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^{15}N-Nitrogen Use Efficiency, Productivity, and Quality of Durum Wheat Integrating Nitrogen Management and an Indigenous Bacterial Inoculant in a Single Growing Season

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Abstract: In the Yaqui Valley, Mexico, the current and estimated annual growth rate of durum wheat (*Triticum turgidum* L. subsp. *durum*) yield is insufficient to satisfy the food demand that the world will be facing by 2050. Furthermore, besides the high doses of fertilizers applied to wheat in this region, nitrogen-use efficiency (NUE) remains low (<34.4%). A sustainable strategy to reduce the use of fertilizers and to increase crop yield and quality is the use of native plant growth-promoting bacteria as microbial inoculants. This study was performed under field conditions during one agricultural season in the Yaqui Valley, Mexico. It aimed to quantify the impact of the inoculation of a native bacterial consortium (BC) composed of *Bacillus cabrialesii* subsp. *cabrialesii* TE3^{T}, *Priestia megaterium* TRQ8, and *Bacillus paralicheniformis* TRQ65 on grain yield, grain quality, and NUE (measured through ^{15}N-isotopic techniques) at different stages of development of durum wheat variety CIRNO C2008 under three doses of urea (0, 120, and 240 kg N ha^{-1}) fractionated at 30%, 60%, and 10%. Results showed that yield, quality, and NUE were highly affected by the N doses, while the inoculation of the BC had a lower impact on these parameters. Nevertheless, the inoculation of the BC on wheat had positive effects at the early stages of growth, on plant height (+1.6 cm), root depth (+11.9 cm), and spikes per square meter (+25 spikes m^{-2}). Moreover, the addition of the BC improved N acquisition by the plants, at different crop growth stages, compared to uninoculated treatments. Finally, our results indicated that reducing the N dose from 240 kg of N ha^{-1} to 120 kg of N ha^{-1} improved the NUE (27.5% vs. 44.3%, respectively) of the crop. Hence, results of this preliminary study showed that the incorporation of bacterial inoculants into the wheat crop requires a simultaneous adequate N management, in order to obtain the desired positive effect on wheat productivity.

Keywords: bacterial consortium; wheat yield; sustainable agriculture; *Bacillus*; *Priestia*

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1. Introduction

Wheat and its derivates, among all crops, are the main source of calories and nutrients to the human diet, grown in 70% of the world's cultivated area, across a wide range of edaphoclimatic conditions, which makes it the most significant grain crop worldwide [1,2]. The Yaqui Valley (birthplace of the Green Revolution) and the Mayo Valley are the main farming regions of the State of Sonora, Mexico. In 2023, a total area of 253,414 hectares of wheat was planted in these valleys, which has an average production of 1,893,260 tons, representing 56% of national wheat production [3]. However, nitrogen-use efficiency (NUE) of the wheat crop in this region has been reported in a range from 25.3 to 34.4% [4,5]. The information above suggests that most of the N applied is lost in the environment through runoff, leaching, deposition, volatilization, and/or denitrification. These cause alterations in the ecosystems and biodiversity, and societal threats related to water, soil, and air quality, greenhouse gas imbalance, adverse health conditions, and economic losses [6].

Considering the demands of the growing population, and the fact that 30% of the costs of wheat production (2022–2023) in Mexico is used in fertilization [7], it is necessary to optimize the use of synthetic fertilizers applied to this crop, while increasing its yield, quality, and NUE [8]. Aiming to satisfy the 2050 estimated food demand, wheat yield needs to increase at an annual rate of 2.4%, but the global average rate of wheat yield increase was only 0.9% per year by 2013, while in Mexico, the annual rate for wheat increment was 1.1% [9]. Moreover, yield growth for the next 20 years in the Yaqui Valley is estimated to be 1.2% per year in the absence of sustainable technologies [10].

A sustainable and promising strategy to enhance plant growth, while increasing the dependency on synthetic fertilizers and pesticides is the use of plant growth-promoting bacteria (PGPB) [11]. These bioproducts have the potential to reduce the use of nitrogen, phosphorus, and potassium fertilizers by 50% and to increase crop yield by up to ~10 to 30% according to recent studies [12]. These bacteria can promote plant growth through direct mechanisms: (i) phytohormones modulation/production; (ii) 1-Aminocyclopropane-1-carboxylate deaminase (ACC deaminase) synthesis; (iii) nitrogen fixation; and (iv) phosphate solubilization, as well as through indirect mechanisms, such as (i) antibiotic production; (ii) lytic enzyme production; (iii) niche competition; (iv) endotoxin production; and (v) mycoparasitism, or mechanisms with dual effects, such as (i) phytohormones production; (ii) siderophores production; and (iii) quorum sensing [13].

To investigate the impact of inoculation and co-inoculation of rhizobacteria on durum wheat growth and productivity in alkaline calcareous soil, Chami et al. [14] established and compared four treatments during two agricultural cycles: an uninoculated control, inoculation on wheat with a *Paenibacillus polymyxa* SGH1 strain, inoculation with *P. polymyxa* SGK2, and their co-inoculation on wheat. Results showed that the co-inoculation of durum wheat with *P. polymyxa* strains had positive effects on wheat morphological traits (collar diameter, +16.9%; tillers plant^{-1}, +89.8%; weight of rhizospheric soil/dry weight of roots, +35.5%), yield (+41.1% in the first season and +16.6% in the second one) parameters, and quality traits, compared to uninoculated treatments, as a result of a synergistic activity [14].

The three native bacterial strains with plant growth-promoting traits used in the present experiment were isolated from the Yaqui Valley and have been studied by our research team under laboratory and field conditions. *Bacillus cabrialesii* subsp. *cabrialesii* TE3^{T} is an endophytic strain isolated from wheat's leaf tissue and reported as a novel bacterial species [15]; *Priestia megaterium* TRQ8 (previously affiliated and reported as *Bacillus megaterium)* and *Bacillus paralicheniformis* TRQ65 [16] were isolated from the rhizosphere of wheat in commercial wheat fields. Rojas Padilla et al. (2020) [17] reported that TRQ8 was able to produce siderophores (production index of 8.17). They also determined the solubilization index of insoluble phosphorus by TRQ8, TRQ65, and TE3^{T}, which resulted

in ranges from 1.37 to 1.43. Finally, they quantified the strains' capacity to produce indoles, with amounts that ranged from 8.21 to 39.29 μg/mL.

Moreover, it was demonstrated that the co-inoculation of TRQ8 and TRQ65 in wheat increased their ability to promote growth in the early stages of development, without antagonistic effects among them. Thus, their co-inoculation showed significant ($p < 0.05$) increments on aerial length = 5.5%, root length = 10.5%, dry weight of the aerial part = 60.7%, root dry weight = 82.3%, and biovolume = 17.6% [17].

In addition, Rojas-Padilla et al. (2022) [18], conducted a greenhouse assay, where wheat seedlings were inoculated with alginate microbeads containing these strains, this treatment improved biometric parameters (vs. the non-inoculated treatment), such as root dry weight (13–77%), root length (8–19%), stem dry weight (31%), stem length (8–12%), and chlorophyll content (7%).

Field assays over four wheat crop cycles in the Yaqui Valley demonstrated that inoculating the consortium with the strains TRQ8, TRQ65, and TE3^T, and combined with different fertilization doses, increased grain yield by 0.5 to 2.0 ton ha^{-1} compared to non-inoculated treatments, while maintaining grain quality required by the industry [5,19]. In those studies, N fertilization fractionated into three equal parts (33.3%). However, current recommendations for wheat production in the Yaqui Valley are to apply N fertilization in splits: 30%, 60%, and 10%; 30%, 50%, and 20%, or 30, 70%, and 0% (in sowing, stem elongation, and booting), among others [20], and it is crucial to assess whether these N management recommendations are compatible with the use of native microorganisms as bacterial inoculants. Thus, this one-crop season study aimed to quantify the impact of inoculating a bacterial consortium (BC) (*Bacillus cabrialesii* subsp. *cabrialesii* TE3^T, *Priestia megaterium* TRQ8, and *Bacillus paralicheniformis* TRQ65) under three urea doses (0, 120, and 240 kg N ha^{-1}) fractionated at 30%, 60%, and 10%, on grain yield, grain quality, and NUE—through ^{15}N-isotopic techniques—at different stages of development of durum wheat (CIRNO C2008) in the Yaqui Valley, Mexico. It is crucial to assess the impact of inoculation on a specific N management strategy in the short term, highlighting the key findings for the studied traits. This is particularly relevant given that farmers are looking for beneficial effects within a single growing season. This study serves as a starting point for establishing broader, longer-term lines of research. The present findings can be used to adjust the experimental design in subsequent cycles.

2. Materials and Methods

2.1. Edapho-Climatic Characteristics of the Study Site and Agronomic Management Conditions

The study was conducted during the fall–winter crop season (2 December 2021 to 22 May 2022) at the Experimental Technology Transfer Center (CETT-910) of the Instituto Tecnológico de Sonora (ITSON) in the Yaqui Valley, Mexico (Latitude: 27°21′57.74″ N, Longitude: 109°54′55.91″ W). Climatic conditions during the crop cycle included 589 accumulated chill hours, an average temperature of 18.6 °C (mean maximum temperature of 29.1 °C, and mean minimum temperature of 9.5 °C, see Supplementary Table S1), total precipitation of 17.5 mm, and average relative humidity of 62.8% (REMAS, https://siafeson.com/remas/ accessed on 1 June 2022).

The physicochemical characteristics of the soil, macro, and micronutrients are shown in Table 1. These values were determined following the protocols outlined in the Official Mexican Standard NOM-021-SEMARNAT-2000. The nitrogen level reported in Table 1 was calculated from the soil sample analysis at UC-Davis (described in Section 2.5) using an Elemental Analyzer coupled with an Isotope Ratio Mass Spectrometer (EA-IRMS, Elementar Analysensysteme GmbH, Hanau, Germany). Total nitrogen was estimated considering the %N in the soil samples, converted into kg of N per hectare; accordingly considering the

volume, bulk density, and the weight of the soil in 1 hectare. Finally, inorganic nitrogen (the available form of N) was calculated by multiplying each value by 0.035, as 3 to 4% of the total N in the Yaqui Valley soils is typically inorganic. The reported value represents an average of 15 samples.

Table 1. Physicochemical and elemental soil analysis.

Property	Value
Texture	Clay soil (sand 45.4%, silt 17.1%, clay 37.5%)
Water content at saturation	48%
Electrical conductivity	1.8 dS m^{-1}
pH	7.9
Organic matter content	1.00%
Calcium carbonates	0.10%
Hydraulic conductivity	1.0 cm h^{-1}
Bulk density (30 cm)	1.2 g cm^{-3}
Macronutrients	**Concentration (kg ha^{-1})**
Inorganic N	183
P	52.7
Mg	2509.2
Ca	22,032
K	2376
Na	3024
Cation exchange capacity	41.8 mEq 100 g^{-1}
Micronutrients	**Concentration (ppm)**
Fe	7.2
Mn	15.4
Zn	0.5
Cu	1.2
B	0.7

The land was prepared using fallow and primary tillage, which included chiseling and plowing. Durum wheat (*Triticum turgidum* L. subsp. *durum*), specifically the commercial cultivar CIRNO C2008, was sown on 2 December 2021, using a seeder (SUB-24) with three rows per furrow, at a seed density of 120 kg ha^{-1}, under field conditions, following the common wheat crop management of the Yaqui Valley on a vertisol soil—without crop rotation.

Disease, insect, and weed management were carried out as follows (all products were diluted in 40 L of water and applied using a backpack pesticide sprayer): for weed control (broadleaf herbs), 2 L ha^{-1} of Paraquat 30.1% (Gramoxone SL 2.0, Syngenta, Basel, Switzerland) were applied 5 days before planting; once the crop germinated (13 days after sowing), a mixture of 2,4-D dimethylamine salt 70.53% (Amina 6, AgroLucava, Celaya, Mexico) (1 L ha^{-1}) and Fluroxypyr: 1-methylheptyl ester 45.52% (Starane, Corteva, Seville, Spain) (0.5 L ha^{-1}) was applied. For the control of aphids, 80 days after sowing: Imidacloprid 18.8% + Lambda Cyhalotrin 13.6% (Corax SC, Lapisa, La Piedad, Mexico) (200 mL ha^{-1}), and to control leaf rust: Propiconazole 25.5% (Sanazole 250 EC, Velsimex, Mexico City, Mexico) (600 mL ha^{-1}).

Mono-ammonium phosphate (100 kg ha^{-1}) was applied by broadcasting on the sowing date, and urea was applied in bands and fractionated as follows: 30% during pre-planting, 60% at the first irrigation, and 10% at the second irrigation. The irrigation water was 14 cm, and the irrigation time was 12 h. Harvest occurred 171 days after sowing (Figure 1).

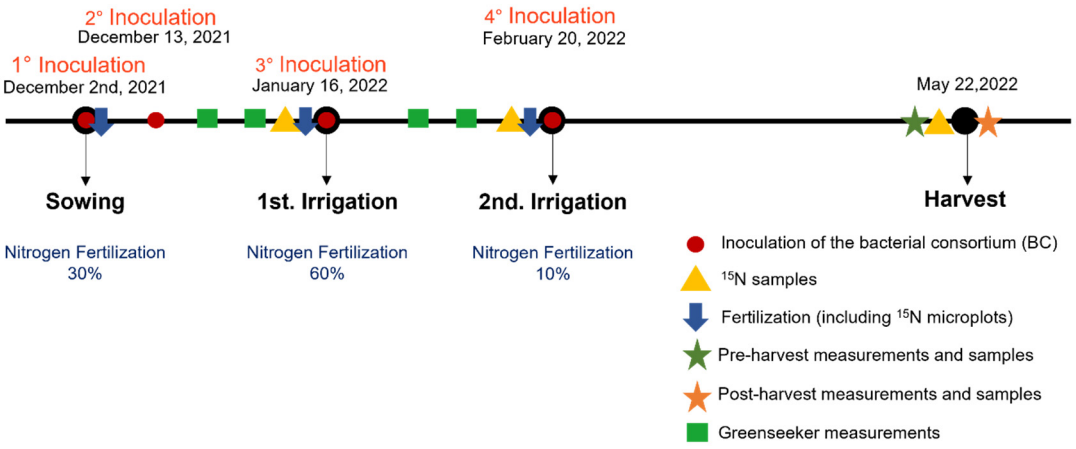

Figure 1. Timeline of the fieldwork developed during the wheat crop cycle.

2.2. Experimental Arrangement of the Field Experiment

The experimental design consisted of a split-plot (6.4 m × 10 m each plot) arrangement of six treatments [three (urea) fertilization doses (0, 120, and 240 kg N ha^{-1}) with and without the inoculation of the studied bacterial consortium (BC)], each replicated four times. Each plot contained an internal microplot (2.4 m × 2 m) exclusively designated for the application of ^{15}N-isotopically enriched urea at 1 atom % ^{15}N, following the rates of 120 and 240 kg N ha^{-1}. Urea of 5.09 atom % ^{15}N (Shanghai Research Institute of Chemical Industry Co., provided by the International Atomic Energy Agency), was isotopically diluted with conventional urea (with a natural abundance of ^{15}N) to achieve the desired enrichment of 1 atom % ^{15}N as stated in the equation described by Zapata and Axmann (1990) [21]. Urea with an enrichment of 1 atom % ^{15}N was then used in this experiment.

2.3. Bacterial Consortium

The native bacterial consortium consisted of *Bacillus cabrialesii* subsp. *cabrialesii* $TE3^T$, *Priestia megaterium* TRQ8, and *Bacillus paralicheniformis* TRQ65. These strains are currently cryopreserved at −80 °C in nutrient broth (NB) culture medium with glycerol (30%), at Colección de Microorganismos Edáficos y Endófitos Nativos (COLMENA, www.itson.mx/COLMENA, accessed on 24 October 2024).

Each bacterial strain was pre-cultured individually on Petri plates with Nutrient Agar (MCD Lab, Cat. 7141) and incubated at a temperature of 28 °C for 24 h. Each strain was also pre-inoculated in a minimal salt medium [in a volume of 20 mL, composed of glucose, 10 g L^{-1}; KH_2PO_4, 6.4 g L^{-1}; K_2HPO_4, 5.32 g L^{-1}; $(NH_4)_2\ SO_4$, 4 g L^{-1}; 0.4 g L^{-1} of $MgSO_4{\cdot}H_2O$; 0.03 g L^{-1} of $FeSO_4{\cdot}7H_2O$; 0.044 g L^{-1} of $MnSO_4{\cdot}H_2O$; and 0.021 g L^{-1} of $CaCl_2$], with a period of incubation of 24–48 h, at 180 rpm and a temperature of 28 °C, until an optical density (absorbance at the wavelength of 630 nm) of ~1.0 was reached. Later, strains were inoculated in 500 mL of the same minimal salt culture medium individually, under the same conditions for 48 h, until reaching $\geq 1 \times 10^7$ cell mL^{-1}.

The three bacterial strains ($\geq 1 \times 10^7$ cell mL^{-1}) were combined to form a consortium and were fermented in a BIOSTAT A bioreactor (Sartorius Stedim Biotech, Göttingen, Germany) in 5 L of a synthetic mineral medium composed of 20 g L^{-1} of glucose, 6.4 g L^{-1} of KH_2PO_4, 23.44 g L^{-1} of K_2HPO_4, 8 g L^{-1} of $(NH_4)_2\ SO_4$, 0.8 g L^{-1} of $MgSO_4{\cdot}H_2O$, 0.06 g L^{-1} of $FeSO_4{\cdot}7H_2O$, 0.088 g L^{-1} of $MnSO_4{\cdot}H_2O$, 0.126 g L^{-1} of $CaCl_2$, and $Na_3C_6H_5O$, 10.08 g L^{-1}. The bacterial consortium was fermented for 48 h, at the following conditions: temperature of 37 °C, pH of 7.0, agitation of 400 rpm, with constant air flow of 0.75 vvm, and silicon antifoam addition (30% p/v).

The number of viable bacterial cells was quantified as colony-forming units per milliliter (CFU mL^{-1}). Once a density of 1×10^{10} CFU mL^{-1} was obtained, the inoculant was applied to the crop (300 mL by each row of 0.8 m × 10 m long), manually directly to the soil (1×10^9 cells $plant^{-1}$) at the sowing date; 11 days after sowing (wheat emergence); 45 days after sowing (tillering stage), hours before the first irrigation; and 80 days after sowing (milk stage of development) before the second irrigation (Figure 1).

2.4. Measurement of Yield and Quality Parameters

The measurements of spikes m^{-2} (n = 12 per treatment), spike length (n = 36 per treatment), and the number of grains per spike (n = 62 per treatment) were registered at physiological maturity (pre-harvest). At the harvest, discarding the furrows of the edges, wheat biomass was collected manually using sickles, from the center of each plot. Every sheaf of wheat (per plot/replicate) was weighed on a scale (to estimate the total aboveground biomass), and then their grains were weighed after using a wheat threshing machine (to record grain weight per sample (n = 12 per treatment), considering the percentage of moisture at harvest, which was 8.8%). Then, straw yield and grain yield (adjusted to 14% moisture) were estimated, extrapolating each weight per area to tons per hectare. The harvest index was calculated by dividing grain yield by above-ground biomass (n = 12 per treatment). Hectoliter weight (n = 12) and 1000-grain weight of grains samples of each plot (n = 12) were estimated with a SeedCount Digital Image Analysis System SC5000 (Next Instruments, Sydney, Australia). On the other hand, a DA 7200 NIR analysis system (Perten Instruments, Sweden) was used to measure the percentage of grain (n = 12) protein at 12.5% humidity by near-infrared spectroscopy, which was calibrated based on official methods AACC 39-10.01 and 45-15.01 [22]. Additionally, wholemeal yellowness (Minolta b* value) was estimated utilizing a colorimeter (Konica Minolta, Tokyo, Japan) (n = 12); the SDS sedimentation index (n = 12) was calculated by dividing the SDS sedimentation volume by the sample protein content; sodium dodecyl sulfate (SDS) sedimentation volume (n = 12) was determined according to the method of Peña et al. (1990) [23].

2.5. ^{15}N-Nitrogen-Use Efficiency

To estimate ^{15}N-nitrogen-use efficiency with isotopic techniques of plant, straw, grain, and soil, samples were collected from the ^{15}N microplots (from the central furrow: an internal area of 0.8 m × 1 m, after excluding the edges) [21]. The collection of plant samples was before each fertilization event (45 and 80 days after sowing, in the tillering stage and the milk stage of development, respectively). Straw, grain, and soil samples were collected at harvest from the same internal sampling area of the microplot. The soil was collected at 30 cm depth, dried at room temperature, and sifted with a No. 100 mesh sieve (to achieve a particle size of 0.149 mm). Plant, straw, and grain samples were milled to obtain smaller sample sizes (straw and plant particle size <2 mm, grain particle size <0.3 mm).

All ^{15}N-enriched samples were processed in the Department of Plant Sciences of the University of California, at the Stable Isotope Facility (SIF) in Davis, with a continuous flow Elemental Analyzer coupled with an Isotope Ratio Mass Spectrometer (EA-IRMS). The equipment determined the percentage of ^{15}N atoms on all samples (plant, soil, straw, and grain; a ^{15}N-enriched urea sample was used as a control). Then, the NUE in the samples was calculated according to Zapata and Axmann (1990) [21], obtaining the nitrogen derived from the soil (Ndfs), subtracting the nitrogen derived from the fertilizer (Ndff), from the total N in the sample.

2.6. In Vitro Assay of the Microbial Consortium Response to Different Rates of N and C (Ammonium Sulfate and Glucose)

To determine the influence of varying carbon and nitrogen concentrations in the culture medium on the growth of the consortium strains, nine treatments comprising three replicates of three distinct N (ammonium sulfate) and C (glucose) concentrations were established in Elisa plates. Each strain of the bacterial consortium (*Bacillus cabrialesii* subsp. *cabrialesii* TE3^T, *Priestia megaterium* TRQ8, and *Bacillus paralicheniformis* TRQ65) was inoculated and fermented in individual centrifuge tubes with 15 mL of a synthetic mineral medium, of composition previously mentioned in the preparation of the bacterial consortium for the field application [glucose, 10 g L^{-1}; KH_2PO_4, 6.4 g L^{-1}; K_2HPO_4, 5.32 g L^{-1}; $(NH_4)_2$ SO_4, 4 g L^{-1}; 0.4 g L^{-1} of $MgSO_4 \cdot H_2O$; 0.03 g L^{-1} of $FeSO_4 \cdot 7H_2O$; 0.044 g L^{-1} of $MnSO_4 \cdot H_2O$; and 0.021 g L^{-1} of $CaCl_2$], at 30 °C and 180 rpm, for ~24 h, until reaching an optical density of ~1.0 (630 nm). Then, they were co-inoculated and co-fermented in a flask with 1 L of the synthetic mineral medium at the same conditions as the preinocula. After the optical density reached ~1.0, 0.1 mL of the inoculum was added to each well of the Elisa plates.

The base conditions of N and C were the original synthetic mineral medium, which was developed and selected due to the observed optimal growth of the microorganisms in the culture medium in those conditions. A total volume of 1 mL of the mineral medium was added to each well to establish the treatments. Later, proportional amounts of glucose and ammonium sulfate were added (Table 2) to form the treatments (3 replicates each one) that were (i) low N + low C; (ii) low N + medium C; (iii) low N + high C; (iv) medium N + low C; (v) medium N + medium C; (vi) medium N + high C; (vii) high N + low C; (viii) high N + medium C; and (ix) high N + high C.

Table 2. Necessary concentrations of ammonium sulfate and glucose to establish each treatment.

Component	N or C Content in the Treatment	Desired Composition (g/L)	Additional Mass to Make Up the Treatment (g/L)	Additional Mass to Make Up the Treatment in Each Well of 1 mL (g/1.0 mL)
$(NH_4)_2SO_4$	Low N	4	0	0
$(NH_4)_2SO_4$	Medium N	9.6000	5.6000	0.0056
$(NH_4)_2SO_4$	High N	19.2000	15.2000	0.0152
$C_6H_{12}O_8$	Low C	10	0	0
$C_6H_{12}O_8$	Medium C	20	10	0.0100
$C_6H_{12}O_8$	High C	27.7778	17.7778	0.0178

The optical density of all treatments was measured at 630 nm each hour for 21 h. At 14 h, when all treatments reached a value of absorbance of around 1.0, a serial dilution technique was used (0.1 mL of the sample in 0.9 mL of sterile water) to count the number of cells and spores (10^{-4} and 10^{-5}) and to count only spores (10^{-1} and 10^{-2}, raising the temperature to 80 °C for 15 min). Then, 0.1 mL of each dilution was added to Petri dishes with nutrient agar (5 g L^{-1} of peptone, 3 g L^{-1} yeast extract, and 15 g L^{-1} of bacteriological agar) and incubated for 24 h and 37 °C. The colony-forming units per milliliter (CFU mL^{-1}) were quantified for each treatment.

2.7. Statistical Analysis

Data components were processed with a two-way analysis of variance (ANOVA) in InfoStat, considering the nitrogen level (kg N ha^{-1} of nitrogen applied), the bacterial consortium, and their interactions as mixed factors. All factors were normally distributed (Shapiro–Wilk test). Levene's test of homogeneity of variances indicated that there are no significant dif-

ferences between the variances of the treatments. Statistical results were validated with a significance level of $p < 0.05$ and $p < 0.01$. Differences between the parameters' means were assessed using Duncan's Multiple Range Test at a 95% confidence level.

3. Results and Discussion

The inoculation of the BC caused a significant increase ($p < 0.05$) in plant height on treatments of 0 and 240 kg N ha^{-1} vs. the uninoculated ones at the same N rate (Supplementary Table S2). Plant height was positively impacted by the inoculation of the bacterial consortium, as well as by the reduction in N fertilization to 0 kg N ha^{-1} (Table 3). Similarly, a remarkable tendency of higher values of root depth in all the inoculated treatments vs. uninoculated was observed (from 12.1 cm more) (Table 3). These positive outcomes could be due to the previously observed positive effects of the studied BC, for example, indole-3-acetic acid (IAA) biosynthesis, which is involved in several physiological processes, such as cell elongation and division, tissue differentiation, germination stimulation, influences root development, photosynthesis, pigment formation, biosynthesis of metabolites, and resistance to stress conditions [24]. In addition, strains contained in the studied BC can solubilize phosphate into a more accessible form to be absorbed by wheat plants. Phosphorus, as a macronutrient for crops, is the second most important mineral, an elemental component of nucleic acids, nucleotides, phospholipids enzymes, and coenzymes. Thus, it is involved in numerous physiological and biochemical reactions, such as photosynthesis, root and stem development, formation of flowers and seeds, crop maturation, and resistance to some diseases [25,26]. Furthermore, Valenzuela-Ruiz et al. (2019) [16] demonstrated significant increments in shoot height (26%), root length (36%), shoot dry weight (100%), stem diameter (53%), and biovolume index (146%) when strain TRQ65 was inoculated in wheat (vs. uninoculated wheat plants). Also, *P. megaterium* TRQ8, *B. cabrialesii* subsp. *cabrialesii* $TE3^T$, and *B. paralicheniformis* TRQ65 share more than 50% of biofertilization-related genes associated with CO_2 fixation, N and Fe acquisition, phosphate and K solubilization, and P assimilation, as well as genes related to the production of siderophores and stress response [5].

The number of spikes per square meter in all inoculated treatments resulted in optimal values for high wheat yield (200–300 spikes m^{-2}), without significant differences among them (Supplementary Table S2) [27]. However, a significant and positive effect of the inoculation of the BC was observed in this study (25 more spikes per square meter) compared to the uninoculated ones (Table 3); for example, a significant increase ($p < 0.05$) of 16.7% in spikes m^{-2} was observed in the 120 kg N ha^{-1} treatment when the CB was inoculated (Supplementary Table S2). Also, not applying N fertilization caused a positive impact on this trait, compared to 120 and 240 kg N ha^{-1} applied (Table 3). In the case of grains per spike, this parameter increased with the highest doses of N applied (55 grains per spike, vs. 51 and 52, Table 3).

In this sense, Oksel et al. (2022) [28] reported that the individual inoculation of seven *Bacillus* strains on wheat in Turkey, compared to an uninoculated control, the inoculation led to an increase in biometric traits; however, the inoculation of the *Bacillus* strains did not show significant increments in the number of grains per spike, suggesting that the positive effect of the inoculation of these strain on wheat yield could be through regulating several physiological traits.

Table 3. Wheat quantitative traits of yield and harvest indexes.

Factor		Plant Height (cm)		Root Depth (cm)		Spikes m^{-2}		Grains $Spike^{-1}$		Spike Size (cm)		Grain Yield (ton ha^{-1})		Straw Yield (ton ha^{-1})		Harvest Index	
		Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error
N Level, kg N ha^{-1} (A)	0 kg N ha^{-1}	79.7 b	0.25	108.3 a	5.61	274 b	6.1	51 a	0.61	6.3 a	0.06	5.01 a	0.14	3.57 a	0.12	0.59 a	0.0032
	120 kg N ha^{-1}	78.2 a	0.25	106.8 a	5.61	239 a	6.0	52 a	0.60	6.4 a	0.06	6.33 b	0.12	3.71 a	0.11	0.61 b	0.0038
	240 kg N ha^{-1}	78.0 a	0.25	96.3 a	5.61	256 a	6.0	55 b	0.58	6.9 b	0.06	6.77 c	0.12	4.22 b	0.10	0.63 c	0.0033
Bacterial consortium (B)	Uninoculated	77.8 a	0.21	97.8 a	4.58	244 a	5.0	53 a	0.49	6.5 a	0.05	6.18 a	0.10	4.01 b	0.08	0.61 a	0.0027
	Inoculated	79.4 b	0.21	109.7 a	4.58	269 b	4.8	53 a	0.48	6.5 a	0.05	5.90 a	0.11	3.66 a	0.09	0.61 a	0.0029
A × B	F value	17.83		0.24		0.91		4.79		21.24		14		9.63		3.25	
	p-value	<0.0001 **		0.7932		0.4095		0.0089 **		<0.0001 **		<0.0001 **		0.0004 **		0.0456 *	

Values in the same column indicate the mean, and different letters indicate a significant difference between treatments of the same parameter, using the Duncan test ($p < 0.05$). The bacterial consortium was composed of *Bacillus cabrialesii* subsp. *cabrialesii* $TE3^T$, *Priestia megaterium* TRQ8, and *Bacillus paralicheniformis* TRQ65. Asterisks indicate the significance level by the Duncan test [$p < 0.05$ (*)], $p < 0.01$ (**)].

In our essays, spike size significantly increased by 9% ($p < 0.05$) on the 240 kg N ha^{-1} + CB treatment compared to the uninoculated treatment at the same rate of N (7.2 vs. 6.6 cm, Supplementary Table S2), but in the unfertilized treatments (0 kg N ha^{-1}) a decrease of 7.7% was observed in the inoculated vs. uninoculated treatment (6 vs. 6.5 cm). In this sense, this trait was more influenced by the application of higher doses of N (240 kg N ha^{-1}), than by the inoculation of the BC (Table 3).

Regarding grain yield, a significant ($p < 0.05$) increase due to the inoculation of the BC was observed at 240 kg N ha^{-1} (7.06 vs. 6.48 tons ha^{-1}). On the other hand, a decrease of 1.26 ton ha^{-1} was observed by the inoculation of BC vs. the uninoculated treatment under 0 kg N ha^{-1} (Supplementary Table S2). In general, in this crop cycle, the doses of N had a greater impact on grain yield than the inoculation of the bacterial consortium (Table 3). This effect of inoculation of the BC on wheat contrasts with that observed in previous field experiments carried out by our group, under similar field conditions. Ayala-Zepeda et al. (2024) [5] reported that the inoculation of strains TRQ8, TRQ65, and TE3^{T} to durum wheat resulted in a yield increase of 1.1 ton ha^{-1} under 0 kg N ha^{-1} (123 kg N ha^{-1} as residual in the soil) compared with its uninoculated control, and an increment of 2.0 ton ha^{-1} under 120 kg N ha^{-1} (104 kg N ha^{-1} a residual in the soil) compared with uninoculated treatment. Our essay was carried out at the same location, agricultural practices, and N applied, as well as similar soil physical properties and climatic conditions compared with Ayala-Zepeda et al. (2024). However, in the present study, the N content in the soil on the sowing date was 183 kg N ha^{-1}, and the N was fractionated differently at 30, 60, and 10%, in comparison with 33%, 33%, and 33% used by Ayala-Zepeda et al. (2024) [5].

Another distinct finding was reported by Ibarra-Villarreal et al. (2023) [19], who inoculated a bacterial consortium (*Bacillus subtilis* TSO9, *Bacillus cabrialesii* subsp. *tritici* TSO2^{T}, *Bacillus subtilis* TSO22, *Bacillus paralicheniformis* TRQ65, and *Priestia megaterium* TRQ8), to durum wheat in the same location, under the same agricultural practices, three different N rates applied, and similar soil and climatic conditions, obtaining a increment of 1 ton ha^{-1} in grain yield in the inoculated treatment under 130 kg N ha^{-1} (medium N fertilization) compared to the uninoculated control.

Various studies have shown increases in crop productivity, yield parameters, or nitrogen-use efficiency with the inoculation of plant growth-promoting bacteria under medium doses of N fertilization. Gaspareto et al. 2023 [29] tested the co-inoculation of winter wheat with *Azospirillum brasilense* and *Bacillus subtilis* under five N doses (0, 40, 80, 120, and 160 kg ha^{-1}, applied from urea) in Brazilian Cerrado. They suggest that N fertilization can be reduced by application of those strains under a no-tillage system; their results show that a medium rate of fertilization of 80 kg ha^{-1}, nitrogen-use efficiency, grain N accumulation, and number of grains spikes^{-1} were significantly increased. On the other hand, Pardo-Díaz et al. (2021) [30] found that the effect of PGPB inoculation on plant growth was observed only with 50% of the N fertilization dose. Moreover, Saia et al. (2015) [31] found that durum wheat inoculation with arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) (alone or in combination) improved plant growth, and N uptake, in comparison with uninoculated controls.

According to these results, regarding the use of bacterial inoculants as a sustainable alternative to wheat production, not only is the nitrogen fertilization dose important, but so is fractionation or the N split application (the total N at specific stages of wheat growth). Moreover, in earlier works, we proposed that positive effects on wheat yield in the Yaqui Valley inoculated with the bacterial consortium used in this study are most likely to be observed with total N concentrations of 123–225 kg N ha^{-1}, considering soil N content and N from fertilization (but with a different N split application: 33% at the sowing date, 33% with the first irrigation event, and 33% with the second irrigation) [5]. In this sense, in this

crop cycle, the treatments of 120 and 240 kg N ha^{-1} applied surpass this range (adding 183 kg N ha^{-1} of the soil).

It has been observed that as N levels surpass 225 kg N ha^{-1}, wheat yield and productivity begin to decrease, which may be due to exceeded crop nutrient requirements, as well as adverse effects on the photosynthesis rate and grain filling potential in cereal crops [32]. Thus, high doses of N in the soil, along with low efficiency, can cause toxicity, salinity, and pollution of soils and water bodies [33]. Also, it has been reported that N fertilizer input in the short term altered the soil NO_3^- contents, consequently impacting negatively the predominant rhizosphere soil bacterial species [34].

In agricultural soils, high doses of N affect growth and some beneficial effects of plant growth-promoting microorganisms [13] and decrease microbial functional diversity [35]. In general, fertilization changes the activity and community structure of soil microorganisms [34,36]. In this sense, results of varying carbon and nitrogen in vitro essay (and the inoculation of the BC that was used in the field), showed that the highest sporulation occurred with the highest nitrogen content in the medium, and with the three different C contents (4.90×10^4 UFC mL^{-1}, 1.07×10^5 UFC mL^{-1}, 4.80×10^4 UFC mL^{-1}: low, medium, and high, respectively) (Figure 2). Although this dormant state (endospore formation) is a desirable trait for the development of cost-effective microbial inoculants, microorganisms also produce spores as a survival mechanism to adverse environmental conditions and different types of stress, resulting in a metabolically inactive state [37].

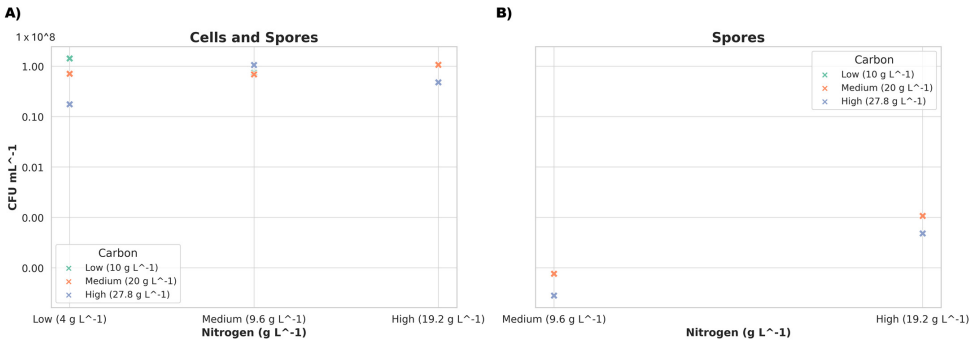

Figure 2. The growth response of the microbial consortium to varying rates of nitrogen and carbon, as detailed in Table 2: (**A**) Quantification of colony forming units (CFU) per milliliter, including both cells and spores. (**B**) Quantification of colony-forming Units (CFU) per milliliter of spores following a 15 min thermal shock treatment.

The above could be a factor limiting the BC beneficial effect at high N doses, considering that there are certain important growth stages of wheat where fertilization and the application of the BC are synchronized and where plant growth-promoting activity is crucial to observe positive impacts on plant biometric parameters, yield, and quality. These results suggest that these strains could be forming endospores when high quantities of N are applied to the soil, instead of being in an active state at those specific and critical moments (sowing date, tillering stage, booting stage, among others).

Thus, medium rates of nitrogen are required for adequate BC growth, since the consortium strains resulted in 7.40×10^7 UFC mL^{-1} (with low C), 6.90×10^7 UFC mL^{-1} (with medium C), and 1.06×10^8 UFC mL^{-1} (along with high C) cells and spores; also, in 2.80×10^3 UFC mL^{-1} of spores with high C content, and 7.60×10^3 UFC mL^{-1} of spores with medium C content (Figure 2).

Other studies have shown how high N fertilization rates affect soil microbial flora. Perez et al. [38] studied the influence of nitrogen in soil microbial population of a *Coffea canephora* Pierre cultivation and found that the application of 90 and 135 kg N ha^{-1} resulted in the highest values of bacteria populations (116–139 $\times$ 10^7 $CFUg^{-1}$); 150 kg N ha^{-1}

reduced the populations to their initial state (107×10^7 CFUg^{-1}); and the highest rate of fertilization, 200 kg N ha^{-1}, depleted the populations of bacteria to 50×10^7 CFUg^{-1}. In comparison with Pardo-Díaz et al. [30], who evaluated *Herbaspirillum* sp. AP21 and *Azospirillum brasilense* D7 (without inoculation, individual, and in combination) with 50% and 75% of urea, vs. controls without PGPB with 0% and 100% of urea on a Perennial ryegrass (*Lolium perenne* L.) var. One 50 and red clover (*Trifolium pratense* L.) intercropping system, they reported that the inoculation of the PGPB decreased the rhizosphere bacterial diversity, but nitrogen doses did not cause significant changes in the bacterial community.

Regarding the quality of the grain, the traits of hectoliter weight and protein percentage (Table 4) were positively ($p < 0.05$) impacted by the increased doses of applied nitrogen dose, more than the inoculation of the studied bacterial strains (Table 4). Similarly, Lerner et al. [39] reported that the protein content in grains of different Argentine varieties of bread wheat was highly dependent on the level of N.

Concerning the trait of 1000-grain weight, no significant difference was observed among treatments due to the inoculation of the BC (Table 4), and it has been stated that increases in spikes per m^2, 1000-grain weight, and number of grains per spike are necessary to achieve an increment of wheat yield [40]. This indicator is consistent with the yields obtained (except for the lowest two values when 0 kg N ha^{-1} was applied) (Supplementary Tables S1 and S2). The range between all results of Minolta b* values of yellowness was 14.20 to 14.73 (Supplementary Table S3), without a significant effect due to the inoculation of the BC, and a tendency of higher values with increasing N rates (Table 4). These results were coherent, as this quality trait is reported to be more stable in different conditions and mainly genetically determined [41]. All values of yellowness in this study met the quality requirements for this wheat variety [42].

However, a low protein percentage in general (among all treatments) was observed, which is the main trait considered to define an appropriate wheat grain quality for industrial purposes in the region. This effect could be due to low N availability in advanced stages of growth (e.g., heading or grain filling) and N was not available for a beneficial impact on quality (protein), considering that the last fractionation of N was 10% in the grain filling stage. It is important to highlight that wheat gluten is composed of proteins divided into gliadins (responsible for dough extensibility and viscosity) and glutenins (main determinants of dough strength), which must be in balance to achieve desirable quality characteristics. Therefore, it has been reported that the γ-gliadin gene family is largely regulated by the N supply in the course of wheat grain development [43,44]. Otherwise, Lerner et al. (2016) [39] reported that the relationship between dough extensibility and recovery efficiency of N in grain was more related to the partition of N to harvestable destinations than to the absorption of N from the soil.

In summary, the inoculation of the bacterial consortium had no significant effect on the studied quality parameters, which were more stable and/or influenced by different N doses. Although beneficial bacteria have the potential to improve plant growth and fertilizer uptake, excess nitrogen can induce sporulation and limit their metabolic activity at critical stages of crop development. This highlights the importance of properly managing nitrogen doses to maximize the benefits of microbial inoculants in crops.

In this study, it was demonstrated that fertilization of 120 kg of N ha^{-1} is more efficient (NUE: 44.32%) than the full fertilization dose of 240 kg of N ha^{-1} (NUE: 27.51%) (Table 5), due to a lower amount of N fertilizer applied. Although the N derived from the fertilizer at physiological maturity (Figure 3) was quantitatively higher in the 240 kg N ha^{-1} treatments (69.1 ± 30.3 kg ha^{-1} and 62.9 ± 11.5 kg N ha^{-1}) compared to the 120 kg of N ha^{-1} treatments (52.4 ± 7.6 kg ha^{-1} and 54.0 ± 23.2 kg ha^{-1}), the difference was not statistically significant.

Table 4. Wheat quality traits of the studied treatments.

Factor		Hectoliter Weight (kg hL^{-1})		1000 Grain Weight (g)		Protein (%)		SDS Sedimentation Test (mL)		SDS/Protein Index		Wholemeal Yellowness (Minolta, b)	
		Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error
N Level, kg N ha^{-1} (A)	0 kg N ha^{-1}	78.85 a	0.13	57.94 b	0.42	9.20 a	0.16	11.31 a	0.17	1.25 b	0.02	14.23 a	0.06
	120 kg N ha^{-1}	80.89 b	0.13	58.08 b	0.42	11.09 b	0.16	11.29 a	0.16	1.02 a	0.02	14.52 b	0.06
	240 kg N ha^{-1}	80.76 b	0.13	56.61 a	0.42	11.81 c	0.16	12.16 b	0.16	1.04 a	0.02	14.68 b	0.06
Bacterial consortium (B)	Uninoculated	80.48 a	0.11	57.69 a	0.35	10.58 a	0.13	11.49 a	0.14	1.10 a	0.01	14.53 a	0.05
	Inoculated	80.52 a	0.11	57.40 a	0.35	10.82 a	0.13	11.68 a	0.14	1.10 a	0.01	14.43 a	0.05
A × B	F value	0.72		3.03		0.8		0.87		2.33		0.12	
	p-value	0.4911		0.0553		0.4538		0.4228		0.1056		0.8851	

Values in the same column indicate the mean, and different letters indicate a significant difference between treatments of the same parameter, using the Duncan test ($p < 0.05$). The bacterial consortium was composed of *Bacillus cabrialesii* subsp. *cabrialesii* TE3^{T}, *Priestia megaterium* TRQ8, and *Bacillus paralicheniformis* TRQ65.

Table 5. Nitrogen (^{15}N) uses the efficiency of wheat under two different doses of urea and the inoculation of a native bacterial consortium.

Factor		Nitrogen-Use Efficiency of ^{15}N Labeled Urea (%)						^{15}N Remnant in the First 30 cm of Soil (%)		Losses of ^{15}N (%)	
		Grain (%)		Straw (%)		Whole Plant (%)					
		Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error
N Level, kg N ha^{-1} (A)	120 kg N ha^{-1}	40.53 b	4.08	3.79 a	0.33	44.32 b	4.30	4.88 a	0.84	47.22 a	3.77
	240 kg N ha^{-1}	24.48 a	4.41	3.03 a	0.35	27.51 a	4.64	6.39 a	0.78	65.85 b	3.77
Bacterial consortium (B)	Uninoculated	33.29 a	4.41	2.95 a	0.35	36.23 a	4.64	4.17 a	0.78	59.33 a	3.77
	Inoculated	31.73 a	4.08	3.87 a	0.33	35.60 a	4.30	7.10 b	0.84	53.73 a	3.77
A × B	F value	0.04		2.75		0.09		9.89		0.17	
	p-value	0.8526		0.1257		0.7652		0.0093 **		0.6931	

One atom % ^{15}N excess labeled urea was used in this experiment. The bacterial consortium was composed of *Bacillus cabrialesii* subsp. *cabrialesii* TE3^{T}, *Priestia megaterium* TRQ8, and *Bacillus paralicheniformis* TRQ65. Different letters in the same column indicate a significant difference using the Duncan test ($p < 0.05$). Asterisks indicate the significance level by the Duncan test [$p < 0.01$ (**)].

STAGE OF DEVELOPMENT	TREATMENT				PARAMETER
	120 kg N ha^{-1}	120 kg N ha^{-1} + BC	240 kg N ha^{-1}	240 kg N ha^{-1} + BC	
Tillering					
	36	**36**	**72**	**72**	**Total N applied up to that stage (kg N ha^{-1})**
	5.8 ± 2.5 a	9.4 ± 1.7 a	3.1 ± 0.6 a	11.9 ± 4.6 b	**Nitrogen Use Efficiency (%)**
	2.1 ± 0.9 a	3.4 ± 0.6 a	2.2 ± 0.4 a	8.6 ± 3.3 b	**Nitrogen derived from the fertilizer (kg N ha^{-1})**
	21.7 ± 6.5 a	23.0 ± 12.3 a	11.5 ± 2.9 a	23.9 ± 4.7 a	**Nitrogen derived from the soil (kg N ha^{-1})**
Milk stage					
	108	**108**	**216**	**216**	**Total N applied up to that stage (kg N ha^{-1})**
	33.4 ± 5.9 a	46.7 ± 6.5 a	35.1 ± 7.2 b	22.5 ± 1.3 a	**Nitrogen Use Efficiency (%)**
	36.1 ± 6.4 a	50.4 ± 7.0 a	75.9 ± 15.6 b	48.5 ± 2.9 a	**Nitrogen derived from the fertilizer (kg N ha^{-1})**
	61.2 ± 14.7 a	65.6 ± 32.1 a	61.1 ± 26.8 a	78.4 ± 7.9 a	**Nitrogen derived from the soil (kg N ha^{-1})**
Physiological maturity					
	120	**120**	**240**	**240**	**Total N applied up to that stage (kg N ha^{-1})**
	43.7 ± 6.3 a	45 ± 19.3 a	28.8 ± 12.6 a	26.2 ± 4.8 a	**Nitrogen Use Efficiency (%)**
	52.4 ± 7.6 a	54.0 ± 23.2 a	69.1 ± 30.3 a	62.9 ± 11.5 a	**Nitrogen derived from the fertilizer (kg N ha^{-1})**
	59.7 ± 10.5 a	88.8 ± 39.5 ab	78.6 ± 1.9 ab	105.1 ± 28.6 b	**Nitrogen derived from the soil (kg N ha^{-1})**

Figure 3. Effect of nitrogen dose and the inoculation of a bacterial consortium on nitrogen-use efficiency (^{15}N) at different durum wheat growth stages. 1 atom % ^{15}N excess labeled urea was applied at planting (30%), 45 days after sowing (60%), and 80 days after sowing (10%) at two different rates of N, with and without the inoculation of a bacterial consortium (BC) formed by *Bacillus cabrialesii* subsp. *cabrialesii* TE3^{T}, *Priestia megaterium* TRQ8, and *Bacillus paralicheniformis* TRQ65. 'Total N applied up to that stage' is the sum of N applications per hectare before the date of the collection of that sample in that stage of development; 'Nitrogen-use Efficiency' is the percentage of the applied nitrogen fertilizer that was recovered by the crop. Different letters in the same row indicate a significant difference, using the Duncan test ($p < 0.05$).

Silveira et al. [45] studied the impact of three diazotrophic endophytic bacteria, IAC-AT-8 (*Azospirillum brasilense*), IAC-HT-11 (*Achromobacter insolitus*), and IAC-HT-12 (*Zoogloea ramigera*), on the metabolism, physiology, and growth of wheat (*Triticum aestivum hard* L.) plants under different levels of nitrogen urea: 0%, 50%, and 100% of the recommended N-fertilizer rate. They found increases in glutamine synthetase (GS) activity in 0% and 50% of the fertilizer (which also exhibited an increment in nitrate reductase—NR—activity) in plants colonized by *A. insolitus.* The GS activity is studied as an auxiliary parameter for the evaluation of N utilization; its increase is related to increments of N content and dry weight of shoot, root, and/or grain.

As previously mentioned, recent works by our group have demonstrated a positive effect of the BC on wheat yield at total N concentrations of 123–225 kg N ha^{-1} in the soil, maintaining good grain quality [5], emphasizing significant differences in inoculated treatments vs. their uninoculated controls (fractionating N: 33%, 33%, and 33% at each fertilization event). Also, increases in nitrogen-use efficiency (14 and 12.5%) at two consecutive agricultural cycles 2018–2019 and 2019–2020 were observed when the N fertilization dose was 120 kg N ha^{-1} (uninoculated and inoculated; NUE of 39.3 and 36.3% in 2018–2019; 46.9 and 42.8% in 2019–2020) vs. the total N rate fertilization of 240 kg N ha^{-1} (both uninoculated and inoculated; NUE of 25.3 and 26.3% in 2018–2019; 34.4 and 21% in 2019–2020) [5].

It is worth noting that fertilization at planting, measured at the tillering stage (Figure 3), showed a very low NUE, with values no higher than 11.9 ± 4.6%, and as low as 3.1 ± 0.6%. During this stage, more N was derived from the soil (from 11.5 ± 2.9% to 23.9 ± 4.7%). This effect could be attributed to the fertilization method, where urea is surface-applied in bands

without irrigation at sowing, combined with root architecture that makes soil-derived N more accessible. These factors likely result in greater N losses from the fertilizer during early growth stages [46].

When high quantities of N are applied at early stages (with low plant uptake capacity), N losses from the soil–plant system are enhanced, which leads to low N recovery efficiency and low yields [32]. In this sense, it is important to note that in this study, when fertilizing with half the recommended N rate with the inoculation of the BC (120 kg N ha^{-1} + BC) significantly fewer nitrogen losses are observed (47.22%) compared to when wheat was fully fertilized (240 kg N ha^{-1}, 65.85%, Table 5 and Supplementary Table S4).

Moreover, it has been reported that the application of 20 to 40% less N (as compared to the recommended dose) can cause a significant increment in NUE of the wheat crop without affecting the yield, and it can reduce N losses [46]. However, at the tillering stage and physiological maturity (Figure 3), all inoculated treatments had a higher total N (from both soil and fertilizer), compared to the uninoculated controls at the same dose. By the milk stage, inoculation of the BC with 120 kg of N ha^{-1} also resulted in higher total N compared to the uninoculated treatment.

It has been reported that PGPB have a great impact on nitrogen use by increasing nitrate (NO_3^-) uptake capacity, whether it is by the direct stimulation of NO_3^- transport systems, or as a result of stimulated lateral root development [47].

Moreover, all treatments at the three studied physiological stages (except the treatment of 240 kg N ha^{-1}, uninoculated, at the milk stage) took more nitrogen from the soil than from the fertilizer (Figure 3), which represents another indicator of the importance of diminishing N fertilizer rates and/or to improve their management, showing, that the use of sustainable agricultural practices is essential.

It is important to highlight that the present investigation is not only about applying the PGPB but also about identifying the suitable conditions that would guarantee their maximum efficiency in the field. One of them is nitrogen management (the dose and fractionation of the applied N). Since there are no significant differences in some parameters due to the inoculation of BC (as the results observed in earlier works [5,19]), the main findings are that the same microorganisms depending on the dose and fractionation of N may or may not have the desired beneficial effect on wheat productivity.

Although conducted over a single agricultural cycle, this study lays the foundation for future research aimed at strengthening the analysis of the observed phenomena through different lines of research. Furthermore, these findings are crucial to refining recommendations on the BC application in conjunction with proper N management, offering farmers potential savings from fertilizer inputs or increased profits from higher wheat productivity in individual crop cycles, while promoting more sustainable practices.

Given that, to increase wheat productivity sustainably, two approaches are of importance: management practices (including crop rotation, cover crops, integrated pest management, N rate, N source, right moment of N fertilization, and N place of application, among others) and plant breeding (developing improved cultivars with desired traits such as tolerance to biotic and abiotic stresses, or high yield and quality, to mention a few). However, to guarantee the expression of genetic potential, most wheat breeding trials are carried out with high levels of N [48]. In this sense, it is recommended to continue testing crop cultivars with low N environments, along with sustainable alternatives such as the inoculation of native microorganisms, to explore their performance on N-limiting conditions, and to harness the potential of the strains, since it has been well documented that the beneficial effect of PGPM on crops is mostly presented with lower doses of N.

4. Conclusions

Bacterial inoculants based on PGPB represent a sustainable alternative to increase crop productivity and diminish the dependence on synthetic inputs, such as nitrogen fertilizers. However, their effectiveness depends not only on the target crop and the microbial inoculant characteristics but also on soil and environmental conditions, as well as management practices. In this regard, the inoculation of wheat with the bacterial consortium used in this study (*Bacillus cabrialesii* subsp. *cabrialesii* TE3^{T}, *Priestia megaterium* TRQ8, and *Bacillus paralicheniformis* TRQ65) had positive effects on the early growth stages, plant height, root depth, and spikes per square meter parameters (when the plants are fertilized at sowing with a fraction of 30% of the total N). However, no increases in grain yield due to the inoculation of the BC were observed when fertilization was reduced completely or by half (0 or 120 kg N ha^{-1}; as already observed in other crop cycles and studies conducted by our research group). This was probably due to the high residual N soil content (183 kg N ha^{-1}) along with the fractionation of N fertilization by 30%, 60%. and 10% (in sowing, stem elongation, and booting), due to the high N input when fertilized with 60% at stem elongation. Furthermore, the BC had no significant effect on the studied quality parameters, which were more stable and influenced by different N doses. Nevertheless, the inoculation of the bacterial consortium on wheat caused more N acquisition from both sources: soil and fertilizer, at different N fertilization rates and growth stages.

Fertilization had a significant effect on nitrogen-use efficiency (NUE) of the whole plant: 120 kg of N ha^{-1} was more efficient (NUE: 44.32%) than the full fertilization dose (NUE: 27.51%). Consequently, significantly greater losses of N from the soil-plant system are observed when wheat was fully fertilized (240 kg N ha^{-1}, 65.85%) compared to fertilization with half the recommended N rate with the BC inoculation (120 kg N ha^{-1} + BC, 47.22%).

Overall, when incorporating the studied bacterial inoculant into wheat crops, it is essential to use appropriate agricultural practices, including N management of no more than 225 kg N ha^{-1} (from both soil and fertilizer) and opt for a nitrogen fertilizer fractionation of 33%, 33%, and 33%. This short-term experiment lays the groundwork for developing more comprehensive and long-term studies that will elucidate effective management practices compatible with bacterial consortium inoculation, ultimately contributing to sustainable agriculture.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app15031429/s1, Supplementary Table S1. Additional climatic conditions during the crop cycle; Supplementary Table S2. Wheat quantitative traits of yield and harvest indexes; Supplementary Table S3. Wheat quality traits of the studied treatments; Supplementary Table S4. Nitrogen (^{15}N) uses the efficiency of wheat under two different doses of urea and the inoculation of a native bacterial consortium.

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