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Research article

SynComs and organic fertilizers positively influenced rhizosphere microbial and crops characteristics of sweet corn

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Abstract: The systematic application of fertilizers and pesticides to increase crop productivity leads to depleted soils with low organic matter content, which increases greenhouse gas emissions and alters profiles of soil microbial communities. Our study investigated the effect of different types of fertilizers on the growth and yield of sweet corn (*Zea mays* L., var. *saccharata*), soil microbial community characteristics, and functional diversity. Five treatments were prepared (untreated control, application of mineral fertilizer, organic fertilizer, combined application of both, and microbial fertilizer-SynComs) with four replicates per treatment. Physicochemical soil parameters, soil carbon dioxide emissions (in situ), and physiological profiles of microbial communities were measured. The highest corn yield was found when microbial fertilizer was applied, followed by mineral and combined fertilizations. The highest microbial activity at ripening, measured by basal and substrate-induced soil respiration, was found in the organic fertilization treatment. The metabolic quotient correlation showed the highest values in the control treatment without any amendments, while the lowest data were observed when SynComs were added. Among the soil enzymes studied, mineral fertilization led to the highest dehydrogenase activity, organic fertilization to the highest phosphatase activity, and combined fertilization to the highest β-glucosidase activity. The most pronounced increase in carbon source utilization capacity between the two sampling periods was observed in the microbial fertilizer and organic variants. Current research has confirmed the excellent effects of combined solutions in agriculture, in which different approaches are applied together to reduce the environmental and climate impacts of interventions.

Keywords: sweet corn; beneficial microorganisms; SynComs; microbial activity; soil enzymes; fertilization; CLPP

1. Introduction

Sweet corn belongs to the grass family (*Poaceae*) and is characterized by higher sugar content and lower starch content, which defines it as a vegetable species (Tracy et al., 2019) [1]. *Zea mays* convar. *saccharata* is cultivated in many regions worldwide, including America, Asia, Europe, the Middle East, Africa, and Australia (Bavec et al., 2013; Dhaliwal et al., 2019; Gao et al., 2020) [2–4]. In 2018, the United States emerged as the world's leading producer of sweet corn, cultivating 240,130 ha, followed by Nigeria, Guinea, and Indonesia (ers.usda.gov). Cultivar choice and harvest date are significant determinants of growth, cob traits, and sugar accumulation in sweet corn [4].

The impact of fertilizers on nutrient availability and on the structure and functionality of the soil microbial community can vary considerably, exerting different effects on crop productivity (Chivenge et al., 2011; Herencia et al., 2008) [5,6]. In vegetable production, excessive use of mineral fertilizers can lead to increased nitrate content, deteriorated soil structure, and groundwater contamination, which is why organic nutrient sources are applied (Zhang et al., 2010) [7]. When investigating the influence of different organic fertilizer sources and inoculation with actinomycorrhizal fungi (AMF), it was found that poultry manure, when inoculated with AMF, can increase sweet corn production on poor soils (Arshad et al., 2015) [8]. Emam et al. (2020) evaluated the effects of compost and organic extracts on the growth and yield of sweet corn and rhizosphere microbial populations, comparing compost extract, vermicompost, organic poultry manure, and mineral fertilizer [9]. Unlike mineral fertilizers, straw and organic manure are expected to help overcome several negative environmental consequences, including loss of organic matter and nutrients, greenhouse gas emissions, and soil degradation (Matson et al., 1997; Guo et al., 2010) [10,11].

Inoculation with beneficial microorganisms is one of the routes for improving nutrient acquisition in sweet corn, and its outcome is conditioned by the soil into which the inoculum is introduced. Inoculation with arbuscular mycorrhizal fungi, together with the tillage practice applied, exerts a positive effect on yield and nutrient uptake in sweet corn, although efficacy may depend on field conditions, existing soil nutrient levels, and native microbial communities (Zinati et al., 2025) [12]. Bacterial inoculants act via the same route: inoculating hybrid sweet corn seeds with *Azospirillum brasilense* positively affects vegetative growth, yield, protein content, and total sugars in the kernels (Numoto et al., 2019) [13].

Nitrogen is the key macronutrient for *Zea mays*, being the element most strongly accumulated in the plants during their development (Okumura et al., 2014) [14]. It improves photosynthetic efficiency and stimulates the development of greater leaf area, which in turn leads to a significant increase in cob length and circumference, the number of first-grade kernels, and kernel weight in super sweet corn (Raja, 2001) [15].

Further studies have shown that organic fertilizers increase crop productivity by enhancing biodiversity and interactions among microbial taxa (Schmid et al., 2018) [16], and that organic matter also positively affects microbial activity (Yaseen and Yossif, 2019) [17]. Soil fertility is to a considerable extent a biological property: the release of nutrients from crop residues, their retention in chelated form, and the stability of soil aggregates all depend on microbial activity (Abbott and Murphy,

2007) [18]. How well a community performs these roles depends on its metabolic repertoire (Nannipieri et al., 2003) [19], which is why respiration-based approaches—quantifying the CO_2 released after defined carbon substrates are supplied—are used to characterize soil communities functionally rather than taxonomically (Campbell et al., 2003) [20]. Nevertheless, there is a lack of research on how mineral, organic, and combined fertilization influenced microbial activity in the soil compared with the applied consortium of beneficial microorganisms.

The aim of the present study is to investigate the impact of SynComs, compared to residual organo-mineral fertilization effect, on functional microbial metrics in the rhizosphere of sweet corn. Our hypothesis is that introducing a beneficial microbial consortium will enhance microbial activity, resulting in a stronger positive physiological response in corn.

2. Materials and methods

2.1. Field description and experimental design

The experiment growing sweet corn GSS 3287/Overland (Syngenta) was established on the experimental field of ISSAPP "N. Pushkarov" in the land of the village of Tsalapitsa, Rhodope municipality, Plovdiv district, Bulgaria (site location: 42°10'48.1"N 24°32'31.1"E) during the vegetation period of 2024. Five variants, each with four replications in a row, were applied, with a plot size of 30 m² (3 × 10 m). The sowing was performed at the end of April 2024 at a rate of 70,000 seeds/ha, while the harvest was made in early August 2024. The soil in the experimental plot (a multi-year vegetable experiment) is alluvial-meadow Fluvisol (FAO, ISRIC World Soils). A block design with five variants, each replicated four times at 30 m^2 each, was used. The climatic characteristics during the precipitation. The corn-growing season was estimated using a proper meteorological station situated in the vicinity of the experimental field. The data were marked by high temperatures (average monthly values between 3.8–5.6 °C above the climatic norm for the multi-year period 1961–1990) and a sharp precipitation deficit combined with drip irrigation. The rainfall from April to August totaled 122 mm (norm 253 mm), with practically no precipitation in June and July. Two irrigations were applied at the critical stages for water demand: the first at flowering (BBCH 61–69) and the second at the early milk stage (BBCH 73). Each irrigation was 450 m^3 ha^{-1}. Under these conditions, irrigation reduced the soil water deficit, but the plants were still exposed to high temperatures and low air humidity. The experimental field is characterized by long-term research involving crop cultivation in rotations while maintaining the specific field's fertilization regime unchanged. Variants were defined as follows: 1. untreated control (without amendments), 2. organic fertilizer (organic chicken manure 1 t/ha, NPK 4-3-3, 62% org. matter, Fertisol, Komeco B.V., Netherlands), 3. mineral fertilizer (266 $kg.ha^{-1}$ $N_{15}K_{15}P_{15}$), 4. combined fertilization, 50% organic fertilizer + 50% mineral fertilizer; and 5. microbial fertilizer (Microbtima Protect, Romb Ltd., Bulgaria). The fertilizers (mineral, organic, and combined) were spread on the soil surface and incorporated (plowed) to a depth of 30 cm. The microbial fertilizer contained the following strains: *Trichoderma harzianum*, *T. asperellum*, *T. viride*, *T. koningii*, *Pseudomonas chlororaphis*, *Ps. putida*, *Azotobacter vinelandii*, *Rhizophagus irregularis*, and *S. cerevisiae*. The working solution was prepared at 5 ml of product per 1 L of water (1: 200), giving approximately 5×10^5 CFU ml^{-1}. The applied dose was 40 ml of product per kg of seed, which corresponds to approximately 4×10^9 CFU kg^{-1} seed (about 10^5–10^6 CFU per seed). The organic, mineral, and combined fertilization treatments were applied at the end of March 2024, before sowing,

and incorporated into the soil. The microbial fertilizer was applied by seed inoculation prior to sowing in April 2024. The corresponding nutrient inputs for each variant are presented in Table 1, while the timeline of soil practices during corn cultivation is presented in Table 2. The applied concentration of N is identical across the three variants—ensuring that they are balanced in this regard—whereas the concentrations of P and K differ to some extent due to variations in the nutrient composition of the different fertilizers. The harvest was made at the beginning of August at the milk R3 stage. Plant protection activities were carried out in accordance with the crop agrotechnology.

Table 1. Nutrients' inputs in the variants supplied by the fertilizers (kg. ha^{-1}).

No	Variant	N	P_2O_5	K_2O
1	Untreated control	0	0	0
2	Organic (Fertisol)	40.00	30.00	30.00
3	Mineral ($N_{15}P_{15}K_{15}$)	39.90	39.90	39.90
4	Integrated (Fertisol + $N_{15}P_{15}K_{15}$)	39.95	34.95	34.95

Table 2. Agricultural practices for sweet corn cultivation during 2024.

Practice	March	April	May	June	July	August
Fertilization						
Soil sampling for MA						
for SPch						
Sowing						
Harvesting						

MA: microbiological analyses, SPch: soil physicochemical analyses.

2.2. Soil physicochemical analyses

Prior to the trial establishment, soil samples were collected at a depth of 0–30 cm from each replicate. The samples were prepared from four subsamples of each replicate. During the ripening, the samples were taken from the plant rhizosphere with roots. After removing excess soil, the soil remaining on the roots was washed off in an aqueous solution of 0.85% NaCl. Sampling was carried out randomly, followed by air drying and sieving (2 mm). The soluble nitrogen was analyzed by the standard method of Bremner and Keeney, 1965 [21]. For the determination of soluble forms of phosphorus and potassium, a standard method in lactate extract was used (Ivanov, 1984) [22]. For the determination of pH, samples were dispersed in deionized water (1:5, w/v) and shaken in a reciprocal shaker for 30 min, then allowed to settle for 10 min and measured with a laboratory pH-EC-meter (Thomas, 1996; Rhoades, 1996) [23,24]. Humus content was determined by the Tyurin titrimetric method (Tyurin, 1931) [25].

2.3. Soil microbiological analyses

Soil samples for microbiological analysis were collected from the rhizosphere at a depth of 0–30

cm after removing the top layer, before sowing, and at the end of the growing season. One sample was taken from each replicate and placed in a sterile plastic bag and immediately transferred to the laboratory for preparation and analysis.

2.3.1. Basal and substrate-induced respiration

For the determination of basal respiration (BR) the methodology described by Alef (Alef, 1995) was used [26]. Fifty grams of moist soil with a corrected water holding capacity of 55% was placed in a closed beaker with 20 ml of 0.05 M NaOH at 22 °C. After incubation for 24 h, 1 ml of $BaCl_2$ was added to precipitate the carbonates. Before being titrated to decolorization with 0.05 M HCl, 1 drop of phenolphthalein was added. To estimate the weight of atmospheric CO_2, a control sample without soil was used. All samples were processed in triplicate.

The CO_2 released from the soil was calculated using the following formula:

$$CO_2\,\langle mg|dw|h\rangle = (V_o - V) * \frac{1.1}{\mathrm{dwt} * 24} \quad (1)$$

where *dw* is the amount of soil dry weight (g), *Vo* is the volume of HCl used for titration of the blanks (mL), *V* is the volume of HCl used for titration of the sample (mL), *dwt* is the dry weight of 1 g of fresh soil, *1.1* is the conversion factor (1 mL 0.05 NaOH equals 1.1 mg CO_2), and *24* is the incubation time in hours.

Substrate-induced respiration (SIR) was determined following the same procedure as basal soil respiration, with the addition of glucose as a readily available carbon source at a concentration of 10,000 mg C kg^{-1} soil. The glucose was dissolved in water and added to the soil to stimulate the active microbial biomass. The maximum initial respiratory response was measured over the first 6 hours of incubation. Soil microbial biomass carbon (SMB-C) was estimated from the SIR values using the relationship established by Anderson and Domsch (1978) [27]. The metabolic quotient (qCO_2) was subsequently calculated as the ratio of basal respiration to microbial biomass carbon, reflecting the metabolic efficiency of the soil microbial community.

2.3.2. Soil enzyme activities

2.3.2.1. Dehydrogenase activity

Dehydrogenase enzyme activity was determined by the method of Thalmann (1968) [28], modified by Alef (1995) [29]. The method is based on the reduction of triphenyltetrazolium chloride (TTC) to triphenylformazan (TPF) by microorganisms after incubation at 30 °C for 24 h. Five grams of moist soil were placed in a test tube, to which 5 ml of TTC solution was added before incubation. After the incubation period, 40 mL of acetone was added, stirred intensively and incubated for two hours in the dark. The soil suspension was filtered, and the optical density of the filtrate was determined against the control at a wavelength of 546 nm.

The results obtained were recalculated using the following formula:

$$\mathrm{TFP}\ (\mu g.g^{-1}) = \mathrm{TPF}\left(\frac{\mu g}{mL}\right) * \frac{45}{\mathrm{dwt} * 5} \quad (2)$$

where dwt is the absolute dry weight of 1 g of moist soil, 5 is the amount of moist soil used in grams, and 45 is the volume of the solution added to the soil sample.

2.3.2.2. Phosphatase activity

Phosphatase activity was measured as described by Eivazi and Tabatabai (1977) [30]. The method is based on the separation of *p*-nitrophenol (pNP) after incubation of soil samples with p-nitrophenyl phosphate for 1 hour at 37 °C. One gram of sieved and moist soil was placed in a 50 mL Erlenmeyer flask; 0.25 mL of toluene, 4 mL of modified universal buffer (MUB) with pH 6, and 1 mL of *p*-nitrophenyl phosphate solution were added, and then incubated for 1 h. Subsequently, 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH were added and mixed, followed by filtration and measurement of absorbance at 400 nm on a spectrophotometer.

The results were calculated using the following formula:

$$p\text{NP}\ (\frac{\mu g}{g}) = \frac{C*V}{dwt*SW*t} \tag{3}$$

where *C* is the concentration of *p*-nitrophenol (μg/mL), *V* is the total volume of final solution, *dwt* is the absolute dry weight of 1 g of moist soil, *v* is the total volume of the soil suspension in milliliters, *SW* is the amount of soil used (1 g), and *t* is the time of incubation.

2.3.2.3. β-glucosidase activity

The activity of β-glucosidases was assessed using the method of Tabatabai (Tabatabai, 1982) [31], modified by Alef and Nannipieri (Alef, K., Nannipieri, P.,1995) [26]. The method consisted of the degradation of p-nitrophenyl-β-D-glucoside (PNG) by microorganisms to p-nitrophenol. 1 g of moist soil was placed in a flask containing 0.25 mL of toluene, 4 mL of buffer, and 1 mL of PNG solution. The mixture was incubated for one hour at 37 °C. Then, 1 mL of $CaCl_2$ and 4 mL of Tris buffer, pH 12, were added, mixed, and filtered. The absorbance was measured at 400 nm on a spectrophotometer, and the final amount of p-nitrophenol was calculated using a standard calibration curve.

2.3.3. Community-level physiological profiling with Biolog^{TM} EcoPlates

The functional diversity of rhizosphere microbial communities was assessed through community-level physiological profiling (CLPP) using Biolog™ EcoPlates (Biolog Inc., Hayward, CA, USA). Each plate contained 31 unique carbon sources arranged in triplicate, along with one water control well. Ten grams of fresh rhizosphere soil were dispersed in 90 mL of 0.85% NaCl solution and agitated for 20 minutes at 250 rpm, followed by 15 min of settling. The resulting suspension was serially diluted and inoculated into the EcoPlate wells (150 μL per well) using a multichannel pipettor. Plates were incubated at 25 C and absorbance was read at 590 nm at 24-h intervals over 120 h using a Biolog™ MicroStation® reader. The average well color development (AWCD) was calculated according to Garland and Mills (1991) [32] as:

$$\text{AWCD} = \Sigma\frac{(A-B)}{n} \tag{4}$$

where *A* is the optical density of the well, *B* is the blank well reading, and *n* is the total number of carbon sources.

2.4. Statistical analysis

All analyses were carried out in four replicates. One-way analysis of variance (ANOVA: Single Factor, Statistix v.10) followed by LSD all-pairwise comparisons test and using Microsoft Excel v.14 were applied for variables at a probability level of $p \leq 0.05$.

3. Results

3.1. Physicochemical analysis

The soil in the experimental plot prior to trial establishment is generally characterized by a neutral reaction (pH 6.7–7.1) and a low humus content (1.32–1.83%). The soluble nitrogen varied from low to medium across treatments (13.3–21.9 $mg.kg^{-1}$), with the highest values recorded under mineral fertilization and the lowest in the SynComs. Mobile phosphorus concentration ranged widely—from low in the SynComs variant (101.6 $mg \cdot kg^{-1}$) to relatively high under combined fertilization (363 $mg.kg^{-1}$), reflecting the N:P ratio and the large application rate of the organic fertilizer. K_2O concentrations were in the range from medium to good stocking (171–223 $mg.kg^{-1}$), with the highest values observed under mineral fertilization (Table 3).

Table 3. Physicochemical properties of the soil of the studied treatments prior to trial establishment on a dry weight basis. Data show mean and standard error (n = 4).

Treatments	pH (H_2O)	$\Sigma N{-}NH_4 + NO_3$ ($mg.kg^{-1}$)	P_2O_5 ($mg.kg^{-1}$)	K_2O ($mg.kg^{-1}$)	Humus (%)
Control	7.1	15.0	128.1	174.0	1.32
Organic	7.0	17.6	304.0	171.0	1.83
Mineral	7.2	21.9	327.0	223.0	1.51
Combined	7.2	17.6	363.0	192.0	1.72
SynComs	6.7	13.3	101.6	200.1	1.38

3.2. Corn biometric analyzes

Figure 1 shows the aspect of the corn plants prior to harvesting.



Figure 1. Photos of the trail before plant harvest.

The yield of sweet corn showed a tendency to increase with the increase of the number of treatments (Figure 2, left). The highest yield was recorded in the microbial fertilizer variant, exceeding the control by approximately 37%, followed by the mineral fertilizer variant at 29%, combined fertilization at 27%, and organic fertilization at 14%. Besides, the yield of corn in the application of a beneficial microbial consortium case was found significantly higher than in organic fertilization.

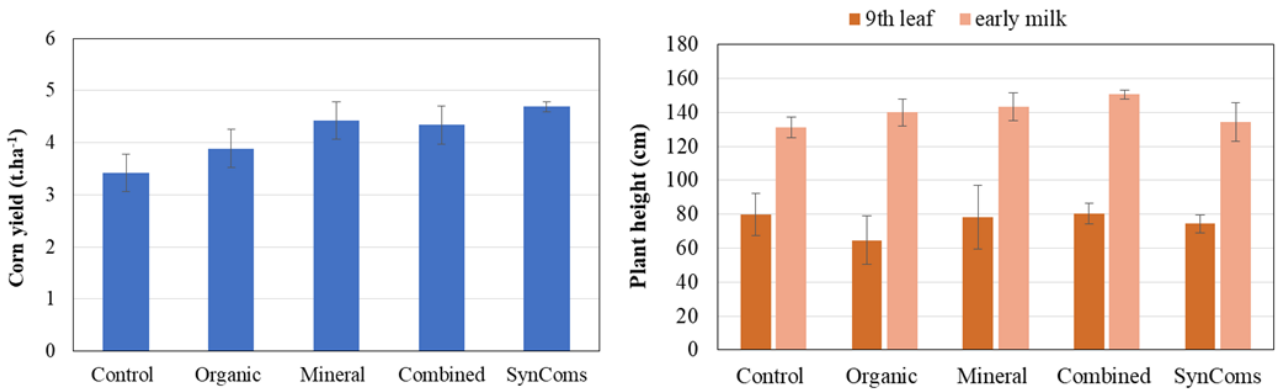


Figure 2. Yield in different (left) and plant height (right) of corn. Results show mean values and standard error (n = 4).

These results suggest a positive trend in yield response to fertilization, particularly in the microbial and mineral variants, which may be attributed to improved nutrient availability and enhanced rhizosphere microbial activity.

At the vegetative stage (9th leaf), no significant differences in plant height were observed among the treatments (Figure 2, right). During milk maturity, all fertilized treatments showed increased plant

height compared to the control. The greatest increment was observed in the combined fertilization variant (+14.6%), followed by the mineral (+9.2%), the organic (+6.6%), and the microbial (2.5%) cases.

The chlorophyll content in sweet corn leaves, expressed as the SPAD index, is presented in Figure 3. An increase was observed in all treatments compared to the control during the milk maturity stage. The highest values were recorded in the combined and mineral fertilization variants, exceeding the control by approximately 26% and 25%, respectively, followed by the microbial variant with 18% and the organic variant with 14%. No statistically significant differences were detected among the treatments at $p \leq 0.05$.

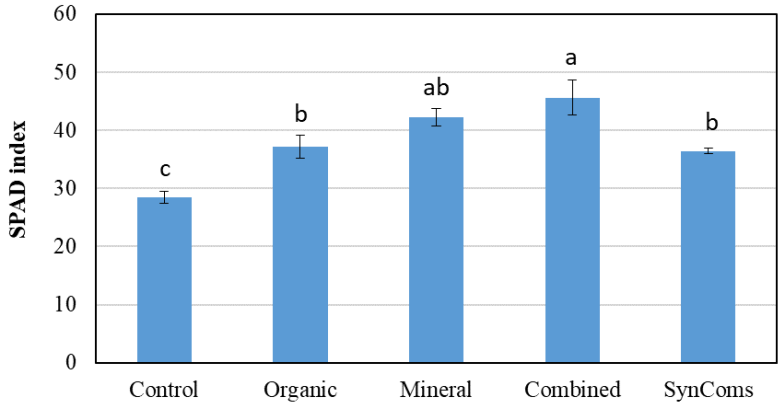


Figure 3. SPAD index measured during the milk maturity stage. Results showed the mean of three replicates and standard error (n = 4).

3.3. Soil microbial analyzes

3.3.1. Basal and substrate-induced respiration

Soil basal respiration was generally higher in the studied soils (Figure 4, left). Data collected before sowing showed existing differences among the studied plots due to the long-term experimental history. Soil basal respiration was highest in the combined and microbial consortium (SynComs) treatments before adding amendments and sowing sweet corn—by 95% in respect to the control—but the results were not significant. All basal respiration values were in the range of 0.67–2.41 μg CO_2-C g^{-1} h^{-1}. Due to the amendments and plant root effects, microbial activity, expressed as BR, increased in all treatments, and the differences were found to be significant. The largest increment was observed in the organic fertilization treatment, followed by the mineral one, and control, with increases of 308.57%, 268q40%, and 261,20%, respectively. In the combined treatment, the respiration rate increased by 191,67%, whereas in the SynComs variant, it increased by 171,79%. Thus, organic fertilizer and plant roots resulted in the highest microbial activity compared to the control (ripening stage)—by 100%—followed by mineral treatment at 38.03% and combined and SynComs at 31.42% and 27.62%. Nevertheless, significant differences were found only between the organic fertilization treatment and the rest.

The microbial activity was affected by the addition of easily degradable carbon substrate in the

study of SIR (Figure 4 right), and in all treatments it was increased in the following order: mineral, organic, SynComs, combined, and control, with increases of 167.86%, 157.14%, 133.33%, 70.87%, and 47.06%, respectively. These differences are related to the potentially mobilizable microbial biomass, representing the capacity of microbial growth rate in each treatment before adding amendments and seeds. The soil of organic plots showed a higher SIR than the other treatments, while the lowest was observed in the combined fertilization treatment. In the ripening stage, the highest respiration was observed in the organic treatment—significantly relative to the rest—followed by mineral and SynComs. The lowest SIR was found in the combined treatment. The comparison between the amended variants and the control showed the greatest difference with organic fertilizer (116%), followed by the mineral (50%), SynComs (40%). It appears that organic fertilization possesses higher potential to mobilize the soil microbiome, followed by mineral fertilization and beneficial populations, than combined fertilization and the control.

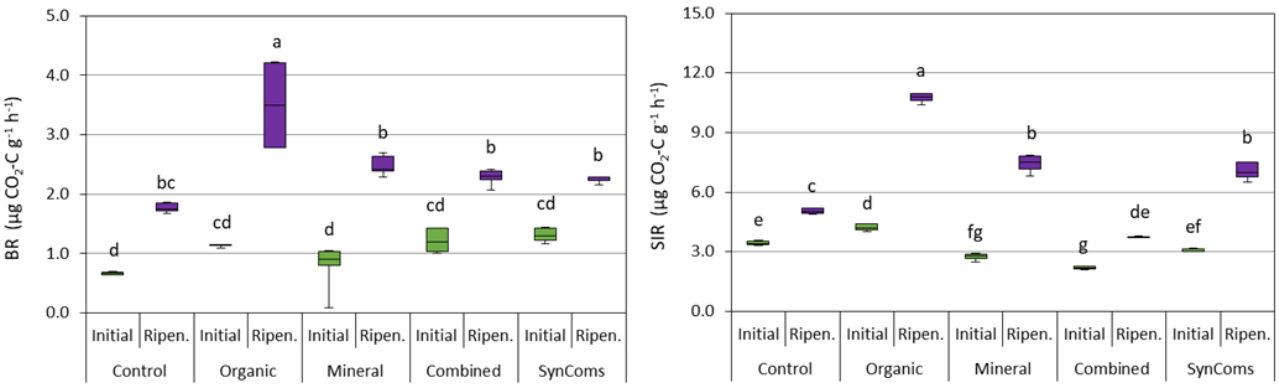


Figure 4. Soil basal (left) and substrate-induced respiration (right) before sowing and during the ripening stage of sweet corn. The results showed the median of four replicates and the standard error. Different letters indicate least significant differences between treatments at significance of $p \leq 0.05$.

The metabolic quotient (qCO_2) in both cases of our study is shown in Figure 5, left. The study was affected by site management over the years and by the continuous application of fertilizers. In this sense, the plots before sowing sweet corn showed certain discrepancies among treatments. Thus, lower qCO_2 was observed in the control, and the difference with the rest of the control and the difference with the rest of the cases was significant, except for the organic one. The highest qCO2 was found in the case of combined treatment, which was significant compared to the rest of the cases, except the SynComs one. Plant development led to changes in mean values that, in some cases, were significant, while in others they were very small. In the ripening stage, most of the qCO2 were in the range of 0.007−0.009 µg CO_2−C and did not show any statistical difference. Only in combined treatment did the qCO2 values exceed 0.015 µg CO_2−C h^{-1} μg^{-1} and were significantly higher than the rest. Both sampling moments differed significantly because of the biological activity in the soils. That is why in some plots a considerable difference was observed. In all studied variants, except SynComs, qCO_2 was higher in rhizosphere soils than in soil before sowing (Figure 5, right), so the values after dividing the means were greater than 1. Thus, in organic, mineral, and combined treatments, the values ranged from 1.05 to 1.14. In the unamended control, qCO_2-correlation was 1.83, which means that plants

contributed to the microbial communities' development and metabolic activity, almost doubling the values of MQ. In that treatment, the data were significantly higher than in the rest of the treatments. Because higher qCO_2 values were found in SynComs before sowing than in the ripening stage, the correlation was lower than 1.

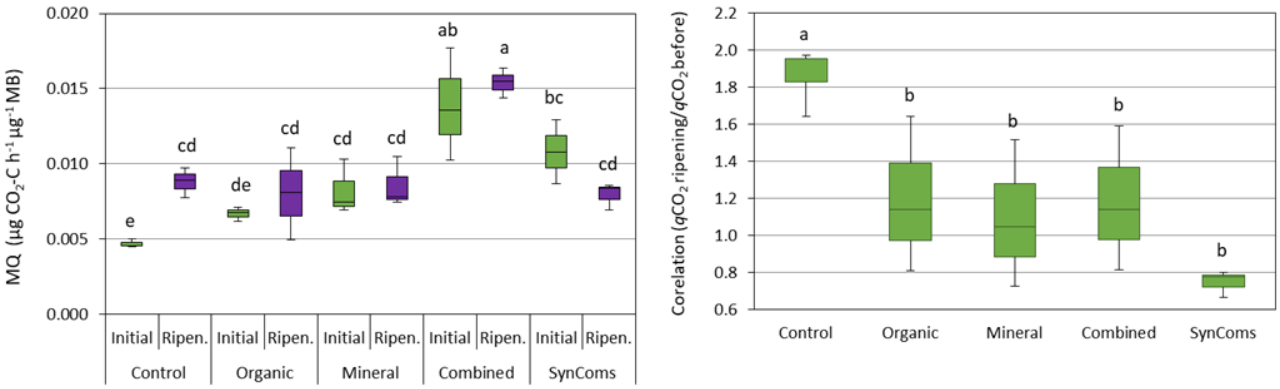


Figure 5. Microbial metabolic quotient calculated for studied treatments before sowing and during ripening of sweet corn (left) and correlation between qCO_2 at ripening stage and before sowing (right). The results showed the median of four replicates and the standard error. Different letters indicate least significant differences between treatments at significance of $p \leq 0.05$.

3.3.2. Soil enzyme activities

Studies on dehydrogenase enzyme activity did not show significant differences between the variants before sowing and before the addition of amendments. During ripening, some differences were noted between individual variants, some of which were statistically significant. In the control, no difference was observed despite the presence of plants. In organic fertilization, an increased activity of this enzyme was found without significance. The increase in mineral fertilizer application reached 79.1%, in combined fertilization 75.3%, and in the microbial variant 45.9%; all three values were significant both in relation to the data before sowing and in relation to the other variants.

Regarding phosphatase activity during the period of milk maturity of sweet corn, a statistically significant difference was established for different amendments (Figure 6). Activity before sowing was demonstrably higher in all amended variants compared to the control, likely due to the long-term addition of amendments over the years. During ripening, all amended variants showed a strong increase in phosphatase activity in the rhizosphere. When comparing these values to the control, we observed statistically significant differences in the following descending order: organic, mineral, microbial, and combined—with increases of 210.5%, 145.8%, 132.9%, and 119.9%, respectively. The highest influence on soil phosphatases was exerted by the organic amendment, with significant differences from all other variants.

For β-glucosidase, a statistical difference was found in both sampling periods. Before the application of amendments, the highest enzyme activity was observed in the microbial (60.5%) and combined (40.9%) variants compared to the control, followed by organic (27.3%) and mineral (19.4%). During ripening, the combined variant showed the highest activity above the control (+50.0%),

followed by organic (+28.9%), microbial (+27.3%), and mineral (+18.6%) variants (Figure 6).

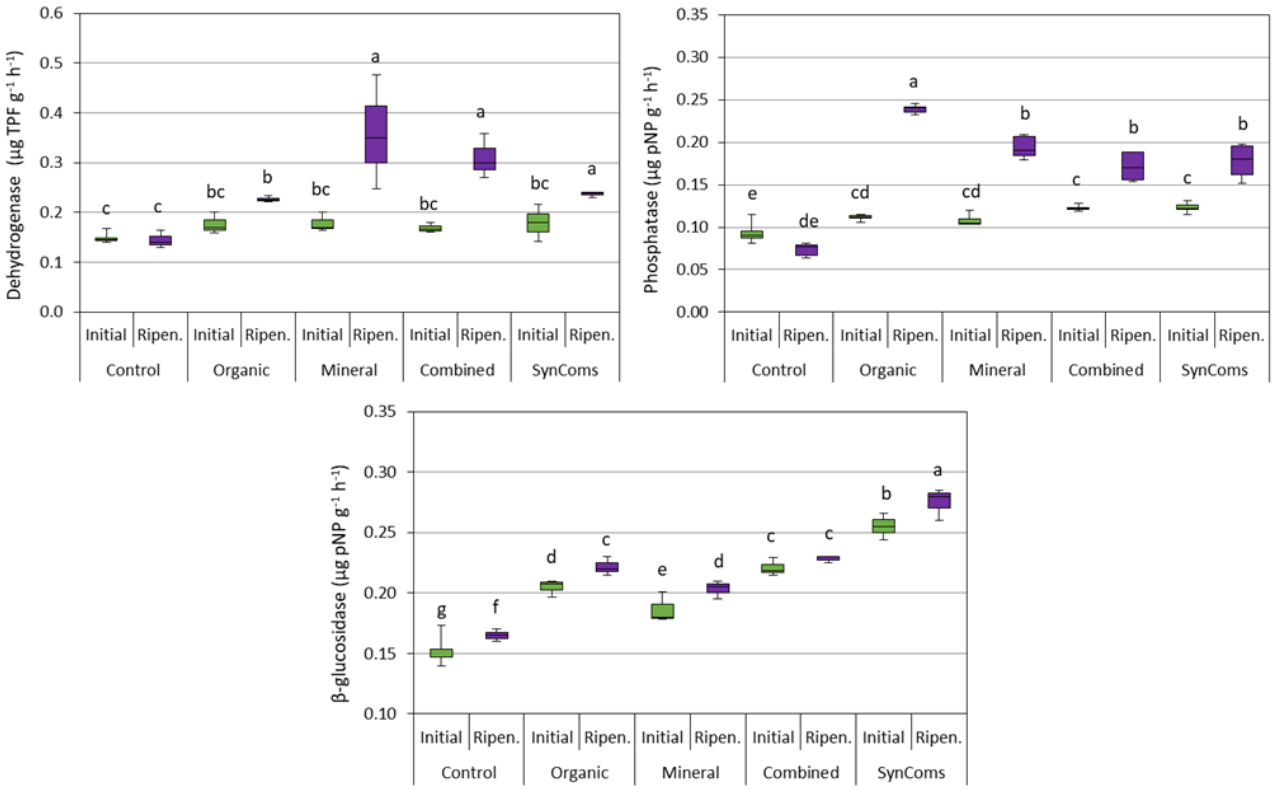


Figure 6. Soil enzyme activities (dehydrogenase, phosphatase, β-glucosidase). The results showed the median of four replicates and the standard error. Different letters indicate least significant differences between treatments at significance of $p \leq 0.05$.

3.3.3. Community-level physiological profiling with Biolog^{TM} EcoPlates

The carbon source utilization capacity of the rhizosphere microbial communities showed different metabolic rates between the two sampling periods and among the treatments (Figure 7). Before sowing (Figure 7, left), the slope of the AWCD curves differed considerably among treatments throughout the incubation period. The highest utilization rate was observed in the mineral and combined fertilization treatments, followed by the control and organic variants. The microbial fertilizer treatment showed the lowest community utilization rate before sowing, reflecting the baseline state of the soil microbiome prior to seed inoculation.

During ripening (Figure 7, right), the stratification among treatments was considerably reduced, with all variants showing similar AWCD values throughout the incubation period. The highest utilization rate at ripening was recorded in the organic treatment, followed by mineral, combined, control, and microbial variants. This convergence is attributed to the homogenizing effect of plant root exudates on the rhizosphere microbial communities.

The increment in AWCD between the two sampling periods was most pronounced in the microbial fertilizer and organic variants, while mineral and combined treatments showed the lowest increase, as their communities were already highly active before sowing (Figure 7, down).

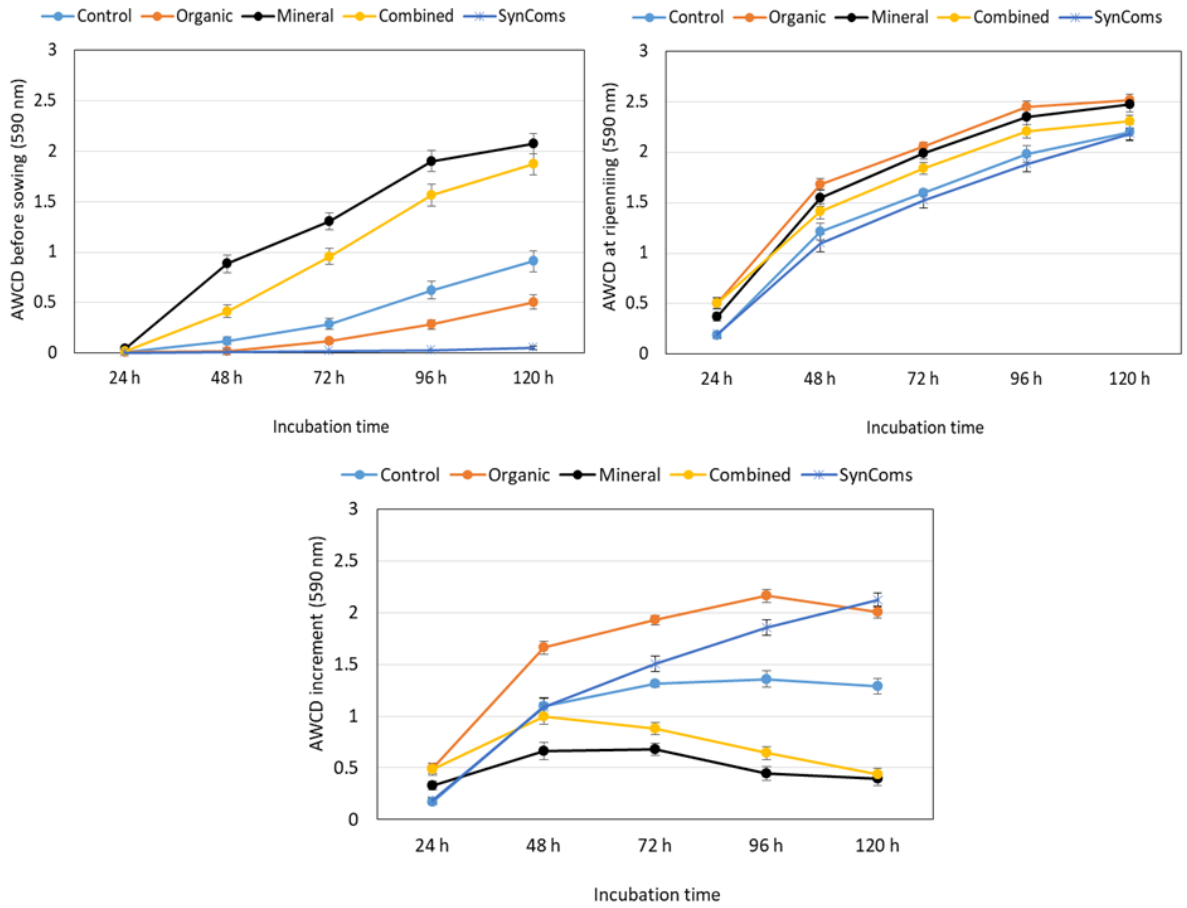


Figure 7. Average well color development measured in the soil samples before sowing, at ripening, and the increment at 590 nm. The data represent the mean and standard deviation (n = 4).

4. Discussion

The soil functioning correlates with the soil microbiome, which actively participates in the formation of soil fertility and crop yields (Shilev et al., 2022) [33]. The recorded yield of sweet corn was 37% higher than the control in the microbial fertilizer treatment (incorporation of microbial consortium), followed by the mineral fertilizer treatment at 29%, combined at 27%, and organic at 14%. Similar results have been obtained upon inoculation with multi-strain PGPR in corn, with yield increases of 22–29% compared to uninoculated control treatments. The improved yield was attributed to increased microbial diversity in the rhizosphere and better nutrient uptake (Chen et al., 2020) [34]. Amogou and co-authors demonstrated that PGPR inoculation combined with mineral fertilization increased corn yields in poor ferruginous soils by approximately 30% [35]. Another study conducted under water stress with PGPR (*Cupriavidus necator* 1C2 and *Pseudomonas fluorescens* S3X) increased yield by 18 to 35%, with the greatest increase recorded under combined inoculation, along with improved nitrogen and phosphorus uptake, higher photosynthetic activity, and better nutrient use efficiency (Dinh et al., 2020) [36]. A field study of a consortium of five growth-promoting bacterial strains applied to corn (*Zea mays*) in semi-arid conditions in Turkey significantly improved soil fertility

(increased organic matter content, more available phosphorus and potassium, increased cation exchange capacity) and led to higher grain yields. The genotype × dose interaction indicated that the effect varies depending on the corn genotype, and some foliar analyses showed elevated trace element values (Zn and Cu), highlighting the need to regulate application rates (Kurt, 2025) [37]. Results from studies with other crop species showed that integrating growth-promoting microorganisms in potato fertilization systems maintains yield at reduced fertilizer rates. When microbial inoculations were applied in combination with 50% of the standard mineral fertilizer dose, yields did not differ statistically from full mineral fertilization, confirming that microbial consortia can effectively compensate for reduced fertilizer quantities (Ollio et al., 2025) [38]. The application of growth-promoting microorganisms in wheat production represents an effective strategy for increasing yield and optimizing nutrient uptake, with yield increases averaging 15–25% compared to uninoculated controls (Rashid et al., 2016) [39]. All these literature testimonials confirm our data in different extents.

During the milk maturity stage, the highest SPAD index values and plant heights were recorded in the combined and mineral fertilization treatments. The application of biostimulants in potatoes leads to higher SPAD values, reflecting improved physiological status and more intensive photosynthesis, confirming that chlorophyll content is directly related to plant growth and vitality (Zarzecka et al., 2021) [40].

Agronomic practices, the type and method of fertilization, have a significant influence on microbial activity and the carbon cycle in the soil (Dhaliwal et al., 2024) [41,42]. In the laboratory measurements of basal and substrate-induced respiration, a general trend was observed: the organic treatment showed the highest microbial activity at ripening for both parameters, while the combined treatment demonstrated the lower potential for microbiome mobilization in the SIR measurements. The microbial and organic treatments showed the greatest increment between the two periods, suggesting higher mobilization potential under rhizosphere influence. This trend is also confirmed by the metabolic quotient (qCO_2), in which the combined treatment showed the highest values at ripening, and in all treatments except the microbial one qCO_2 was higher than before sowing, reflecting the substantial contribution of the rhizosphere to the metabolic activity of microbial communities. These results are consistent with the observations of Lazcano and co-workers in sweet corn, according to which organic fertilization is associated with higher microbial biomass and activity; it is not necessary to fully replace mineral fertilizers with organic ones (up to 25% of nutrients were supplied via organic material—rabbit manure or vermicompost) to have a positive effect on enzyme activity, basal soil respiration, and microbial biomass [43]. On the other hand, when compared only with nitrogen fertilizer in a no-till corn system, increasing nitrogen fertilization does not lead to a significant increase in soil respiration (Grandy et al., 2013) [44]. Basal soil respiration showed significant dependence on the type of fertilization, with organic fertilization leading to higher values compared to mineral fertilization. According to research reported by Zhang et al., organic fertilizers increase basal soil respiration by an average of 81.45%, while mineral fertilizers increase it by 47.78% compared to the control [45]. Chadwick et al. demonstrated that soils fertilized with organic manure shown higher organic carbon content and more intensive CO_2 emissions compared to minerally fertilized soils [46]. This confirms that the type of fertilizer used directly influences the carbon balance and microbial activity in agroecosystems.

The three studied enzymes showed a differentiated response to the type of fertilization, reflecting the specific biochemical processes in which each participates. The highest dehydrogenase activity was recorded under mineral fertilization, consistent with the results of Muhammad et al., who concluded

that mineral nitrogen stimulates microbial activity in the rhizosphere by increasing available nitrogen in the soil and altering its physicochemical properties [47]. The highest phosphatase activity in corn rhizosphere was recorded under organic fertilization with animal manure, since organic matter provides an additional substrate for phosphatase-producing microorganisms [48]. In addition, we found the highest β-glucosidase activity under combined fertilization. The results are consistent with those of other researchers who found in a wheat–corn rotation within a 34-year field experiment that the combination of balanced NPK fertilization with straw incorporation led to the most significant increase in soil β-glucosidase activity [49]. These findings also confirm the observations of Yang et al. that integrated organic–mineral fertilization regimes synergistically stimulate different groups of soil enzymes involved in the carbon, nitrogen, and phosphorus cycles [50].

The capacity for carbon source utilization by rhizosphere microbial communities, measured by CLPP with Biolog EcoPlates, showed different metabolic rates between the two sampling periods and among the treatments. Before sowing, the highest utilization rate was recorded in the mineral and combined fertilization treatments, while the microbial fertilizer treatment showed the lowest activity, reflecting the baseline state of the soil microbiome prior to seed inoculation. At ripening, the differences between treatments were considerably reduced, with the highest activity recorded in the organic fertilization treatment, attributed to the homogenizing effect of root exudates on rhizosphere microbial communities during the period of active growth. The greatest AWCD development between the two periods was observed in the microbial fertilizer and organic fertilization treatments, while mineral and combined fertilization showed the smallest increase due to their already high baseline microbial activity. Similar results have been reported in corn where the type of fertilization and the phenological stage of the plants were found as key factors determining the functional diversity and metabolic activity of rhizosphere microbial communities [49, and Kong et al. (2024)].

5. Conclusions

The application of fertilizers supported enhanced dehydrogenase and phosphatase activities, higher yields, and higher chlorophyll content. Mineral fertilization was characterized by the highest dehydrogenase, while organic fertilization showed the highest phosphatase activity. The organic fertilizer exhibited the highest microbial activity, as measured by both respiration types. The highest yields in amended variants were accompanied by the highest AWCD development between the two sampling periods in SynCom and organic variants, reflecting significant activation of the rhizosphere microbiome. Nevertheless, the highest microbial activity during ripening, in both basal and substrate-induced respiration, was observed in the organic variant, making it the most favorable from the perspective of the agroecosystem's carbon balance.

The results suggested the importance of alternative solutions to mineral fertilizers, such as organic amendments or microbial inoculants, that can ensure similar yields with lower environmental impacts. The research highlights the ever-growing need for in-depth studies of the soil microbiome, soil health, and balanced fertilization, which are key to maintaining optimal yields and preserving soil fertility. Thus, the sustainability of agroecosystems and the reduction of adverse environmental impacts require a comprehensive approach to soil management that considers microorganisms as a key supporting element.

6. The limitations of this study

Our study was conducted on plots where the fertilization regime has been applied for over 20 years. This factor should be taken into account when evaluating the study results. Nevertheless, we believe that the findings can also be interpreted positively, specifically regarding the long-term application of the given fertilizer type and its cumulative, as well as current, impact on the soil–microbiome–plant system. Another limitation is that the experiment presented here spans only one year, although it is highly representative given the long-term history of fertilization on these plots.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Teodora Tomova: Data curation (lead), Visualization, Writing — original draft. Ivelina Neykova: Data curation, Investigation, Validation. Veselina Vasileva: Data curation, Investigation. Nikolay Dinev: Funding acquisition (lead), Supervision. Stefan Shilev: Conceptualization (lead), Methodology, Writing — original draft, Writing — review & editing, Funding acquisition.

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