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Original Research

An exploratory assessment of the impact of *Methylobacterium symbioticum* on grain quality and dough rheology of Argentinean bread wheat cultivars

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Abstract: Biological fertilization has emerged as a promising strategy to enhance wheat quality while reducing reliance on synthetic nitrogen fertilizers. This study evaluated the effects of supplemental biological fertilization using *Methylobacterium symbioticum* strain SB23, combined with conventional nitrogen fertilization, on grain quality and dough rheology in three Argentine bread wheat genotypes representing distinct industrial quality groups. A split-plot design with three fertilization treatments—control (C), nitrogen (N), and nitrogen plus biological (NB)—was conducted over a single growing season. Grain quality parameters (protein content, gluten content, Zeleny index, TKW, and TW) and dough properties (SDSS, W, P, L, and P/L) were analyzed. Significant effects of genotype, fertilization, and genotype × fertilization interactions were detected. NB increased protein content in the high-quality genotype (G1) by 1.21% compared to N, and tended to improve gluten and sedimentation values. Dough strength (W) reached its highest values in G1 under NB, suggesting enhanced gluten polymerization in this genotype. G2 also showed improvements under NB, while the low-quality genotype (G3) maintained limited rheological performance regardless of treatment. Kernel traits (TKW, TW) were mostly unaffected by fertilization, likely due to favorable grain-filling conditions. Overall, biological fertilization demonstrates the ability to improve protein quality and dough functionality in genotypes with higher quality potential. These findings suggest that complementing biological and conventional fertilization can improve gluten functionality and dough strength depending on the genotype and the environmental conditions, supporting further investigation of biological fertilization as a component of sustainable, quality-oriented wheat production.

Keywors: Wheat quality, Dough rheology, Biofertilizers, *Methylobacterium*, Gluten strength, Sustainable agriculture

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1. Introduction

Wheat is one of the most important cereals worldwide for human consumption [1]. It is the staple food for 35% of the world's population and represents the main source of plant protein. Additionally, biofortification programs have improved its nutritional profile to combat malnutrition and hidden hunger, particularly in impoverished regions [2]. Its adaptability to diverse production environments and the versatility of its grains, suitable for a wide range of foods—from simple breads to more complex products—have made it a dietary foundation in numerous cultures [3].

Each wheat-derived product requires flour with specific technological properties, including variations in protein percentage and gluten content, ash content, dough strength, tenacity and extensibility, water absorption, and dough stability. Thus, there is no single definition of wheat quality, but rather multiple flour qualities depending on end-use requirements [4].

Wheat quality is largely determined by the quantity and composition of storage proteins—gliadins and glutenins—which form gluten, a viscoelastic matrix responsible for gas retention during fermentation in breadmaking. These protein fractions depend on genotype, environment, agronomic practices, and genotype × environment interactions [5].

In the context of a growing global population, food security requires not only increasing yield per unit area but also maintaining grain and flour quality [6]. Traditionally, achieving high yields and quality has relied on the intensive application of synthetic fertilizers, which can negatively impact water bodies and increase greenhouse gas emissions. Consequently, food security demands shifting beyond raw agronomic yield toward sustainable intensification [7], the circular recovery of value from agro-industrial by-products, and the application of advanced bioprocessing and stabilization techniques to enhance and preserve functional nutrition. In this scenario, evaluating innovative biological tools that optimize plant-microbe interactions during crop development represents a crucial step in exploring strategies to maintain grain industrial quality while potentially reducing environmental trade-offs.

In recent years, the use of biological fertilizers in cereals has expanded, aiming to increase both yield and grain quality while reducing environmental impacts [8]. Combining biological and chemical fertilizers has also demonstrated benefits under water-deficit conditions [9]. Foliar application of nitrogen-fixing bacteria has gained particular relevance due to their ability to associate with plant tissues, potentially contributing to nitrogen availability, producing phytoregulators, and enhancing nutrient uptake [10].

Studies have reported increases in vegetative vigor, yield, and protein content in wheat treated with biofertilizers [10-11]. Additionally, biofertilizers can influence the biochemical composition of wheat grains. Biostimulants combined with conventional fertilization have been shown to improve nutritional quality, including cationic mineral content (Ca, Mg, K), without reducing yield [12]. Under limited N availability, inoculation with nitrogen-fixing bacteria can maintain or improve N and P uptake, thereby contributing to nutrient-use efficiency and supporting reductions in synthetic fertilizer use [13].

Although the benefits of biofertilizers for yield are well documented, their specific effects on the rheological properties that define industrial quality remain poorly understood. Most studies have focused on protein quantity, leaving a significant knowledge gap about how biological inputs influence gluten functionality, such as dough strength and stability, across different genetic backgrounds.

Considering that genotype × environment interactions strongly influence dough quality in bread wheat [14], it is essential to investigate the impact of biological fertilization on grain and flour quality in genotypes with different inherent technological potentials. The objective of this study was to conduct a preliminary assessment of the industrial performance of Argentinean bread wheat genotypes under a sustainable fertilization strategy within a single growing season and regional field conditions. Specifically, we aimed to: (i) identify the impact of *M. symbioticum* on selected grain quality and milling-related indicators; (ii) determine how biological fertilization influences dough strength responses across genotypes with different quality potentials; and (iii) evaluate the potential contribution of the biofertilizer in enhancing gluten functionality compared to conventional nitrogen-only fertilization.

2. Materials and methods

2.1 Field experiment

The analyzed grains were obtained from an exploratory experiment sown during the 2024/2025 growing season, at the experimental farm of the Faculty of Agronomy of Azul (National University of the Center of the Province of Buenos Aires), located at km 307, National Route No. 3 (36º 49' 53'' S latitude, 59º 53' 23'' W longitude). This single-campaign trial was designed as an initial assessment in wheat production conditions in one of the main wheat-growing regions of Argentina, to elucidate the response trends of different Argentine wheat genotypes to supplemental biological fertilization under specific regional conditions.

Sowing was carried out in the first week of July using an experimental seeder. Each plot measured 13.3 m^2, with seven rows at 20 cm spacing. The soil was a typical Argiudoll, with low nitrate and organic matter content; soil analysis prior to sowing (0-20 cm) showed an organic matter content of 3.58 %, available nitrates of 24.42 kg N/ha, and available P of 17.24 ppm. Three bread wheat genotypes (G) released by different seed breeders were used, one from each quality group (QG) determined by the National Seed Institute: Genotype 1: Klein Valor (G1) belonging to QG1 (high baking quality), Genotype 2: Buck Colihue (G2) corresponding to QG2 (intermediate quality), and Genotype 3: Klein Ballesta (G3) corresponding to QG3 (lower baking quality) [15] (Table 1).

Table 1. Expected values of quality attributes for commercial Argentinian genotypes belonging to the three quality groups (QG1, QG2, and QG3)

	TW (Kg/hl)	% P	% G	W (Alveogram, 10E-4J)	P/L (Alveogram)	Stability (Farinogram, minute)	Bread volume (cc)
QG1	> 79	> 11	> 27	340-600	> 1	15-40	> 800
QG2	> 76	> 10.5	> 25	240-340	0.7-1	10-15	> 700
QG3	> 73	> 10	> 22	180-240	0.5-0.8	> 7	> 600

TW: Test weight; %P: Protein percentage; %G: Gluten percentage; W: Dough strength; P/L ratio: Tenacity and extensibility ratio

The experimental design corresponded to Randomized Complete Block Design (RCBD) with a split-plot arrangement and three blocks (B), where the main plot was the genotype (G) and the subplot was the fertilization treatment (F). Blocks were considered random effects in the statistical analysis.

The experiment consisted of three manually applied fertilization treatments. The Control (C) remained unfertilized. The Nitrogen (N) treatment received 150 kg ha^{-1} of nitrogen applied as a urea topdressing, split into 40% at crop emergence (Zadoks 1) [16] and 60% at tillering (Zadoks 2). The Nitrogen plus Biological (NB) treatment received the same urea application as the N treatment, followed by a supplemental application of the bacterial biofertilizer *Methylobacterium symbioticum* strain SB23 (1×10^7 CFU g^{-1}). This biofertilizer was applied at a dose of 333 g ha^{-1} during a more advanced crop stage (stem elongation, Zadoks 3). At physiological maturity, spikes from each plot were harvested manually and threshed using a stationary machine.

2.2 Grain quality

The thousand kernel weight (TKW) was determined manually by counting (four replicates of 100 grains each) and weighing the grains [17]. The protein percentage (%P), test weight (TW), gluten percentage (%G), and Zeleny index were determined by near-infrared transmittance (NIT) with a double-beam monochromator using an Agrichek® device (Bruins Instruments, Germany).

2.3 Dough quality

Milling was carried out in an experimental mill with horizontal blades to obtain whole wheat flour. Then, the sedimentation volume was determined by the sodium dodecyl sulfate sedimentation (SDSS) test, as described by Dick and Quick [18]. The test consisted of hydrating the flour (1 g), mixing it with a lactic acid-sodium dodecyl sulfate reagent, shaking to hydrate all particles, and allowing sedimentation. The starch and soluble proteins (albumins and globulins) dissolve in the solution, while the proteins that form gluten (gliadins and glutenins) absorb water, swell, and precipitate. Therefore, the higher the sedimentation volume obtained, the greater the amount of gluten-forming proteins and the greater their potential contribution to breadmaking quality. The result is expressed as sedimentation volume, which is related to gluten quality.

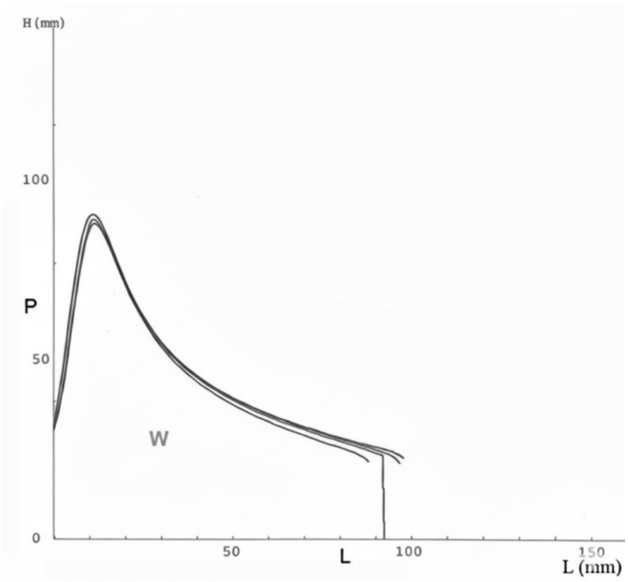

Figure 1. Alveogram indicating its main parameters: dough strength (W), tenacity (P), extensibility (L), and tenacity-to-extensibility ratio (P/L). This analysis corresponded to one replicate of Genotype 1 under the NB treatment

The rest of the grains harvested from each plot were milled in a laboratory mill with grooved rollers (Granotec®, Argentina), and on 250 g of white flour, alveograph tests were performed using a Chopin Alveograph [19], obtaining rheological quality parameters: P or dough tenacity, L or extensibility, W or dough strength, and the P/L ratio. The analysis process consisted of kneading the flour at constant hydration for 8 minutes, then laminating the dough, and subsequently cutting 5 discs, which were left to rest in a special compartment until 28 minutes had elapsed. After that time, air was blown into each disc, producing a bubble, and the pressure required to inflate the bubble until it broke was recorded. The result obtained was a graph called an alveogram, representing the average of the replicates of each analyzed sample, in which the main parameters can be observed: W, P, L, and dough balance ratio (P/L) (Figure 1).

2.4 Statistical analysis

The relative response (RR) of the dough strength (W) was calculated to quantify the variation in the NB treatment relative to treatments C and N, using the formula:

$$RR = (W_{NB} - W_N)/(W_N) \times 100$$

$$RR = (W_{NB} - W_C)/(W_C) \times 100$$

where W_{NB} is dough strength in the biological fertilization treatment (NB); W_N is dough strength in nitrogen fertilizer treatment (N); W_C is dough strength in control treatment (C).

Data normality was assessed using the Shapiro-Wilk test and inspection of Q-Q plots, while homogeneity of variances was evaluated using Levene's test. All measured parameters were subjected to ANOVA, and means were compared using Fisher's LSD test ($\alpha = 0.05$).

To complement the ANOVA and quantify non-significant differences, Cohen's d was calculated as a standardized measure of effect size. The effect size was determined by the difference between treatment means divided by the pooled standard deviation (s_p), where $s_p = \sqrt{MSE}$ (Root Mean Square Error) from the ANOVA. The magnitude of the effect was interpreted following Cohen's benchmarks as general reference values: small ($d = 0.2$), moderate ($d = 0.5$), and large ($d = 0.8$). Statistical analyses were performed using the InfoStat software package [20].

3. Results

Both the thousand kernel weight (TKW) and the test weight (TW) are indirect estimators of milling yield, representing the amount of flour obtainable from the grains. According to the analysis of variance (ANOVA), TKW was significantly influenced only by the genotype effect ($p < 0.01$), with G3 consistently exhibiting the highest grain weight values (Table 2). Conversely, neither the fertilization treatments nor the G × F interaction exerted a significant effect on TKW ($p > 0.05$). However, a numerical difference of approximately 2 g was observed in G3 between the NB and N treatments, although it was not statistically significant. Although this difference was not statistically significant ($p > 0.05$), a moderate effect size was observed (Cohen's $d = 0.67$). While this value indicates an observed numerical tendency toward increased grain weight under NB treatment, the limited scale of the trial does not allow conclusions regarding the consistency of this response. Further studies would be required to confirm this observation.

Table 2. Mean squares and significance levels, where applicable, for parameters of grain quality and dough quality, according to sources of variation: genotype (G), fertilization treatment (F), and their interaction (G × F)

	TKW	%P	TW	%G	Z	SDSS	W	P	L	P/L
G	138.35**	3.47**	54.9	23.3	490.37*	310.78**	30160.7***	19434.04***	8551.59***	114.07***
F	8.36	4.48***	24.52	48.85***	620.43**	100**	11368.26***	5223.81***	229.59***	11.57***
G × F	2.71	0.77**	21.22	2.14	56.57	4.94	4140.43***	2105.7***	75.15***	4.64***

TKW: thousand kernel weight; %P: protein percentage; TW: test weight kg/hl; %G: gluten percentage;
Z: zeleny index; SDSS: sedimentation volume; W: dough strength; P: tenacity; L: extensibility; P/L ratio;
Significance levels: "***" $p < 0.001$; "**" $p < 0.01$; "*" $p < 0.05$; ns: not significant ($p > 0.05$)

Test weight (TW) did not differ significantly among genotypes or fertilization treatments, indicating stability of this parameter under the conditions tested. Grain weight in wheat is influenced by both genotype and environmental conditions, particularly during the grain filling period, which occurs after flowering. In this experiment, flowering took place during the first 10 days of November, when water availability was relatively favorable due to cumulative rainfall of 234 mm (Figure 2).

Regarding the environmental conditions during the crop cycle, it is important to note that although the grain-filling stage was well-supplied with water and experienced moderate maximum temperatures (below 30 °C)—conditions considered optimal for protein and carbohydrate accumulation—the vegetative stage (from sowing in July until flowering) also saw moderate temperatures but only accumulated slightly more than 122 mm of rainfall. This limited water availability during the early stages may have restricted overall crop yield per unit area (g·m^{-2}) due to a potential reduction in spikes per unit area, rather than affecting individual grain parameters (data not shown).

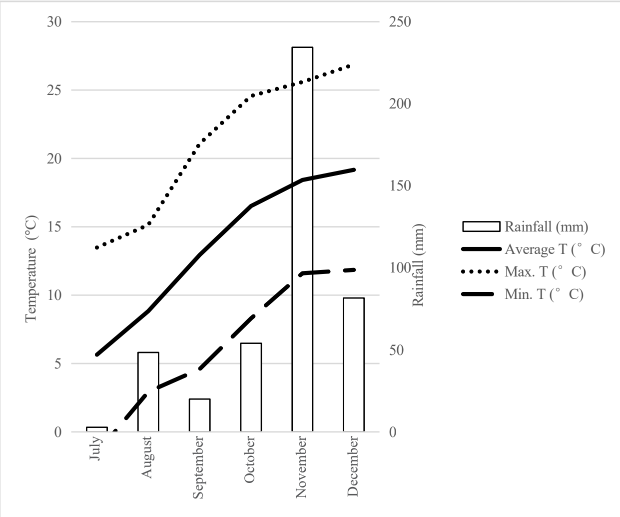

Figure 2. Maximum, mean, and minimum temperatures (°C) and cumulative rainfall (mm) during the crop cycle (July to December) at the experimental site

In terms of grain quality, significant G × F interactions reflected differential protein and gluten accumulation (Table 2). The NB treatment was associated with improved protein content in high-quality genotypes; specifically, G1 achieved *a* 1.21% increase in protein content compared to the N treatment (Figure 3A). While increases in gluten content and sedimentation volumes (SDSS and Zeleny) under biological fertilization were not always statistically significant, the NB group showed numerically higher values than urea alone across these parameters (Figure 3B, 3C).

Rheological parameters obtained from the Chopin Alveograph also varied significantly among genotypes, fertilization treatments, and their interaction (G × F). Significant differences were found in dough strength (W), tenacity (P), extensibility (L), and P/L ratio, indicating that flour quality, and consequently its suitability for specific baked products, depends on both genotype and fertilization practice (Table 2).

As expected for a QG1 genotype, G1 showed the highest W values and a favorable P/L balance under the NB treatment (Table 1, Figure 4A-C).

Genotype 2 also displayed significant differences in W among treatments, with NB showing higher values than both N and Control treatments. The P/L ratio ranged from 2.16 to 3.41, reaching its highest values under NB. Conversely, Genotype 3, which has lower inherent technological quality, showed the lowest W values under NB and Control, while W under N was slightly higher (272 × 10^{-4} J). This genotype exhibited the highest P values in both fertilized treatments and the lowest L values across all treatments, resulting in the highest P/L ratios and indicating greater dough tenacity (Figure 4A-C).

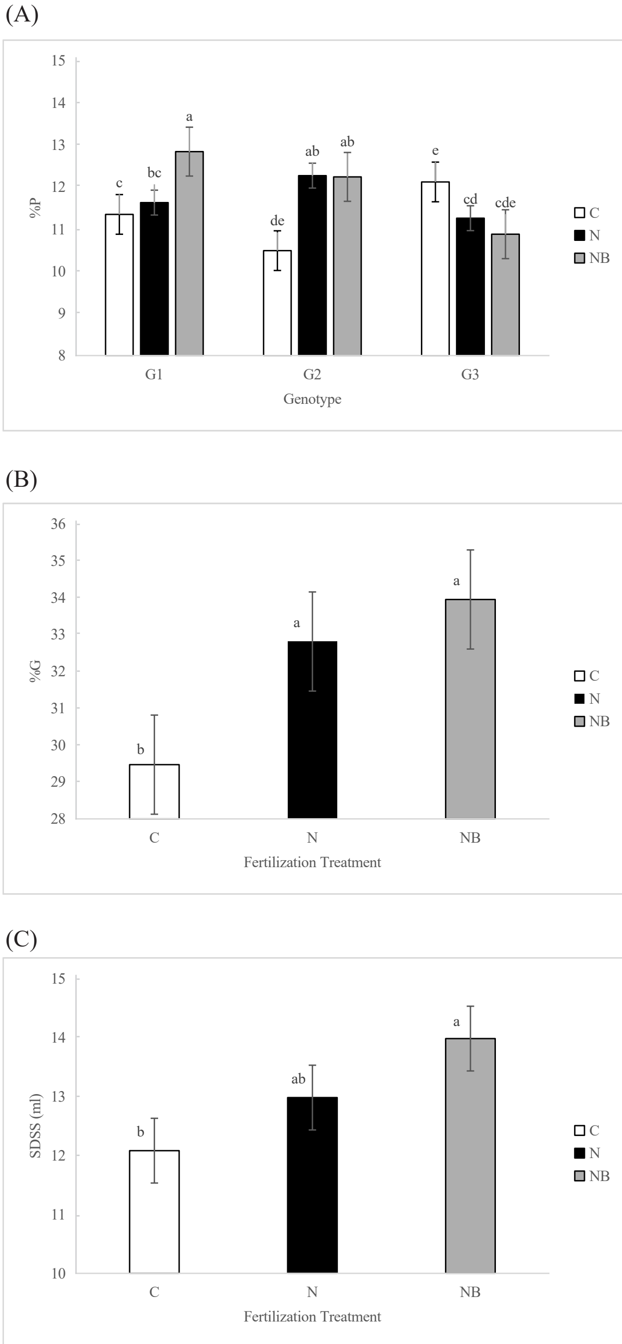

Figure 3. A-Protein percentage (%P) for genotypes 1, 2, and 3 across fertilization treatments (C, N, NB); B-Gluten percentage (%G); C-Sedimentation volume (SDSS, mL). Panels B and C highlight the main effect of fertilization. The letters above the bars indicate differences among treatments according to Fisher's least significant difference (LSD) test ($\alpha = 0.05$). Means sharing a common letter are not statistically different

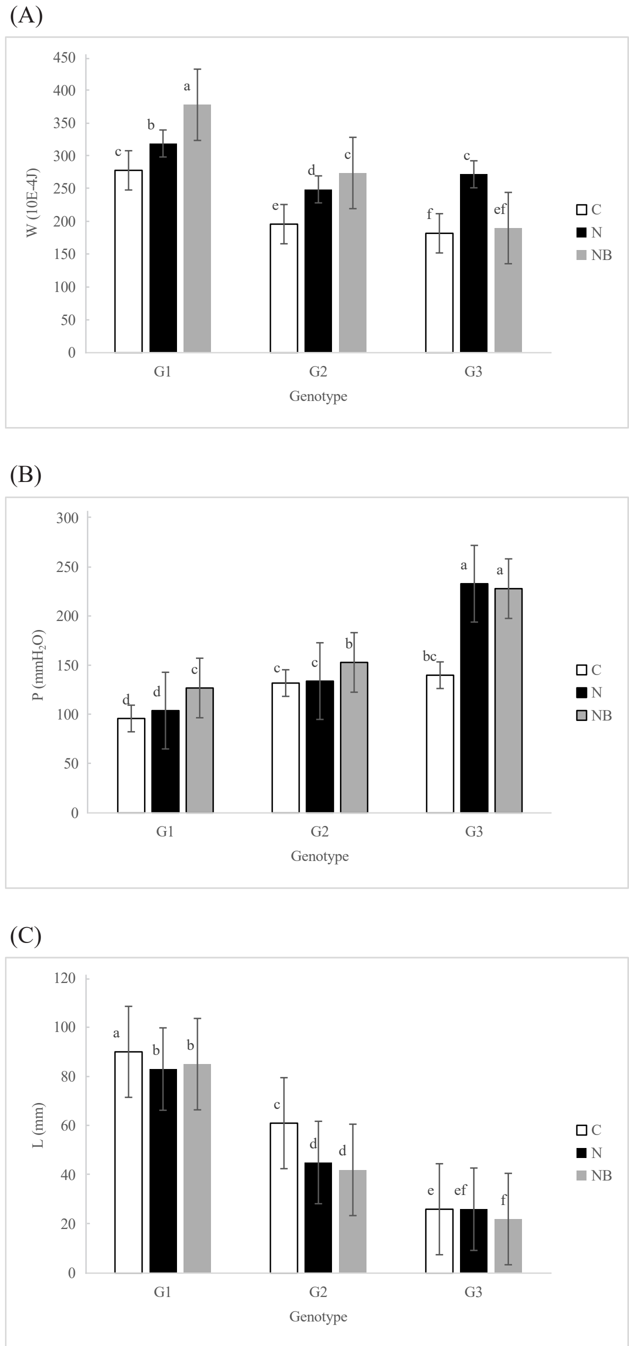

Figure 4. A-Dough strength (W); B-Tenacity (P); C-Extensibility (L) for genotypes (G) 1, 2, and 3 across fertilization treatments (C, N, NB). The letters above the bars indicate significant differences among treatments according to Fisher's least significant difference (LSD) test ($\alpha = 0.05$). Means sharing a common letter are not statistically different

Furthermore, considering the importance of dough strength (W) for the industrial processing of wheat flour, the response of the NB treatment was calculated with respect to treatments C and N for each genotype. The relative response to the biofertilizer (NB) treatment varied among the three genotypes evaluated. In G1, RR values were positive and ranged from 17.45% to 36.20%, indicating positive numerical changes in W. G2 also exhibited positive responses, within a wider range (8.16% to 43.59%), across all comparisons, although the magnitude was lower than that observed in treatment N. In contrast, G3 exhibited a heterogeneous response, including declines compared to the N treatment. Overall, these results suggest that the two genotypes with higher potential quality (quality groups 1 and 2) responded more favorably under the NB treatment, while the genotype belonging to quality group 3 showed a variable response, with moderate increases in some cases and declines in others.

4. Discussion

This study analyzed the effect of supplemental biological fertilization alongside conventional nitrogen fertilization, commonly used in Argentina, on grain quality and dough rheological properties in three Argentinian bread wheat genotypes, representing different quality groups, i.e., with varying potential quality (Table 1). Although the experiment was carried out during a single agricultural campaign, which inherently limits the assessment of multi-year environmental stability, the results on quality address a little-explored knowledge gap, showing potential effects associated with the complementary application of biological products on industrial quality parameters. By framing this work as an exploratory case study, the focus is on highlighting physiological and technological trends associated with the application of *Methylobacterium symbioticum* under specific regional conditions, serving as a necessary basis for future research.

Results showed significant effects of genotype, fertilization treatments, and the G × F interaction for most evaluated parameters (Table 2), confirming the strong genetic influence on final wheat quality and its interaction with environmental and agronomic management factors [14].

The TKW differed mainly due to genotype, with G3 showing the highest kernel weight. Although the biofertilizer treatment (NB) slightly increased the grain weight of G3 compared to the nitrogen-only treatment (N), these differences were not statistically significant. However, the moderate effect size (Cohen's $d = 0.67$) suggests a meaningful biological trend, though this single-season result warrants cautious interpretation given the limited replication. In these preliminary results, the use of effect size indices provided key insights into the magnitude of treatment differences, although further multi-year studies are required to confirm this response. Similar findings have been reported by Fasani et al. [12], who demonstrated that biofertilizer combined with conventional fertilization improved aspects of grain composition without necessarily altering grain size.

The absence of differences in test weight (TW) between treatments and genotypes indicates high stability for this parameter, which is often determined more by environmental and sanitary conditions during grain filling than by fertilization [4]. Environmental and edaphic conditions at the experimental site partially explain this stability. The crop was grown on a typical Argiudoll soil, with low initial nitrate and organic matter content—conditions that favor crop responsiveness to microorganism-based biofertilizers [21]. However, grain filling coincided with exceptionally high water availability (234 mm accumulated in November, Figure 2), which promoted dry matter accumulation and contributed to high TKW values regardless of treatment. Previous studies indicate that, in wet years, water availability can exert a dominant control over grain size and weight, often outweighing fertilization effects [3]. This suggests that, in this study, treatments were more closely associated with changes in dough rheological quality, while environmentally sensitive parameters, such as grain weight, remained stable due to favorable climatic conditions. Furthermore, Basile et al. [22] analyzed the period from 1931 to 2014 for the study region, estimating increases of up to 42.7% (152 mm) for accumulated precipitation during the flowering stage of wheat (November). Overall, under climate change scenarios with increased spring precipitation, treatment effects on grain weight may be masked by climatic conditions, whereas fertilization strategies and biological inputs may become more relevant for modulating grain quality rather than yield components.

Significant increases in protein percentage (%P) were observed in G1 under the NB treatment compared to N and C (Figure 3A). This implies higher commercial value for the grains under Argentine INASE standards [23], since protein content above 11% receives a price premium (Figure 3A). This aligns with studies showing that nitrogen-fixing bacteria applied during the wheat growth cycle can enhance grain protein accumulation by improving nitrogen availability and assimilation at critical stages [12]. Although the gluten percentage (%G) also increased under NB (Figure 3B), differences were not statistically significant compared to N, suggesting that biological fertilization may have contributed to reserve protein accumulation. This trend was further supported by sedimentation values in both Zeleny and SDSS tests, where both fertilized treatments exceeded the control (Figure 3C). This response likely stems from the ability of *M. symbioticum* to colonize the phyllosphere and actively participate in nitrogen metabolism, thereby enhancing nitrogen availability during grain protein accumulation.

The potential contribution of *M. symbioticum* in improving wheat quality may be associated with its unique colonization mechanism. As an aerobic, endosymbiotic α-proteobacterium, it enters the plant through the stomata and colonizes the intercellular spaces of the mesophyll. This bacterium establishes a systemic presence within the phyllosphere, utilizing methanol—a byproduct of pectin metabolism during cell wall expansion—as a carbon and energy source. This endophytic niche has been reported to enable the bacteria to fix atmospheric nitrogen directly within the leaf tissues through the nitrogenase enzyme complex. In this study, the foliar application at the stem elongation stage (Zadoks 3) coincided with the period of high nitrogen demand. The observed increases in protein and gluten strength may suggest that biologically fixed nitrogen contributed to nitrogen availability during the critical window of grain protein deposition, potentially reducing the dependence on root-derived nitrogen sources.

Rheological parameters measured by the Chopin Alveograph (W, P, L, and P/L) differed significantly between genotypes and treatments, with a marked G × F interaction (Table 2). These results indicate that biological fertilization not only altered grain protein composition but also influenced gluten functionality, a key factor in bread-making quality [8].

Dough strength (W) is highly valued by the industrial baking sector. Together with the P/L ratio, it determines the potential end-use of the flour. Flour blends are often required to achieve target W values for specific products. Moreover, wheat producers may receive a premium for grain exceeding a defined W threshold.

Genotype 1, classified as having high potential baking quality, exhibited the highest dough strength (W), particularly under the NB treatment (Figure 4A). This increase suggests that biological fertilization may have promoted changes associated with the synthesis or polymerization of high-molecular-weight glutenins (HMW-GS), which are responsible for gluten strength. The significant increase in W and sedimentation volume (SDSS) observed, particularly in genotypes with high-quality potential (G1 and G2), suggests a targeted effect on gluten protein accumulation and functionality. Wheat technological quality is fundamentally governed by the ratio and composition of gliadins and glutenins. While gliadins contribute to dough viscosity and extensibility (L), glutenins—specifically HMW-GS—are the primary determinants of the elastic network and gluten strength (W) [14]. The complementary nitrogen provided by the endophytic fixation of *M. symbioticum* may have contributed to improved nitrogen availability and partitioning toward the grain during late development stages. This additional N availability could have favored the synthesis of HMW-GS over gliadins or promoted a higher degree of glutenin polymerization. As established by Arrigoni and Arata [14], the presence of specific allelic combinations in modern Argentinean cultivars, such as those in QG1, provides a genetic scaffold that is highly responsive to nitrogen management. Similar results were reported by El-Akhdar et al. [21], showing that microbial consortia can modify gluten subunit ratios, improving dough viscoelasticity without increasing total gluten.

Genotype 2 also showed a significant increase in W and P/L under NB (Figure 4A, B), indicating stronger and more balanced dough—desirable characteristics for certain bakery products. The positive response across genotypes of differing potential quality suggests a genotype-dependent effect of the biofertilizer, as reported in previous studies on cereal biofertilization [11-12]. Conversely, G3, classified as having lower industrial quality by INASE, exhibited the lowest W and L values and the highest P and P/L values, regardless of treatment (Figure 4A, B, C). This confirms that certain rheological traits are strongly genetically determined and less sensitive to nutritional practices [24]. Nevertheless, the NB treatment did not deteriorate these rheological parameters, indicating that biological fertilization is compatible with lower-quality genotypes without compromising dough quality.

From an agronomic perspective, these results are promising for systems aiming to advance sustainable agriculture without compromising food security. Combining nitrogen fertilization with biofertilizers may improve grain quality without increasing synthetic fertilizer doses, thereby reducing environmental impacts associated with excessive fertilization [8].

The results of this study show that the relative response of the bread-making quality parameter (W) to the biofertilizer (NB) treatment was genotype-dependent, demonstrating consistent increases in two groups and a variable response in the third. In the groups with uniformly positive responses, the observed increases suggest improved nitrogen utilization or enhanced grain protein accumulation, an effect documented in crops inoculated with growth-promoting microorganisms [7].

For the milling and baking industry, improvements in dough strength (W) and P/L ratio through biofertilizer use represent an opportunity to obtain flours that produce higher-quality dough without relying solely on superior genetics or high nitrogen inputs.

The improvements observed in dough strength (W) and gluten polymerization stability after the application of *M. symbioticum* suggest that the treatment may support nitrogen assimilation and partitioning to the grains. Although this study did not evaluate gene expression, recent literature [10] indicates that endophytic colonization by these bacteria activates metabolic pathways linked to glutamine synthetase, increasing nitrogen use efficiency (NUE). In the present work, the significant changes in rheological parameters and electrophoretic profiles act as functional indicators of increased synthesis of high molecular weight glutenins. The variability in response among genotypes could be attributed to a differential genotype-bacteria interaction, where the canopy architecture and foliar receptivity of each cultivar would determine the rate of biological nitrogen fixation and its subsequent impact on technological quality under the specific conditions tested.

Although this study was conducted in a single location and season, making it desirable to evaluate response stability under different environments and climatic conditions—especially in years with lower water availability—these preliminary findings are promising for one of the most important food sources for human populations.

5. Conclusion

The results suggest that biological fertilization can enhance protein quality and gluten functionality, with especially promising effects in genotypes with high potential quality. Although these findings derive from a single-season exploratory assessment, they indicate that biofertilizers could constitute a tool to support the sustainability of the Argentinian wheat system while potentially improving industrial grain quality. In this case, supplementing conventional nitrogen fertilization with biological fertilization not only improved grain protein-related quality traits but also enhanced flour functionality and dough rheology, which could potentially contribute to better breadmaking performance. This aligns with previous research demonstrating that specific agronomic strategies can influence the technological properties of wheat flour and provide practical benefits for the baking industry. Ultimately, this case study establishes a valuable baseline for future multi-year regional networks aimed at maintaining industrial wheat grain quality while potentially reducing environmental impacts through more efficient nutrient management, aligning with sustainable intensification goals, which is especially important in a global context where wheat quality stability depends on more efficient and resilient management practices amidst climate change.

Author contributions

A. Arrigoni, A. Arata, M. Martínez, and L. Lázaro: Conceptualization, Methodology, Formal analysis, Investigation, and Writing (Original Draft, Review & Editing). A. Arrigoni and L. Lázaro also provided project supervision. Y. Blando, M. Sañico, M. Lugo, and J. Allende: Participated in the experimental investigation and data collection. All tables and figures were prepared by the authors.

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Conflicts of interest

The authors declare no conflicts of interest.

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