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Effects of a microbial plant biostimulant on soil microbial activity, photosynthetic performance, and grain productivity of winter wheat under greenhouse conditions

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Abstract

Plant biostimulants are widely recognized as an eco-friendly alternative to chemical fertilizers due to their beneficial effects on soil health and plant growth. This study evaluated the effects of a microbial plant biostimulant on soil microbial activity, photosynthetic performance, and grain productivity of winter wheat grown under greenhouse conditions. Four treatments were tested: control (C), microbial plant biostimulant (MPB, 1 kg/ha), chemical fertilizer (CF, 100 kg/ha), and combined application (CF+MPB, CF dose reduced by 25%). The experiment used winter wheat variety KWS Lazuli and each treatment comprised six replications at completely randomized design. Studied parameters included soil chemical analysis, microbial metabolic activity, photosynthetic performance, and wheat grain productivity. The results showed that application of microbial plant biostimulant (MPB) did not affect soil chemical composition but increased soil dehydrogenase activity. Microbial plant biostimulant (MPB) also affected plant growth parameters and grain productivity. The combined application (CF+MPB) showed no synergistic effects and for net photosynthetic rate and grain productivity did not exceed the results obtained with the CF. However, the overall positive trend across studied parameters supports further research on the partial replacement of chemical fertilizers with microbial plant biostimulants under field conditions.

Keywords: Grain productivity, microbial biostimulant, soil microbial activity, photosynthetic performance, winter wheat

Introduction

According to the European Union (2019), plant biostimulants can be components, mixtures, or microorganisms that do not contain nutrients but can stimulate the natural processes of plant nutrition (EU Regulation 2019/1009). Plant biostimulants are considered an eco-friendly alternative to chemical fertilizers and a promising tool stimulating soil natural processes and nutrients cycling. Thus they can contribute to soil health and fertility, soil biodiversity, improved plant resilience towards abiotic stress and sufficient crop yield (Calvo et al., 2014; Daniel et al., 2022; Khan et al., 2023; Roche et al., 2024). Due to various effects of biostimulants on plant physiology and morphology, along with stimulation of diverse soil processes, they can be invaluable tool in sustainable agriculture (Rouphael and Colla, 2018; Hamid et al., 2021). Utilization of products and agricultural practices that increase the number, activity and/or diversity of beneficial microorganisms can contribute significantly to both soil fertility and health, and consequently to affect positively plant growth and productivity (de Vries et al., 2013; Reicosky, 2015). Microbial biofertilizers, as a type of plant biostimulants, contain different species of plant growth promoting bacteria (PGPB) and/or arbuscular mycorrhizal fungi (AMF). Beneficial microorganisms possess a variety of properties such as biological nitrogen fixation, phosphate solubilization, synthesis of iron chelating

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compounds, synthesis of plant phytohormons and biologically active substances (Kumari et al., 2023). Depending on their properties and intended use microorganisms can be classified as biofertilizers, bioherbicides, biopesticides, and biocontrol agents (Alori and Babalola, 2018). Under current European Union legislation (EU Regulation 2019/1009), biostimulants are clearly defined, whereas biofertilizers are not formally recognized. Biostimulants are intended to stimulate plant physiological processes, while biofertilizers primarily function to supply nutrients through microbial activity. Nevertheless, in the scientific literature the terms biostimulants and biofertilizers are often used interchangeably. Microbial biostimulants contribute to nutrient bioavailability through natural processes, improve soil health, increase yield, and reduce dependency of chemical fertilizers (Saa et al., 2015; Abioye et al., 2024; Verma and Pandey, 2023). Sun et al. (2020) found that application of microbial biofertilizer reduced the nitrogen loss by 54% and increased nitrogen utilization and maize yield by 11.2% and 5.0%, respectively. Similarly, Chen et al. (2021) reported a 15.2–33.4% increase in wheat yield after application of microbial inoculants. Positive results related to either partial replacement of chemical fertilizers, or combined application with biofertilizer were also obtained (Nascimento et al., 2020). Microbial plant biostimulants significantly improved grain yield, leaf greenness index, photosynthetic rates, and soil nutrient content in winter wheat (Stępień et al., 2021; Rahman et al., 2022). Several studies also focused on evaluating the effect of microbial biostimulants varying in their species composition on wheat growth and productivity (Liu et al., 2023; Aechra et al., 2022). Due to the positive perception of plant biostimulants and the projected increase in market demand, the variety of plant biostimulants is expanding. Additionally, the diverse range of potentially useful microorganisms that can be used as plant biostimulant further contribute to increasing number of available on the market products (Critchley et al., 2021). However, the proper dose and timing of application, assessment of effectiveness of biostimulant at different crops, interactions with soil chemical properties and indigenous soil microorganisms should be assessed both in pot and field experiments. Such assessment is necessary for every newly formulated biostimulant intended for use in agriculture (Liu et al., 2023). This task is further complicated by the fact that the biostimulant's mechanisms are still not fully understood due to their complexity and variable effects. Assessing the effectiveness of biostimulants on plant growth and yield at different climatic and edaphic conditions would further strengthen the justification of their utilization. Furthermore, their broader application will be important element in sustainable agricultural practices (Berg, 2009; Bernabeu et al., 2018; Kumawat et al., 2021).

The aim of this study was to assess the effects of a microbial plant biostimulant, formulated with a selected strain of *Priestia megaterium* recently introduced to the Bulgarian market. The biostimulant was applied either alone or in combination with reduced doses of chemical fertilizer. Its impact was evaluated in a wheat pot greenhouse experiment by measuring soil microbial activity, photosynthetic performance and grain productivity.

Material and Methods

Experimental design

The greenhouse experiment was conducted in the Agricultural University-Plovdiv, Bulgaria between November 2023 and June 2024 using winter wheat variety KWS Lazuli. KWS Lazuli is a well-adapted to Bulgarian conditions high-yielding wheat variety with an early to medium maturation. The pot experiment was conducted in a greenhouse that relied only on natural sunlight. Since the facility had no artificial lighting or temperature control, conditions such as light, temperature, and humidity changed with the outdoor weather. Pots were arranged on a single table and repositioned after watering to minimize uneven light exposure. The soil used for the experiment was collected at depth of 0 to 20 cm from the experimental field near main buildings of the university (Latitude 42.1416 N, Longitude 24.7561 E), and its characteristics are indicated in Table 1.

Table 1. Soil characteristics

Soil parameters	Value	Unit
pH	8.82	-
Electrical conductivity	197.6	µS/cm
Soil texture	560 sand, 320 silt, 120 clay	g/kg
Total Nitrogen	32.82	mg/kg
Soil organic matter/humus	18.5/32	g/kg
Phosphorus (P_2O_5)	812.2	mg/kg
Potassium (K_2O)	511.6	mg/kg
Exchangeable cations (Ca+Mg)	12.50	meq/100 g
$CaCO_3$	10.0	g/kg

The preliminary soil analysis indicated soil pH of 8.82 (1:2.5 soil water extract), and an electrical conductivity of 197.6 µS/cm. Based on the content – 560 g/kg sand, 320 g/kg silt, and 120 g/kg clay the soil was classified as sandy clay loam (USDA soil textural triangle). The soil was mixed with perlite at a 3:1 volume ratio, and 1.5 kg of small size stones were laid at the bottom of the 5-liter pot to allow adequate drainage of water. The perlite used in the experiment was newly purchased from the local garden center and stones was washed before use. The pots were sown in November 2023. The field capacity of sieved soil (coarse sieve) was estimated in advance and the 55-65% of maximum water holding capacity during experiment was maintained gravimetrically. After seed germination, plant density was adjusted to seven plants per pot. Weeds were removed manually. The experimental design (completely randomized design, CRD) included four variants with six replications (pots) and their description is provided in Table 2. The analysis indicated soil with moderate nitrogen content (Tomov et al., 2009) and very high phosphorus and potassium quantity (Roy et al., 2006).

Table 2. Experimental variants

Variant (abbreviation and description)	Chemical fertilizer, kg/ha	Microbial plant biostimulant, kg/ha
C (control)	–	–
MPB (microbial plant biostimulant)	–	1
CF (chemical fertilizer)	100	–
CF + MPB (chemical fertilizer and microbial plant biostimulant)	75	1

Microbial plant biostimulant

The study used biostimulant Nuptak (Daymsa, Spain), which is available on the Bulgarian market. The biostimulant (power form) contains a selected strain of free-living nitrogen-fixing bacteria *Priestia megaterium* (CB2001) at quantity of 1x10^7 CFU/g, according to the manufacturer. The list of ingredients includes also amino acids (17.5%) and sucrose (77.5%). The pH value of the product is in the range 4-5. The biostimulant was applied to the soil at seed germination at quantity of 30 ml per pot, equivalent to the recommended field application rate of 1 kg ha^{-1}.

Soil chemical analyses

Soil analysis was performed at the Accredited Laboratory Centre at the Agricultural University-Plovdiv, Bulgaria. Soil pH was determined in a soil-water solution (1:2.5). Total mineral nitrogen was determined using the modified Kjeldahl method. The organic carbon concentration was analyzed by Walkey-Black wet digestion (Nelson and Sommers, 1982). Organic matter content was computed by multiplying the estimated soil organic carbon by 1.724 according to the assumption that OM is composed of approximately 58% carbon (Brady and Weil, 1999). Exchangeable cations (Ca^{2+} and Mg^{2+}) were measured using NH_4OAc filtrate according to Thomas (1982). Soil phosphorus analysis was conducted according to the method of Egner et al. (1960) with $SnCl_2$ as indicator and the measurement at a wavelength of 700 nm. Available potassium content was determined with a flame photometer.

Metabolic activity of soil microbial community

Soil microbial activity was assessed by the 96-well EcoPlate of BIOLOG (Biolog Inc., USA) at 25ºC ±2 incubation for 168 hours as described in Dimitrova et al. (2024). The calculations for average well-color development (AWCD) and separately for each guilds were based on the optical density (OD) measured at 590 nm and 750 nm according to the procedure described by Sofo and Ricciuti (2019). The data normalisation was done by subtracting each measurement from the corresponding OD at 24th hour according to Urakawa et al. (2013). The negative values obtained at any stage of data normalisation was set to zero (Garland, 1996). Microbial communities' structure was characterized by functional indexes indicating biodiversity or species evenness (Table 3). Due to relatively low microbial activity, calculation of indexes was based on the values obtained at the 120 hours of incubation and all wells which OD exceeded 0.100.

Enzyme analysis

Soil dehydrogenase enzyme activity was determined according to Alef (1995) with TTC as a substrate and incubation at 30ºC for 24 hours. The measurements were done at 546 nm wavelength.

Plant analysis

Leaf nitrogen, phosphorus, and potassium (NPK) content was measured at the heading phase. The determination of the total nitrogen in plants was done using the Kjeldahl method. Plant potassium content was measured by flame photometry and phosphorus content was estimated by Egner et al. (1960). Plant photosynthetic parameters included: net photosynthetic rate (A), transpiration rate (E), stomatal conductance (Gs) and chlorophyll content. Leaf gas exchange parameters were estimated using the flag leaf of the main stem, and measurements were taken between 9:00 – 11:00 am under natural light conditions using the LCpro+ open photosynthetic system (Analytical Development Company Ltd., Hoddesdon, UK). A portable Chlorophyll Content Meter, model CCM-300 (ADC BioScientific Ltd.), was used for chlorophyll measurements. At harvest, grain productivity and morphological parameters such as shoot height, ear length, spikelets number per ear were measured.

Table 3.Formulas for functional indexes calculation

Functional index	Formula	References
Shannon index, H'	$H' = -\sum p_i \times (\ln p_i)$ where p_i is C_i, divided by the sum of C_i, values ≥ 0.100	Jurkšienė et al. (2020)
Pielou index, E	$E = \frac{H'}{\ln S}$ where H' is Shannon index S – number of values ≥ 0.100	Pielou (1966), Jurkšienė et al. (2020)
Margalef index, d	$d = \frac{(S-1)}{\ln N}$ where, S – number of wells ≥ 0.100, N – number of substates i.e. 31	Türkmen and Kazanci (2010)
McIntosh index, U	$U = \sqrt{\sum P_i^2}$ where P_i is C_i, divided by the sum of C_i values ≥ 0.100	McIntosh (1967) Huang et al. (2012)
McIntosh evenness, McI	$McI = N - U/N - (N/\sqrt{S})$ where U – McIntosh diversity index, N – sum of values ≥ 0.100, S – number of substrates i.e. 31	Xu et al. (2015)

Data analysis

The three sets of substrates in each EcoPlate were considered replicates (n = 3). Calculation of AWCD, functional indexes, and graphs was done with Microsoft Excel. Analysis of variance (ANOVA) with factor treatment and significance level at $p < 0.05$ was performed with SPSS (IBM version 26). Further comparisons among treatment means were conducted using Tukey's post hoc test. Wheat biometric data and grain productivity were based on measurements of all plants per variant.

Result

Soil parameters

The evaluated soil chemical parameters were pH levels, total nitrogen, available phosphorus, and potassium. The pH values ranged between 8.88 and 9.00 no significant difference was observed as a result of different fertilization approaches (Table 4). The combined treatment of chemical fertilizer and biostimulant had the highest nitrogen content of 15.16 mg/kg. The content of soil mobile phosphorus (P_2O_5) ranged between 837.5 to 1122.5 mg/kg with the highest estimated level at combined treatment (CF+MPB). Potassium (K_2O) levels ranged from 1159.1 to 1247.1 mg/kg. Because only one measurement was obtained per variant, statistical analysis was not possible. Consequently, differences among treatments cannot be interpreted as statistically significant and should be considered descriptive.

Table 4. Soil chemical composition at the end of the wheat cultivation

Treatment	Total Nitrogen (NH_4^+, $N0_3^-$) mg/kg	Mobile phosphorus (P_2O_5), mg/kg	Mobile potassium (K_2O), mg/kg	pH
C	12.12	837.5	1247.1	8.88
MPB	11.33	1023.5	1159.1	8.96
CF	10.28	893.0	1169.2	9.00
CF+MPB	15.16	1122.5	1176.4	8.88

Legend: C – control, no treatment, MPB – microbial plant biostimulant, CF – chemical fertilizer

Effect of biofertilizer on soil microbial activity

The measurements of optical density (OD) indicated a prolonged lag phase. After 72 hours, there was a very slow increase in OD which at the average value varied between 0.035 and 0.062 units per measured period. The changes in the OD slightly increased between 120 and 144 hours, but after that the estimated metabolic activity, declined again (Figure 1). After 120 hours the metabolic activity in the control soil was higher than the other experimental variants. Thus, at the end of the incubation period its OD was approximately 1.5-fold higher than the other treatments. Utilization of amines was characterized with uneven start among treatments and was accompanied with significant standard deviation of the mean. At the end of the incubation period the soil treated with MPB had the highest value – OD 0.263 ±0.188. Carboxylic acids were utilized slowly by the microorganisms in the soil treated with chemical fertilizer. At the end of the incubation the OD in the control reached 0.416 ±0.096, and for chemical fertilized treated soil only 0.196 ±0.140. Metabolic activity towards carbohydrates were lower in the soil treated with MPB. On the contrary, the carbohydrates utilization was almost identical for soil treated either with chemical fertilizer or combined treatment. Polymers were more vigorously utilized in not-treated soil and much lower in soil treated either with MPB or CF. Utilization of phenolic compounds were detected only in the soil under combined treatment with the mean value of 0.377 on the 168 hours due to utilization of 4-hydroxy benzoic acid exclusively.

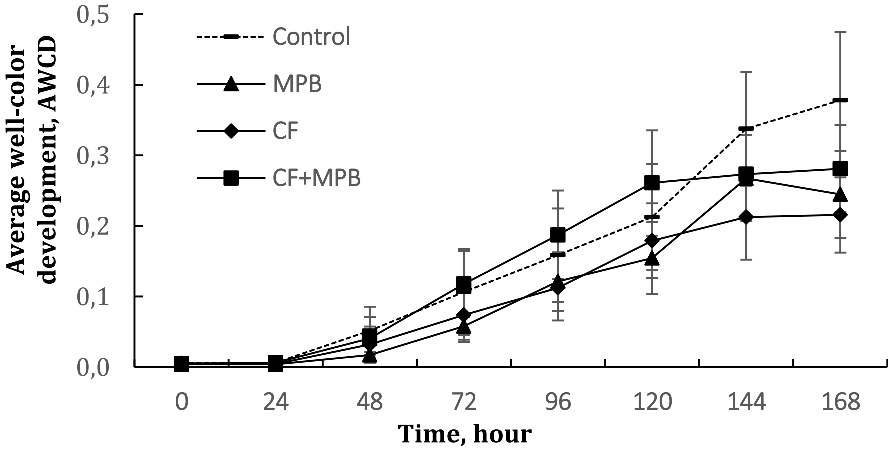

Figure 1. Microbial metabolic activity in soil subjected to different fertilization approaches

Effect of treatments on soil microbial community structure

In the current study, the highest mean values for Shannon index – 2.579 (p=0.07), Margalef index – 3.009 (p=0.573), and McIntosh Index – 0.506 (p=0.072) were estimated for soil microbial communities subjected to combined treatment (CF+MPB) (Table 5). It should be noted, the variant treated solely with MPB has the highest Pielou evenness index (0.938, p=0.062). Although the mean values of the CF+MPB treatment indicated a tendency toward a more balanced microbial community structure, the generally low OD values used in the index calculations, together with the absence of statistically significant differences, hamper a conclusive estimation of the effect.

Table 5. Functional indexes characterizing soil microbial community structure based on the 120 hours of EcoPlate incubation

Treatment	Functional indexes				
	Shannon diversity	*Pielou evenness*	*McIntosh index*	*McIntosh evenness*	*Margalef index*
C	2.014 ±0.25	0.919 ±0.03	0.386 ±0.04	1.127 ±0.05	2.426 ±0.94
MPB	1.997 ±0.30	0.938 ±0.01	0.388 ±0.05	1.092 ±0.07	2.233 ±0.73
CF	2.096 ±0.29	0.882 ±0.02	0.390 ±0.05	1.115 ±0.04	2.912 ±0.87
CF+MPB	2.579 ±0.15	0.900 ±0.03	0.506 ±0.06	1.132 ±0.04	3.009 ±0.44
p-value	0.070	0.062	0.072	0.810	0.573

Effect of treatments on soil dehydrogenase activities (DHA, µg TPF g^{-1} dwt h^{-1})

The highest DHA value (mean = 5.527 µg TPF g^{-1} dwt h^{-1}, SD = 0.417) was observed in soil treated solely with the microbial plant biostimulant, followed by the control soil (mean = 5.391, SD = 0.535), with no statistically significant difference between them. In contrast, soils treated with chemical fertilizer (mean = 4.604, SD = 0.209) and combined treatment (CF + MPB; mean = 4.596, SD = 0.495) showed lower values. ANOVA indicated significant differences among treatments (p=0.015).

Effect of treatments on leaf NPK content

The estimated total nitrogen content across the treatments varied in a narrow range of 0.63 to 0.70% (Table 6). Plants subjected to the combined treatment of chemical fertilizer and microbial plant biostimulant (CF+MPB) have nitrogen content of 0.70%. Phosphorus content ranged between 0.63 to 1.06%. The highest phosphorus content was estimated for the plant cultivated in soil with combined CF+MPB treatment (1.06%), followed by the CF and MPB variants with content of 0.87 and 0.84%, respectively (Table 6). Potassium content of wheat plants ranged between 1.55 and 1.83%. Plants cultivated on non-treated soil have potassium content of 1.83% followed by CF and MPB treatments with content of 1.82% and 1.68%, respectively.

Table 6. NPK content in wheat plant leaves

Treatment	Total Nitrogen (%)	Phosphorus (%)	Potassium (%)
C	0.63	0.63	1.83
MPB	0.68	0.84	1.68
CF	0.66	0.87	1.82
CF+MPB	0.70	1.06	1.55

Legend: C – control, no treatment, MPB – microbial plant biostimulant, CF – chemical fertilizer.

Effect of treatments on wheat photosynthetic parameters

Leaf gas exchange parameters indicated the highest values in plants treated with chemical fertilizer, except for chlorophyll concentration, which was higher in plants subjected to the combined treatment (Table 7). Photosynthetic rate (A) ranged between 14.28 to 16.67 µmol CO_2 m^{-2} s^{-1} with the highest rates for treatment with chemical fertilizer (16.75, p=0.03). The transpiration rate (E) ranged between 2.03 and 2.59 mmol H_2O m^{-2} s^{-1} except the treatment with chemical fertilizer which reached 2.67 mmol H_2O m^{-2} s^{-1}. The estimated stomatal conductance (gs) ranged insignificantly between 0.08 and 0.09 mol m^{-2} s^{-1} with the highest value in plants treated solely with chemical fertilizer – 0.11. Chlorophyll content of the plant in non-treated soil was 347 mg m^{-2}. The highest values were measured for CF and CF+MPB treatments with 390 and 393 mg m^{-2} values, respectively (Table 7).

Table 7. Leaf gas exchange parameters of wheat plants

Treatment	Photosynthetic parameter A µmol CO_2 m^{-2} s^{-1}	 E mmol H_2O m^{-2} s^{-1}	 Gs mol m^{-2} s^{-1}	 Chlorophyll concentration, mg m^{-2}
C	15.19^{ab}	2.03^{a}	0.09	347^{a}
	±2.55	±0.46	±0.03	±24
MPB	14.28^{a}	2.09^{a}	0.08	371^{ab}
	±1.37	±0.39	±0.02	±26
CF	16.75^{b}	2.67^{b}	0.11	390^{ab}
	±1.87	±0.42	±0.03	±29
CF+MPB	16.67^{ab}	2.09^{a}	0.08	393^{ab}
	±2.17	±0.58	±0.02	±16
p-value	*0.003*	*0.001*	*0.005*	*0.000*

Legend: net photosynthetic rate (A), transpiration rate (E), stomatal conductance (Gs), NT– no treatment, BF – Biofertilizer, CF – Chemical fertilizer. Superscripts denote significant differences at a 5% probability level. Treatments with the same superscript are not significantly different from each other.

Effect of treatment on grain productivity and agromorphological traits

The obtained data indicated, that in comparison to the control variant employed treatments positively influenced all wheat growth parameters and in some cases the difference was proven statistically – grain productivity (p=0.01), shoot high (p <0.05) ear length (p=0.08), spikelets number per ear (p=0.02) (Table 8). However, the observed trend for a positive effect of CF, MPB, and CF+MPB compared with the control was not consistent across all estimated parameters, and in some cases no statistical differences were detected. This may be explained by physiological variability among plants, the limited number of replicates, or potential interaction effects between factors influencing the measured responses.

Table 8. Grain productivity and selected agromorphological traits of wheat plants

Treatment	Grain productivity and agromorphological traits GP	 PT	 SH (cm)	 EL (cm)	 SNE	 SdNE	 TKW, g	 WSE (g)
C	13.38^{a}	2.07	52^{a}	8.06^{a}	14^{a}	31	47.53	1.460
	±0.98	±0.54	±1.93	±0.83	±2	±7	±1.62	±0.37
MPB	17.01^{b}	2.33	56^{b}	8.78^{b}	16^{b}	36	48.32	1.697
	±0.85	±0.82	±1.51	±0.83	±1	±7	±2.61	±0.36
CF	17.02^{b}	2.36	57^{b}	8.39^{ab}	15^{ab}	35	47.29	1.648
	±1.85	±2.59	±2.12	±1.18	±2	±9	±2.46	±0.49
CF+MPB	16.24^{b}	2.39	58^{b}	8.65^{ab}	16^{b}	34	46.12	1.583
	±1.73	±1.21	±1.38	±0.82	±1	±7	±0.79	±0.35
p-value	0.001	0.093	0.000	0.008	0.002	0.089	0.258	0.076

Legend: GP – grain productivity (g per pot); PT – productive tillering; shoot height; SH – shoot length; EL – ear length; SNE - Spikelets number per ear; SdNE – seeds number per ear; TKW – thousand kernels weight (g), WSE - weight of seeds per ear (g); C – control, MPB – microbial plant biostimulant, CF – chemical fertilizer, CF+MPB - combined treatment. Data are presented as mean ±SD. Treatments with the same superscript are not significantly different from each other at p<0.05.

Discussion

Soil nutrients availability

The study evaluated the effects of various fertilization approaches on selected soil and plant parameters. Soil physicochemical analysis showed that the pH of the soil was not affected by either type of fertilization employed in the current study (Table 4). This is in line with observation of Aechra et al. (2022) who used biofertilizers and found non-significant changes in soil pH levels. The initial soil phosphorus content in the current experiment was relatively high (812.2 mg kg^{-1}). However, some other researchers reported range of 400 to 1200 mg kg^{-1} (Halpern et al., 2015) and some other even higher values 660–1870 mg kg^{-1} (Saarela et al., 2003). The slight increase in phosphorus content in sample treated with microbial plant biostimulant is in line with the findings of Ciopińska et al. (2019) who noted that the release of bioavailable nutrients such as phosphorous in the soil is associated with increased bacterial activity. Aechra et al. (2022) observed that biofertilizer application improved the soil NPK contents in comparison to the untreated control and attributed this to enhanced microbial activity, which affect decomposition, mineralization, and nutrient release processes in the soil. However, in the current study the content of potassium was not affected by the applied treatments. This can be due to the high baseline K content, pot effect and short duration of the experiment.

Soil microbial activity and enzyme activity

Soil treated only with microbial biostimulant showed higher dehydrogenase activity (DHA) in comparison to chemical fertilizer treatment ($p=0.015$) which is often linked to better soil quality and the cycling of nutrients (Ali et al., 2023). However, the general metabolic activity estimated by the Biolog method was relatively low. The reported AWCD values usually reached an OD of 1.500–1.800 at 168 hours of incubation, measured at 590 nm (Weng et al., 2022; Wei et al., 2022). Using a similar approach for blank correction (OD_{590} nm – OD_{750} nm), Sofo and Ricciuti (2019) reported values ranging from 0.200 to 0.400 OD for AWCD after 144 hours of incubation, and from 0.200 to 0.700 OD per substrate guild after 96 hours of incubation. In the current study, the OD was almost three times lower and et the end of incubation and varied between 0.216 and 0.378. Such low activity can obscure objective estimation of substrates utilization. The results from the current study are more in line with the low activity observed by Zhao et al. (2019). Additionally, the lack of significant difference between treatments are in compliance with results obtained by Gomez et al. (2000) who did not find variation in metabolic activity of microbial communities in samples collected from four locations with different native flora, crops, management practices. On the contrary, Chandra et al. (2021) considered that application of bioformulations improved wheat rhizospheric biological activity and enhanced both bacterial colonization and enzymatic activities.

Calculation of functional indexes using the OD values of Biolog EcoPlates is based on the number of positive wells and specific pattern of substrate utilization. Most indexes have a specified range and present an aspect of a species' community structure defined as diversity or evenness. In the current study, the Shannon, Margalef, and McIntosh indexes, which denote the diversity in the analyzed sample, have relatively higher values for soil under combined treatment (CF+MPB) suggesting a formation of balanced and richer microbial community. Similarly, Hui et al. (2018) reported changes in the composition of the microbial community after biofertilizer application. However, the difference between observed indexes were not proven statistically. Li et al. (2022) reported that the community composition of rhizosphere fungi shifted following biofertilizer application, although no significant differences in the species diversity or richness of either bacteria or fungi were observed. Similarly, Dal Cortivo et al. (2020) observed no changes in microbial biodiversity. The authors considered that the seed-applied biofertilizers may be effectively exploited in sustainable wheat cultivation without altering the microbial biodiversity. Despite that, the authors warrant for more attention towards composition of the microbial consortia in order to maximize their benefits in crop cultivation.

Plant photosynthetic performance

A trend toward increased net photosynthetic rate (A) was observed in parallel with higher chlorophyll content under the CF and CF+MPB treatments. Notably, the net photosynthetic rate was lower in the control and MPB variant; however, when MPB was applied in combination with mineral fertilization, it enhanced photosynthetic performance and compensated for reduced nitrogen availability, as evident when comparing variants CF and CF+MPB. The relatively smaller positive effects of mineral and combined fertilization on A, compared to their effects on overall productivity, may be attributed to the longer-lasting residual influence of these treatments on yield formation. These results are similar to the findings of Kubar et al. (2022) who observed that the photosynthetic rate and transpiration rate increased with the increase in nitrogen rate supplied. Overall, the results showed that the combined application of chemical fertilizer and biofertilizer improved wheat physiological performance more than the single application of each component. Singh et al. (2023) also reported that at different water regimes microbial-inoculated wheat plants showed substantially higher photosynthetic rate, transpiration rate, stomatal conductance, and internal CO_2 levels than uninoculated wheat plants. Kang et al. (2023) acknowledged the importance of nitrogen fertilization and at conditions of sufficient or reduced irrigation and reported photosynthetic rate and stomatal conductance of 15-25 μmol m^{-2} s^{-1}, of 0.15, and 0.50 μmol CO_2 mol^{-1}, respectively, at two different varieties of wheat. Zhang et al. (2017) discussed that reduction of nitrogen rates affect net photosynthetic rates and stomatal conductance but the yield was dependent on the photosynthetic capacity of plant during specific stage of growth i.e. the grain filling stage. The authors also considered the relationships between photosynthesis and biological factors including non-stomatal limitation of photosynthesis which are important for understanding the mechanisms of yield reduction due to reduction in N and provide scientific basis for application of different fertilization approaches. The soil pH value of 8.82 in the current experiment may stimulate nitrification by the strain in the biostimulant, as alkaline conditions generally favor this process. Although photosynthesis was slightly improved in the CF+MPB variant, photosynthetic parameters reflect only a momentary measurement, while grain productivity is the ultimate outcome of plant development. Improved photosynthesis is most critical during the grain-filling stage and can be influenced by protein remobilization from older leaves. Moreover, grain productivity is affected by multiple factors, since nitrogen is required for both photosynthetic enzymes and pigments.

Grain productivity and agromorphological traits

The data from the current study showed that under both sole CF and combined application with MPB, grain productivity was 21 to 27% higher compared to the control. This can be regarded as an integrated result of the effects of greenhouse conditions and pot experimental design on productive tillering, the number of spikelets, grain weight per spike, and thousand grain weight. Increased nutrient availability resulted in increase in plant morphometrics (Hyles et al., 2020). In the current study both CF and application of microbial plant biostimulant positively affected the observed growth parameters (Table 8). These results are in line with Mohanta et al. (2020), Bayraklı (2022) and Jabran et al. (2024). Tkaczyk et al. (2018) studied the effect of application of nitrogen fertilizer and phosphorus and concluded that the winter wheat yield was affected by these factors but at varying extend. On the contrary, Khadka et al. (2022), did not report a statistical difference in the grain yield after inoculation with biofertilizer. However, the authors noted that the use of soil-applied and seed-applied biofertilizer increased the grain yield in comparison to the uninoculated plots. Rossini et al. (2025) considered that the application of biostimulants have the most significant impact on grain yield only at low and medium nitrogen doses. In the current study, the preliminary soil analysis revealed a moderate level of nitrogen which can hamper the effect of MPB to some extent. Li et al. (2023) considered that sufficient nitrogen fertilization masked the positive effects of biostimulants on wheat yield and nitrogen use efficiency. Dal Cortivo et al. (2020) reported that the biofertilizers studied significantly enhanced plant growth and nitrogen accumulation during stem elongation and heading; however, this translated into only small yield gains of 1–4% compared with the control. In the current study, the grain productivity per pot also was 1.3% higher than the control. The thousand kernel weight (g) was not significantly different between treatments. Some authors reported significantly higher grain yield (40.41%) after application of microbial consortia (Pathak et al., 2024). Ayed et al. (2022) found that microbial biostimulant enhanced development of several durum wheat cultivars by influencing various traits. El Habasha et al. (2013) and Abd El-Razek and El-Sheshtawy (2013) noticed that different wheat varieties responded differently to biostimulants application. Furthermore, Nguyen et al. (2019) observed inconsistent effects of microbial inoculation on plant biomass despite insignificant differences in average air temperature in the greenhouse across two consecutive experiments. In some cases, microbial inoculants increased root biomass by 31% compared to the non-inoculated control, while other treatments promoted root growth by 17% without statistical significance. However, in the second experiment, the author did not find significant differences in biomass between inoculated plants and the control. Since the current experiment used non-sterile soil, the lack of statistically significant differences in plant agromorphological parameters could be related to the low average microbial activity detected by the Biolog EcoPlate method. In a greenhouse pot experiment Hett et al. (2022) found that inoculation with two different types of microbial inoculants did not affect any of the observed vegetation indexes at any measurement date.

Conclusion

The current study evaluated the effect of different fertilization approaches to a broad range of traits that included both soil microbial activity and plant photosynthetic and growth parameters in a pot-based greenhouse experiment. No clear or consistent relationships among the observed parameters were found. For example, higher photosynthetic activity and grain productivity were not associated with increased soil microbial activity under the combined treatment (CF+MPB). This may be due to the complex and overlapping effects of treatments during plant growth and development. In general, the applied microbial plant biostimulant or its combined treatment with chemical fertilizer at reduced dose (by 25%) increased dehydrogenase activity and contributed to higher microbial biodiversity. None of the fertilization approaches contributed to significant change of leaf mineral content but photosynthetic parameters were positively affected in comparison to non-treated control. The utilized fertilization approaches beneficially affected wheat biometric parameters (shoot and ear length, spikelets number per ear) and grain productivity and the differences were statistically significant. According to the general view, biofertilizers are more effective in plant subjected to environmental stress or in unfavorable conditions. Because this study did not impose environmental stress, the results should be considered indicative rather than conclusive regarding traits influenced by microbial biostimulant application. Future field experiments under variable soil and climatic conditions are required to validate these greenhouse findings.

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