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RESEARCH ARTICLE
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Synergy between *Methylobacterium symbioticum* and Nitrogen to Improve the Yield of Brassica oleracea var. italica under Andean Conditions

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ABSTRACT

The cultivation of *Brassica oleracea* var. *italica* plays a crucial role in the Ecuadorian Andes' economy, yet its productivity relies heavily on synthetic nitrogen fertilization, raising environmental and economic concerns. This study evaluated the efficacy of BlueN technology (*Methylobacterium symbioticum*) as a phyllosphere nitrogen-fixing inoculant to enhance broccoli yield and Nitrogen Use Efficiency (NUE). A completely randomized block design with ten treatments and three replications was employed, interacting three BlueN doses (0, 1, and 2g L^{-1}) with four nitrogen fertilization levels (0%, 50%, 75%, and 100% of the recommended 150kg N ha^{-1}). Results demonstrated a significant non-linear response: foliar application of 1g L^{-1} of *M. symbioticum* combined with 100% soil nitrogen yielded the highest head weight (1060.85g), a 45.56% increase over the control. Notably, the intermediate dose (1g L^{-1}) consistently outperformed the higher dose (2g L^{-1}), suggesting a niche saturation effect in the phyllosphere. Furthermore, the combination of 1g L^{-1} with 75% nitrogen fertilization-maintained yields statistically comparable to the 100% nitrogen treatment (1040.31g). These findings validate *M. symbioticum* as a disruptive biotechnological tool for Andean horticulture, capable of substituting up to 25% of synthetic nitrogen inputs, thereby promoting sustainable intensification and reducing the carbon footprint of broccoli production systems.

Keywords: Nitrogen, Biofertilization, *Methylobacterium*, Yield, Sustainability.

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INTRODUCTION

Agricultural production is intrinsically linked to the efficient management of nitrogen fertilization, a critical driver of global food security. Howevr, modern agriculture faces a dual challenge: the escalating cost of synthetic inputs, exacerbated by geopolitical tensions in major producing nations like Russia and China (Bunce & Yaselga, 2023) and the environmental imperative to reduce nitrate leaching and greenhouse gas emissions (Conversa et al., 2019; Ammar et al., 2024; Qaisar et al., 2024; Sarwar & Asif, 2024). In the high-altitude ecosystems of the Ecuadorian Andes, these challenges are magnified. Agriculture remains the backbone of the local economy, ensuring food sovereignty and providing raw materials for the agro-industrial sector (Pacheco et al., 2018). Within this context, the cultivation of *Brassica oleracea* var. *italica* (broccoli) has emerged as a strategic commodity. In 2022, broccoli exports from Ecuador surged by 23%, reaching a valuation of USD 74 million, driven by high demand from markets such as Japan, the United States, and the European Union (FEDEXPOR, 2022). Despite this economic success, the sustainability of Andean broccoli production is threatened by its heavy reliance on intensive nitrogen inputs.

Nitrogen (N) is the most limiting macronutrient for plant growth, serving as a fundamental constituent of amino acids, nucleic acids, and chlorophyll (López et al., 2007). *Brassica* crops, in particular, are classified as high nutrient consumers, requiring substantial N supply to maximize head biomass and maintain post-harvest quality (Gutezeit, 2004). Current agronomic practices in the Andes often involve excessive urea applications sometimes exceeding 400kg N ha^{-1} (Castellanos et al., 2001)

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to guarantee yield. However, this "insurance fertilization" approach leads to low Nitrogen Use Efficiency (NUE), soil acidification, and water contamination (Moniruzzaman et al., 2007). Consequently, there is an urgent need to validate disruptive biotechnological tools that can decouple yield maximization from synthetic N dependence. Recent research has shifted focus towards Plant Growth Promoting Rhizobacteria (PGPR) and, more innovatively, phyllosphere colonizing bacteria as sustainable alternatives to mineral fertilization (Ollio et al., 2024; Nazli & Zahra, 2024).

While rhizosphere biofertilizers (e.g., *Azospirillum*, *Bacillus*) have been extensively studied, their efficacy is often constrained by soil edaphic factors and competition with native microbiota (Mahanty et al., 2017). In contrast, the phyllosphere the aerial surface of plants represents an underutilized niche for biological nitrogen fixation (BNF). Recent breakthroughs in agricultural microbiology have identified *Methylobacterium symbioticum* as a key player in this domain (Pascual et al., 2020). Unlike root associated bacteria, *M. symbioticum* is a pink pigmented facultative methylotroph (PPFM) that colonizes the leaf stomata. Its mechanism of action is symbiotic and unique: the bacterium metabolizes methanol released by the plant during pectin degradation in cell wall expansion (Sy et al., 2005) and in exchange, fixes atmospheric nitrogen (N_2) via the nitrogenase enzyme complex, converting it into ammonium (NH_4^+) directly available to the plant mesophyll (Madhaiyan et al., 2015). This metabolic synergy allows the plant to acquire nitrogen without the energetic cost of root uptake and translocation, theoretically offering a higher efficiency pathway under abiotic stress conditions typical of the Andes.

The agronomic potential of *M. symbioticum* has gained traction in the literature between 2020 and 2025. Torres Vera et al. (2024) demonstrated that foliar inoculation with *M. symbioticum* allowed for a 25-50% reduction in synthetic nitrogen fertilization in maize and strawberry without compromising yield. Similarly, Pascual et al. (2020) reported significant biomass increments in rice and grapes, validating the bacterium's capacity to act as a robust biofertilizer across diverse photosynthetic metabolisms (C_3 and C_4). However, the response to *M. symbioticum* is not universally positive and appears to be crop specific. For instance, Arrobas et al. (2024) found limited efficacy of *M. symbioticum* in enhancing lettuce yield under certain soil conditions, suggesting that the "stomatic colonization capacity" may vary depending on leaf morphology and waxy cuticle properties. Furthermore, studies specifically targeting *Brassica oleracea* remain scarce. While Ollio et al. (2024) and Saini et al. (2025) recently highlighted the benefits of microbial consortia in broccoli, the specific interaction between *M. symbioticum* (commercialized as BlueN technology) and broccoli's high nitrogen demand in high-altitude environments has not been systematically evaluated.

This knowledge gap is critical for Andean agriculture. The physiological behavior of *Methylobacterium* at altitudes above 2,900m.a.s.l., where UV radiation is intense and diurnal temperature variations are extreme, remains unknown. Does the bacterium maintain its nitrogenase activity under these conditions? Can it effectively replace a significant portion of the 150-250kg N ha^{-1} typically required by broccoli? This study addresses these questions by evaluating the synergy between different doses of *Methylobacterium symbioticum* and reduced mineral nitrogen gradients. The hypothesis posits that phyllosphere inoculation will enhance Nitrogen Use Efficiency (NUE), allowing for a reduction in synthetic fertilizer without penalizing the morphometric quality or weight of the broccoli head. Therefore, the objective of this research was to determine the effect of BlueN technology on the agronomic performance of *Brassica oleracea* var. *italica* under the specific edaphoclimatic conditions of the Ecuadorian Andes, providing a scientific basis for more sustainable fertilization protocols.

MATERIALS & METHODS

Experimental Area

Between December 2023 and March 2024, an experiment was conducted at the San Francisco Experimental Center, belonging to the Universidad Politécnica Estatal del Carchi, located in Huaca, Ecuador (latitude north 00°36′55", longitude west 77°45′07") (Fig. 1). This center is located in an area characterized by a cold climate, with temperatures ranging between 3°C and 18°C, averaging around 10°C. The region has an average annual precipitation of 1100mm and is at an altitude of 2959 meters above sea level. The relative humidity in this place is 76%. Additionally, the farm's terrain presents an undulating relief and varied topography that includes gentle to moderate slopes. The soils in this area are mainly sandy-loam and loam-silt, with good water retention capacity and adequate drainage, ideal for developing various crops. Predominant crops in this region include potatoes, corn, beans, and various vegetables. These agroclimatic and edaphological conditions make the San Francisco Experimental Center an ideal site for agricultural research and the implementation of sustainable farming practices (Peña, 2012).

Edaphoclimatic Characterization of the Experimental Area

To carry out the edaphological characterization at the San Francisco Experimental Center, a physical-chemical soil analysis was performed following the methods described in Table 1. To obtain a representative soil sample, 10 points of the plot were randomly selected, and a soil auger was used to extract subsamples at a depth of 30 cm. These subsamples were mixed to form a homogeneous sample. Before packing, clods were broken, and organic matter residues were removed. Finally, one pound of soil was packed in a properly labeled plastic bag for analysis. These analyses provided a detailed characterization of the soil properties at the Center, allowing an understanding of the soil conditions for agricultural research and the implementation of sustainable management practices.

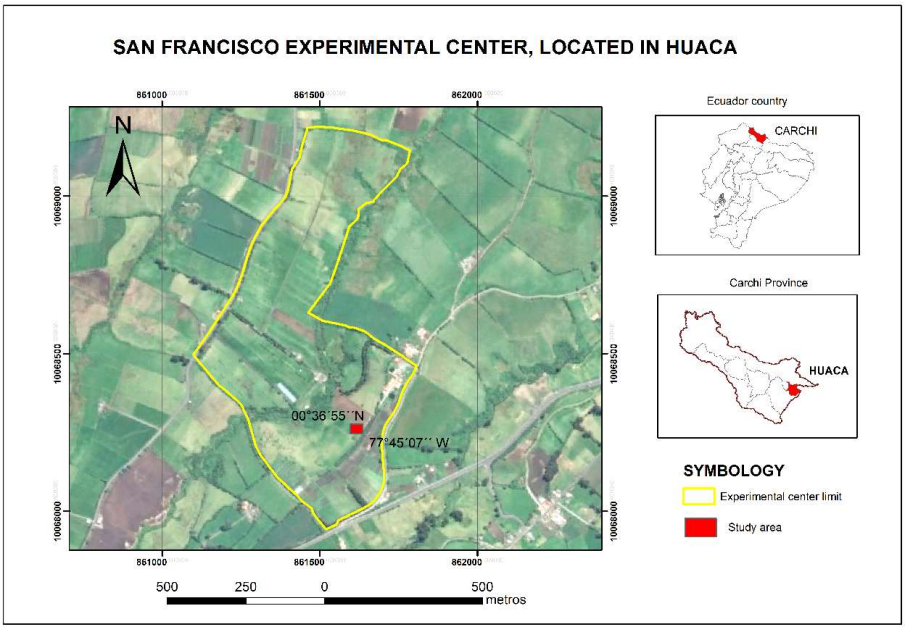

Fig. 1: Geographic location of the study area. The panels illustrate the national (Ecuador) and provincial (Carchi) context. The main map details the San Francisco Experimental Center in Huaca, where the red box indicates the specific site of the experiment (00°36′55" N, 77°45′07" W).

Table 1: Specifications of the physical-chemical soil analysis

Nutrient	Unit	Method Used
N	ppm	Spectrophotometry (Abs vs C)
P	ppm	Spectrophotometry (Abs vs C)
K	meq/100mL	Atomic absorption spectrum
Ca	meq/100mL	Atomic absorption spectrum
S	ppm	Spectrophotometry (Abs vs C)
Mg	meq/100mL	Atomic absorption spectrum
Zn	ppm	Atomic absorption spectrum
Cu	ppm	Atomic absorption spectrum
Fe	ppm	Atomic absorption spectrum
Mn	ppm	Atomic absorption spectrum
B	ppm	Spectrophotometry (Abs vs C)
MO	%	Sulfochromic oxidation
CE	mS/cm	Conductivity meter
pH	escala pH	Potentiometer

In the climatic characterization, we can indicate that the Huaca Canton presents a varied climate in its territory. In most parts, which exceed 3000 meters above sea level, a high mountain equatorial climate prevails. However, in lower altitude areas, the predominant climate is semi-humid mesothermal equatorial (GAD Huaca, 2023). Regarding the climate in the study area, measurements of solar radiation (MJ), precipitation (mm), average temperature (°C), and environmental humidity (%) were taken into account. These data were collected from the portable meteorological station brand NEI (Nippon Electric Instrument) owned by the Universidad Politécnica Estatal del Carchi located at the San Francisco Experimental Center.

Experimental Procedure

Soil samples were taken for analysis. Then, the land was prepared by passing the harrow twice, without using the plow. A total of 30 plots were marked, each with an area of 30 square meters. Based on the soil analysis results, agricultural lime was applied to the soil one month before transplanting. Subsequently, 72 plants of Brassica oleracea var. italica, 20 days old, of the Zafiro hybrid, were acquired and transplanted in each plot. The planting distance was 0.50 meters between plants and 0.80 meters between rows.

According to the soil analysis results, a single soil fertilization was performed 21 days after transplanting. The fertilization consisted of a uniform mixture of urea (46–0–0), triple superphosphate (0–46–0), and potassium chloride (0–0–60), applied directly to the root zone of each plant. Four nitrogen fertilization levels were established (0, 50, 75, and 100%), based on a reference dose of 150kg N ha^{-1}, corresponding to the local agronomic recommendation for *Brassica oleracea* var. *italica* under Andean conditions. The nitrogen source was urea, equivalent to 22g $plant^{-1}$ for the 100% treatment (≈326kg urea ha^{-1}), 16.5g $plant^{-1}$ for the 75% treatment (≈244.5kg urea ha^{-1}), and 11g $plant^{-1}$ for the 50% treatment (≈163kg urea ha^{-1}). In all treatments, phosphorus and potassium were applied at constant doses of 10g $plant^{-1}$ of triple superphosphate (≈105kg P_2O_5 ha^{-1}) and 8g $plant^{-1}$ of potassium chloride (≈120kg K_2O ha^{-1}), respectively. The fertilizers were manually mixed immediately before application to ensure homogeneity and were incorporated into the soil near the plant base to minimize nutrient losses. No further fertilization was carried out during the crop cycle.

The BlueN product (*Methylobacterium symbioticum*, strain SB23, 3.3 × 10^7 CFU/g) was applied using a Royal Condor manual sprayer with a 20L capacity and a bronze nozzle delivering 1000cc min^{-1} at a pressure of 40psi. The product was applied in doses of 0, 1, and 2g L^{-1} of water, ensuring homogeneous coverage of the foliage until incipient runoff. Two foliar applications were performed during the crop cycle. The first application was carried out 21 days after transplanting, when plants had developed four fully expanded leaves. The second application was conducted at the beginning of flowering, 85 days after transplanting (approximately 25 days before harvest). Each treatment was applied early in the morning under low wind conditions to minimize evaporation and optimize bacterial adhesion to the leaf surface.

The experiment considered two study factors: BlueN product dose applying doses of zero, one, and two grams per liter of water. Nitrogen fertilization levels with urea

using percentages of 0%, 50%, 75%, and 100%. The following variables were evaluated: plant height (90 DDT), number of leaves (90 DDT), stem diameter, head diameter, and head weight at harvest (110 DDT).

To determine the growth stages of Brassica oleracea var. italica, the Crop Coefficient (Kc) proposed by Allen et al. (2006) was used as a theoretical reference to describe the phenological phases and potential water requirements of the crop under Andean conditions. The Kc values considered were 0.70 for the initial stage (25 days), 1.05 for the development stage (50 days), and 0.95 for the final stage (35 days). In this study, the corresponding phenological periods were as follows: Initial stage, from December 11, 2023, to January 5, 2024; development stage, from January 6 to February 24, 2024; and final stage, from February 25 to March 30, 2024. However, no supplementary irrigation was applied during the experimental period, since natural rainfall adequately met the crop's water demand throughout all phenological stages. Precipitation events were evenly distributed, maintaining sufficient soil moisture to avoid water stress conditions.

Statistical Analysis

As part of the study, an exploratory data analysis was performed to determine the initial distribution and detect patterns or trends. To identify outliers, the Mahalanobis distance (D^2) was used, which measures the multivariate distance of each point relative to the centroid of the dataset. The equation 1 applied for outlier detection was as follows:

$$D^2 = (x-\mu)^T \Sigma^{-1} (x-\mu) \quad (1)$$

Where (x) is a data vector, (μ) is the mean vector, and (Σ^{-1}) is the inverse covariance matrix. No outliers were detected in the analyzed data using the Mahalanobis distance.

After the exploratory analysis, the experimental design was carried out considering two factors: the BlueN product dose at three levels (0, 1, and 2g) and nitrogen fertilization at four levels (0%, 50%, 75%, and 100%).

According to Nassis & Gruffi (2023), factorial designs are essentially useful when the number of factors varies between 2 and 5 ($2 \leq k \leq 5$). The assumptions of normality, homoscedasticity, and independence must be maintained in each cross.

The effects of the design are estimated by sample means, the mean effect of each factor is estimated by row and column means, and the differences between the global mean and row and column means estimate the individual effects of each treatment so that the means of the crosses estimate the possible interaction between factors A and B and the difference in means in the response variable (height, number of leaves, stem diameter, head diameter, weight).

A 3 x 4 factorial design was used to evaluate the individual contribution of each factor to the observed response according to the following model in equation 2:

$$Y_{ijt} = \mu + \alpha_i + \beta_j + (\alpha\beta)ij + \varepsilon ijt \quad (2)$$

The analysis of variance assumes that the residuals follow a normal distribution and that variances are homogeneous between groups. To verify these assumptions, the following tests were performed:

1. Kolmogorov-Smirnov (K-S) Test: used to verify the fulfillment of the normality assumption of the residuals, being more effective in medium to large samples (n > 30).
2. Levene's Test for Homoscedasticity: used to verify that the variances of the groups are homogeneous.
3. Runs Test for Randomness: applied to check that the residuals of the model present a random pattern.

Since the assumptions of normality and homoscedasticity were not met in the response variables in the study, the non-parametric Kruskal-Wallis test, which does not assume normality, was used. The Kruskal-Wallis statistic was calculated according to equation 3.

$$H = \frac{12}{N(N+1)} \sum_{i=1}^{k} \frac{R_i^2}{n_i} - 3(N+1) \quad (3)$$

For the variables where ANOVA or the Kruskal-Wallis test detected significant differences, post hoc tests were performed to identify between which specific levels there were differences. For parametric data, the Tukey test was used, and for non-parametric data, the Wilcoxon test adjusted with the Bonferroni correction was employed to avoid Type I errors and compare all possible pairs of level crossings and their significance.

The analysis was conducted using the R software and its RStudio environment, which allowed for the combination of parametric and non-parametric analyses, strengthening the results and confirming the significance of the main and interaction effects, despite the presence of values that did not meet the ANOVA assumptions.

RESULTS

Edaphoclimatic Characterization of the Experimental Area

Table 2 shows the results of the physical-chemical soil analysis used in the research. Below are the obtained values, their corresponding units, and the interpretation of each nutrient:

According to the soil analysis report, a sandy-loam texture was determined with the following proportions: 53.20% sand, 36% silt, and 10.80% clay. Additionally, the data reveal that the soil has high levels of calcium (8.27meq/100mL), iron (191.36ppm), and organic matter (14.5%), which is favorable for plant growth due to the importance of these nutrients in various physiological and structural functions. However, the high concentration of some nutrients can lead to nutritional imbalances and environmental problems if not properly managed. For example, excess nitrogen can cause leaching and groundwater contamination.

On the other hand, the analysis report shows medium levels of nitrogen (43.75ppm), potassium (0.32meq/100mL), and magnesium (0.82meq/100mL), low levels of phosphorus (9.57ppm), sulfur (10ppm), zinc (3.08ppm), copper (0.87ppm), manganese (2.81ppm), and boron (0.32ppm). These deficiencies could limit the growth and development of some crops, as these nutrients are essential for various metabolic functions, including root formation, flowering, and disease resistance. The electrical conductivity (0.16mS/cm) indicates low salinity,

Table 2: Results and interpretation of the soil analysis used in the research

Result			Interpretation				
Nutrient	Value	Unit		Low	Medium	High	
N	43.75	ppm	Medium	< 20.00	20.00 – 60.00	> 60.00	
P	9.57	ppm	Low	< 10.00	10.00– 20.00	> 20.00	
K	0.32	meq/100mL	Medium	< 0.20	0.20 – 0.40	> 0.40	
Ca	8.27	meq/100mL	High	< 2.00	2.00 – 5.00	> 5.00	
S	10.00	ppm	Low	< 15.00	15.00 – 30.00	> 30.00	
Mg	0.82	meq/100mL	Medium	< 0.50	0.50 – 1.50	> 1.50	
Zn	3.08	ppm	Low	< 5.00	5.00 – 10.00	> 10.00	
Cu	0.87	ppm	Low	< 1.00	1.00 – 3.00	> 3.00	
Fe	191.36	ppm	High	< 50.00	50.00 – 150.00	> 150.00	
Mn	2.81	ppm	Low	< 5.00	5.00 – 15.00	> 15.00	
B	0.32	ppm	Low	< 0.50	0.50 – 2.00	> 2.00	
MO	14.50	%	High	< 2.00	3.00 – 5.00	> 5.00	
CE	0.160	mS/cm	Low	< 1.00	1.00 – 2.00	> 2.00	
pH	5.05	pH scale	Acidic	Acidic	Slightly acidic	Neutral	Slightly alkaline	Alkaline
				0.00 – 5.50	5.50 – 6.50	6.50 – 7.50	7.50 – 8.00	> 8.00
Textural class		Sandy loam		Sand: 53.20%, Silt: 36.00%, Clay: 10.80%				

which is favorable for most crops, while the soil pH (5.05) is acidic, which can affect nutrient availability and microbial activity. Although the soil at the San Francisco Experimental Center presents considerable fertility due to the high levels of some nutrients and organic matter, the deficiencies of other nutrients and the acidic pH imply adjusting fertilization to correct phosphorus, sulfur, and micronutrient deficiencies, and considering liming to neutralize soil acidity. To ensure optimal growth and yield of Brassica oleracea var. italica, it is essential to consider several factors. This crop adapts well to various soil types, preferring those that are loamy to sandy-loam, fertile, with high organic matter content, deep, with excellent drainage and moisture retention, and with a pH between 5.7 and 6.8 (Molina, 2015).

Table 3 and Fig. 2 show the results of the climatic variables in the study area from December 11, 2023, to March 31, 2024. The initial stage, which lasted 25 days (from December 11, 2023, to January 5, 2024), recorded a maximum temperature of 22.20°C and a minimum of 6.10°C, with an average of 12.79±2.83°C; the environmental humidity reached a maximum of 99.33% and a minimum of 49.12%, with an average of 92.13±10.13%; the solar radiation had a maximum of 3.97MJ, with no minimum value applicable at night, and an average of 0.59±0.90MJ. The development stage, which extended for 50 days (from January 6, 2024, to February 24, 2024), showed a maximum temperature of 21.70°C and a minimum of 0.10°C, with an average of 12.79±2.83°C; the environmental humidity had a maximum of 99.33% and a minimum of 36.01%, with an average of 88.99±13.36%; the solar radiation reached a maximum of 4.55MJ, with no minimum value applicable at night, and an average of 0.72±1.08MJ. The final stage, which lasted 35 days (from February 25, 2024, to March 30, 2024), recorded a maximum temperature of 21.00°C and a minimum of 3.90°C, with an average of 12.52±3.12°C; the environmental humidity reached a maximum of 99.33% and a minimum of 48.82%, with an average of 90.88±11.44%; the solar radiation had a maximum of 4.01MJ, with no minimum value applicable at night, and an average of 0.59±0.90MJ. During the 110 days of the crop, the accumulated precipitation was 287.50mm, distributed as follows: 159.50mm in the initial stage, 63.00mm in the development stage, and 65.00mm in the final stage.

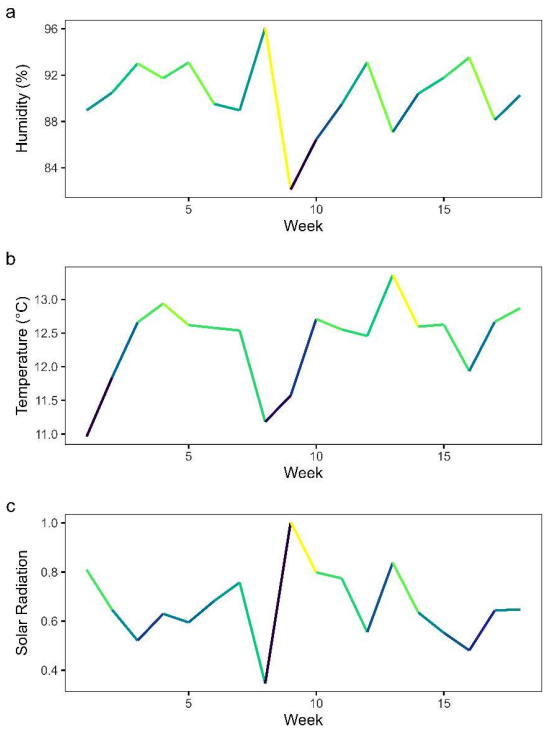

Fig. 2: Climatic conditions during the growing cycle.

Table 4 shows the results of evapotranspiration detailing the daily water requirement in mm per plant for the growth stages of Brassica oleracea var. italica, from December 11, 2023, to March 31, 2024. The initial stage, which lasted 25 days (from December 11, 2023, to January 5, 2024), recorded a reference evapotranspiration (ETo) of 0.16mm/day/plant, a crop evapotranspiration (ETc) of 0.11mm/day/plant with a crop coefficient (Kc) of 0.7, reaching an accumulated crop evapotranspiration (ETc) of 2.75mm/plant during this stage. The development stage, which extended for 50 days (from January 6, 2024, to February 24, 2024), showed an ETo of 0.24mm/day/plant, an ETc of 0.25mm/day/plant with a Kc of 1.05, reaching an accumulated ETc of 12.5mm/plant during this stage. The final stage, which lasted 35 days (from February 25, 2024, to March 30, 2024), recorded an ETo of 0.19mm/day/plant, an ETc of 0.18mm/day/plant with a Kc of 0.95, reaching an accumulated ETc of 6.3mm/plant during this stage.

Table 3: Climatic variables obtained from the UPEC - Huaca meteorological station

Statistic	Environmental Humidity (%)			Solar Radiation (MJ)			Temperature (°C)		
	Initial Stage	Development Stage	Final Stage	Initial Stage	Development Stage	Final Stage	Initial Stage	Development Stage	Final Stage
Mean	92.13±10.13	88.99±13.36	90.88±11.44	0.59±0.90	0.72±1.08	0.59±0.90	12.79±2.83	12.29±3.80	12.52±3.12
Median	97.94	97.14	97.73	0.007	0.01	0.00	12.20	11.80	11.09
Max	99.33	99.33	99.33	3.97	4.55	4.01	22.20	21.70	21.00
Min	49.12	36.01	48.82	NA*	NA*	NA*	6.10	0.10	3.90
Range	50.21	63.32	50.51	3.97	4.55	4.01	16.10	21.60	17.10
CV	11.00%	15.02%	12.59%	152.09%	148.78%	152.53%	22.20%	30.92%	24.99%

Note: data collected from December 11, 2023 to March 31, 2024 at each stage of crop development. NA* (no solar radiation at night)

Table 4: Crop Evapotranspiration (ETc)

Growth Stages of Broccoli	Crop Coefficient (Kc)	Reference Evapotranspiration (ETo) mm/day/plant	Crop Evapotranspiration (ETc) mm/day/plant	Crop Evapotranspiration (ETc) mm/stage/plant
Initial (25 days)	0.70	0.16	0.11	2.75
Development (50 days)	1.05	0.24	0.25	12.5
Final (35 days)	0.95	0.19	0.18	6.30

Table 5: Descriptive Analysis of the Evaluated Variables

FACTOR A (Bacteria Dose in g)	FACTOR B (Percentage of Soil Fertilizer)	EVALUATED VARIABLES				
		Plant Height (cm)	Number of Leaves	Stem Diameter (cm)	Head Diameter (cm)	Head Weight (g)
0	0					
Mean		50.48±1.92	13.58±0.74	3.23±0.23	14.73±0.85	524.68±19.49
Coefficient of Variation		3.81	5.44	7.05	5.78	3.71
0	100					
Mean		55.62±1.51	14.08±0.74	4.11±0.36	16.60±0.93	728.82±9.01
Coefficient of Variation		2.72	5.25	8.81	5.58	1.24
1	0					
Mean		56.01±1.30	14.29±0.54	3.80±0.35	16.08±0.45	712.84±11.80
Coefficient of Variation		2.32	3.81	9.16	2.78	1.66
1	50					
Mean		60.12±1.47	14.15±0.62	4.09±0.35	17.11±0.68	942.69±14.90
Coefficient of Variation		2.45	4.37	8.61	4.00	1.58
1	75					
Mean		63.92±2.07	14.40±0.64	5.21±0.29	21.21±0.78	1040.31±35.55
Coefficient of Variation		3.24	4.47	5.59	3.68	3.42
1	100					
Mean		57.70±1.81	14.40±0.64	5.12±0.31	20.42±1.42	1060.85±65.15
Coefficient of Variation		3.14	4.47	5.98	6.96	6.14
2	0					
Mean		54.62±1.82	14.29±0.68	3.81±0.30	16.32±0.62	715.67±9.98
Coefficient of Variation		3.33	4.78	7.77	3.81	1.39
2	50					
Mean		58.40±1.41	14.23±0.59	3.94±0.26	18.20±0.77	935.70±13.65
Coefficient of Variation		2.41	4.16	6.60	4.21	1.46
2	75					
Mean		57.40±1.38	14.27±0.68	4.70±0.57	19.90±1.16	1036.83±36.46
Coefficient of Variation		2.40	4.74	12.19	5.83	3.52
2	100					
Mean		56.62±1.49	14.25±0.64	4.90±0.41	20.40±1.35	1044.76±30.85
Coefficient of Variation		2.64	4.46	8.34	6.64	2.95

Statistical Analysis of the Agronomic Variables of the Study

According to Gomes (1985), in field agricultural experiments, coefficients of variation are considered low if they are less than 10%, medium if they are between 10% and 20%, high if they vary between 20% and 30%, and very high if they exceed 30% Ospina (2001), indicates that coefficients of variation above 30% indicate notable heterogeneity in the data group. Additionally, Patel et al. (2001), suggest that values greater than 30% reflect low precision in the experiment, implying that these data should be discarded. In the present research, the low variability observed in all evaluated variables, without extreme fluctuations, with coefficients of variation values below 10%, increases confidence in the obtained results.

Table 5 shows the results of the descriptive analysis of the study variables. Forthe variable plant height, the maximum value was 63.92±2.07 cm with 1g of BlueN and 75% soil fertilizer, while the minimum was 50.48±1.92 cm without BlueN or fertilizer. The maximum number of leaves was 14.40±0.64 with 1g of BlueN and 75% or 100% soil fertilizer, and the minimum was 13.58±0.74 without BlueN or fertilizer. The maximum stem diameter was 5.21±0.29cm with 1g of BlueN and 75% soil fertilizer, and the minimum was 3.23±0.23cm without BlueN or fertilizer. The maximum head diameter was 21.21±0.78cm with 1g of BlueN and 75% soil fertilizer, and the minimum was 14.73±0.85cm without BlueN or fertilizer. The maximum head weight was 1060.85±65.15g with 1g of BlueN and 100% soil fertilizer, and the minimum was 524.68±19.49g without BlueN or fertilizer. The maximum yield was 26.50±0.35t/ha with 1g of BlueN and 100% soil fertilizer, and the minimum was 13.12±0.47t/ha without BlueN or fertilizer.

Fig. 3, 4, 5 and 6 show that for the variable's height, stem diameter and flower head diameter, the median value is higher in the treatment of 1g BlueN and 75% soil fertilizer, but not for the variable weight in which the maximum value corresponds to 1g BlueN and 100% soil fertilizer.

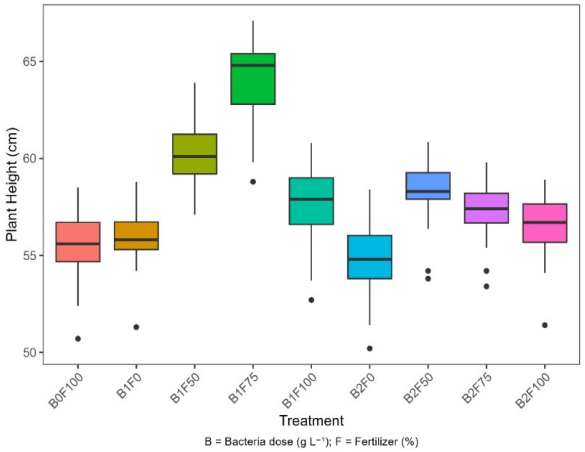

Fig. 3: Box-and-whisker plot for the height variable by treatments.

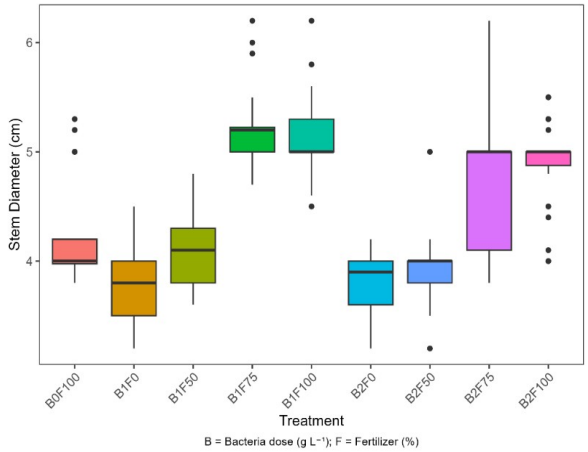

Fig. 4: Box-and-whisker plot for the stem diameter variable by treatments.

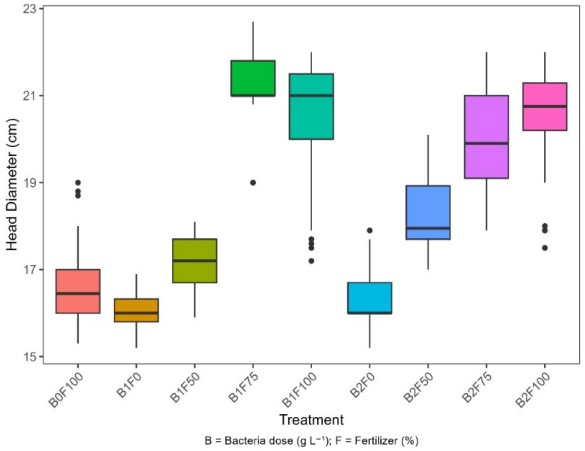

Fig. 5: Box-and-whisker plot for the flower head diameter variable by treatments.

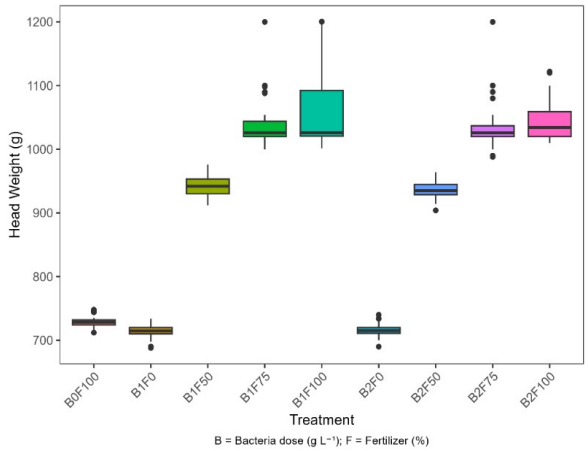

Fig. 6: Box-and-whisker plot for the weight variable by treatments.

Table 6: ANOVA of the Evaluated Variables

FUENTES	Plant Height (cm)		Number of Leaves		Stem Diameter (cm)	
	Value F	Value p	Value F	Value p	Value F	Value p
Bacteria	493.79	< 0.001***	18.47	< 0.001***	203.86	< 0.001***
Fertilizer	254.47	< 0.001***	2.93	0.033*	346.44	< 0.001***
Bacteria*Fertilizer	60.82	< 0.001***	2.55	0.039*	10.31	< 0.001***
FUENTES	Head Diameter (cm)		Head Weight (g)			
	Value F	Value p	Value F	Value p		
Bacteria	392.60	< 0.001***	4134.85	< 0.001***		
Fertilizer	484.14	< 0.001***	2904.29	< 0.001***		
Bacteria*Fertilizer	43.73	< 0.001***	82.49	< 0.001***		

Table 6 shows the results of the analysis of variance, indicating significant differences in all evaluated variables. When analyzing plant height, number of leaves, and head weight, significant differences are observed in each factor as well as in their interaction. However, it is shown that the factor related to bacteria has a greater influence, given by the higher F statistic. Additionally, when considering stem diameter and head diameter, significant differences are also evident in both factors and their interaction, particularly highlighting the influence of the fertilizer due to its higher F value.

Table 7 presents the results of the Tukey mean comparison test at 5% significance. It is observed that for the variables of plant height, number of leaves, stem diameter, and head diameter, the interaction B1 - F75 (1g of Bacteria + 75% Fertilization) achieved the highest means in each case. However, for the variable head weight, this same interaction was placed in the third group (b) of six groups, with a value of 1040.31g, following the group (ab) interaction B2 – F100 (2g of Bacteria + 100% Fertilization) which reached 1044.76g, while in the first group (a), the interaction B1 – F100 (1g of Bacteria + 100% Fertilization) obtained 1060.85g. On the other hand, in the same variable, the interaction B0 – F100 (0g of Bacteria + 100% Fertilization) was situated in the fifth group (d) with 728.81g, while the interaction B0 – F0 (0g of Bacteria + 0% Fertilization) was placed in the sixth group (e) with 524.68g.

Table 7: Tukey Test at 5% Significance for the Evaluated Variables

Interaction	Plant Height (cm)	Number of Leaves	Stem Diameter (cm)	Head Diameter (cm)	Head Weight (g)
B1 - F100	57.70 c	14.39 a	5.12 ab	20.41 b	1060.85 a
B2 - F100	56.62 de	14.25 a	4.90 bc	20.39 b	1044.76 ab
B1 - F75	63.91 a	14.39 a	5.21 a	21.20 a	1040.31 b
B2 - F75	57.39 cd	14.27 a	4.69 c	19.89 b	1036.83 b
B1 - F50	60.12 b	14.14 a	4.09 d	17.10 d	942.69 c
B2 - F50	58.40 c	14.22 a	3.95 de	18.19 c	935.69 c
B0 - F100	55.62 ef	14.08 a	4.11 d	16.60 de	728.81 d
B2 - F0	54.62 f	14.29 a	3.80 e	16.32 e	715.67 d
B1 - F0	56.01 e	14.29 a	3.79 e	16.07 e	712.83 d
B0 - F0	50.47 g	13.58 b	3.22 f	14.72 f	524.68 e

Note: Bacteria dose in grams: B0=0g, B1=1g, B2=2g. Percentage of soil fertilizer: F0=0%, F50=50%, F75=75%, F100=100%. Values bearing different letters in a column differ significantly ($P<0.05$).

DISCUSSION

Nitrogen fertilization is a critical determinant of yield and quality in Brassica oleracea var. italica, particularly in Andean soils where mineralization rates can be limited by lower temperatures. This study demonstrates that the foliar application of *Methylobacterium symbioticum* (BlueN) significantly enhances agronomic performance, offering a viable pathway to improve Nitrogen Use Efficiency (NUE). The results indicate that integrating 100% mineral fertilization with 1g L^{-1} of *M. symbioticum* resulted in the highest head weight (1060.85g), representing a 45.56% increase compared to the control. These findings align with recent studies by Pascual et al. (2020) and Torres Vera et al. (2024) who reported biomass increments in maize and strawberry using similar methylotrophic inoculants. The mechanism driving this synergy is likely the bacterium's ability to fix atmospheric nitrogen directly in the phyllosphere, bypassing soil-based limitations such as leaching or immobilization, which are common in the sandy-loam soils of the study area.

However, one of the most intriguing findings of this research was the non-linear response to the biofertilizer dose. Contrary to the assumption that "more is better," the intermediate dose of M. symbioticum (1g L^{-1}) consistently outperformed the higher dose (2g L^{-1}) across several variables, including head weight and diameter. This phenomenon can be explained by the "niche saturation hypothesis" within the phyllosphere. Methylobacterium species are obligate or facultative methylotrophs that rely on methanol released by plant stomata during cell wall pectin demethylation as their primary carbon and energy source (Sy et al., 2005; Dourado et al., 2015). It is plausible that at the 1g L^{-1} dose, the bacterial population reached an optimal density that matched the carrying capacity of the available methanol supply provided by the broccoli leaves. Conversely, at the 2g L^{-1} dose, intraspecific competition for limited methanol and colonization sites may have induced stress responses in the bacterial colony or triggered plant defense mechanisms, thereby reducing the net nitrogen fixation efficiency per unit of leaf area. Similar saturation effects have been described in other biological systems, where excessive inoculum density fails to translate into proportional vegetative gains (Arrobas et al., 2024).

Furthermore, our data revealed that the combination of M. symbioticum (1g L^{-1}) with a reduced nitrogen dose (75%) yielded heads of 1040.31g, which is statistically comparable to the 100% N treatment. This suggests that BlueN technology can effectively substitute up to 25% of synthetic nitrogen inputs without penalizing yield. This aligns with the findings of Ollio et al. (2024) and Saini et al. (2025) who observed that biofertilizers could offset mineral nitrogen reductions in broccoli production. However, unlike soil-applied PGPRs (e.g., Azospirillum), which are subject to rhizosphere competition, the phyllosphere application of *M. symbioticum* appears to offer a more direct and rapid nutrient delivery system, which is particularly advantageous during the rapid head expansion phase of broccoli.

The impact of environmental conditions on *M. symbioticum* efficacy must also be considered. The study was conducted at 2959m.a.s.l. with high relative humidity (average >88%), conditions that theoretically favor phyllosphere colonization by preventing bacterial desiccation (Madhaiyan et al., 2015). This contrasts with the findings of Arrobas et al. (2024), who reported limited efficacy of *M. symbioticum* in lettuce under different climatic conditions. Our results suggest that the high-humidity, cool environment of the Ecuadorian Andes may act as a "catalyzer" for methylotrophic activity, enhancing the bacterium's persistence and nitrogenase function. This highlights the importance of regional validation for microbial technologies; what works in Mediterranean climates may behave differently in high-altitude tropical ecosystems.

Despite the promising results, limitations exist. The study did not quantify the exact amount of nitrogen fixed by the bacteria using isotopic methods (^{15}N), nor did it assess the post-harvest nutritional quality of the heads (e.g., glucosinolates or vitamin C content). Future research should focus on these physiological markers to fully understand the metabolic trade-offs of biofertilization. Additionally, long-term studies are needed to evaluate the persistence of M. symbioticum in the phyllosphere throughout the crop cycle and its potential interaction with native Andean microbiota.

In conclusion, this study validates *Methylobacterium symbioticum* as a potent biotechnological tool for Andean horticulture. By optimizing the synergy between biological fixation and mineral fertilization, farmers can maintain high yields while reducing synthetic nitrogen dependance. Specifically, the adoption of BlueN technology contributes to sustainable agriculture by improving NUE, potentially lowering the carbon footprint associated with urea production and transport, and mitigating the risk of nitrate contamination in highland water sources. This technology

represents a significant step towards the ecological intensification of Brassica oleracea var. italica production systems.

Conclusion

This study confirms that the phyllosphere inoculation of *Methylobacterium symbioticum* creates a synergistic effect with mineral fertilization, significantly enhancing the agronomic performance of Brassica oleracea var. italica under high-altitude Andean conditions. The optimal response was observed at the dose of 1g L^{-1}, which outperformed the 2g L^{-1} dose, supporting the hypothesis of phyllosphere niche saturation where excessive bacterial density may limit nitrogen fixation efficiency. Crucially, our data demonstrate that BlueN technology allows for a 25% reduction in synthetic nitrogen fertilization (from 100% to 75%) without compromising head weight or quality. This implies that M. symbioticum is not merely a yield enhancer but a strategic tool for sustainable agriculture, enabling farmers to improve Nitrogen Use Efficiency (NUE), reduce production costs associated with urea, and mitigate the environmental risks of nitrate leaching in sensitive highland ecosystems. Future research should focus on the long-term persistence of the inoculum and its impact on post-harvest nutritional quality.

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Data Availability: The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Statement: This study focused exclusively on plant research and did not involve any human or animal subjects.

Author's Contribution: Conceptualization, GJS, GRS and OMQ; methodology, GJS, GRS and OMQ; research, GJS, GRS and OMQ; data curation, GJS, GRS and OMQ; writing: preparation of original draft, GJS, GRS and OMQ; writing: revising and editing, GJS, GRS and OMQ; visualization, GRS and OMQ; supervision, OMQ; All authors have read and accepted the published version of the manuscript.

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