

# Impact of the microalgae-bacteria interaction on maize (*Zea mays* L.) health and yield

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**Abstract.** Microbial biofertilizers, which include microorganisms that improve soil nutrients and make them easier to cultivate, are eco-friendly alternatives to chemical fertilisers, encouraging plant growth and supporting sustainable agriculture. The purpose of the study was to evaluate the health of crops measured by the normalized difference vegetation index (NDVI) and yield, influenced by the combination of biomass from specific cyanobacteria (MACC-612, *Nostoc linckia*) and plant growth promoter bacteria (PGPB). Using a factorial design in a complete randomized block configuration, four replications were performed. The experimental design included the testing of three concentrations of microalgae (untreated, 0.3 g/L *N. linckia*, and 1 g/L *N. linckia*) and two PGPBs (untreated, *Azospirillum lipoferum*, and *Pseudomonas fluorescens*). Experiments in the field were conducted for three consecutive years (2021, 2022, and 2023). The results show that the combined application of *N. linckia* and PGPB to soil treatment has significantly improved plant health and yield characteristics. The combined use of 0.3 g/L *N. linckia* and *A. lipoferum* has improved the health of plants (NDVI), seed count per cob, thousand-seed weight, and total yields, achieving a significant increase of yield by 1.4 fold for 2021, 1.37 fold for 2022, and 1.39 fold for 2023. These results demonstrate that applying low concentrations of *N. linckia* (0.3 g/L) along with *A. lipoferum* provide a cost-effective solution without compromising the benefits. Consequently, the integration of cyanobacteria and PGPB represents a promising approach to improve crop growth and yield while minimizing environmental impacts.

## 1 Introduction

Increased demand for agricultural goods will put greater pressure on further expansion of crop production and should simultaneously focus on mitigation of negative environmental effects. Maize (*Zea mays* L.) is one of the most widely grown field crops in the world, growing in many regions. Given the high demand for nitrogen fertilizers, it is crucial to research other fertilizer options that provide agricultural gains, promote eco-friendliness and are economically viable [1, 2]. To meet the coming climate, economic and social challenges, agriculture needs to improve to ensure productivity, stability and resilience while minimizing

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environmental impacts [3]. In today's context, microbial sources are increasingly being supported as a sustainable and environmentally friendly method of promoting plant growth. Plant growth promoting bacteria (PGPBs) are effective strategies for stimulating growth and nutrient absorption with plants [4]. The possible advantage of *Azospirillum* can be largely attributed to its biochemical and anatomical improvements within the roots of host plants, which facilitate the uptake of minerals and water [5]. *Azospirillum* bacteria affects the growth rate and length of root hair, promotes lateral root development, and expands the surface of the root [6, 7]. *Pseudomonas* is a Gram-negative bacterium of the *Pseudomonadaceae* family, establishes symbiotic relationships with plant roots and serves as an effective biological inoculant, recognized for enhancing plant growth, nutrient uptake, and stress tolerance in the root zone [8, 9]. These uses various mechanisms to promote agricultural crop growth, including biological nitrogen fixation (BNF), plant growth hormones such as indoleacetic acid (IAA), gibberellic acid, cytokinins, and ethylene, along with solubility of crucial minerals such as, potassium, zinc, and phosphorus [10-12].

The *Nostoc* family, a cyanobacterium, has remarkable abilities for photosynthesis, polysaccharide secretion and atmospheric nitrogen fixation [13-16]. It offers a promising opportunity to address agricultural obstacles, particularly in unfavourable environments such as arid regions. In these harsh conditions, cyanobacteria can effectively mitigate challenges such as water scarcity, extreme temperatures, salt levels and soil infertility. Cyanobacteria are an innovative way of modulating plant growth and development [17]. Cyanobacteria have significantly contributed to optimising plant growth patterns by using their ability to fix nitrogen in the atmosphere, produce compounds that promote growth and improve the availability of nutrients [18]. Studies have consistently shown that cyanobacteria and PGPB are very effective in promoting plant growth and productivity. However, the effects of combining cyanobacteria with PGPB on the physiological processes and productivity of corn plants under field conditions are not yet well understood. Consequently, our goal was to evaluate their effects alone and in combination on the growth and yield of cereals (*Z. mays* L.) in the field.

## 2 Methodology

### 2.1 Experimental design

From 2021 to 2023, the trial took place over three years in three different locations of the farm of the University of Széchenyi István in Mosonmagyaróvár, Hungary. The study used four replications of the randomized complete block design (RCBD), including nine treatments. The study examined two main components: cyanobacteria (MACC-612, *Nostoc linckia*) and two types of PGPBs (*A. lipoferum* and *P. fluorescens*). The experiment involved three concentrations of cyanobacteria biomass treatment (untreated, 0.3 g/L *N. linckia*, and 1.0 g/L *N. linckia*) and three different PGPB treatment (untreated, *A. lipoferum*, and *P. fluorescens*).

The study used the MACC-612 strain (*N. linckia*) obtained from the Mosonmagyaróvár Algal Culture Collection (MACC) at the Albert Kázmér Faculty of Agriculture and Food Sciences of the University of Széchenyi István in Hungary. Meanwhile, *A. lipoferum* (NF5) and *P. fluorescens* (NCAIM B01666) are obtained from Biofil Microbiological, Genetic Technology, and Biochemical LLC in Budapest. A total of 36 plots were created by randomly matching each bacterial species to one of the three concentration levels of the cyanobacteria species, repeated four times. During sowing, cyanobacteria (*N. linckia*) and PGPB treatments were applied with a 15 L hand-held sprayer at a dosage of 300 L/ha. The experiment chose a hybrid variety of *Zea mays* L. from the Saaten Union-Körner grains. Plantations were held

on 4 May 2021 and 5 May 2022 and 2023. The test species *Zea mays* L. was sown in rows spaced 75 cm apart, with individual plants 20 cm apart and planted at a depth of 6 cm. Each plot measures 28.5 m<sup>2</sup> (3 × 9.5 m), totaling 256.5 m<sup>2</sup> per replication and 1026 m<sup>2</sup> for four replications. Plots are separated by 0.5 m, with 1 m between blocks.

## 2.2 Data collected and analysed

### 2.2.1 Normalised difference vegetation index (NDVI)

NDVI allows farmers to assess nutrient and crop biomass using indirect reflectance assessment [19]. The spectral reflection of leaves was measured on days 50, 65, and 80 of the DAS using the PSI PolyPen RP 410 handheld device. To evaluate each treatment, three measurements were recorded from fully developed leaves on the main stem of five plants, and the results were compared to average accuracy. NDVI scale vary between +1 to -1, with positive figures showing healthy vegetation and negative figures showing incomplete soil or no vegetation.

### 2.2.2 Yield parameters of plants

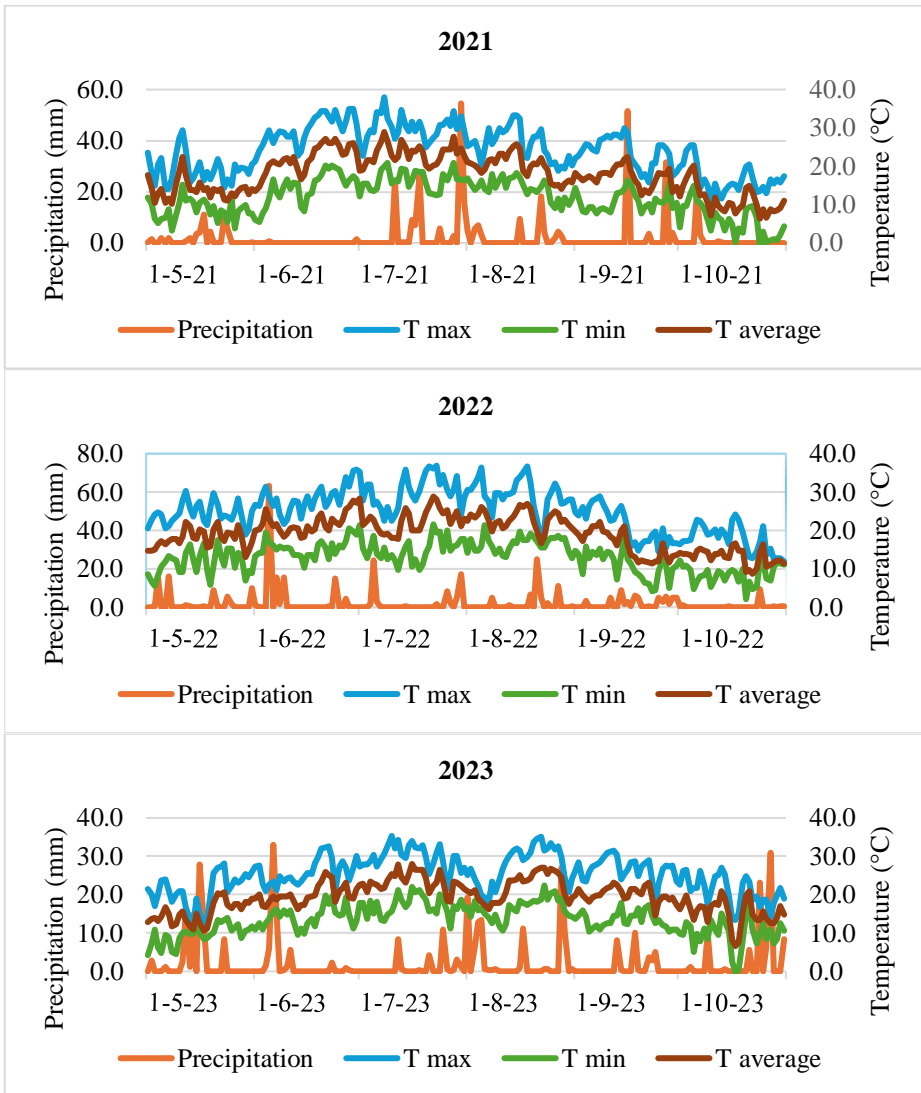
After corn matures in the study, several factors were thoroughly evaluated. This study included extensive assessment of several parameters after corn maturity. Four plants of each central site were chosen for detailed analysis, including measuring plant height, counting grains per ear, and determining the weight of thousands of grains. The weight of 1000 grains were determined by counting a sample of 1 000 grains and measuring the total weight with precision scales. The total production per hectare, expressed as tons, was evaluated on the basis of the harvest data of each parcel.

## 2.3 Statistical analysis

All data obtained from the experimental results are characterized by a standard distribution model, and two-way ANOVA is performed to evaluate the parameters associated with crop vegetation and yield attributes. The results are expressed as the average treatment values (n=4) for each treatment. Then Tukey's honest statistically significant difference (HSD) was analyzed at a level of significance of P = 0.05. Statistical analyses were carried out in R programming [20].

## 3 Result

The meteorological records in the study area show that the annual rainfall in 2021, 2022, and 2023 was 373.5 mm, 369.5 mm, and 403.9 mm respectively in the research site. In the 2022 and 2023 field trials, precipitation rose significantly, mainly during the vegetative growth period, compared to 2021 trials (Figure 1). However, during the reproductive period, the rainfall between 2021 and 2023 was relatively consistent, as opposed to 2022 (Figure 1).



**Fig. 1.** In the 2021, 2022 and 2023 production years, daily temperature and precipitation measurements from experimental fields were collected during the harvest period from planting to harvest. The temperature parameters recorded include T min (minimum temperature), T average (average temperature) and T max (maximum temperature).

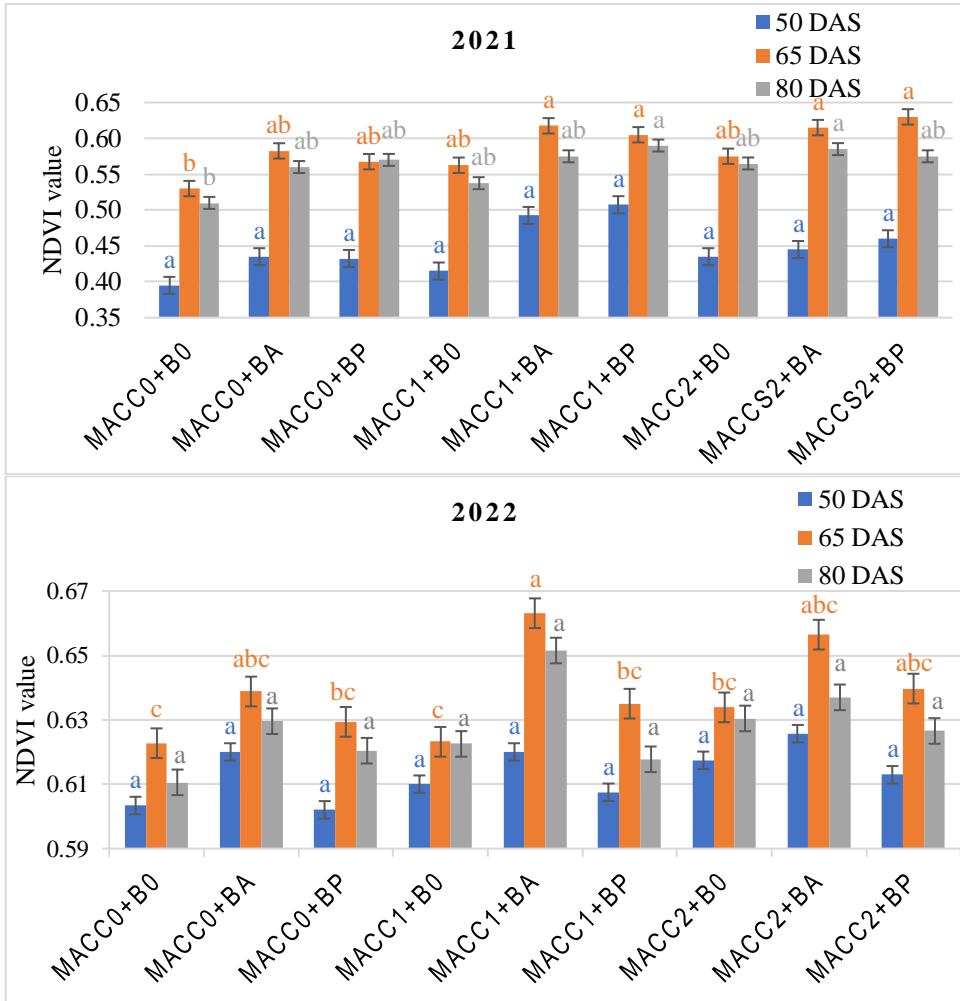
### 3.1 The normalised difference vegetation index (NDVI)

NDVI is a valuable method for measuring vegetation greenness, enabling vegetation density assessment and detection of changes in plant health. These indirect reflectivity measurements are usually used to estimate crop growth and yields. NDVI values showed significant variations ( $P \leq 0.05$ ) between the various measurement periods. The maximum mean measurements were seen at 65 days after sowing (Figure 2 and 3). There were significant differences ( $P \leq 0.05$ ) in the NDVI value at 65 DAS were noted, except in 2023.

In 2021, except for 50 DAS measurements (Figure 2), the NDVI measurements were significant ( $P \leq 0.05$ ). At 65 DAS, the highest average NDVI values were recorded when *N.*

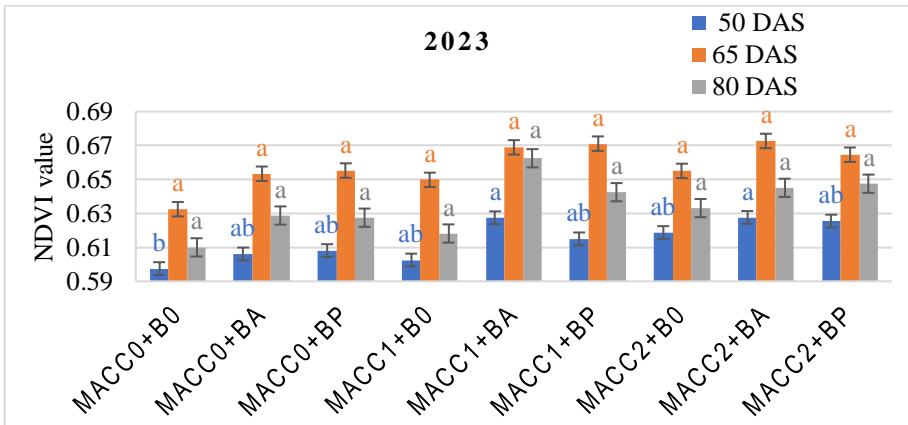
*linckia* was applied at 0.3 and 1 g/L alongside *A. lipoferum* and *P. fluorescens*, respectively, and the minimum values were recorded in the untreated group (MACC0+B0). At 80 DAS, the highest NDVI values were obtained when treating *N. linckia* at 0.3g/L g/L with *P. fluorescens* and *N. linckia* at 1g/L alongside *A. lipoferum*.

In 2022, the NDVI value was insignificant ( $P \leq 0.05$ ), except 65 days after seeding (Figure 2). The highest NDVI was recorded when *N. linckia* 0.3g/L and *A. lipoferum* were combined, while the lowest value was observed when *N. linckia* 0.3g/L was applied alone.



**Fig. 2.** The NDVI value of corn (*Zea mays* L.) was evaluated on several days after seeding of corn in 2021 and 2022. Treatments include MACC0, MACC1 and MACC2, respectively, with concentrations of 0 (control), 0.3g/L, and 1g/L of *N. linckia*. The bacterial treatment was B0 (control), BA (*A. lipoferum*) and BP (*P. fluorescens*).

In 2023, statistical significance of the treatment was observed only for 50 DAS measurements, and there was no significant difference on other observation days ( $P \leq 0.05$ ) (Figure 3). The highest NDVI values were obtained by adding 0.3 g/L and 1 g/L *N. linckia* alongside *A. lipoferum* and *P. fluorescens*.



**Fig. 3.** NDVI value of *Zea mays* L. was determined on various DAS (days after seeding) in 2023. The treatments included MACC0, MACC1, and MACC2, representing *N. linckia* concentrations (control, 0.3 g/L, and 1 g/L, respectively). The bacterial treatments were B0 (control), BA (*A. lipoferum*), and BP (*P. fluorescens*).

### 3.2 Plant yield attributes

Table 1 shows the effects of various concentrations of *N. linckia* and PGPB strains on corn yield parameters. The application of *N. linckia*, PGPB, and its combined interactions have significantly influenced the yield and characteristics of the species. The statistical analysis exhibited that applying *N. linckia* and PGPB, either sole or together over the production years, notably increases the seed count per cob, the weight of thousands of grains and the total yield. However, the impact of *N. linckia* and PPB on plant height are generally non-significant during the experiment, with the exception of the main important effects observed by PPB in 2023.

Throughout the experimental periods, the use of *N. linckia* at 0.3 g/L alongside *A. lipoferum* produced the maximum number of the seed count per cob, while the minimal amount was obtained by untreated control trials. In 2021, a similar crop yield per ear was observed when 0.3 g/L *N. linckia* alongside *A. lipoferum* and *P. fluorescens* and *N. linckia* at 1 g/L alongside *P. fluorescens*. The association between *N. linckia* and PGPB shows a various difference in each year,  $P \leq 0.05$  in 2021 and 2023, and  $P \leq 0.001$  in 2022 (see Figure 1).

The association between *N. linckia* and PGPB was generally not affected by thousands of grain weights, except in 2022, where  $P \leq 0.001$  was significant. The highest weights in 2021 and 2023 were obtained from *N. linckia* with 0.3 g/L and 1 g/L with *P. fluorescens* and *A. lipoferum*. In 2022, the association of *N. linckia* 0.3g/L with *A. lipoferum* lead to in higher weights, while the control (MACC0+B0) was the lowest. The treatment of 0.3 g/L of *N. linckia* alongside *A. lipoferum* resulted in enhancement of the thousand grain weight by 99%, 83.3%, and 90.9% in 2021, 2022, and 2023 respectively.

*N. linckia* and PGPB significantly influenced maize yields in all years except 2022, indicating that they had a major impact on crop productivity. The combination of 0.3 g/L of *N. linckia* and *A. lipoferum* triggered significant increases in grain yields, improved 33% in 2021 and significantly increased 32% in 2022 and 33% in 2023, compared to untreated plots (see Table 1). Overall, the third year recorded the highest grain yield compared to the previous years.

**Table 1.** Effects of single versus combined treatments of microalgae and PGPB on components of maize yield

Treatments	Plant height (cm)					Seed count per cob					Thousand-seed weight (kg)					Yield (ton/ha)		
	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023
MACCO+B0	213.15	171.75	206.38	385.10 <sup>b</sup>	400.34 <sup>d</sup>	421.00 <sup>d</sup>	0.26 <sup>b</sup>	0.35 <sup>h</sup>	0.30 <sup>b</sup>	5.20 <sup>d</sup>	5.97 <sup>bcd</sup>	5.97 <sup>bcd</sup>	5.20 <sup>d</sup>	5.93 <sup>b</sup>	6.22 <sup>c</sup>			
MACCO+BA	214.40	178.98	215.33	473.47 <sup>a</sup>	476.50 <sup>bc</sup>	503.88 <sup>abcd</sup>	0.43 <sup>ab</sup>	0.57 <sup>f</sup>	0.59 <sup>ab</sup>	7.16 <sup>ab</sup>	7.16 <sup>ab</sup>	7.16 <sup>ab</sup>	5.97 <sup>bcd</sup>	7.16 <sup>ab</sup>	6.81 <sup>abc</sup>			
MACCO+BP	216.84	180.03	211.47	472.68 <sup>a</sup>	441.33 <sup>cd</sup>	491.63 <sup>bcd</sup>	0.41 <sup>ab</sup>	0.52 <sup>g</sup>	0.56 <sup>ab</sup>	6.85 <sup>ab</sup>	6.85 <sup>ab</sup>	6.85 <sup>ab</sup>	5.78 <sup>cd</sup>	6.85 <sup>ab</sup>	6.65 <sup>bc</sup>			
MACC1+B0	217.00	182.03	210.67	472.68 <sup>a</sup>	435.80 <sup>cd</sup>	487.38 <sup>bcd</sup>	0.38 <sup>ab</sup>	0.61 <sup>e</sup>	0.52 <sup>ab</sup>	7.13 <sup>ab</sup>	7.13 <sup>ab</sup>	7.13 <sup>ab</sup>	6.03 <sup>bcd</sup>	7.13 <sup>ab</sup>	6.37 <sup>c</sup>			
MACC1+BA	217.95	184.69	223.12	517.05 <sup>a</sup>	548.60 <sup>a</sup>	589.00 <sup>a</sup>	0.77 <sup>a</sup>	0.85 <sup>a</sup>	0.80 <sup>a</sup>	8.15 <sup>a</sup>	8.15 <sup>a</sup>	8.15 <sup>a</sup>	7.27 <sup>a</sup>	8.15 <sup>a</sup>	8.62 <sup>a</sup>			
MACC1+BP	218.03	181.75	215.98	514.88 <sup>a</sup>	510.20 <sup>ab</sup>	548.90 <sup>abc</sup>	0.72 <sup>a</sup>	0.72 <sup>b</sup>	0.71 <sup>a</sup>	7.99 <sup>abc</sup>	7.99 <sup>abc</sup>	7.99 <sup>abc</sup>	7.09 <sup>ab</sup>	7.71 <sup>a</sup>	7.99 <sup>abc</sup>			
MACC2+B0	214.40	182.81	213.25	482.53 <sup>a</sup>	504.67 <sup>ab</sup>	463.63 <sup>cd</sup>	0.47 <sup>ab</sup>	0.68 <sup>c</sup>	0.67 <sup>ab</sup>	7.04 <sup>abc</sup>	7.04 <sup>abc</sup>	7.04 <sup>abc</sup>	6.02 <sup>bcd</sup>	7.33 <sup>ab</sup>	7.04 <sup>abc</sup>			
MACC2+BA	215.70	183.63	219.42	461.90 <sup>ab</sup>	508.50 <sup>ab</sup>	577.25 <sup>ab</sup>	0.67 <sup>a</sup>	0.73 <sup>b</sup>	0.81 <sup>a</sup>	8.31 <sup>ab</sup>	8.31 <sup>ab</sup>	8.31 <sup>ab</sup>	7.07 <sup>ab</sup>	7.96 <sup>a</sup>	8.31 <sup>ab</sup>			
MACC2+BP	218.10	180.31	217.97	475.08 <sup>a</sup>	472.50 <sup>bc</sup>	551.88 <sup>abc</sup>	0.64 <sup>ab</sup>	0.64 <sup>d</sup>	0.73 <sup>a</sup>	7.89 <sup>abc</sup>	7.89 <sup>abc</sup>	7.89 <sup>abc</sup>	6.75 <sup>abc</sup>	7.03 <sup>ab</sup>	7.89 <sup>abc</sup>			

Notes: The data are presented as mean values (n=4), with different superscript letters indicating significant differences based on Tukey's test at P ≤ 0.05. Notations are as follows: ns denotes no significance; \* signifies significance at P ≤ 0.05; \*\* at P ≤ 0.01; and \*\*\* at P ≤ 0.001. The control groups are represented by MACCO and B0; MACC1 and MACC2 correspond to 0.3 g/L and 1 g/L of *N. linckia*, respectively; BA and BP refer to *A. lipoferum* and *P. fluorescens*, respectively.

## 4 Discussion

Exploring the use of beneficial microorganisms in plants is essential to improve plant health (normalized differential plant index, NDVI) and improve yields in several agricultural environments. The goal of research was to assess the synergistic effects of *N. linckia* and PGPR on the crop growth and production of corn at various applications of cyanobacteria and bacteria. Analysis showed various results in assessing the sole and joint impacts of *N. linckia* and PGPR. The NDVI has been widely applied in the assessment of plant cover [21] and in the monitoring of crop growth [22]. Leaf spectral measurement, affected by crop biomass, growth phase, and stress influences are a strong indicator and provide valuable insights into plant current state. Our results show that the combination of *N. linckia* and PGPB has had a substantial influence on NDVI values through the experiment period. In 2021, the NDVI value was lower than the following year, likely due to a decrease in rainfall during the vegetative phase. The highest NDVI values observed when *N. linckia* was mixed with *A. lipoferum* at concentrations of 0.3 g/L and 1 g/L, indicating a significant improvement in plant health and vitality. The NDVI reflects plant greenness and photosynthetic activity, showing that these treatments increase crop growth and canopy development. This improvement is due to the synergistic effect of *N. linckia* and *A. lipoferum*, which can increase the availability and absorption of nutrients, promote better root growth, and improve the overall health of plants. On the other hand, the lower NDVI values recorded in the control group emphasize that these beneficial effects are not obtained without microbial treatment. Without the help of *N. linckia* and *A. lipoferum*, control plants are likely to experience limited nutrient availability and reduced growth efficiency. NDVI provides farmers with a unique tool to estimate shoot biomass and the absorption of nutrients during the initial stage of the crop's growth and provides them with an estimated growth and nutritional status [19]. Reflectivity indicators linked to plant biomass, specific physiological processes and plant biochemical composition are essential for plant monitoring in short and long term [23]. Our research has shown that the joint use of *N. linckia* and PGPB improves the growth and development of maize plants, resulting in improved productivity and yields.

The study showed that *N. linckia* either alone or combined with PGPR had enhanced maize yield properties. This effect has a dramatic effect on the growth of seed per ear, the weight of thousands of grains and the total yield evaluated to the untreated conditions. The observed enhancement in crop growth parameters is due to increased plant absorption of important nutrients from the soil. This improvement is due to the presence of *N. linckia* and *A. lipoferum*, known for promoting the absorption of nutrients. In particular, these microbes increase the solubility and mobilization of nutrients, making them more accessible to plant roots. A significant number of soil microorganisms could improve plant nutrient absorption by providing sustainable and environmentally friendly methods for plant nutritional needs [24, 25]. However, we found that *N. linckia* and PGPB in the study period produced non-significant effects on plant height. On the contrary, the application of cyanobacteria and rhizobacteria to promote plant growth in maize and wheat crops has significantly outperformed untreated in terms of plant height, highlighting the effectiveness of cyanobacteria in promoting vertical growth of maize plants [26-29].

The significant increase in seedlings per ear was observed by mixing 0.3 g/L *N. Linckia* with *A. lipoferum*, suggesting the synergistic effect of these treatments on seedling production. This indicates that combined use of these two bio-fertilizers can improve the availability of nutrients or improve the physiological responses of plants, resulting in improved reproductive outcomes. Statistics indicate that, with the exception of 2022, the association impact of *N. linckia* and PGPB on the thousand-seed weight was non-significant. This suggests that the combined application of these treatments does not result in synergistic

effects on grain weight, but the individual contributions of *N. linckia* and PGPB are still crucial. During the experiment, *N. linckia* and PGPB had a significant major impact on grain weight and demonstrated a consistent positive impact. The highest weight of a thousand grains was achieved by the combination of 0.3 and 1 g/L *N. linckia* with *A. lipoferum*, underscoring the effectiveness of these treatments in increasing seed mass. This shows the importance of *N. linckia* and PGPB to improving crop yields, even without a significant interaction effect.

Our study showed that *N. linckia*, 0.3g/L concentration paired with *A. lipoferum* were positively influenced by corn yields, resulting in a significant increase in grain yields of 7.09 tons (1.4-fold) in 2021, 7.71 tons (1.37-fold) in 2022, and 8.62 tons (1.39-fold) in 2023 compared to control. Previous studies have shown that the use of cyanobacteria and PGPB individually or in combination leads to increased growth and production of corn. This improvement is achieved through direct mechanisms such as better resource utilization and regulatory plant hormone levels, and indirect mechanisms such as mitigating the effects of various harmful substances [30, 31]. The integration of cyanobacteria and PGPB is a promising strategy for stimulating crop growth and increasing yields in important crops such as maize [30-34]. This method not only improves the efficiency of the use of nutrients (NUE), but also increases the uptake of crucial nutrients, especially nitrogen (N) [35, 36]. In 2022, the interaction effects of 0.3 g/L of *N. linckia* along with *A. lipoferum* and *P. fluorescens* on grain yields, as well as 1g/L of *N. linckia*, and *A. lipoferum*, showed that both treatment combinations had similar effects on the measured parameters. It is thus suggested that an increase in *N. linckia* concentration from 0.3g/L to 1g/L with *A. lipoferum* does not significantly enhance the beneficial effects, and that addition of *P. fluorescens* does not show enhancement. This analysis showed that a lower concentration of *N. linckia* (0.3 g/L) in combination with *A. lipoferum* was as effective as a higher concentration (1 g/L), potentially providing a cost-effective solution without compromising the benefits. It also demonstrates the robustness of microbial combined treatments in improving the performance of plants at different concentrations.

## 5 Conclusion

The result of the experiment highlighted that combining *N. linckia* and PGPB significantly affected the NDVI values throughout the experiment, and that the health of plants was enhanced. Furthermore, our observations showed that the use of *N. linckia* and PGPB alone or together improved corn yield parameters, including the number of seed count per cob, thousand-seed weight and total yields. In particular, the combination of 0.3g/L *N. linckia* and *A. lipoferum* increased the health and yield of the plant during the experimentation season. These results suggest that a lower concentration of *N. linckia* (0.3 g/L), when used with *A. lipoferum*, can achieve outcomes comparable to the higher concentration (1 g/L), offering a cost-effective solution without sacrificing benefits. This integrated approach harnesses the synergistic effects of microorganisms to foster a balanced and sustainable agricultural system, addressing food security challenges while reducing environmental impacts.

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