:637196. doi: 10.3389/fagro.2021.637196
- Janusauskaite, D. (2025). Physiological responses of pea (*Pisum sativum* L.) to nutrient management. *Plants* 14:2020. doi: 10.3390/plants14132020
- Jhu, M.-Y., and Ledermann, R. (2025). Division of labor in the nodule: plant GluTRs fuel heme biosynthesis for symbiosis. *Plant Cell* 37:koaf156. doi: 10.1093/plcell/koaf156
- Kaur, J., Srivignesh, S., Vishnu, and Walia, S. S. (2025). Imidacloprid- and carbendazim-tolerant nitrogen fixing *Azospirillum* improved soil health, growth and yield of rice crop in pesticide amended soils. *Cereal Res. Commun.* 53, 1881–1890. doi: 10.1007/s42976-024-00622-3
- Kaziūnienė, J., Gegeckas, A., Lapinskienė, L., Razbadauskienė, K., Mažlytė, R., and Supronienė, S. (2025). Competitiveness and nitrogen fixation efficiency analysis of *Rhizobium leguminosarum* strains in different field pea (*Pisum sativum* L.) genotypes. *Agriculture* 15:1784. doi: 10.3390/agriculture15161784

- Kebede, E. (2021). Contribution, utilization, and improvement of legumes-driven biological nitrogen fixation in agricultural systems. *Front. Sustain. Food Syst.* 5:767998. doi: 10.3389/fsufs.2021.767998
- Kunal, and Sharma, P. (2011). Compatibility of *Mesorhizobium* sp. *Cicer* with seed treatment of fungicide and insecticide in chickpea. *Arch. Agron. Soil Sci.* 58, 115–123. doi: 10.1080/03650340.2010.506483
- Lama-Isminio, D. A., Schaefer, C. E. G. R., Amaral, E. F., Lopes, D. V., Rocha-Francolino, M. R., and Senra, E. O. (2024). Soil–landform interplays from the highlands of the eastern Andes cordillera to the lowlands of the Peruvian Amazon. *Catena* 244:108248. doi: 10.1016/j.catena.2024.108248
- Lê, S., Josse, J., and Husson, F. (2008). FactoMineR: an R package for multivariate analysis. *J. Stat. Softw.* 25, 1–18. doi: 10.18637/jss.v025.i01
- Leceta, F., Binder, C., Mader, C., Mächtle, B., Marsh, E., Dietrich, L., et al. (2024). The impact of agriculture on tropical mountain soils in the western Peruvian Andes: a pedo-geoarchaeological study of terrace agricultural systems in the Laramate region (14.5° S). *Soil* 10, 727–761. doi: 10.5194/soil-10-727-2024
- Lenth, R. V. (2024). Emmeans: estimated marginal means, aka least-squares means (Versión 1.10.1) [software de computación]. Available online at: <https://r-project.org/10.32614/CRAN.package.emmeans> (Accessed May 18, 2026).
- Lindström, K., and Mousavi, S. A. (2019). Effectiveness of nitrogen fixation in rhizobia. *Microb. Biotechnol.* 13, 1314–1335. doi: 10.1111/1751-7915.13517
- Liu, A., Contador, C. A., Fan, K., and Lam, H. M. (2018). Interaction and regulation of carbon, nitrogen, and phosphorus metabolisms in root nodules of legumes. *Front. Plant Sci.* 9:1860. doi: 10.3389/fpls.2018.01860
- López-Alcocer, E., Ferrera-Cerrato, R., Alarcón, A., and Díaz-Franco, A. (2020). Eficiencia de fijación biológica de nitrógeno en cepas de *Rhizobium* spp. *Terra Latinoam.* 38, 841–852. doi: 10.28940/terra.v38i4.654
- Mahmoud, G. A. E., Abeed, A. H., Mostafa, H. H., and Abdel Monsef, O. (2023). Responses of pea (*Pisum sativum* L.) to single and consortium bio-fertilizers in clay and newly reclaimed soils. *Plants (Basel, Switz.)* 12:3931. doi: 10.3390/plants12233931
- Massa, F., Defez, R., and Bianco, C. (2022). Exploitation of plant growth promoting Bacteria for sustainable agriculture: hierarchical approach to link laboratory and field experiments. *Microorganisms* 10:865. doi: 10.3390/microorganisms10050865
- Masson-Boivin, C., and Sachs, J. L. (2018). Symbiotic nitrogen fixation by rhizobia—the roots of a success story. *Curr. Opin. Plant Biol.* 44, 7–15. doi: 10.1016/j.pbi.2017.12.001
- Ministerio de Desarrollo Agrario y Riego (2023). Comportamiento de cultivos transitorios y permanentes Available online at: <http://repositorio.midagri.gob.pe:80/jspui/handle/20.500.13036/1646> (Accessed May 16, 2026).
- Ministerio de Desarrollo Agrario y Riego (2024). Precios en ciudades: Mercados mayoristas y minoristas - Arveja Grano Verde en Ayacucho. Sistema de Información de Abastecimiento y Precios (SISAP). Available online at: <https://siea.midagri.gob.pe/publicaciones/datos-y-estadisticas-agrarias> (Accessed May 21, 2026).
- Nisha, F. A., Tagoe, J. N., Pease, A. B., Horne, S. M., Ugrinov, A., Geddes, B. A., et al. (2024). Plant seedlings of peas, tomatoes, and cucumbers exude compounds that are needed for growth and chemoattraction of *Rhizobium leguminosarum* bv. *Viciae* 3841 and *Azospirillum brasilense* Sp7. *Can. J. Microbiol.* 70, 150–162. doi: 10.1139/cjm-2023-0217
- Nishida, H., and Suzuki, T. (2018). Nitrate-mediated control of root nodule symbiosis. *Curr. Opin. Plant Biol.* 44, 129–136. doi: 10.1016/j.pbi.2018.04.006
- NOM-021-RECNAT-2000. (2002). Norma Oficial Mexicana que establece las especificaciones de fertilidad, salinidad y clasificación de Suelos. Estudios, Muestreos y Análisis. Available online at: <https://platiica.economia.gob.mx/normalizacion/nom-021-semarnat-2000/> (Accessed May 15, 2026).
- Oleńska, E., Imperato, V., Małek, W., Włostowski, T., Wójcik, M., Swiecicka, I., et al. (2020). *Trifolium repens*-associated bacteria as a potential tool to facilitate phytostabilization of zinc and lead polluted waste heaps. *Plants* 9:1002. doi: 10.3390/plants9081002
- Ott, T., van Dongen, J. T., Gu, C., Krusell, L., Desbrosses, G., Vigeolas, H., et al. (2005). Symbiotic leghemoglobins are crucial for nitrogen fixation in legume root nodules but not for general plant growth and development. *Curr. Biol.* 15, 531–535. doi: 10.1016/j.cub.2005.01.042
- Palomino Espinoza, C. C. (2023). Co-inoculación (*Rhizobium* y *Azospirillum*), en el rendimiento de forraje de avena Mantaro 15 (*Avena sativa* L.), en la comunidad de Cochabamba 4100 msnm - Ayacucho [Tesis de grado, Universidad Nacional de San Cristóbal de Huamanga]. Available online at: <https://repositorio.unsch.edu.pe/handle/20.500.14612/6764> (Accessed May 28, 2026).
- Peoples, M. B., Brockwell, J., Herridge, D. F., Rochester, I. J., Alves, B. J. R., Urquiaga, S., et al. (2009). The contributions of nitrogen-fixing crop legumes to agricultural productivity. *Symbiosis* 48, 1–17. doi: 10.1007/BF03179980
- Pereira, L. C., De Carvalho, C., Suzukawa, A. K., Correia, L. V., Pereira, R. C., Santos, R. F. D., et al. (2020). Toxicity of seed-applied pesticides to *Azospirillum* spp.: an approach based on bacterial count in the maize rhizosphere. *Seed Sci. Technol.* 48, 241–246. doi: 10.15258/sst.2020.48.2.11
- Remans, R., Ramaekers, L., Schelkens, S., Hernandez, G., Garcia, A., Reyes, J. L., et al. (2008). Effect of *Rhizobium*–*Azospirillum* coinoculation on nitrogen fixation and yield of two contrasting *Phaseolus vulgaris* L. genotypes cultivated across different environments in Cuba. *Plant Soil* 312, 25–37. doi: 10.1007/s11104-008-9606-4
- Salvagiotti, F., Cassman, K. G., Specht, J. E., Walters, D. T., Weiss, A., and Dobermann, A. (2008). Nitrogen uptake, fixation and response to fertilizer N in soybean: a review. *Field Crop Res.* 108, 1–13. I: doi: 10.1016/j.fcr.2008.03.001
- Santos, M. S., Nogueira, M. A., and Hungria, M. (2019). Microbial inoculants: reviewing the past, discussing the present and previewing an outstanding future for the use of beneficial bacteria in agriculture. *AMB Express* 9:205. doi: 10.1186/s13568-019-0932-0
- Santos, M. S., Rodrigues, T. F., Nogueira, M. A., and Hungria, M. (2021). The challenge of combining high yields with environmentally friendly bioproducts: a review on the compatibility of pesticides with microbial inoculants. *Agronomy* 11:870. doi: 10.3390/agronomy11050870
- Seidenglanz, M., Rotrekl, J., Smýkalová, I., Poslušná, J., and Kolářik, P. (2010). Differences between the effects of insecticidal seed and foliar treatments on pea leaf weevils (*Sitona lineatus* L.) in the field pea (*Pisum sativum* L.). *Plant Prot. Sci.* 46, 25–33. doi: 10.17221/34/2009-pps
- Senthilkumar, M., Amarasena, N., and Sankaranarayanan, A. (2021). “Quantitative estimation of leghemoglobin content in legume root nodules,” in *Plant-Microbe Interactions: Laboratory Techniques*, eds. M. Senthilkumar, N. Amarasena and A. Sankaranarayanan (New York, NY, United States: Springer US).
- Shah, I., Sarim, K. M., Sikka, V. K., Dudeja, S. S., and Gahlot, D. K. (2024). Developed *Rhizobium* strains enhance soil fertility and yield of legume crops in Haryana, India. *J. Basic Microbiol.* 64:e2400327. doi: 10.1002/jobm.202400327
- Sharifi, R., and Ryu, C.-M. (2018). Revisiting bacterial volatile-mediated plant growth promotion: lessons from the past and objectives for the future. *Ann. Bot.* 122, 349–358. doi: 10.1093/aob/mcy108
- Somasegaran, P., and Hoben, H. J. (1994). *Handbook for Rhizobia*. Estados Unidos: Springer-Verlag.
- Spaepen, S., Vanderleyden, J., and Remans, R. (2007). Indole-3-acetic acid in microbial and microorganism–plant signaling. *FEMS Microbiol. Rev.* 31, 425–448. doi: 10.1111/j.1574-6976.2007.00072.x
- Star, L., Matan, O., Dardanelli, M. S., Kapulnik, Y., Burdman, S., and Okon, Y. (2012). The *Vicia sativa* spp. *Nigra*—*Rhizobium leguminosarum* bv. *viciae* symbiotic interaction is improved by *Azospirillum brasilense*. *Plant Soil* 356, 165–174. doi: 10.1007/s11104-010-0713-7
- Takahashi, W., Galvão, C., Urrea-Valencia, S., Gonçalves, D., Hyeda, D., Caires, E., et al. (2021). Impact of seed-applied fungicide and insecticide on *Azospirillum brasilense* survival and wheat growth-promoting ability. *Lett. Appl. Microbiol.* 74, 604–612. doi: 10.1111/lam.13645
- Tsegaye, Z., Alemu, T., Desta, F. A., and Assefa, F. (2022). Plant growth-promoting rhizobacterial inoculation to improve growth, yield, and grain nutrient uptake of teff varieties. *Front. Microbiol.* 13:896770. doi: 10.3389/fmicb.2022.896770
- Tsyganova, A. V., Seliverstova, E. V., and Tsyganov, V. E. (2023). Comparison of the formation of plant–microbial interface in *Pisum sativum* L. and *Medicago truncatula* Gaertn. Nitrogen-fixing nodules. *Int. J. Mol. Sci.* 24:13850. doi: 10.3390/ijms241813850
- USDA (2021). *Using the Appropriate Legume Inoculant for Conservation Plantings* Washington, D.C.: United States Department of Agriculture. Plant Materials Program. Available online at: <https://www.nrcs.usda.gov/plantmaterials/natpmtn13723.pdf> (Accessed May 27, 2026).
- USEPA Method, 9045D. (2004) Soil and Waste pH 2004. Recuperado de. Available online at: <https://www.epa.gov/sites/default/files/2015-12/documents/9045d.pdf> (Accessed May 27, 2026).
- Vejan, P., Abdullah, R., Khadiran, T., Ismail, S., and Nasrullah Boyce, A. (2016). Role of plant growth promoting rhizobacteria in agricultural sustainability. *Molecules* 21:573. doi: 10.3390/molecules21050573
- Vincent, J. M. (1970). A Manual for the Practical Study of Root nodule Bacteria. [Published for the] International Biological Programme [by] Blackwell Scientific. Available online at: <https://books.google.com.pe/books?id=dcQcAQAAIAAJ>
- Visscher, A. M., Vanek, S., Huaraca, J., Mendoza, J., Ccanto, R., Meza, K., et al. (2024). Traditional soil fertility management ameliorates climate change impacts on traditional Andean crops within smallholder farming systems. *Sci. Total Environ.* 912:168725. doi: 10.1016/j.scitotenv.2023.168725
- Wisniewski-Dyé, F., Lozano, L., Acosta-Cruz, E., Borland, S., Drogue, B., Prigent-Combaret, C., et al. (2012). Genome sequence of *Azospirillum brasilense* CBG497 and comparative analyses of *Azospirillum* core and accessory genomes provide insight into niche adaptation. *Genes* 3, 576–602. doi: 10.3390/genes3040576
- Zahrán, H. H., Räsänen, L. A., Karsisto, M., and Lindström, K. (1994). Alteración de los perfiles de lipopolisacáridos y proteínas en SDS-PAGE de rizobios por estrés osmótico y térmico. *World J. Microbiol. Biotechnol.* 10, 100–105. doi: 10.1007/BF00357572
- Zhang, H., Sun, H., Tang, Z., and Wang, G. (2025). Integrated agronomy of pea (*Pisum sativum* L.): a review on cultivation, harvesting, and storage for sustainable agriculture. *Front. Plant Sci.* 16:1670445. doi: 10.3389/fpls.2025.1670445