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RESEARCH PAPER

Bio-seed dressing agent promotes wheat growth and improves fertilizer use efficiency

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Abstract

Efficient and safe bio-seed dressing agents are urgently needed to replace traditional chemical ones in wheat production. Here, we evaluated and compared the field performance of a novel bio-seed dressing formulated with five bacterial strains typically used as animal probiotics against a conventional chemical seed dressing (comprising epoxiconazole, imidacloprid, and chlorpyrifos) in wheat plot trials. Compared with both the undressed control and the chemical treatment, the bio-dressing significantly increased wheat biomass as well as N, P and K accumulation, raising the theoretical grain yield by 8.3% and 10.2%, respectively. Further analyses revealed that the bio-dressing increased both the diversity and abundance of rhizosphere soil bacteria. The Chao1 index (5258.27 ± 87.14) was significantly higher than that of the undressed control (5086.20 ± 29.38) and the chemical treatment (4891.56 ± 87.66). The Shannon index (6.87 ± 0.04) was also significantly higher than that of the undressed control (6.80 ± 0.03) and the chemical treatment (6.82 ± 0.03). The bio-dressing enhanced total N and improved the availability of P and K in rhizosphere soil, thereby promoting mineral nutrient uptake by wheat. These results demonstrate that the bio-seed dressing agent can effectively replace its chemical counterpart to promote wheat growth and increase fertilizer use efficiency.

Keywords

Animal probiotics, bio-seed dressing agent, mineral element accumulation, soil microorganisms, wheat

Introduction

Seed dressing or coating with seed dressing agents is an innovative and sustainable approach for improving seed quality and crop performance (Javed et al. 2022). Chemical seed dressing agents are predominantly employed. While these agents promote uniform and vigorous seedling establishment and protect against pests and diseases (Lacerda et al. 2021; Yadav et al. 2023; Rossini et al. 2024), they also have significant drawbacks. These include toxicity to humans, livestock, and non-target organisms, incompatibility with beneficial organisms and high pesticide residues (Douglas and Tooker 2016; Van Hoesel et al. 2017; Lamichhane et al. 2020). Consequently, there has been a rapid global development of beneficial microbial seed dressings based on biofertilizers and biopesticides (Afzal et al. 2020).

Wheat is the second most widely grown grain crop in the world. Seed dressings prepared with different microbial consortia have varying effects on wheat. One of the earliest bio-seed dressing agents used on wheat consisted of *Bacillus subtilis*, *Paenibacillus mucilaginosus*, and *Streptomyces microflavus*. This dressing improved the germination rates, promoted tillering, increased the number of productive spikes, and enhanced yields by up to 22.8% (Zhang et al. 2012). Microencapsulation of *Pontibacter actiniarum*, which was isolated from kelp and introduced

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with wheat seeds, promotes seed germination and seedling growth under salt stress (Gong et al. 2023). *Trichoderma atroviride* HB20111 seed dressing effectively reduces soil-borne diseases, though wheat yield is similar to the control or chemical seed dressing (Sui et al. 2022). A bio-coating agent containing *Azotobacter vinelandii*, *B. megaterium*, *B. mucilaginosus*, and *S. jingyangensis* improves nutrient absorption in wheat, increasing yield by 13.8% and protein content by 0.6%–1.5% (Fang et al. 2004). Seed dressing with the biocontrol fungus *T. harzianum* increases wheat emergence and tiller number, achieves an average control efficacy of over 60% against wheat sheath blight and over 65% against wheat basal stem rot, and improves yield by 4.3%–6.34% (Hu et al. 2021).

Similar to plant growth-promoting microorganisms, the application of animal probiotics, including *Lactobacillus*, *Bifidobacterium*, *Bacillus*, and yeasts, has become increasingly widespread. These microorganisms contribute to host nutrition by producing essential digestive enzymes and vitamins, thereby enhancing digestion and nutrient absorption (Bhogoju and Nahashon 2022; Madhulika et al. 2025). Upon colonizing or transiting through the gastrointestinal tract, animal probiotics produce antimicrobial and metabolic compounds that suppress pathogenic microorganisms (Anee et al. 2021). In addition, they interact with intestinal immune cells to modulate both innate and adaptive immune responses (Kober et al. 2022).

Accumulating evidence indicates that certain animal probiotics also possess plant growth-promoting attributes. For example, *B. subtilis* has been reported to function as an effective biocontrol agent against plant diseases (Leclère et al. 2005). *B. licheniformis* demonstrates the potential to stimulate plant growth while suppressing fungal and bacterial pathogens (Medison et al. 2023). Moreover, *B. velezensis* can efficiently colonize the plant rhizosphere, thereby promoting root development and nutrient uptake (Yao et al. 2006), and providing strong biocontrol activity against phytopathogens (Elanchezhiyan et al. 2018). However, it is unclear whether the commonly used animal probiotics *Weizmannia coagulans* and *Saccharomyces cerevisiae* var. *boulardii* have this potential. This study investigates the field performance of a bio-seed dressing formulated with the animal probiotics *B. subtilis*, *B. licheniformis*, *B. velezensis*, *W. coagulans*, and *S. cerevisiae* var. *boulardii* in wheat cultivation.

Materials and methods

Wheat variety and seed dressings

The wheat variety used was Bainong 207 (Suppl. material 1: table S1). The self-prepared bio-seed dressing (GB) contained *B. subtilis*, *B. licheniformis*, *B. velezensis*, *W. coagulans*, and *S. cerevisiae* var. *boulardii*, with no antagonistic effects observed among the five strains. The commercial chemical seed dressing (SW) consisted mainly of prothioconazole, imidacloprid, and chlorpyrifos.

Experimental design

The field experiment was conducted at the Yuanyang Science and Education Park of Henan Agricultural University. The soil used in the experiment was a typical sandy fluvo-aquic soil with the following basic physicochemical properties: 17.2 g/kg of organic matter, 1.0 g/kg of total nitrogen, 71.5 g/kg of available nitrogen, 21.3 mg/kg of available phosphorus, 106.4 mg/kg of available potassium, and a pH of 7.2. Prior to sowing wheat, a wheat-specific compound fertilizer ($N:P_2O_5:K_2O = 20:16:16$) was applied at a rate of 750 kg/hm^2 as a basal fertilizer. Three treatments were established: CK (control), GB (bio-seed dressing), and SW (chemical seed dressing). Each treatment was replicated three times, for a total of 18 plots. Each plot measured 1.8 m × 20 m.

Sample collection

Wheat plants and their rhizosphere soils were sampled at the jointing, flowering, and maturity stages. A five-point sampling method was employed in each plot. At maturity, wheat plants were randomly collected from three 1 m^2 areas within each plot.

Determination of nutrient elements contents in soil and wheat

The nitrogen content of the rhizosphere soil and the wheat were determined using the Kjeldahl method. The available potassium and available phosphorus in rhizosphere soil were analyzed using ammonium acetate extraction and atomic absorption spectrometry (Suppl. material 1: table S1), and the sodium bicarbonate extraction and molybdenum-antimony colorimetric methods, respectively. Wheat potassium and phosphorus contents were measured using flame photometry and the molybdenum-antimony colorimetric method, respectively. Soil microbial biomass nitrogen was determined using the chloroform fumigation-extraction method.

Determination of agronomic traits in wheat

Wheat plants with roots thoroughly washed were first inactivated at 105 °C for 1 h and subsequently dried at 75 °C to a constant weight to determine root and shoot dry biomass. At maturity, the number of effective spikes per plant was recorded. After drying and threshing, the number of grains per spike and the thousand-grain weight were measured. The theoretical grain yield was then estimated based on the effective spike number, grains per spike, and thousand-grain weight as follows:

$$Y = S \times G \times T$$

where Y is the theoretical grain yield, S is the number of effective spikes per plant, G is the number of grains per spike, and T is the thousand-grain weight.

The accumulation of nitrogen, phosphorus, and potassium in wheat shoots or roots (kg/hm²) was calculated as:

Nutrient accumulation (kg/hm²) = Dry matter accumulation (kg/hm²) × Nutrient content (g/kg)/1000

Metagenomic analysis of rhizosphere soil at the wheat flowering stage

A 0.5 g soil sample was used for DNA extraction with a soil DNA isolation kit. Universal primers 338F and 806R (Suppl. material 1: table S2) were used to amplify the V3–V4 region of the bacterial 16S rRNA gene to analyze the bacterial community structure. Universal primers ITS1 and ITS2 (Suppl. material 1: table S2) were used to amplify the ITS region of the fungal gene to analyze the fungal community structure. Sequencing libraries were constructed, and the raw reads underwent quality control, chimera removal, and subsequent taxonomic classification for downstream community analysis. Alpha diversity of the bacterial and fungal communities in the rhizosphere soil was analyzed using the Chao1 and Shannon indices with QIIME2 software and visualized with the ggplot2 package (Wickham 2016).

Data analysis

Data are presented as the mean ± standard deviation (SD) of three independent biological replicates. Data organization, statistical analysis, and visualization were performed using GraphPad Prism (v10.1.2). Differences among treatments were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Pearson's correlation analysis between rhizosphere soil parameters and wheat agronomic traits was performed using the TUTOOL online platform. Statistical significance was defined at $P < 0.05$ (95% confidence level).

Results and discussion

Impact of bio-seed dressing agent on mineral nutrients and microbial biomass nitrogen in the wheat rhizosphere

Rhizosphere soil samples were collected at the jointing, flowering, and maturity stages to determine soil mineral elements and microbial biomass nitrogen (MBN). Total nitrogen showed no significant difference between the control (CK) and GB-treated soil at the jointing stage; however, it was higher than in the SW-treated soil. At the flowering stage, no differences were observed among the treatments. At the maturity stage, the GB treatment was 11.1–14.2% higher than the CK and SW treatments (Fig. 1A). MBN in GB-treated soil was consistently higher than in CK and SW soil at all growth stages. Increases were 23.8–56.4% at jointing, 28.3–65.8% at flowering, and 17.5–21.3% at maturity (Fig. 1B). Available phosphorus showed no difference between GB and SW at jointing; both were 15.0–26.9% higher than CK. At flowering and maturity, GB was slightly higher than CK and SW, though not significantly (Fig. 1C). Avail-

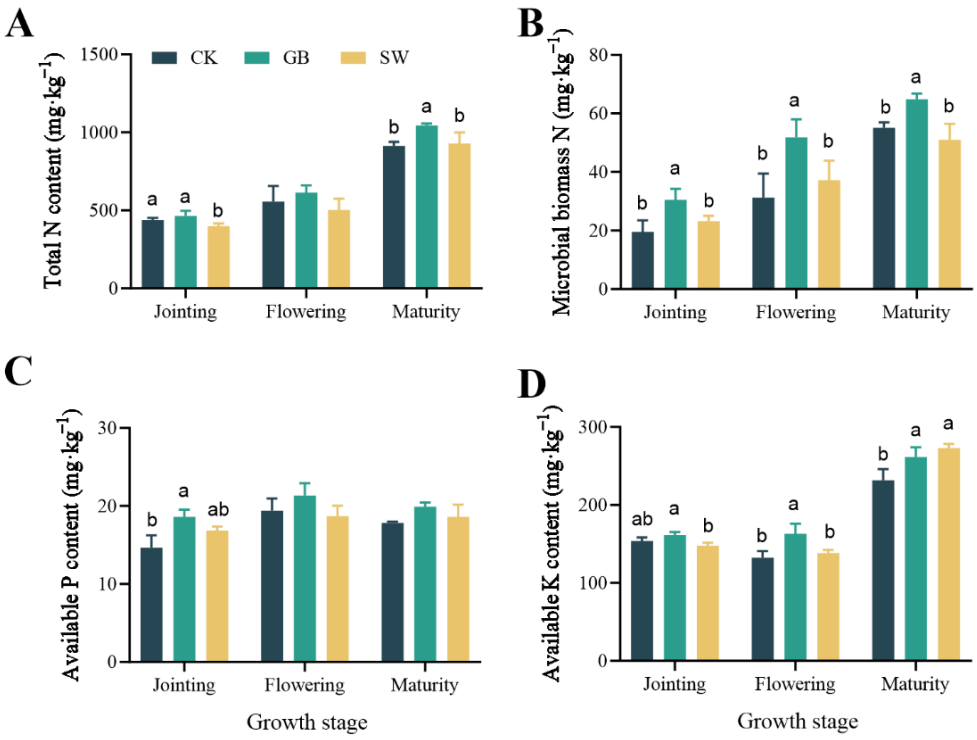

Figure 1. Mineral elements and microbial biomass nitrogen content in rhizosphere soil of wheat at different growth stages. CK, undressed control; GB, bio-seed dressing; SW, chemical seed dressing. Data are presented as the mean ± standard deviation (n = 3). Different lowercase letters indicate significant differences within the same growth stage ($p < 0.05$).

able potassium in GB-treated soil was 8.4% higher than in SW-treated soil at jointing and did not differ from CK. At flowering, GB exceeded CK and SW by 15.2–23.3%, and at maturity, GB and SW were similar, both being 12.9–17.8% higher than CK (Fig. 1D).

Impact of bio-seed dressing agent on wheat growth

To determine shoot and root dry weight, as well as N, P, and K accumulation, wheat plants were sampled at the jointing, flowering, and maturity stages. At all stages, the order of shoot dry weight was GB > SW > CK. GB and SW were 7.9%–25.4%, 13.8%–36.7%, and 18.0%–27.1% higher than CK, respectively (Fig. 2A). Root dry weight under GB was 21.0% higher than CK and 26.7%–38.0% higher than SW at the jointing and flowering stages. At maturity, GB and SW were 14.8%–26.2% higher than CK (Fig. 2B). Shoot N accumulation under GB exceeded that under CK and SW by 22.1%–38.7% and 27.3%–79.6%, respectively, at jointing and flowering and by 13.2% at maturity (Fig. 2C). Root N accumulation was 20.8%–21.4% higher than CK at jointing, 37.7% higher at flowering, and 33.9%–38.9% higher at maturity (Fig. 2D). Shoot P under GB was 15.7%–20.4%, 24.2%–38.1%, and 16.2%–23.0% higher than CK and SW at the three stages (Fig. 2E). Root P under GB was 14.9%–17.2%, 19.6%–44.2%, and 23.7%–57.0% higher (Fig. 2F). Shoot K increased by 16.8%–21.0% (jointing: GB vs. CK and SW), 9.9%–36.2% (flowering: GB vs. CK and SW), and 13.6%–44.4% (maturity: GB vs. CK and SW) (Fig. 2G). Root K increased by 27.0%–35.1%, 21.5%–42.7%, and 21.8%–67.6% under GB compared to CK and SW at the three stages (Fig. 2H).

Impact of bio-seed dressing agent on the yield and quality of wheat

The theoretical yield of wheat under GB was 8.3% and 10.2% higher than under CK and SW, respectively (Fig. 3A). Grain N and P contents were slightly higher in GB than in CK and SW, but the differences were not significant (Fig. 3B, D). Grain K content under GB was 43.3% higher than under SW (Fig. 3C).

Correlation analysis between rhizosphere soil indices and wheat traits

Correlation analysis revealed significant positive relationships between rhizosphere soil mineral elements, microbial biomass N, as well as most wheat traits. Shoot dry weight as well as shoot N, P, and K accumulation were positively correlated with soil and wheat indices ($p < 0.01$), except for root K accumulation. Root dry weight and root N, P, and K accumulation also showed positive correlations ($p < 0.05$) with the exception of soil available K. Soil total N was positively correlated with wheat traits ($p < 0.05$) with the exceptions of soil available P, root K accumulation, and root P accumulation. Soil available K was positively correlated ($p < 0.05$) with most wheat traits, except for root dry weight, root N accumulation, and root K accumulation. Soil available P was positively correlated ($p < 0.01$) with most wheat traits, except for soil total N, available K, and root K accumulation (Fig. 4).

Impact of bio-seed dressing agent on the rhizosphere microbial community at wheat flowering stage

Soil microbial community α diversity

The α diversity of bacterial and fungal communities in wheat rhizosphere soil at the flowering stage was analyzed based on 16S rRNA and ITS amplicon sequencing data. The Chao1 index, which indicates species richness, showed that bacterial richness followed the order GB (5258.27 ± 87.14) > CK (5086.20 ± 29.38) > SW (4891.56 ± 87.66) (Fig. 5A), whereas fungal richness followed the order SW > GB > CK (Fig. 5B). The Shannon index, which evaluates species diversity, showed that bacterial diversity was higher in GB (6.87 ± 0.04) than in CK (6.80 ± 0.03) and SW (6.82 ± 0.03) (Fig. 5C). However, fungal diversity was higher in CK and GB than in SW (Fig. 5D).

Microbial species composition

During the flowering stage, no differences in the relative abundance of bacterial taxa were observed at the phylum level in the wheat rhizosphere soil among CK, GB, and SW. The most abundant phyla were Pseudomonadota, Acidobacteriota, Actinomycetota, and Bacteroidota (Fig. 6).

During the flowering stage, the most abundant fungal genera in the wheat rhizosphere soil were *Alternaria*, *Fusarium*, *Linnemannia*, and *Pyrenochaetopsis*. *Alternaria* was more abundant in SW than in CK and GB, while *Sporothrix* was more abundant in GB than in CK and SW (Fig. 7).

The effects of a bio-seed dressing agent on rhizosphere microbial communities have not been reported previously. Chemical seed dressings, such as a mixture of 10% fluquinconazole, fluopyram, and thiamethoxam, increase fungal Shannon diversity without significantly affecting bacterial alpha diversity or fungal richness (Chen et al. 2024). In this study, the chemical dressing SW (pyraclostrobin-imidacloprid-chlorpyrifos) decreased bacterial Chao1 richness and increased fungal Chao1 richness. It also reduced fungal Shannon diversity. In contrast, the bio-seed dressing GB enhanced the bacterial Chao1 and Shannon indices. It increased fungal Chao1 richness and had no significant effect on fungal Shannon diversity. These results suggest that bio-seed dressings promote bacterial α diversity and have varying effects on fungi. Furthermore, GB significantly increased MBN in the wheat rhizosphere. MBN was positively correlated with most soil and wheat traits, except for root K accumulation. This suggests that increased microbial abundance may contribute to higher levels of soil total N, available P and K, wheat biomass, and nutrient accumulation.

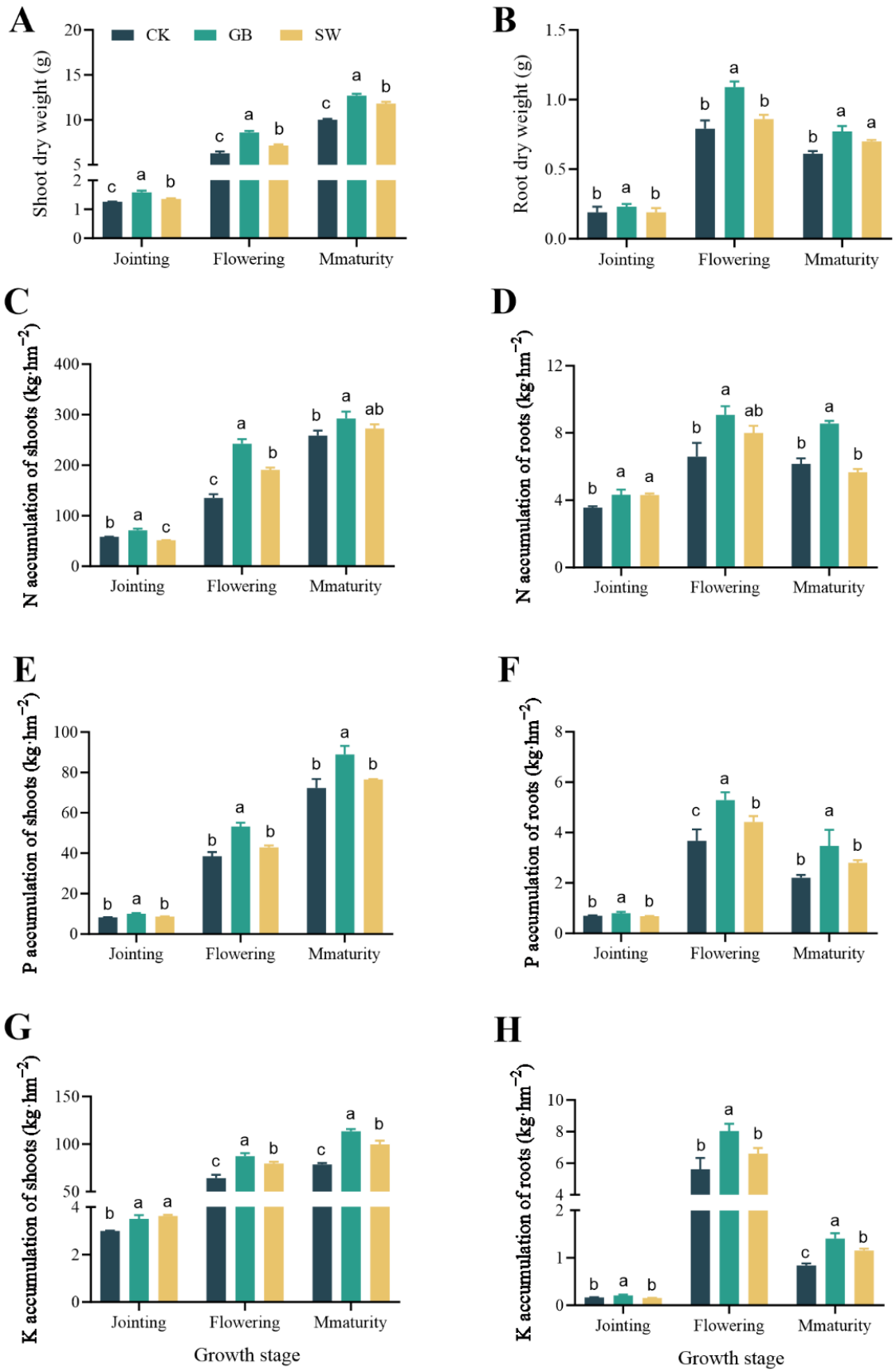

Figure 2. Shoot and root dry weights and their nitrogen, phosphorus, and potassium accumulation in wheat at different growth stages. CK, undressed control; GB, bio-seed dressing; SW, chemical seed dressing. Data are presented as the mean ± standard deviation (n = 3). Different lowercase letters indicate significant differences within the same growth stage ($p < 0.05$).

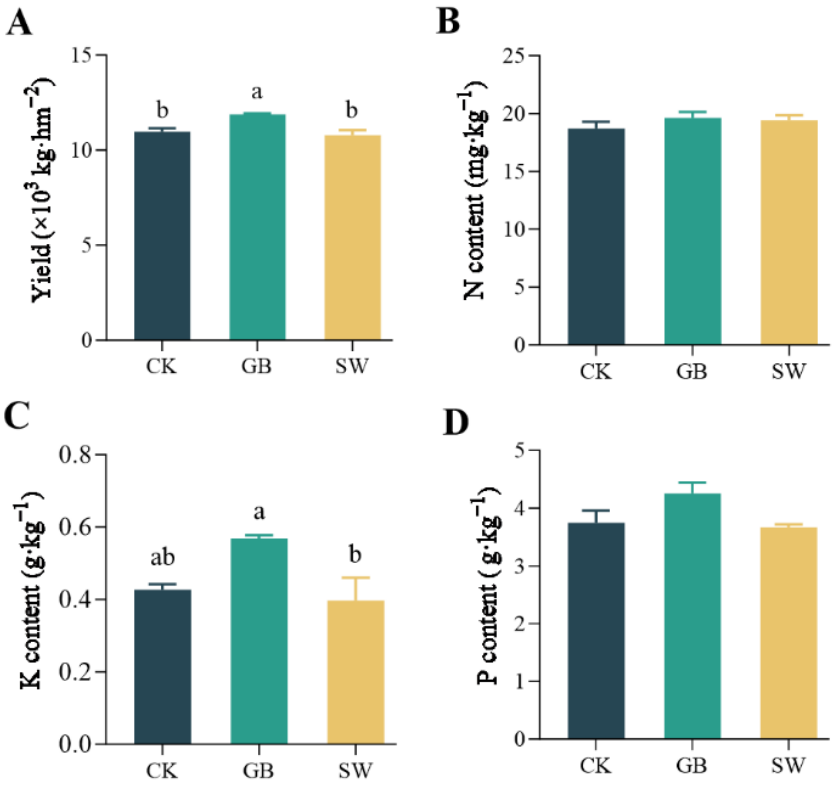

Figure 3. Wheat yield and its nitrogen, phosphorus, and potassium contents. CK, undressed control; GB, bio-seed dressing; SW, chemical seed dressing. Data are presented as the mean ± standard deviation (n = 3). Different lowercase letters indicate significant differences ($p < 0.05$).

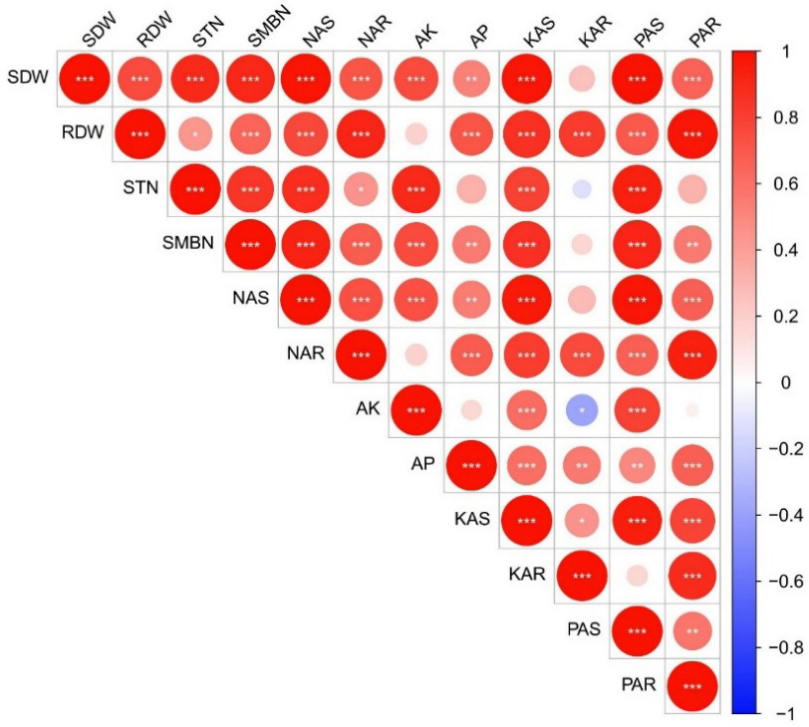

Figure 4. Pearson correlation analysis between rhizosphere soil indices and wheat traits. SDW, shoot dry weight; RDW, root dry weight; STN, soil total nitrogen content; SMBN, soil microbial biomass nitrogen; NAS, N accumulation in shoot; NAR, N accumulation in root; AK, availability K content in soil; AP, availability P content in soil; KAS, K accumulation in shoot; KAR, K accumulation in root; PAS, P accumulation in shoot; PAR, P accumulation in root. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

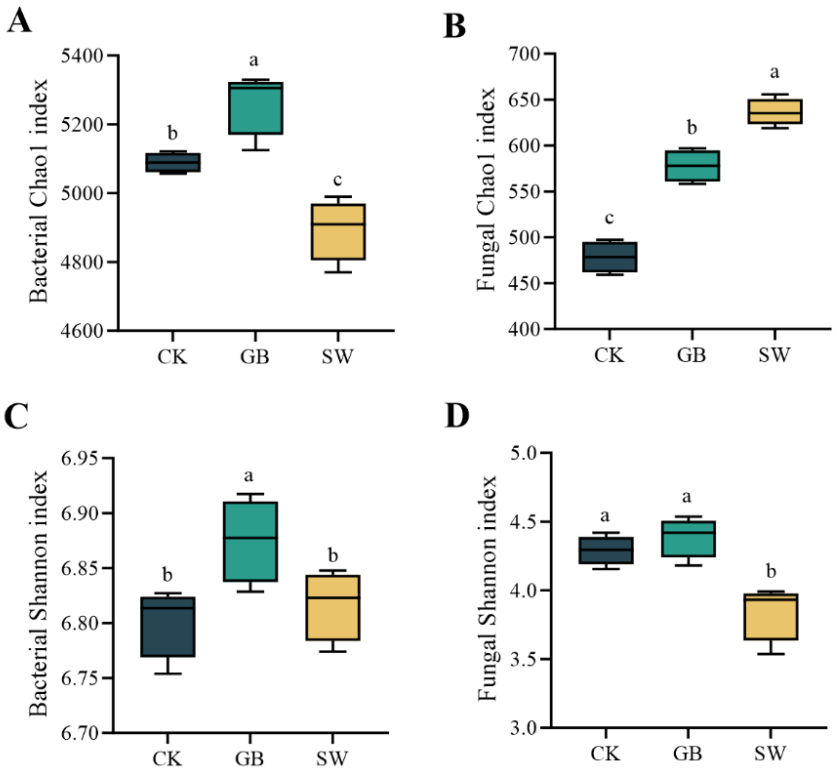

Figure 5. The α diversity of the microbial community in wheat rhizosphere soil at wheat flowering stage. CK, undressed control; GB, bio-seed dressing; SW, chemical seed dressing. Data are presented as the mean ± standard deviation (n = 3). Different lowercase letters indicate significant differences ($p < 0.05$).

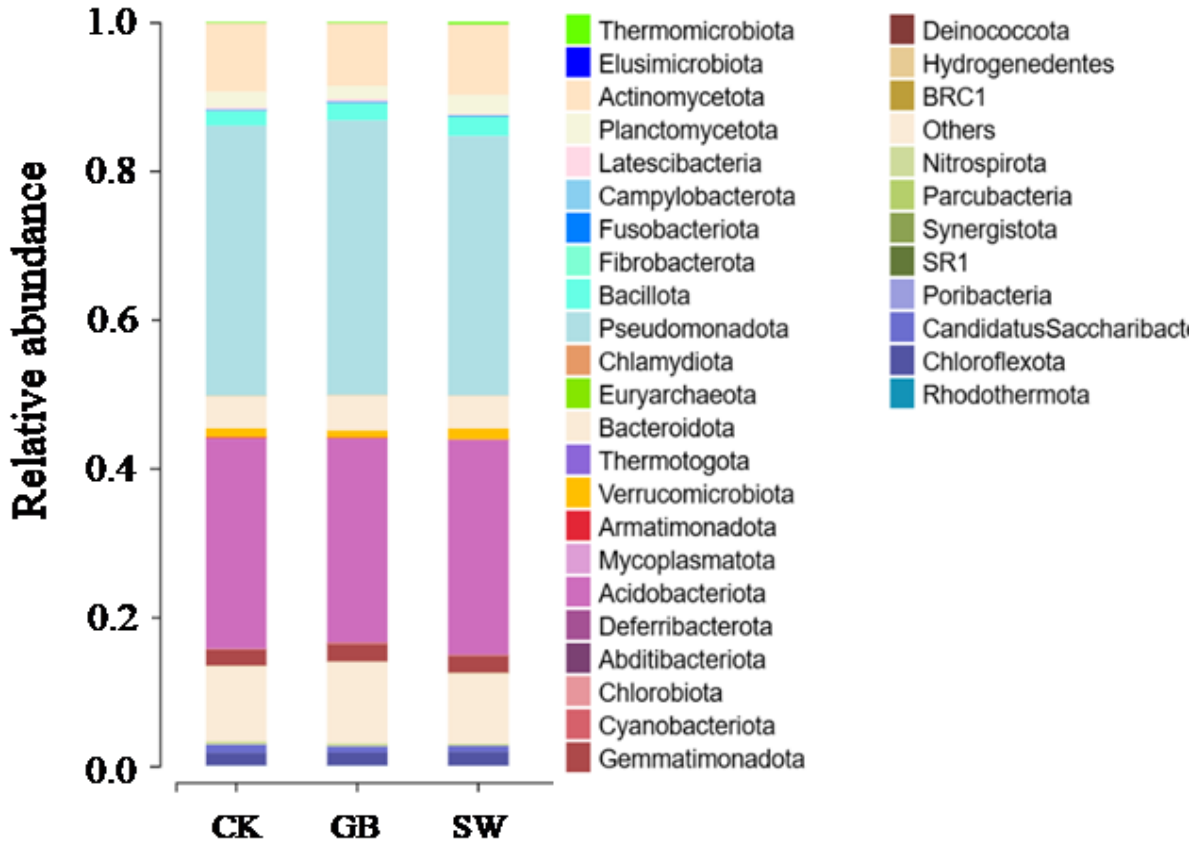

Figure 6. Relative abundance of bacterial phyla in wheat rhizosphere soil at the flowering stage. CK, undressed control; GB, bio-seed dressing; SW, chemical seed dressing.

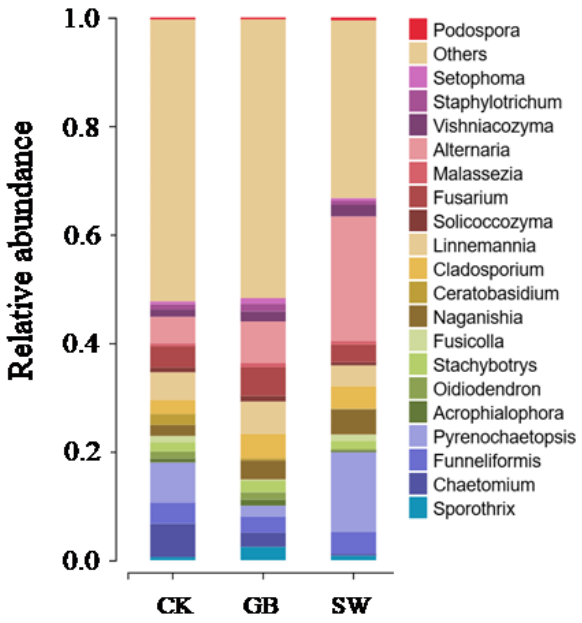

Figure 7. Relative abundance of fungal genera in wheat rhizosphere soil at the flowering stage. CK, undressed control; GB, bio-seed dressing; SW, chemical seed dressing.

Conclusions

In summary, bio-seed dressing formulated with animal probiotics increased wheat biomass, mineral accumulation, and yield. This is likely because the dressing increased the diversity and abundance of rhizosphere soil microorganisms. These microorganisms enhanced the nitrogen supply to wheat, probably through nitrogen retention. Additionally, improving the availability of phosphorus and potassium increased the supply of these nutrients to wheat. Animal probiotics may be an additional source of microorganisms that promote plant growth. Although this study was limited to one site and one growing season, it allowed for a rigorous evaluation of the microbial seed dressing's effectiveness by mitigating environmental variables. The uniformity of the soil's physicochemical properties allowed for precise characterization of the interactions between the inoculant and the wheat rhizosphere. Since the climatic conditions during the experimental period closely aligned with regional historical averages, the observed growth-promoting effects are compelling proof of concept. Nevertheless, further multi-environment trials (METs) are essential to ascertain this agent's functional stability across diverse pedo-climatic gradients.

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Conflict of interest

The authors declare that they have no conflict of interest.

Data availability statement

The 16S rRNA gene and ITS amplicon sequence data associated with this study have been deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.brv15dvq8.

Author contributions

L.D., T.S. and L.Q. designed the experiment; Y.L., C.Z., and D.Z. executed the field research; Y.L. and Y.G. analyzed the data statistically; T.S. and L.Q. wrote the manuscript.

Artificial Intelligence (AI) use

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI.
No AI tools were used in the preparation of this manuscript.

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Supplementary material

Supplementary material 1
Supplementary tables (.docx)
Link: https://doi.org/10.3897/ejfa.2026.173547.suppl1