



original scientific paper

Ratar. Povrt. 2026, 63(3): 129-137

doi: 10.5937/ratpov63-70524

Manuscript submitted: 31 August 2026

Manuscript accepted: 21 September 2026

Published online: 23 September 2026

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Comparative effects of conventional rhizobial inoculants and novel PGPR consortia on rhizosphere microbial parameters and soybean performance

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Summary: *Bradyrhizobium*-based inoculants are widely used in soybean production. Under the field conditions, numerous abiotic and biotic factors can compromise strain survival and significantly reduce their effectiveness. The application of PGPR consortia represents a promising approach for improving the quality of conventional inoculants and soybean performance while supporting beneficial microorganisms in the rhizosphere. This study aimed to comparatively examine the effects of three bacterial treatments: *Bradyrhizobium japonicum* consortium alone and in combination with two distinct PGPR consortia, which differ in strain composition and origin. The inoculants were evaluated on sterile chernozem soil in a pot experiment under semi-controlled conditions by assessing soybean growth, biomass, and nodulation, as well as rhizosphere microbial abundance and activity. The conventional rhizobial inoculant supplemented with a drought-adapted PGPR consortium originating from the soybean rhizosphere significantly increased the abundance of total bacteria, free-living nitrogen-fixing bacteria, azotobacters, and ammonifiers, as well as dehydrogenase activity and soil respiration, compared with *Bradyrhizobium* inoculation alone or in combination with the other PGPR consortium. The greatest effect on soybean growth and biomass was also observed with this bacterial treatment, indicating the potential of novel PGPR strains to improve the quality and effectiveness of conventional inoculants. The obtained findings could contribute to the development of more effective soybean inoculation strategies through the selection of functionally diverse but complementary bacterial strains adapted to the host plant and specific environmental conditions.

Keywords: *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Glycine max*, inoculants, PGPR, plant growth, rhizosphere

Introduction

Soybean (*Glycine max* (L.) Merrill) is one of the most important leguminous crops worldwide, cultivated on approximately 133.7 million ha globally (Faostat, 2025) and over 200000 ha in Serbia (Statistical office of the Republic of Serbia, 2026). It has considerable agro-economic importance due to its favourable grain chemical composition (40% protein and 20% oil content) and other valuable nutrients and bioactive compounds, such as vitamins, minerals, and antioxidants (Baisch et al., 2024). As a legume, soybean establishes a symbiotic association with rhizobia, particularly *Bradyrhizobium* spp. (e.g., *Br. japonicum*, *Br. elkanii*, and *Br. diazoefficiens*) (Thilakarathna

and Raizada, 2017). These bacteria colonize the roots, inducing the formation of nodules, in which atmospheric dinitrogen (N_2) is reduced to ammonium (NH_4^+) and subsequently assimilated by the host plant. Symbiotic nitrogen fixation (SNF) provides the majority of fixed nitrogen, which in soybean ranges from zero to over 300 kg N ha^{-1} of arable soil annually (Ciampitti and Salvaggiotti, 2018). Through SNF, soybean plants can satisfy a substantial portion of their nitrogen demands, thus reducing dependence on external nitrogen inputs. Negative environmental impacts of mineral nitrogen fertilizers, particularly in the context of climate change, impose a need for a broader application of inoculants. Consequently, inoculation with effective *Bradyrhizobium* strains has long been used as a standard practice in soybean production to improve its nutrition, growth, and productivity, while contributing to the sustainability of agricultural systems (Leggett et al., 2017). However, nitrogen fixation efficacy depends on numerous factors, primarily the effectiveness of the rhizobial strains and their ability to establish beneficial interactions with the host plant. Additionally, numerous abiotic and biotic constraints, including temperature extremes, drought, salinity, unfavorable soil pH and nutrient levels, heavy metals, plant pathogens, and pests, can significantly determine SNF efficiency (Nakei et al., 2022).

The effectiveness of *Bradyrhizobium* strains and soybean performance can potentially be enhanced through the use of plant growth-promoting rhizobacteria (PGPR) (Qi et al., 2026). These bacteria originate from the rhizosphere, i.e., the zone in the immediate vicinity of the plant roots. They are directly influenced by root exudates, much more abundant compared to bacterial communities in bulk soil, and therefore have the greatest influence on plant-microbe interactions. Their mechanisms of action include production of plant hormones, biological nitrogen fixation, nutrient solubilization, and other processes that contribute to improved plant growth and development (Yang et al., 2024). In addition, PGPR produce versatile antimicrobial compounds, such as antibiotics, hydrolytic enzymes, siderophores, and volatiles, through which they regulate the presence of pathogenic microorganisms and control plant diseases naturally (Iqbal, 2026). *Azotobacter* and *Bacillus* are among the most important PGPR genera that are widely used as inoculants in agricultural production (Aasfar et al., 2021; Tsotetsi et al., 2022). Besides their effect on plants, PGPR can affect the microorganisms and associated processes in the rhizosphere (Xing et al., 2022). Therefore, the evaluation of both plant and microbial responses can provide a more comprehensive understanding of the effects of soybean inoculation with different bacterial consortia.

The origin and previous plant growth-promoting (PGP) characterization of bacterial strains may be equally important for inoculation outcomes. For instance, bacterial isolates from the rhizosphere of the plant species to be inoculated may be more effective than those isolated from the bulk soil or the rhizosphere of other plant species (Gao et al., 2019). Moreover, if the major limitation factors affecting soybean production, such as drought, are considered during the bacterial isolation, such strains may represent a valuable resource for the development of novel inoculants. The use of multi-species and specifically adapted PGPR consortia may enhance inoculation success by increasing the survival and establishment of applied bacteria in the rhizosphere as well as the complementarity and diversity of their functions (Zeffa et al., 2020). Despite the growing interest in improving the quality of microbial inoculants, the effects of conventional rhizobial inoculation and PGPR consortia with different origins on soybean growth, nodulation, and rhizosphere microorganisms remain limited (Kolanoś et al., 2026). In particular, the potential of newly isolated PGPR strains, adapted to adverse environmental conditions, has been poorly understood. To better assess the effects of bacterial treatments, this study aimed to examine the effects of conventional *Bradyrhizobium* inoculant alone or combined with two PGPR consortia of different origins on soybean and rhizosphere microbial parameters on sterile chernozem soil under semi-controlled conditions.

Materials and Methods

This study was performed with soybean cultivar “NS Olympus” (maturity group 000) and three bacterial treatments. The first treatment (T1) comprised a consortium of highly effective *Bradyrhizobium japonicum* strains routinely used for soybean inoculation for several decades as a standard inoculant (NS Nitragin). *Bradyrhizobium* consortium was also used as a basal inoculum for the other two treatments. The second treatment (T2) was supplemented with a consortium of the PGPR strains: *Azotobacter chroococcum* (A3, A6, A12), *Bacillus megaterium* (B8, B12, B15, and B17), and *Bacillus subtilis* (B5, B7, B13, and B32). These PGPR strains, originally isolated from the soil and the rhizosphere of different plant species (sunflower, maize, wheat, and pepper), were selected for this experiment based on their PGP properties and beneficial effect on soybean during the germination stage. In the third treatment (T3), newly isolated bacterial strains, namely *Azotobacter* spp. (A3.1, A4.1, A5.1, A5.2), *Bacillus* spp. (B1.1, B2.1, B2.2, B4.1, B6.2), and *Pseudomonas* spp. (P1.2, P5.3, P5.5) were applied as an additional inoculum. These strains were obtained from the rhizosphere of drought-tolerant soybean genotypes exposed to several consecutive drought cycles and selected for their drought adaptation and PGP properties (data not shown).

All bacterial strains belong to the collection of the Section for Microbiological Preparations of the Legumes Department, Institute of Field and Vegetable Crops Novi Sad (IFVCNS), Serbia. *Bradyrhizobium*, *Azotobacter*, *Bacillus*, and *Pseudomonas* strains were cultured in yeast extract mannitol, Burk's nitrogen-free, nutrient, and King B broths, respectively, at 28 ± 2 °C and 120 rpm (IST-4075, Jeio Tech, South Korea) for 96 h (*Bradyrhizobium*) and 48 h (*Azotobacter*, *Bacillus*, and *Pseudomonas*). Prior to application, the bacterial suspensions were standardized to a concentration of 10^9 colony-forming unit (CFU ml⁻¹). For consortium treatments, respective bacterial culture suspensions were mixed uniformly. Before formulation of the consortia, the compatibility of the selected PGPR was assessed using a strain compatibility assay on agar plates.

The experiment was conducted in pots (10 dm³ capacity) at Rimski šančevi, Department of Legumes (IFVCNS), using chernozem soil mixed with sand at a 3:1 ratio. The soil used had the following basic chemical properties: pH_(KCl)—7.08; CaCO₃—1.43%; humus—2.70%; total N—0.201%; AL-P₂O₅—44.81 mg 100g⁻¹; and AL-K₂O—24.76 mg 100g⁻¹. Before the experiment, the soil was sterilized to better assess the effects of the introduced consortia on the investigated parameters. Soybean seeds (obtained from the Legume Department, IFVCNS) were surface-sterilized in 2% NaOCl (Sigma Aldrich, St. Louis, MO, USA), washed five times with sterile water, and dried at room temperature. At sowing, each seed of soybean was inoculated with a 1 ml culture suspension of the *Bradyrhizobium japonicum* consortium. Plant density was reduced to six plants per pot a week after emergence, when a 100 ml culture suspension of the PGPR and drought-adapted PGPR consortium was applied per pot for the second and third treatment, respectively. Simultaneously, the first treatment received the same amount of tap water. Each treatment included five pots as replications. The plants were exposed to environmental factors but watered regularly. The effects of applied consortia were observed at the flowering stage (between R1 and R2) of the soybean.

Rhizosphere samples were collected from each pot for the assessment of microbial abundance and activity. The microbial abundance was determined using the indirect dilution plate method and included the total bacteria (soil agar), free nitrogen (N₂) fixing bacteria and azotobacters (nitrogen-free medium), ammonifiers (nutrient agar) and fungi (czapek-dox agar) (Trolldenier, 1996). The average microbial number of microorganisms was calculated for 1.0 g of absolutely dry soil (CFU g⁻¹ soil). The microbial activity was determined based on dehydrogenase activity (DHA) and soil respiration. DHA activity (mU g⁻¹ soil) was determined spectrophotometrically (Agilent Cary 60, Agilent Technologies, Santa Clara, CA, USA) according to ISO 23753-1:2019/Amd 1:2020, by measuring the absorbance of triphenylformazan (TPF) formed through the reduction of 2,3,5-triphenyltetrazolium chloride (ITC) at 485 nm. Soil respiration was determined according to SRPS EN ISO 16072:2013. Soil samples were incubated

in closed vessels, and the released CO₂ was absorbed in 0.5 M NaOH and quantified by back-titration with 0.05 M HCl after the addition of 1 M BaCl₂ and an indicator. The results were expressed based on the amount of CO₂ released per unit mass of soil and incubation time (μg CO₂-C g⁻¹ soil day⁻¹). All microbiological analyses were performed in three replicates.

The effect of applied bacterial consortia was also determined based on the growth (shoot and root length), biomass (shoot and root dry weight), and nodulation (nodule number and dry weight) of soybean. The roots and nodules were gently washed with water, after which all nodules were carefully removed from the roots. The dry weight of shoots, roots and nodules were determined after oven-drying the plant material at 50 °C to a constant weight. Plant parameters were measured for all six plants per pot, and the mean values were calculated per plant.

The data obtained were statistically analyzed using Statistica 12 software (StatSoft Inc., Tulsa, OK, USA). A one-way analysis of variance (ANOVA) was performed to assess differences among treatments, and means were compared using Tukey's Honest Significant Difference (HSD) post hoc test at a significance level of ($p < 0.05$). The relationship between microbial and soybean parameters was determined by Pearson's correlation analysis using R 4.2.2 software (R Foundation for Statistical Computing, Vienna, Austria).

Results and Discussion

The application of bacterial treatments significantly affected microbial parameters in the soybean rhizosphere, with the exception of fungi (Table 1). Total bacterial abundance was significantly higher in T2 and T3 compared to T1, whereas no significant difference was observed between the two PGPR-amended consortia. The highest abundance of free-living N₂-fixing bacteria and azotobacters was observed in T3, with significant differences compared with T1 for N₂-fixers and with both T1 and T2 for azotobacters. A particularly evident response was found for ammonifiers, whose abundance in T3 was noticeably higher compared to other treatments. Conversely, fungal abundance did not differ significantly among bacterial treatments. Microbial activity was also enhanced by the PGPR consortia, as reflected by dehydrogenase activity and soil respiration. Both parameters had the highest values in T3. Dehydrogenase activity measured in this treatment was significantly higher compared to T1 and T2. Soil respiration in T2 showed an intermediate response, while significant differences were observed between values recorded in T1 and T3. The obtained results indicate that supplementation of the *Bradyrhizobium*-based inoculant with PGPR consortia substantially changes the microbial profile of the soybean rhizosphere. Additional incorporation of *Azotobacter* spp., *Bacillus* spp., and *Pseudomonas* spp. strains led to an increase in several bacterial groups and a more bacteria-enriched rhizosphere compared to conventional inoculant alone. Higher microbial abundance observed under T3 was accompanied by increased metabolic activity. Dehydrogenase activity is commonly used as an indicator of overall microbial oxidative activity, while soil respiration shows the intensity of microbial metabolism and carbon mineralization. Similarly, the combination of *Bradyrhizobium* with *Bacillus* and *Paenibacillus* has been shown to alter the composition and functional potential of the bacterial community in the soybean rhizosphere (Xing et al., 2022). The results of Zhou et al. (2024) indicated that synthetic microbial communities comprising several bacterial genera induced significant shifts in the structure and composition of the rhizosphere and endosphere microbiota, while also increasing microbial abundance and diversity.

This effect may be attributed to complementary interactions between *Bradyrhizobium* and PGPR strains, followed by enhancement of rhizosphere colonization and production of diverse microbial compounds (de Andrade et al., 2023). Soybean roots release signaling compounds, particularly flavones and isoflavones into the rhizosphere (e.g., daidzein, genistein, coumestrol) that are species-specific and act as chemoattractants for compatible rhizobia (Liu et al., 2016). Moreover, PGPR like *B. velezensis* may also stimulate flavonoid secretion and thus enhance *B. japonicum*–soybean symbiosis (Cheng et al., 2025). The observed effects of PGPR consortia on

beneficial microbial groups may be partly attributed to PGPR-induced changes in plant metabolism and root exudate composition, which can influence the availability of carbon sources and signaling molecules involved in microbial recruitment, colonization, and interactions within the rhizosphere (Shafi et al., 2025). The ability of PGPR to form biofilms and produce extracellular polymeric substances (EPS) may enhance their survival and colonization in the rhizosphere while facilitating closer cell-to-cell interactions and quorum sensing, potentially supporting the establishment of other beneficial bacteria (Santoyo et al., 2021). Microbial biocontrol agents, particularly *Bacillus* spp. and *Pseudomonas* spp., may promote healthy rhizosphere and microbial balance through the production of antibiotics, siderophores, lytic enzymes, and other antagonistic compounds, suppressing pathogens while favouring beneficial microorganisms (Iqbal, 2026).

Table 1. Effect of different bacterial treatments on microbial abundance and activity in the rhizosphere

Microbial parameters	Treatment		
	T1	T2	T3
Total bacteria (CFU $\times 10^7$ g ⁻¹ soil)	17.34 $\pm$ 7.48 ^b	44.32 $\pm$ 12.96 ^a	45.52 $\pm$ 20.96 ^a
Free N ₂ fixers (CFU $\times 10^6$ g ⁻¹ soil)	65.14 $\pm$ 28.13 ^b	107.4 $\pm$ 36.08 ^{ab}	135.98 $\pm$ 25.97 ^a
Azotobacters (CFU $\times 10^2$ g ⁻¹ soil)	86.84 $\pm$ 13.39 ^b	100.00 $\pm$ 6.11 ^b	125.46 $\pm$ 11.98 ^a
Ammonifiers (CFU $\times 10^6$ g ⁻¹ soil)	24.72 $\pm$ 14.28 ^b	40.84 $\pm$ 11.28 ^b	154.44 $\pm$ 105.64 ^a
Fungi (CFU $\times 10^4$ g ⁻¹ soil)	13.88 $\pm$ 4.12 ^a	10.20 $\pm$ 4.64 ^a	9.86 $\pm$ 4.55 ^a
Dehydrogenase (mU g ⁻¹ soil)	0.076 $\pm$ 0.009 ^b	0.089 $\pm$ 0.007 ^b	0.114 $\pm$ 0.016 ^a
Soil respiration (μ g CO ₂ -C g ⁻¹ soil day ⁻¹)	9.46 $\pm$ 1.10 ^b	14.51 $\pm$ 3.88 ^{ab}	18.32 $\pm$ 5.59 ^a

Values are presented as mean $\pm$ SD. Different letters within the same row indicate statistically significant differences according to Tukey's HSD test at $p \leq 0.05$. T1: *Bradyrhizobium japonicum* consortium (BJ); T2: BJ + PGPR consortium; T3: BJ + drought-adapted PGPR consortium.

Applied bacterial consortia significantly affected parameters of soybean growth and biomass accumulation (Table 2). The most pronounced effects were observed in soybean treated with T3, which consistently showed the highest values for measured growth parameters. A similar pattern was recorded for shoot and root length, with T3 producing longer shoots (+16% and +14%) and roots (+23% and +20%) than in T1- and T2-treated plants. The same treatment had the highest effect on dry biomass accumulation, leading to significant increase of shoot dry weight (+49% and +23%) and root dry weight (+31% and +17.5%) compared to T1 and T2, respectively. However, statistical analysis indicated a significant difference only between T1 and T3, whereas T2 again caused an intermediate effect on soybean biomass. In contrast, neither nodule number nor nodule dry weight differed significantly among investigated bacterial treatments.

Applied bacteria, including *Bradyrhizobium*, *Azotobacter*, *Bacillus*, and *Pseudomonas*, are widely recognized for their diverse plant growth-promoting functions. One of the most important mechanisms underlying their activity is the production of indole-3-acetic acid (IAA) and other phytohormones (e.g., gibberellins and cytokinins), which can increase the development of the root system and the area available for colonization (Kurepa and Smalle, 2022). In addition, PGPR can solubilize phosphorus, produce siderophores, and mineralize organic compounds, potentially creating more favorable conditions and nutrients for both bacteria and plants (Sun et al., 2024). PGPR strains possessing these characteristics have been reported to enhance seed germination, root development and architecture, and plant biomass, with improved root system development contributing to more efficient nutrient uptake and, consequently, higher soybean growth and yield (Araujo et al., 2021; Kangsopa et al., 2025). Moreover, PGPR inoculants comprising multiple species or strains with complementary functions have been shown to modify rhizosphere microbial communities and promote soybean growth (Zhou et al., 2024). A meta-analysis of showed that co-inoculation of soybean with *Bradyrhizobium* and PGPR significantly increased root and shoot

biomass as well as nodule number and nodule biomass, although these improvements did not translate into significant increases in shoot N content or grain yield (Zeffa et al., 2020).

Table 2. Effect of different bacterial treatments on soybean growth, biomass accumulation, and nodulation

Plant parameters	Treatment		
	T1	T2	T3
Shoot length (cm plant ⁻¹)	32.66 ± 1.24 ^b	33.25 ± 0.97 ^b	37.97 ± 3.61 ^a
Root length (cm plant ⁻¹)	38.48 ± 2.64 ^b	39.61 ± 1.86 ^b	47.37 ± 6.74 ^a
Shoot dry weight (g plant ⁻¹)	1.228 ± 0.10 ^b	1.478 ± 0.15 ^{ab}	1.825 ± 0.14 ^a
Root dry weight (g plant ⁻¹)	0.412 ± 0.05 ^b	0.461 ± 0.08 ^{ab}	0.542 ± 0.08 ^a
Nodule number (No plant ⁻¹)	24.26 ± 4.61 ^a	25.13 ± 6.49 ^a	24.11 ± 3.62 ^a
Nodule dry weight (g plant ⁻¹)	0.606 ± 0.10 ^a	0.603 ± 0.21 ^a	0.470 ± 0.14 ^a

Values are presented as mean ± SD. Different letters within the same row indicate statistically significant differences according to Tukey's HSD test at $p \leq 0.05$. T1: *Bradyrhizobium japonicum* consortium (BJ); T2: BJ + PGPR consortium; T3: BJ + drought-adapted PGPR consortium.

Although both PGPR consortia improved investigated parameters, the consortium composed of newly isolated strains from drought-exposed, tolerant soybean genotypes (T3) had a stronger effect on microbial groups involved in nitrogen transformations such as N₂-fixers, azotobacters, and ammonifiers as well as soybean growth and biomass accumulation. These bacteria may have established particularly favorable interactions within the rhizosphere, which may be related to the origin and selection history of its component strains (Munoz-Ucros et al., 2022). Isolates obtained from the rhizosphere of soybean genotypes exhibiting drought tolerance following repeated drought cycles may possess traits that facilitate their persistence under adverse environmental conditions. This is particularly important given the sensitivity of rhizobial strains and the various factors that influence the extent of nitrogen fixation under natural conditions. The greater adaptability and resilience of novel inoculants may therefore enhance the quality of conventional inoculants and their effectiveness in soybean.

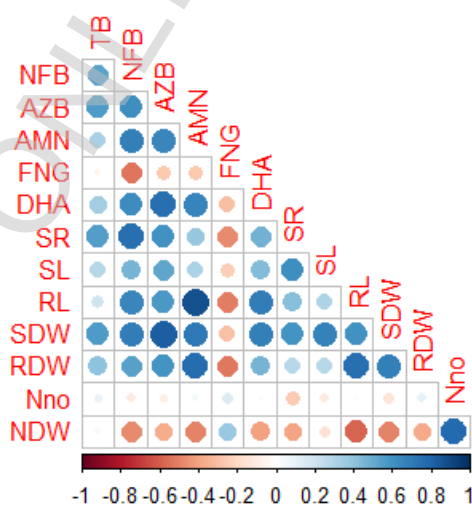


Figure 1. Correlation heat map of microbial and soybean parameters. Blue colors indicate positive relationships, and red colors indicate negative relationships. TB – total bacteria, NFB – free-living nitrogen-fixing bacteria; AZB – azotobacters, AMN – ammonifiers; FNG – fungi, DHA – dehydrogenase; SR – soil respiration, SL - shoot length, RL - root length, SDW - shoot dry weight, RDW - root dry weight, NNo - nodule number, NDW - nodule dry weight.

Correlation analysis between examined parameters confirmed the positive effects of applied bacterial treatments on soybean and rhizosphere microbiota under semi-controlled conditions (Figure 1). Most microbial parameters were positively associated with soybean growth and biomass traits, whereas fungi generally showed weak to negative relationships. In particular, the strong positive correlations between nitrogen-fixing bacteria, azotobacters, ammonifiers, root growth and soybean biomass suggest that higher abundance of bacterial groups involved in nitrogen transformations may be associated with improved plant growth and biomass accumulation. Dehydrogenase activity and soil respiration also showed positive correlations with shoot and root length as well as shoot and root dry weight, indicating that increased microbial activity in the rhizosphere soil was closely associated with enhanced soybean growth. Similarly, shoot and root lengths were positively correlated with shoot and root dry weights, demonstrating a consistent relationship between vegetative growth and biomass production. Nodule number and nodule dry weight showed comparatively weak correlations with most microbial and plant-growth parameters. Overall, the heat map shows that beneficial bacterial groups and soil microbial activity were more strongly associated with soybean growth and biomass than fungal abundance, highlighting the potential importance of bacterial-mediated soil processes in supporting soybean development.

Conclusions

Co-application of *Bradyrhizobium* strains with PGPR consortia had positive effects on most rhizosphere microbial and plant parameters compared to their individual use, indicating the potential of evaluated PGPR strains to complement conventional rhizobial inoculants. The greatest improvement in rhizosphere microbial status, plant growth, and development was observed after application of consortia consisting of *Bradyrhizobium* and drought-adapted PGPR strains isolated from the rhizosphere of drought-tolerant soybean genotypes exposed to several consecutive drought cycles. These findings suggest that the origin, functional characteristics, and compatibility with the rhizobial strains and host plant may be important determinants of inoculation outcomes. The present study highlights the importance of selecting inoculation strains, taking into account the host plant and its interaction with introduced bacterial strains. Improving the quality of conventional inoculants through the addition of multi-species PGPR consortia can increase microbial activity in the rhizosphere, potentially contributing to improved soybean growth and productivity. Further field trials are needed to validate the effectiveness and consistency of conventional rhizobial inoculants and novel PGPR consortia across different soybean cultivars and environmental conditions. Research should also be directed toward the characterization of the rhizosphere microbiome using advanced molecular approaches, including 16S rRNA gene and shotgun metagenomic sequencing, to elucidate changes in microbial community composition, diversity, and functional potential of inoculation strains.

Author contributions: Conceptualization: D.M., J.M. (J. Marinković), M.V. and B.T.; Methodology: D.M., J.M. (J. Marinković), M.V., B.T. and V.Đ.; Project administration: D.M. and J.M. (J. Marinković); Software: M.V., A.U. and V.Đ.; Investigation: D.M., J.M. (J. Marinković), M.V., B.T. and A.U.; Resources: D.M., J.M. (J. Marinković), M.V., V.Đ. and J.M. (J. Miladinović); Data Curation: D.M., J.M. (J. Marinković), M.V., A.U. and V.Đ.; Writing – Original Draft Preparation: D.M., J.M. (J. Marinković) and B.T.; Writing – Review & Editing: D.M., J.M. (J. Marinković), M.V., B.T., A.U., V.Đ. and J.M. (J. Miladinović); Visualization: D.M., J.M. (J. Marinković), M.V., B.T. and A.U.; Validation: D.M., J.M. (J. Marinković), A.U. and V.Đ.; Supervision: J.M. (J. Marinković), V.Đ. and J.M. (J. Miladinović); Funding Acquisition: D.M., J.M. (J. Marinković), M.V., V.Đ. and J.M. (J. Miladinović).

Competing interests` statement: No competing interests were disclosed.

Funding statement: This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, grant number: 451-03-33/2026-03/200032; and the Provincial

Secretariat for Higher Education and Scientific Research, AP Vojvodina, long-term project “SOYMICROBREED - Advancing drought resilience in soybean through rhizosphere microbiome selection”, grant number: 003872014 2025 09418 003 000 000 001 04 004.

Data availability statement: The data presented in this study are available from the corresponding author upon reasonable request.

Ethical issues statement: No AI tools were used in the preparation of this manuscript.

References

- Aasfar, A., Bargaz, A., Yaakoubi, K., Hilali, A., Bennis, I., Zeroual, Y., & Meftah Kadmiri, I. (2021). Nitrogen Fixing *Azotobacter* Species as Potential Soil Biological Enhancers for Crop Nutrition and Yield Stability. *Frontiers in Microbiology*, 12, 628379. <https://doi.org/10.3389/fmicb.2021.628379>
- Araujo, F. F., Bonifacio, A., Bavaresco, L.G., Mendes, L.W., & Araujo, A. S. F. (2021). *Bacillus subtilis* changes the root architecture of soybean grown on nutrient-poor substrate. *Rhizosphere*, 18, 100348. <https://doi.org/10.1016/j.rhisph.2021.100348>
- Baisch, J. S., Grohs, M., Ferreira, P. A. A., Ugalde, G. A., Tres, M. V., & Zabet, G. L. (2024). Protein and Oil Contents, Micro- and Macronutrients, and Other Quality Indicators of Soybean Cultivated in Lowland Fields. *Foods*, 13(23), 3719. <https://doi.org/10.3390/foods13233719>
- Cheng, Y., Jiang, X., He, X., Wu, Z., Lv, Q., Zhao, S., Zhang, X., Wang, S., He, H., & Liu, J. (2025). *Bacillus velezensis* 20507 promotes symbiosis between *Bradyrhizobium japonicum* USDA110 and soybean by secreting flavonoids. *Frontiers in Microbiology*, 16, 1572568. <https://doi.org/10.3389/fmicb.2025.1572568>
- Ciampitti, I. A., & Salvagiotti, F. (2018). New Insights into Soybean Biological Nitrogen Fixation. *Agronomy Journal*, 110(4), 1185-1196. <https://doi.org/10.2134/agronj2017.06.0348>
- de Andrade, L. A., Santos, C. H. B., Frezarin, E. T., Sales, L. R., & Rigobelo, E. C. (2023). Plant Growth-Promoting Rhizobacteria for Sustainable Agricultural Production. *Microorganisms*, 11(4), 1088. <https://doi.org/10.3390/microorganisms11041088>
- Food and Agriculture Organization of the United Nations (2025). FAOSTAT. <https://www.fao.org/faostat/en/#compare>
- Gao, J., Luo, Y., Wei, Y., Huang, Y., Zhang, H., He, W., Sheng, H., & An, L. (2019). Screening of plant growth promoting bacteria (PGPB) from rhizosphere and bulk soil of Caragana microphylla in different habitats and their effects on the growth of Arabidopsis seedlings. *Biotechnology & Biotechnological Equipment*, 33, 921-930. <https://doi.org/10.1080/13102818.2019.1629841>
- Iqbal, M. (2026). Microbial biocontrol agents and the rhizosphere microbiome: integrating ecological function and climate resilience in sustainable agriculture. *Frontiers in Microbiology*, 17, 1771649. <https://doi.org/10.3389/fmicb.2026.1771649>
- Kangsopa, J., Singsopa, A., & Thawong, N. (2025). Seed coating with bacteria-producing indole-3-acetic acid (IAA) on germination, seedling growth and nutrient contents of vegetable soybean [*Glycine max* (L.) Merrill]. *Legume Research*, 48, 1130-1138. <https://doi.org/10.18805/LRF-847>
- Kolanoš, A., Panasiewicz, K., Faligowska, A., Szymańska, G., & Ratajczak, K. (2026). Potential of PGPR to Enhance Soybean Productivity in Europe. *Agriculture*, 16(5), 497. <https://doi.org/10.3390/agriculture16050497>
- Kurepa, J., & Smalle, J. A. (2022). Auxin/Cytokinin Antagonistic Control of the Shoot/Root Growth Ratio and Its Relevance for Adaptation to Drought and Nutrient Deficiency Stresses. *International Journal of Molecular Sciences*, 23(4), 1933. <https://doi.org/10.3390/ijms23041933>
- Leggett, M., Diaz-Zorita, M., Koivunen, M., Bowman, R., Pesek, R., Stevenson, C., & Leister, T. (2017). Soybean Response to Inoculation with *Bradyrhizobium japonicum* in the United States and Argentina. *Agronomy Journal*, 109, 1031-1038. <https://doi.org/10.2134/agronj2016.04.0214>
- Liu, C. W., & Murray, J. D. (2016). The Role of Flavonoids in Nodulation Host-Range Specificity: An Update. *Plants (Basel)*, 5(3), 33. <https://doi.org/10.3390/plants5030033>
- Munoz-Ucros, J., Wilhelm, R. C., Buckley, D. H., & Bauerle, T.L. (2022). Drought legacy in rhizosphere bacterial communities alters subsequent plant performance. *Plant and Soil*, 471, 443-461. <https://doi.org/10.1007/s11104-021-05227-x>
- Nakei, M. D., Venkataramana, P. B., & Ndakidemi, P. A. (2022). Soybean-Nodulating Rhizobia: Ecology, Characterization, Diversity, and Growth Promoting Functions. *Frontiers in Sustainable Food Systems*, 6, 824444. <https://doi.org/10.3389/fsufs.2022.824444>
- Qi, P., Haoyue, L., Zhao, L., Khoso, M. A., Wagan, S., Zhang, J., Wang, Q., Jiang, B., Huang, F., Yuxin, W., Ma, J., Khan, I., Brohi, N.A., Dinislam, K., & Li, S. (2026). Plant growth-promoting rhizobacteria: dual roles in enhancing nutrition and mitigating climate-related abiotic stresses. *Frontiers in Microbiology*, 17, 1800561. <https://doi.org/10.3389/fmicb.2026.1800561>
- Santoyo, G., Urtis-Flores, C. A., Loeza-Lara, P. D., Orozco-Mosqueda, M. d. C., & Glick, B. R. (2021). Rhizosphere Colonization Determinants by Plant Growth-Promoting Rhizobacteria (PGPR). *Biology*, 10(6), 475. <https://doi.org/10.3390/biology10060475>

- Shafi, Z., & Shahid, M. (2025). Root exudates as molecular architects shaping the rhizobacterial community: A review, *Rhizosphere*, 36, 101212. <https://doi.org/10.1016/j.rhisph.2025.101212>
- Statistical Office of the Republic of Serbia. (2026). *Crop production—data by municipalities*. <https://data.stat.gov.rs/Home/Result/130102?languageCode=en-US>
- Sun, W., Shahrajabian, M. H., & Soleymani, A. (2024). The Roles of Plant-Growth-Promoting Rhizobacteria (PGPR)-Based Biostimulants for Agricultural Production Systems. *Plants (Basel, Switzerland)*, 13(5), 613. <https://doi.org/10.3390/plants13050613>
- Thilakarathna, M. S., & Raizada, M. N. (2017). A meta-analysis of the effectiveness of diverse rhizobia inoculants on soybean traits under field conditions. *Soil Biology and Biochemistry*, 105, 177–196. <https://doi.org/10.1016/j.soilbio.2016.11.022>
- Trolldenier, G. (1996). Plate count technique. In F. Schinner, R. Öhlinger, E. Kandeler, & R. Margesin (Eds.), *Methods in soil biology* (pp. 20–26). Springer-Verlag.
- Tsotetsi, T., Nephali, L., Malebe, M., & Tugizimana, F. (2022). *Bacillus* for Plant Growth Promotion and Stress Resilience: What Have We Learned? *Plants (Basel)*, 11(19), 2482. <https://doi.org/10.3390/plants11192482>
- Xing, P., Zhao, Y., Guan, D., Li, L., Zhao, B., Ma, M., Jiang, X., Tian, C., Cao, F., & Li, J. (2022). Effects of *Bradyrhizobium* Co-Inoculated with *Bacillus* and *Paenibacillus* on the Structure and Functional Genes of Soybean Rhizobacteria Community. *Genes (Basel)*, 13(11), 1922. <https://doi.org/10.3390/genes13111922>
- Yang, P., Condrich, A., Scranton, S., Hebner, C., Lu, L., Ali, M. A. (2024). Utilising Plant Growth-Promoting Rhizobacteria (PGPR) to Advance Sustainable Agriculture. *Bacteria*, 3(4), 434–451. <https://doi.org/10.3390/bacteria3040030>
- Zeffa, D. M., Fantin, L. H., Koltun, A., de Oliveira, A. L. M., Nunes, M. P. B. A., Canteri, M.G., & Gonçalves, L.S.A. (2020). Effects of plant growth-promoting rhizobacteria on co-inoculation with *Bradyrhizobium* in soybean crop: a meta-analysis of studies from 1987 to 2018. *Peer J.*, 8, e7905. <https://doi.org/10.7717/peerj.7905>
- Zhou, J. X., Liu, Y., Xu, W. H., Wang, Z. G., Chen, W. J., & Hu, Y. L. (2024). Effect of synthetic microbial communities on rhizosphere and root-endophytic microbiota of soybean [J]. *Chinese Journal of Eco-Agriculture*, 32(4), 571–581. <https://doi.org/10.12357/cjca.20230496>

Usporedni efekti konvencionalnih rizobialnih inokulanata i novih PGPR konzorcijuma na mikrobiološke parametre rizosfere i rast i razvoj soje

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Sažetak: Inokulanti na bazi *Bradyrhizobium*-a imaju široku primenu u proizvodnji soje. U uslovima spoljašnje sredine, brojni abiotički i biotički faktori mogu značajno uticati na preživljavanje i efikasnost ovih sojeva. Primena PGPR konzorcijuma predstavlja obećavajući pristup za unapređenje kvaliteta konvencionalnih inokulanata i performansi soje, uz istovremenu podršku korisnim mikroorganizmima u rizosferi. Cilj ovog istraživanja bio je komparativno ispitivanje efekata tri bakterijska tretmana: konzorcijuma *Bradyrhizobium japonicum* primenjenog samostalno i u kombinaciji sa dva PGPR konzorcijuma različitog sastava i porekla. Inokulanti su ispitivani na sterilnom zemljištu tipa černoze, u ogledu koji je izveden u sudovima i polukontrolisanim uslovima, pri čemu su praćeni rast, biomasa i nodulacija soje, kao i brojnost i aktivnost mikroorganizama u rizosferi. Konvencionalni rizobialni inokulant, obogaćen konzorcijumom PGPR sojeva adaptiranih na sušu, poreklom iz rizosfere soje, značajno je povećao ukupan broj bakterija, brojnost slobodnih azotofiksatora, azotobaktera i amonifikatora, kao i aktivnost dehidrogenaze i respiraciju zemljišta, u poređenju sa primenom *Bradyrhizobium* sojeva pojedinačno ili u kombinaciji sa drugim PGPR konzorcijumom. Najizraženiji efekat na rast i masu soje, takođe je zabeležen primenom ovog bakterijskog tretmana, što ukazuje na potencijal novih PGPR sojeva da poboljšaju kvalitet i efikasnost konvencionalnih inokulanata. Dobijeni rezultati mogu doprineti razvoju efikasnijih strategija inokulacije soje kroz selekciju funkcionalno različitih, ali komplementarnih bakterijskih sojeva, prilagođenih biljci domaćinu i specifičnim uslovima sredine.

Ključne reči: *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Glycine max*, inokulanti, PGPR, rast biljaka, rizosfera