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Fertigation with *Pseudomonas putida* and *Bacillus subtilis*: impact on growth and productivity of off-season quinoa grown in coastal Peru

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Quinoa (*Chenopodium quinoa* Willd.) is a nutritionally valuable crop adapted to adverse agroclimatic conditions, but its production in arid regions such as the Peruvian coast is limited by water scarcity, low soil fertility, and heat stress. Plant growth-promoting rhizobacteria from *Pseudomonas* and *Bacillus* offer a sustainable strategy to improve growth and yield. This study evaluated the impact of fertigation with *Pseudomonas putida* (P3 strain) and *Bacillus subtilis* (BacF strain) on the growth and yield of the Salcedo INIA quinoa variety cultivated out of season under arid environment. A split-plot design was implemented, in which the main-plot factor was microbial inoculation [inoculated (+) *vs*. non-inoculated (–)], while the subplot factor was synthetic fertilization (75% *vs*. 100% of the recommended NPK dose). Inoculation was performed at two time points during the crop cycle at a concentration of 1×10^9 CFU mL^{-1}, whereas fertigation was applied at four-day intervals. Rhizobacterial inoculation significantly improved plant biometric characteristics, resulting in a 12% increase in dry biomass accumulation. Photosynthetic capacity increased, as indicated by higher leaf area index and SPAD values than uninoculated plants. Thus, the main effect of inoculation was the significant increase of yield potential (i.e. panicle weight increasing by 12% and thousand-grain weight increasing by 19%) with yield improvements significant at both fertilization levels. Despite these positive effects, high temperatures (>30 °C) during the growing season limited commercial yields [(+)100%: 2.20 $\pm$ 0.30 t ha^{-1}, (+)75%: 1.42 $\pm$ 0.19 t ha^{-1}, (-)100%: 1.50 $\pm$ 0.30 t ha^{-1}, (+)75%: 1.02 $\pm$ 0.13 t ha^{-1}]. This reduction is likely due to heat stress during flowering, which may have compromised pollen viability and grain set efficiency. The findings suggest that *P. putida* (P3 strain) and *B. subtilis* (BacF strain) are promising biotechnological tools for improving quinoa productivity in arid climates.

KEYWORDS
arid environment, *Bacillus*, *Chenopodium quinoa*, fertigation, PGPR, *Pseudomonas*

Highlights

- Fertigation with *Pseudomonas putida* and *Bacillus subtilis* enhances quinoa growth and biomass.
- Heat stress limits commercial yield; sowing date is critical.
- Sustainable strategy to optimize nutrient use and reduce fertilizer dependency in arid environments.

1 Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a crop of global significance due to its high nutritional value and remarkable adaptability to adverse agroclimatic conditions (Zurita-Silva et al., 2014; Fuentes et al., 2009). These attributes have driven its increasing agronomic importance, positioning it as a key contributor to food security (Ruiz et al., 2014). Since the 1980s, quinoa exports from South America have increased, and its cultivation has expanded to various countries worldwide (Bazile et al., 2016; Ruiz et al., 2014). In Peru, quinoa has become one of the most important Andean grains, with a steadily growing production and export value that reflects its increasing global demand (MIDAGRI, 2025).

Nationally, 97.5% of quinoa production is concentrated in eight departments: Puno, Ayacucho, Apurímac, Cusco, Arequipa, Junín, Huancavelica, and La Libertad (MIDAGRI, 2023). In the Arequipa department, the cultivated area has expanded significantly in recent years. Notably, the Majes, Santa Rita, and La Joya irrigation systems account for 60% of this area (Zegarra Alarcon, 2018). However, these irrigation systems are located along the Peruvian coast, where agricultural production is constrained by water scarcity and soil limitations. Coastal soils are often characterized by high salinity, low organic matter content, and poor humidity retention capacity, which hinder optimal crop development. To overcome these challenges, the adoption of efficient irrigation technologies, such as drip irrigation, and sustainable soil management practices are essential for maintaining agricultural productivity.

Due to these soil limitations, Peruvian coastal agriculture relies heavily on synthetic chemical fertilizers as the primary source of nutrients, which are indispensable in conventional farming (Krasilnikov et al., 2022). Over the past decade, disruptions in global supply chains—including natural disasters, geopolitical conflicts (such as the war in Ukraine), and the COVID-19 pandemic—have impacted the production and distribution of fertilizers, thereby threatening food security (Ben Hassen and El Bilali, 2022; Penuelas et al., 2023). Additionally, climate change has introduced new challenges, including rising annual and seasonal temperatures, shifts in precipitation patterns, and an increased frequency of extreme weather events, all of which significantly impact agricultural productivity (Grigorieva et al., 2023). Changes in key agroclimatic indexes—such as Growing Degree-Days, the Temperature-Humidity Index, and variations in the growing season length—have been observed, further influencing crop yields and production stability (Mu et al., 2013; Kipriyanov and Savinykh, 2019). Addressing these challenges necessitates adaptive strategies, including the development of more resilient crop varieties and the optimization of sowing schedules. In this context, implementing multiple sowing cycles or year-round cultivation has been proposed as a viable approach to sustaining food production in the face of changing climatic conditions (Gao et al., 2023; Zou et al., 2024).

Additionally, plant growth-promoting rhizobacteria (PGPR) have emerged as an innovative, natural, and environmentally sustainable technology for optimizing fertilizer use and mitigating abiotic stress (Ikiz et al., 2024; Shah et al., 2021). PGPR enhances plant growth through multiple mechanisms, including biological nitrogen fixation, nutrient solubilization, siderophore production, and phytohormone synthesis. Furthermore, PGPR improves plant resilience to abiotic stress by promoting the accumulation of specific osmolytes and by reducing ethylene levels in roots through the activity of 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Ahemad and Kibret, 2014; Vurukonda et al., 2016; Braud et al., 2009; Hayat et al., 2010; Hartman and Tringe, 2019; Etesami and Adl, 2020). Among the diverse soil bacterial taxa, species belonging to the genera *Pseudomonas* and *Bacillus* are particularly abundant in strains with well-documented PGPR effects (Zahir et al., 2003; Shah et al., 2021; Hasan et al., 2024).

The growth-promoting effects of *Pseudomonas putida* include nitrogen fixation, siderophore production, and phosphate solubilization (Sah et al., 2021). Similarly, *Bacillus subtilis* enhances plant nutrition through nitrogen fixation, phosphate solubilization, and the production of key phytohormones such as auxins, cytokinins, and gibberellic acid (Arkhipova et al., 2005; Gutiérrez-Mañero et al., 2001; Přikryl et al., 1985; Testen et al., 2022). Studies have identified *P. putida* and *B. subtilis* in the rhizosphere of quinoa plants, with functional capacities associated with biological nitrogen fixation, phosphorus solubilization, and indole-3-acetic acid biosynthesis (Ortuño Castro et al., 2014; Rafique et al., 2025). Furthermore, *Bacillus* isolates associated with *Chenopodium* species have been shown to possess plant growth-promoting traits (Testen et al., 2022).

PGPR applications can be tailored to different crops and agricultural practices. Standard inoculation methods include seed treatment, soil drenching, foliar spraying, and root dipping (Yang et al., 2024). However, current PGPR research in quinoa is largely limited to conventional growing seasons, highland or rainfed systems, and non-fertigation inoculation methods, which restricts its applicability to intensive coastal agriculture. Although previous studies have demonstrated the beneficial effects of *P. putida* and *B. subtilis* on quinoa growth and nutrient acquisition (Ortuño Castro et al., 2014), no studies have explicitly evaluated their co-application via fertigation under arid, off-season coastal conditions in Peru. This lack of evidence represents a clear research gap, particularly in regions such as the Peruvian coast, where drip irrigation is essential and quinoa production is increasingly expanding into non-traditional sowing periods. Fertigation-based PGPR delivery offers several agronomic advantages, including targeted inoculum placement in the rhizosphere, enhanced root colonization,

and compatibility with reduced fertilizer inputs. Nevertheless, the effectiveness of fertigation-applied PGPR in improving quinoa performance under arid and off-season conditions remains unknown.

Therefore, a critical knowledge gap exists regarding whether fertigation-based co-inoculation with *P. putida* and *B. subtilis* can sustain quinoa growth and yield under reduced NPK fertilization in arid coastal environments. Addressing this gap is essential for developing sustainable microbial-based management strategies adapted to climate variability and resource-limited agroecosystems.

The objective of this study was to evaluate the effect of fertigation-based co-inoculation with *Pseudomonas putida* (P3 strain) and *Bacillus subtilis* (BacF strain) on quinoa growth and yield under arid, off-season coastal conditions in Peru, in combination with reduced synthetic NPK fertilization, in order to assess its potential as a sustainable strategy to optimize fertilizer use and improve productivity.

2 Materials and methods

2.1 Study area

The study was conducted at the Santa Rita Experimental Centre of the Arequipa Agrarian Experimental Station (EEA), part of the Instituto Nacional de Innovación Agraria (INIA), located in the district of Santa Rita de Siguas, province, and department of Arequipa (Figure 1). The experimental plot was established at 16° 28'26.8" S, 72°06'41.5" W, at an altitude of 1,280 m.a.s.l. The study area has a mean annual rainfall of 2.9 mm, with minimum temperatures reaching 7 °C in July and maximum temperatures ranging from 27 to 28 °C between September and April. These averages were calculated based on historical data (1966–2013) from the La Joya meteorological station (16°35'0.91"S, 71°55'28.69"W), managed by the National Service of Meteorology and Hydrology (SENAMHI) of Peru (SENAMHI, 2024). Additionally, meteorological variables during the study period were recorded using data from the Santa Rita EEA meteorological station (16°28'24.3"S, 72°06'44.1"W), also managed by SENAMHI (Figure 2).

2.2 Design and treatments

A split-plot design with 2 x 2 factorial arrangement was implemented with four blocks. The main-plot factor was microbial inoculation (inoculated (+) *vs.* non-inoculated (−)) and the subplot factor was synthetic fertilization (75% *vs.* 100% of the recommended NPK dose). This arrangement generated four treatment combinations (T1–T4), each replicated four times, for a total of 16 experimental plots (4 blocks × 4 treatments) (Table 1). Main plots measured 18 × 7 m, subdivided into two subplots, with randomization within each block. The experimental unit consisted of a plot measuring 18 m in length and 7 m in width, comprising nine beds, as illustrated in Figure 3.

2.3 Soil physicochemical characteristics

Prior to establishing the experimental plot, a composite and representative soil sample was collected from the arable layer at a depth of 0.30 m for physicochemical characterization at the Soil, Water, and Foliar Analysis Laboratory of INIA Arequipa. The following parameters were analyzed: soil texture (Bouyoucos, 1962), pH (McLean, 1982), electrical conductivity (EC) (ISO, 1994), organic matter (OM) (Walkley and Black,

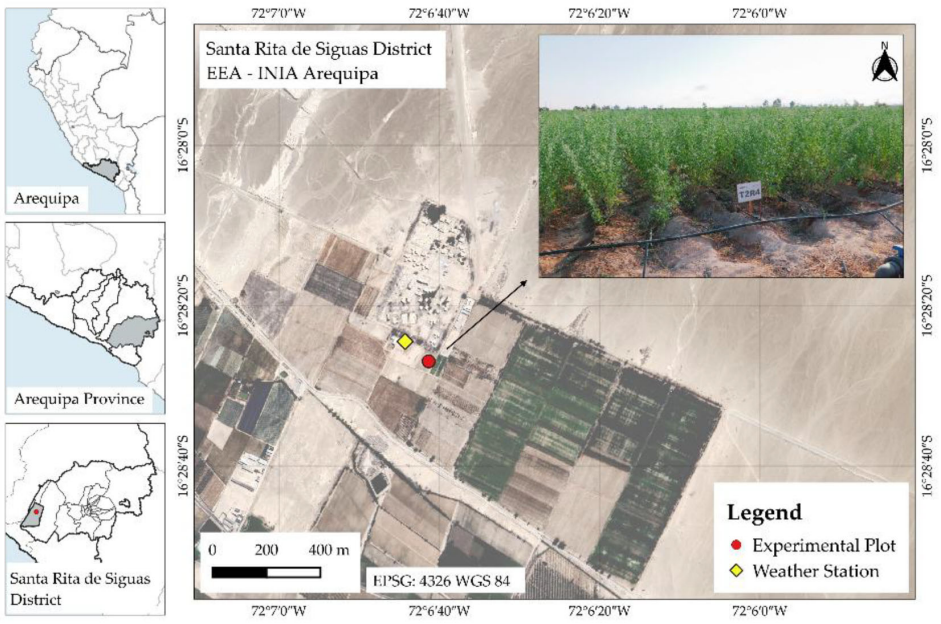

FIGURE 1
Study area, location map of the Arequipa department within Peru. The red dot indicates the area where the crop was installed.

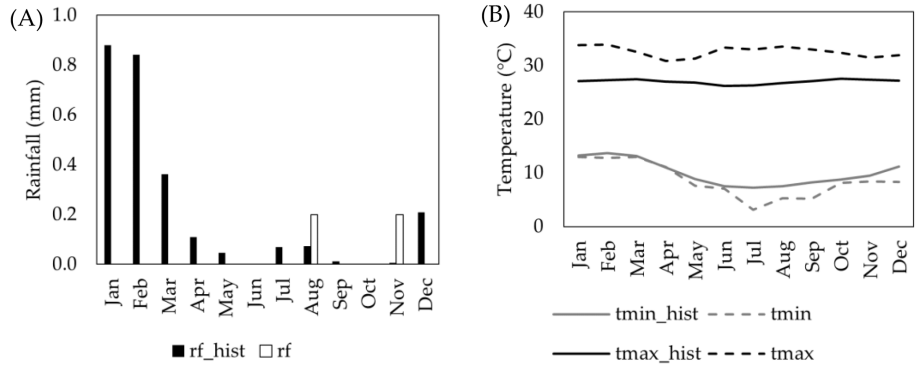

FIGURE 2
Local meteorological conditions during the experimental period (August 2023–July 2024). **(A)** Rainfall, where rf represents monthly accumulated rainfall; **(B)** Temperature, where tmin represents the average minimum temperature and tmax represents the average maximum temperature._hist refers to historical values for each parameter during the period 1966–2013.

1934), total nitrogen (ISO, 1995), available phosphorus (Olsen and Sommers, 1982), and available potassium (Semarnat, 2002) as shown in the Table 2.

2.4 Experimental plot management

The experimental plot was established in November 2023 and harvested in April 2024. This sowing occurred outside the regular growing season, as the typical sowing period at Santa Rita Irrigation falls between April and June. Prior to implementation, a drip irrigation system was installed using 16 mm outside diameter laterals with built-in emitters delivering a nominal flow rate of 1 L h^{-1} at 60 kPa, spaced at 0.20 m intervals. Fertigation arches equipped with ¾" Venturi Wade Rain injectors were installed at the entrance of each main plot. The land was subsoiled, plowed, and leveled using a John Deere 6110D farm tractor. Raised beds measuring 0.30 m in height and spaced 0.80 m apart were constructed using a furrower with a track attached to the tractor. Irrigation laterals were positioned along the central axis of each bed. Sowing was conducted in double rows on the beds using a hand seeder model Clean Seeder JP-2 (Jang Automation Co., Ltd). Seeds of the Salcedo INIA quinoa variety (Apaza Mamani et al., 2013) provided by INIA were used. Following sowing, the first irrigation was applied for two hours to facilitate seed germination. Subsequent irrigation was carried out every two days. The crop water requirement was determined based on crop evapotranspiration (ETc), calculated as the product of reference evapotranspiration (ETo) and the crop coefficient (Kc). The ETo was derived from meteorological data recorded at the Santa Rita EEA, while the Kc values were based on those proposed by García Villanueva et al. (2017) for quinoa cultivation.

Based on the physicochemical characterization of the soil, fertilizer requirements were established, and ammonium nitrate (375 kg ha^{-1}), monoammonium phosphate (200 kg ha^{-1}), and potassium nitrate (400 kg ha^{-1}) were applied. Fertilization was delivered through drip fertigation at four-day intervals, starting 15 days after sowing (DAS). This fertigation frequency was selected to ensure a continuous and readily available nutrient supply in the root zone, particularly under arid conditions and in soils with limited water and nutrient retention capacity, thereby improving nutrient use efficiency and minimizing leaching losses. Nutrient application was synchronized with crop demand following a stage-based uptake pattern derived from published quinoa nutrient absorption curves (Ulloa and Valle, 2021) and practical experience in quinoa crop management, while phenological stage definition was based on Núñez Chávez (2018) (Supplementary Tables S3, S4). Accordingly, the 100% NPK treatment received 200–120–180 kg ha^{-1} of N, P_2O_5, and K_2O, respectively, whereas the 75% NPK treatment received 150–90–135 kg ha^{-1} (Supplementary Tables S5, S6). Agricultural management practices included manual

TABLE 1 Treatment combinations in the 2 × 2 split-plot factorial design (microbial inoculation × fertilization levels).

Main plot (Inoculation)	Subplot (Fertilization)	Treatments	Treatment code
Inoculated (+)	100% NPK	Inoculated x 100% NPK	T1
Inoculated (+)	75% NPK	Inoculated x 75% NPK	T2
Non-inoculated (–)	100% NPK	Non-inoculated x 100% NPK	T3
Non-inoculated (–)	75% NPK	Non-inoculated x 75% NPK	T4

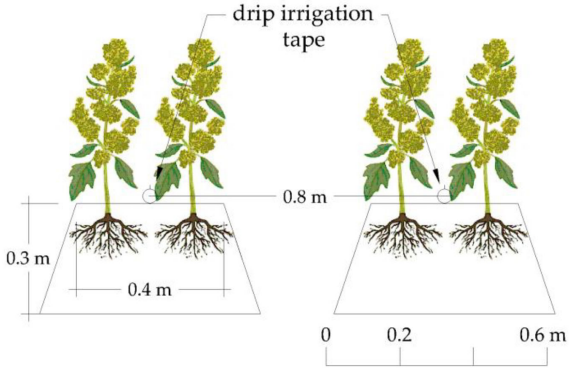

FIGURE 3
Schematic representation of the planting pattern in beds.

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TABLE 2 Physicochemical characteristics of the soil at the study site.

Parameter	Unit	Value
Textural class	–	Sandy loam
Sand	%	63.3
Clay	%	5.4
Silt	%	31.3
pH (1:1)	–	7.70
EC (1:5)	dS m^{-1}	0.27
OM	%	1.10
N	%	0.06
Available P	mg kg^{-1}	10.00
Available K	mg kg^{-1}	651.67

weeding at 25 DAS, leaving approximately 10 plants per linear meter. Phytosanitary control involved the application of SERENADE® SOIL (Bayer CropScience) at a rate of 2 L ha^{-1} during the second phenological stage, characterized by rapid root development (Garcia et al., 2015), to prevent and control *Pythium* spp. and *Fusarium* spp. Additionally, CURTINE-V® 720 WP (1 kg ha^{-1}) was applied during early grain filling for the control of *Peronospora variabilis* (Garcia et al., 2015).

2.5 Microbial inoculation

Pseudomonas putida (P3 strain) and *Bacillus subtilis* (BacF strain) were provided by the Santa Ana EEA of INIA and were preliminarily characterized according to Solórzano-Acosta and Quispe (2024). Both strains were molecularly identified by sequencing of the 16S rRNA gene. Bacterial isolates were reactivated in nutrient broth and incubated at 37 °C for 18 h. Genomic DNA was extracted using the JetFlex™ Genomic DNA Purification Kit (Thermo Scientific), following the manufacturer's instructions. The 16S rRNA gene was amplified by PCR using universal bacterial primers. PCR products were purified and commercially sequenced by Macrogen Inc. (Seoul, South Korea).

Raw sequences were edited and assembled using CodonCode Aligner v. 8.02, and multiple sequence alignments were performed with Clustal W v. 2.0 (Larkin et al., 2007). Taxonomic identification was carried out by comparison with reference sequences available in the NCBI database using the BLASTn algorithm (Altschul et al., 1990). Based on sequence similarity (>99%), the strains were identified as *Pseudomonas putida* (P3) and *Bacillus subtilis* (BacF). The corresponding 16S rRNA gene sequences were deposited in the GenBank database under accession numbers MT982624 and MT982637, respectively.

The selection of this bacterial combination was based on the synergistic effects previously reported in promoting plant growth by Solórzano-Acosta and Quispe (2024), as well as on the ability of these same strains to enhance stress tolerance as demonstrated in prior studies (Solórzano-Acosta et al., 2023; Solórzano-Acosta and Quispe, 2024). A microbial inoculum of these strains was independently prepared in nutrient broth and incubated at 28 °C for 48 hours, reaching a final concentration of 1×10^9 CFU mL^{-1}. Two inoculation events were carried out during the crop cycle: the first at 10 DA and the second at 60 DAS, both at a rate of 6 L ha^{-1}. In each application, a total irrigation water volume of 62 m^3 ha^{-1} was applied, resulting in an approximate concentration of 1×10^4 CFU mL^{-1} at the irrigation emitters. Inoculations were applied through the irrigation system, independently and not concurrently with fertilizer application, in order to avoid potential negative effects on the microbial population associated with increased electrical conductivity of the solution (Li D. et al., 2023; Zhang et al., 2019).

2.6 Biometric and physiological parameters

At 130 DAS, corresponding to the stage of maximum vegetative development, the leaf area index (LAI, cm^2cm^{-2}), SPAD index, and dry weight (g) were evaluated. All biometric measurements were performed on three representative plants per experimental unit. The weight of the aerial and root parts of the plant was determined using an electronic scale (Explorer™ Pro Precision, Ohaus, USA) with a measurement accuracy of 0.001 g. The dry weight was measured after the samples were dried in an oven (Memmert Schwabach 854) at 80 °C until a constant weight was achieved. LAI was measured in the field using a 0.8 m-long ceptometer (AccuPAR model LP-80 PAR/LAI Ceptometer, Decagon Devices Inc.) between 11:00 and 13:00 h under clear sky conditions. Four measurements were taken in the central zone of the middle furrows for each experimental unit. During each measurement, the probe was positioned diagonally across two crop rows at the soil surface level, with the external radiation sensor placed above the crop. Chlorophyll content was assessed using a chlorophyll meter (SPAD 502, Minolta Camera Co. Ltd., Tokyo, Japan). For each leaf sample, measurements were taken at four different points on fully developed leaves located in the upper third of the plant. Plant height (cm) was recorded at harvest (160 DAS) on three representative plants per experimental unit.

2.7 Yield parameters

At 160 DAS, harvesting and evaluation of yield parameters, including panicle length (cm), panicle dry weight (g), thousand-seed weight (g), and commercial yield (t ha^{-1}), were conducted. Yield was determined by harvesting a central area of 2 m × 2.4 m within each experimental unit. For measurements of panicle length and panicle dry weight, three representative plants were selected from the cultivated area of each experimental unit.

2.8 Statistical analysis

Data were analyzed using split-plot analysis of variance for biometric, physiological, and yield variables. Microbial inoculation (M) was assigned to main plots and fertilization levels (F) to subplots. Model assumptions were corroborated using the Shapiro-Wilk test for normality and Bartlett's test for homoscedasticity, as implemented in the stats package (v. 4.3.0) for the R Interactive document, which facilitates basic statistical analysis (Bolar et al., 2019). The statistical model was constructed using the sp.plot function from the agricolae package (v. 1.3-7) (Mendiburu, 2019). For variables showing significant differences,

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mean comparisons were performed using Tukey's Honestly Significant Difference (HSD) test ($\alpha = 0.05$) via the HSD.test function from the same package.

3 Results

3.1 Biometric and physiological parameters

Among the biometric and physiological variables evaluated, only plant height, and the SPAD chlorophyll index exhibited significant interactions between fertilization dose and microbial inoculation. In terms of plant height, microbial inoculation did not result in significant differences when full fertilization was applied. However, at the reduced fertilization dose (75%), plants receiving microbial inoculation displayed greater height (Figure 4A; Supplementary Table S1).

Regarding plant biomass, significant differences were observed for the main effects of microbial inoculation and fertilization dose. Microbial inoculation resulted in a significant increase in dry weight (DW) compared to non-inoculated treatments, with an average enhancement of 12% (Figure 4B). Additionally, the fertilization dose significantly influenced root dry weight (RDW), with the 100% NPK treatment exhibiting 22% higher values than the 75% NPK treatment (Figure 4C). The leaf area index (LAI) exhibited significant differences, with the 100% NPK treatment showing values 12% higher than the 75% NPK treatment (Figure 5A), consistent with the trend observed in the crop´s total fresh weight. Regarding the chlorophyll index (SPAD), a significant interaction was observed between microbial inoculation and fertilization levels. Microbial inoculation with *Pseudomonas putida* and *Bacillus subtilis* significantly increased the SPAD index in plants receiving 75% NPK fertilization, aligning their values with those of the 100% NPK treatment (Figure 5B). However, in plants fertilized with 100% NPK, microbial inoculation did not induce significant changes in the SPAD index (Figure 5A).

3.2 Yield parameters

Microbial inoculant application had a significant effect on panicle dry weight (PW) and thousand-seed weight. PW increased by an average of 19% in treatments inoculated with *Pseudomonas putida* and *Bacillus subtilis* compared to non-inoculated treatments (Figures 6A, C). A similar trend was observed for thousand-seed weight, depending on the inoculation treatment. Regarding the

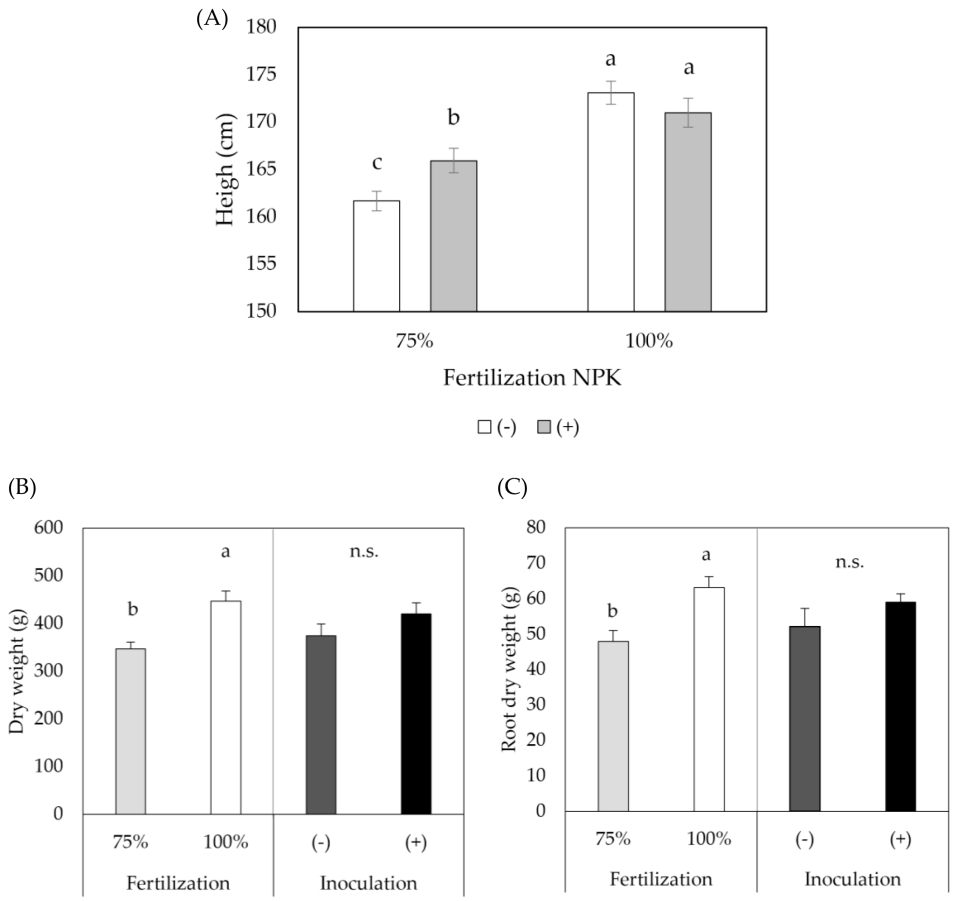

FIGURE 4
Effects of microbial inoculant application and two fertilization levels on quinoa crop biomass. **(A)** Height (cm), **(B)** Dry weight (g), and **(C)** Root dry weight (g). Means with different lowercase letters (a, b) indicate statistically significant differences among treatments at $p < 0.05$ for the HSD test. n.s. denotes a non-significant difference. Error bars represent the standard error of the mean (n = 4).

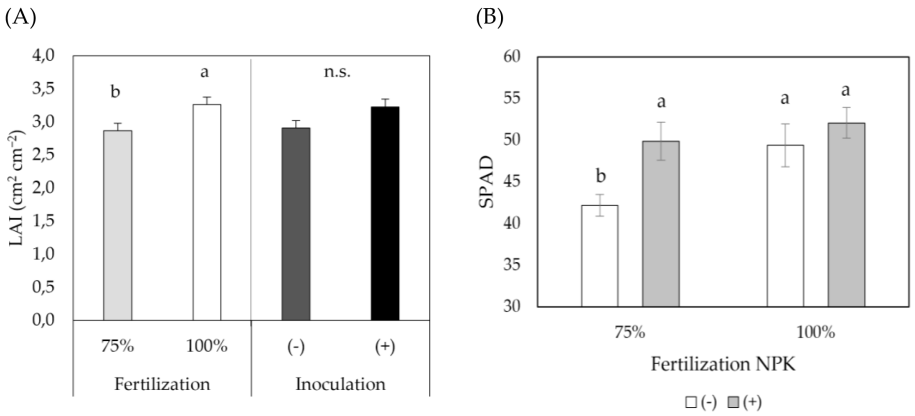

FIGURE 5
Effects of microbial inoculant application and two fertilization levels on quinoa crop biomass. **(A)** Leaf area index ($cm^2 \cdot cm^{-2}$) and **(B)** SPAD. Means with different lowercase letters (a, b) indicate statistically significant differences among treatments at $p < 0.05$ for the HSD test. n.s. denotes a non-significant difference. Error bars represent the standard error of the mean (n = 4).

commercial yield, only fertilization showed a significant main effect on it. The 75% fertilization treatments yield, on average, 34% less than the 100% NPK treatment (Figure 6D). The average yield for all the treatments was 1.53 t ha^{-1} (T1: 2.20 ± 0.30 t ha^{-1}, T2: 1.42 ± 0.19 t ha^{-1}, T3: 1.50 ± 0.30 t ha^{-1}, T4: 1.02 ± 0.13 t ha^{-1}) (Supplementary Table S2).

4 Discussion

This study evaluated the fertigation-based application of a microbial inoculant composed of rhizobacteria from the genera *Pseudomonas* and *Bacillus* in the Salcedo INIA quinoa variety, cultivated out of season. The inoculant's effect was analyzed as a

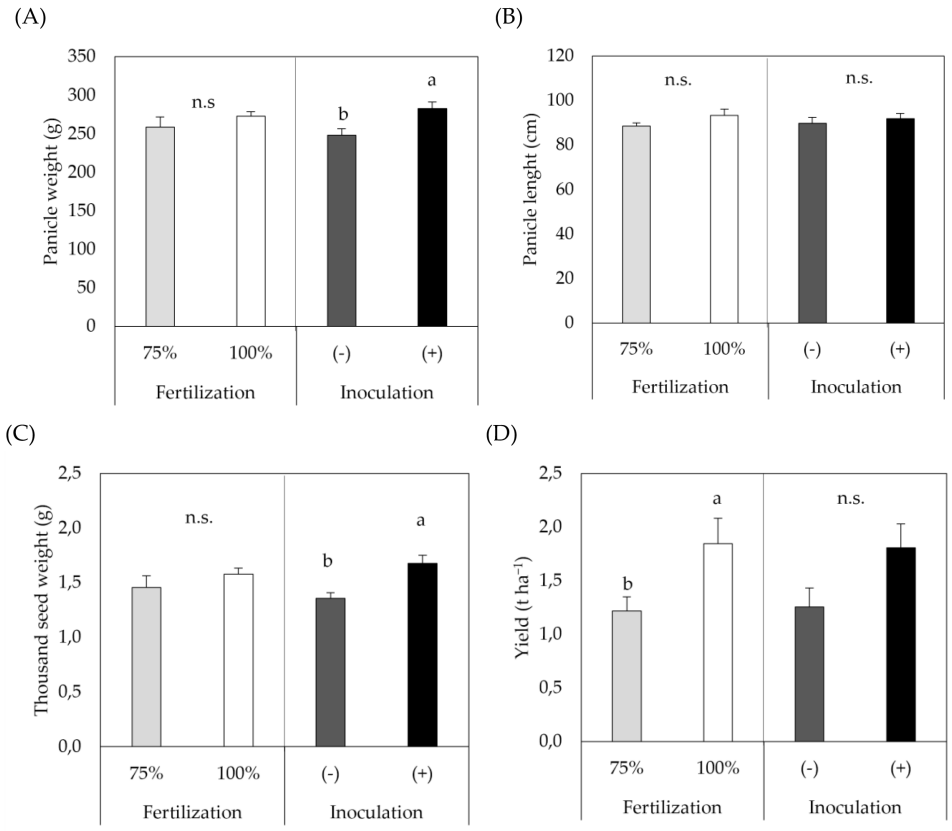

FIGURE 6
Effect of microbial inoculant application and two fertilization levels on quinoa crop performance. **(A)** Panicle weight (g), **(B)** Panicle length (cm), **(C)** Thousand-seed weight (g), and **(D)** Yield ($t \cdot ha^{-1}$). Means with different lowercase letters (a, b) indicate statistically significant differences among treatments at $p < 0.05$ for the HSD test. n.s. denotes a non-significant difference. Error bars represent the standard error of the mean (n = 4).

growth and yield promoter under arid climatic conditions with persistent moisture deficiency (SENAMHI, 2020). The beneficial effects of plant growth-promoting rhizobacteria (PGPR) are well-documented and include enhanced nutrient uptake, phytohormone production, and the release of substances that improve resistance to abiotic stress through the accumulation of specific solutes (Sarti et al., 2023; Sarti and Miyazaki, 2013; Wang et al., 2024).

In line with these findings, plants treated with microbial inoculant and 75% NPK fertilization exhibited greater height than those without inoculation. Additionally, PGPR application led to a higher accumulation of dry biomass (12%). Solórzano-Acosta and Quispe (2024) reported that the *Bacillus subtilis* strain used in their study enhances soil NPK extraction and improves nitrogen uptake efficiency. Likewise, *Pseudomonas putida* can solubilize poorly soluble phosphate compounds by releasing low-molecular-weight organic acids, such as gluconic acid, and producing phosphatases and phytases, which break down organic phosphate compounds. This process acidifies the external environment, chelates phosphate-bound cations, and increases phosphate availability (Khoso et al., 2024; Rafique et al., 2022).

Based on these mechanisms, microbial inoculation can induce vegetative development, including increased root biomass (Rafique et al., 2022; Solórzano-Acosta and Quispe, 2024). However, in contrast to the findings of Solórzano-Acosta and Quispe (2024), our results did not show significant differences in root dry biomass despite a tendency toward higher values in inoculated treatments (Figure 4C). This discrepancy may be related to the characteristics of the Salcedo INIA quinoa variety, which prioritizes biomass development under stress conditions (Antezana, 2019). Temperature effects may also have influenced vegetative development, as quinoa's adaptability to high temperatures without water deficit has been linked to the development of larger stomata and an increased number and length of lateral branches, often at the expense of better root development (Becker et al., 2017; Hinojosa et al., 2019; Serrat et al., 2024).

This thermal effect was reflected in the leaf area index (LAI), which showed the highest values in treatments receiving full fertilization, whereas microbial inoculant treatments did not exhibit significant differences (Figure 5A). This suggests that optimal leaf distribution was favored under conditions of sufficient water and nutrient availability (Becker et al., 2017). Additionally, studies such as those by Kakabouki et al. (2019) indicate that increasing nitrogen doses to 200 kg ha^{-1} in quinoa cultivation can enhance LAI, thereby improving yield. The resulting increase in LAI may facilitate greater light capture, potentially boosting photosynthesis and ensuring an adequate supply of assimilates to the developing organs of the quinoa plant. Plant chlorophyll content is closely linked to photosynthetic capacity and nutritional status, as nitrogen is a fundamental component of this biomolecule (Su et al., 2017). The SPAD chlorophyll index serves as an indicator of plant nitrogen status, demonstrating a positive correlation with nitrogen availability (Ribeiro Da Cunha et al., 2015). Accordingly, the values recorded for the 75% fertilization treatments tended to be lower than those for full fertilization. However, under fertilization stress conditions, microbial inoculation enabled chlorophyll content to remain statistically comparable to treatments receiving full NPK doses, with an average increase of 10% (Figure 5B).

Studies such as that by Ferioun et al. (2024) reported increases of 13–30% in SPAD values following the application of formulations containing *Achromobacter insolitus*, *Pseudomonas putida*, *Providencia rettgeri*, and *Alcaligenes* sp. Similarly, Li J. et al. (2023) observed higher chlorophyll levels in quinoa crops treated with *Bacillus*-based inoculants. This trend has also been documented in maize, tomato, strawberry, oats, alfalfa, and cucumber using *Bacillus* and *Pseudomonas* as microbial inoculants (Abbasi et al., 2011; Chebotar et al., 2022; Li et al., 2020; El hjouji et al., 2025). Similar to plant height, once the crop's nutritional requirements were met, microbial inoculation did not lead to further significant increases in chlorophyll content. This suggests that quinoa has an upper limit for nutrient uptake, beyond which additional inputs do not further enhance its physiological potential (Cárdenas-Castillo et al., 2021).

The capacity of *Pseudomonas putida* (P3 strain) and *Bacillus subtilis* (BacF strain) to enhance yield-related traits is evident in the Figure 6, where microbial inoculation led to increased panicle dry weight at both fertilization levels, as well as higher thousand-grain weight. These findings demonstrate that these inoculants positively influence grain yield, a key agronomic trait, possibly through the production of growth hormones and/or nutrient mobilization (Rafique et al., 2025). Solórzano-Acosta and Quispe (2024) reported that the *Pseudomonas putida* strains used in their study can solubilize phosphates and produce siderophores, while *Bacillus subtilis* enhances nitrogen uptake efficiency—mechanisms that align with their potential to improve crop yields. Similarly, Castillo et al. (2022) highlighted the ability of *Bacillus* sp. to enhance nitrogen fixation and phosphorus solubilization in quinoa, resulting in improvements in growth parameters, including length, weight, diameter of the panicle, and grain yield. Additionally, Rafique et al. (2022) reported that *Pseudomonas* spp. significantly enhance quinoa growth and yield by improving relative water content, quantum flux, diffusive resistance, and crop transpiration rate.

Despite a general trend toward higher yields through microbial inoculation, this did not translate into significant improvements in commercial yield. However, the positive effect of full fertilization on commercial yields was confirmed, reflecting quinoa's strong ability to assimilate fertilizers at the proposed doses. Cárdenas-Castillo et al. (2021) suggested that quinoa grown in low-fertility areas, such as the Peruvian coast, can achieve yields exceeding 3.6 t ha^{-1} after assimilating approximately 240 kg ha^{-1} of nitrogen. Furthermore, fertilization rates of 90 kg ha^{-1} phosphorus and 105 kg ha^{-1} potassium have been reported to enhance both yield and nutritional content in quinoa (Kakabouki et al., 2019; Taaime et al., 2023; Van Minh et al., 2022).

Despite improvements in vegetative development and a positive trend in yield potential (i.e., greater panicle and thousand-seed weights), the obtained commercial yields were lower than the typical 4 t ha^{-1} reported for the Salcedo INIA quinoa variety in coastal production areas (INIA, 2014). This outcome may be primarily attributed to the atypical sowing season, which coincided with high temperatures (33 °C) during the flowering stage. Such conditions have been reported to reduce quinoa grain-

setting efficiency by altering the final number of grains per abortion (Bertero and Ruiz, 2008; Lesjak and Calderini, 2017). Given that the Salcedo INIA variety is adapted for coastal environments with average temperatures of 24–25 °C (FAO, 2015), it is likely that heat stress during flowering negatively impacted grain set efficiency. Similarly, Hinojosa et al. (2019) investigated quinoa growth and pollen morphology in response to high temperatures, reporting a 30–70% reduction in pollen viability under heat stress. However, they found that seed size and leaf greenness were unaffected, while photosynthesis rates increased, demonstrating quinoa's high plasticity in response to elevated temperatures and environmental stresses (Alzahrani et al., 2024).

Based on these findings, the use of microbial inoculants *Pseudomonas putida* (P3 strain) and *Bacillus subtilis* (BacF strain) has a positive effect on quinoa cultivation in low-fertility, arid environments such as the Peruvian coast. This effect is particularly significant under reduced fertilization conditions, suggesting a potential strategy to lower production costs without compromising biomass, especially in fields that employ drip irrigation systems. On one hand, microbial inoculants enhance nutrient uptake efficiency; on the other, they represent an underutilized technology in the country that could be integrated with more widely adopted practices in coastal agriculture, such as pressurized irrigation systems.

However, careful management of sowing seasons is crucial for this variety, as its ability to tolerate temperatures above 30 °C during the full flowering and grain-setting stages is limited, which ultimately compromises commercial yield. In this context, beyond the use of PGPR, it may also be necessary to incorporate varietal selection or breeding programs aimed at improving heat tolerance, so that microbial strategies are complemented by greater crop resilience.

It should be acknowledged that the application of SERENADE SOIL (*Bacillus subtilis* QST 713 strain) to all treatments, including the non-inoculated controls, represents a methodological limitation of this study. Its use was necessary to prevent severe phytosanitary losses and ensure uniform plant survival. Therefore, the non-inoculated treatment cannot be considered a completely Bacillus-free control. Importantly, this experimental condition implies that the treatment comparisons should not be interpreted as "PGPR versus no PGPR", but rather as "BacF + *B. subtilis* QST 713" versus "*B. subtilis* QST 713 alone". Although *B. subtilis* QST 713 is primarily recognized for its antifungal activity, its presence may have partially masked or attenuated the exclusive effects of the BacF PGPR strain. Therefore, the physiological and agronomic responses observed in this study reflect the additional or synergistic contribution of BacF under a baseline biofungicide application, rather than its isolated effect. Despite this limitation, the consistent differences between inoculated and non-inoculated treatments indicate that BacF exerted functional effects beyond those provided by *B. subtilis* QST 713 alone. Future studies should include treatments without Bacillus-based fungicides and, where possible, monitor microbial populations to more precisely distinguish the individual contributions of biocontrol agents and PGPR inoculants.

5 Conclusions

This study demonstrates that the integration of *Pseudomonas putida* (P3 strain) and *Bacillus subtilis* (BacF strain) through fertigation promotes quinoa growth and biomass accumulation, particularly under reduced fertilization in arid coastal environments. Microbial inoculation enhanced nutrient uptake and yield components such as panicle weight and thousand-grain weight, highlighting its potential to optimize production while reducing dependence on chemical fertilizers. However, commercial yield was constrained by heat stress during flowering ((+)100%: 2.20 ± 0.30 t ha^{-1}, (+)75%: 1.42 ± 0.19 t ha^{-1}, (-)100%: 1.50 ± 0.30 t ha^{-1}, (+)75%: 1.02 ± 0.13 t ha^{-1}), underscoring the importance of proper sowing date management and the need to complement microbial strategies with variety selection or breeding for heat tolerance.

From a sustainability perspective, fertigation with microbial inoculants represents an environmentally friendly alternative that can reduce fertilizer use, improve soil health, and strengthen the resilience of agroecosystems. Broader validation across different environments, cost–benefit evaluations, and assessments of grain nutritional quality are required to confirm its broader applicability. Extending this approach to other regional crops such as maize and rice could further increase agricultural productivity while safeguarding natural resources in Peru.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

AQ: Writing – review & editing, Conceptualization, Methodology, Writing – original draft. RP-C: Investigation, Methodology, Writing – original draft. RF-M: Methodology, Writing – review & editing, Supervision, Writing – original draft. RS-A: Supervision, Writing – review & editing.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2026.1733699/full#supplementary-material

SUPPLEMENTARY TABLE 1
Biometric and physiological parameters at different fertilization and microbial inoculation levels.

SUPPLEMENTARY TABLE 2
Yield parameters at different fertilization and microbial inoculation levels.

SUPPLEMENTARY TABLE 3
Nitrogen, phosphorus, and potassium requirements of quinoa at different phenological stages at 100%.

SUPPLEMENTARY TABLE 4
Fertilizer application schedule and nutrient supply (N, P_2O_5, and K_2O) of quinoa expressed as days after sowing (DAS) at 100%.

SUPPLEMENTARY TABLE 5
Nitrogen, phosphorus, and potassium requirements of quinoa at different phenological stages at 75%.

SUPPLEMENTARY TABLE 6
Fertilizer application schedule and nutrient supply (N, P_2O_5, and K_2O) of quinoa expressed as days after sowing (DAS) at 75%.

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