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Impact of microalgae-bacteria interaction on maize (*Zea mays* L.) growth and soil fertility

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MOSONMAGYARÓVÁR

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Impact of microalgae-bacteria interaction on maize (*Zea mays* L.) crop yield and soil fertility

Written by

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GLOSSARY

Rhizosphere: The soil surrounding plant roots forms a narrow area housing a diverse underground ecosystem, with his significant observation indicating a greater abundance of microorganisms in the immediate vicinity of the roots compared to the surrounding soil.

Microbial inoculant: Bacteria, fungi, or other microorganisms, typically in pure cultures, are deliberately introduced into an environment to improve a specific function. Instances include the intentional use of microorganisms for biological pest control or to stimulate plant growth. This can involve either a single strain or a group of microorganisms working together.

Biofertilizer: are substances containing living microorganisms that, when applied to seeds, plant surfaces, or soil, can colonize the rhizosphere or interior of the plant and promote growth by increasing the availability or uptake of essential nutrients. These microorganisms fix atmospheric nitrogen, solubilize phosphate, or facilitate other nutrient uptake processes, enhancing plant health and productivity. They're considered environmentally friendly alternatives to chemical fertilizers, as they improve soil fertility and promote sustainable agricultural practices.

Biostimulant: are substances or microorganisms applied to plants or soil to enhance their natural processes, growth, and nutrient uptake, without serving as a conventional fertilizer or pesticide. They typically contain various compounds, such as amino acids, seaweed extracts, humic acids, or beneficial microorganisms, that stimulate plant

development, improve nutrient absorption, strengthen stress tolerance, or enhance overall plant health.

Biopesticides: are naturally derived substances or microorganisms used to control pests, diseases, or weeds in agriculture, forestry, or public health. They include microbial pesticides (such as bacteria, fungi, or viruses), biochemical pesticides (compounds naturally found in plants, animals, or minerals), and plant-incorporated protectants (proteins derived from genetically modified plants). Unlike conventional chemical pesticides, biopesticides often have lower toxicity to non-target organisms, degrade more quickly in the environment, and are considered more environmentally friendly alternatives for pest control.

Microbiome: The intricate community of microorganisms residing in a specific environment, such as soil, while it's commonly associated with bacteria and fungi, encompasses archaea, viruses, protists, and various other organisms. All these microorganisms can potentially influence the establishment of an inoculant.

Formulation: The process of preparing and stabilizing microbial cells so that they can be stored before being used.

Consortium: Several microbial groups are co-cultured and/or co-inoculated together within a specific environment. These groups can either have the potential to work together in a complementary manner or be capable of performing the same function, albeit in distinct environmental contexts.

ACRONYMS

Table 1. Abbreviations

Acronyms	
A. lipoferum	*Azospirillum lipoferum*
P. fluorescens	*Pseudomonas fluorescens*
N. linckia	*Nostoc linckia*
CFU	Colony forming unit
DAS	Days after sowing
PGPB	Plant growth promoting bacteria
PGPMs	Plant growth promoting microorganisms
MACC	Mosonmagyaróvári Algal Culture Collection
P	Phosphorus
K	Potassium
SMO	Soil microorganism
NUE	Nitrogen use efficiency
g	gram
kg	Kilogram
L	Liter
N	Nitrogen
NO_3^-	Nitrate
NO_2^-	Nitrite
NDVI	Normalized Difference Vegetation Index

ABSTRACT

Intensive chemical usage in agriculture to maximize yields results in soil degradation, impacts soil microorganisms, and disrupts ecological balance. Biofertilizers harboring living organisms hold allure due to their prospective favorable influence on plant growth, coupled with a diminished environmental footprint and cost-effective in contrast to conventional mineral fertilizers. The aim of the present study was to assess the capacity of a specific cyanobacterium (MACC-612, *Nostoc linckia*) biomass and plant growth promoting bacteria (PGPB) together, to enhance crop growth, increase grain yield, and promote soil health. The study followed a factorial approach of completely randomized block design with four replications. The three levels of the microalgae (control, 0.3 g/L of *N. linckia,* MACC-612, 1 g/L of *N. linckia,* MACC-612) and three levels of bacteria strains (control, *Azospirillum lipoferum*, and *Pseudomonas fluorescens*) were used for the experiment. Field experiments were established for three years (2021, 2022, 2023). The result demonstrated that the utilization of *N. linckia* and PGPB alone or in combination in soil treatment resulted in a significant enhancement in the chlorophyll, plant biomass, number of seeds per ear, the weight of a thousand seeds, and overall crop yield while also enhancing soil properties including pH, humus, (NO_3^-+ NO_2^-)-nitrogen and total nitrogen. Furthermore, there were statistically significant differences in the activity of bacteria and actinomycete populations. Using *N. linckia* at 0.3 g/L along with A. *lipoferum* positively influenced yield of maize, leading to a significant

enhancement in grain yield by 7.09 tonha^{-1} (33.20%) during 2021, 7.71 tonha^{-1} (31.53 %) in season 2022, and 8.62 tonha^{-1} (32.34%) in season 2023, as compared to the control. The result revealed that the combined application of *N. linckia* at the concentration of 1 g/L with *A. lipoferum* resulted increases the (NO_3^-+ NO_2^-)-N content by 27.05%, 59.20%, and 51.54% in 2021, 2022, and 2023, respectively compared to the untreated. Moreover, the studies show that the synergistic application of *N. linckia* at a concentration of 0.3 g/L, in conjunction with *A. lipoferum*, led to significant improvements in total nitrogen levels, registering increments of 40%, 20.69%, and 27.59% for the years 2021, 2022, and 2023, respectively, when compared to untreated control trials. The formulation of biofertilizers through synergistic combinations of two or more microorganisms, such as algae-bacteria, holds promise for enhancing crop productivity. Hence, optimal synergistic groupings were identified by combining *N. linckia* at a concentration of 0.3 g/L with *A. lipoferum*, leading to enhanced maize growth, increased yield, improved soil fertility, and increased microbial populations.

Key words: Soil fertility; Microorganisms; Plant growth promotion, Interaction of cyanobacterium biomass and soil bacteria.

1. INTRODUCTION

There has been a global tension between ever-growing demand for food, water, and energy sources, which calls for novel and sustainable approaches to increase agricultural productivity and maintain the environment. It is generally believed that sustainable agricultural intensification should be considered the issues of increasing production and reducing environmental damage. However, the current crop production system has become strongly dependent on agrochemicals, which have caused considerable damage to global ecological security such as acidification and hardening, decreasing beneficial soil microorganisms, and increasing disease incidence (Chandini et al., 2019; Meena et al., 2020b). Modern agriculture must assess its methods by integrating new systems to produce food sustainably. A novel and eco-friendly approach to addressing these challenges involves the development of microalgae-bacteria based products such as biofertilizers, biostimulants, and biopesticides, which reduce reliance on agrochemicals and achieve higher production and sustainable value in modern agriculture with minimalised the negative effects on agroecosystem. Microalgae and beneficial bacteria can be used alone or in consortiums as an alternative source of chemical fertilizers to enhance plant growth, nutrient cycling, plant protection, productivity, and soil fertility (Garcia-Gonzalez and Sommerfeld, 2016; Holajjer et al., 2013; Niu et al., 2020; Singh et al., 2011).

1.1 Enhancing sustainable agriculture with beneficial microorganisms

A wide range of beneficial microorganisms engage in intricate partnerships with plants, acting as growth facilitators and playing essential roles in promoting plant health and enriching soil fertility, with some microorganisms possessing known capabilities while others remain subjects of ongoing research. Prior research has demonstrated the advantageous potential of collaboration among diverse microorganisms, a concept of growing importance due to contemporary apprehensions surrounding the adverse consequences of agrochemicals, leading to heightened curiosity about advancing our comprehension of cooperative interactions within rhizosphere microbial communities and their potential applications in agriculture (Mahmud et al., 2021; Meena et al., 2020b).

The health, productivity, and fertility of soil are influenced by the interactions between plants and microbes in the rhizosphere (Souza et al., 2015). Soil microbiomes, led by plant growth-promoting bacteria (PGPB) like rhizospheric bacteria (Sharma and Kumawat, 2022) and symbiotic rhizobia (Jaiswal et al., 2021), are driving the emergence of a new era in sustainable agriculture, and these bacteria are recognized as plant health-promoting bacteria (PHPB) agents (Chen et al., 2022; Khalil and Shinwari, 2022). Additionally, cyanobacteria (blue-green algae) play a central role in sustainable agriculture by enhancing soil properties, providing nutrients, promoting plant growth, and acting as biocides against soil-borne pathogens, making them valuable

biofertilizers and contributors to agricultural sustainability (Eman et al., 2023; Singh et al., 2016). Microalgae and cyanobacteria, functioning as a primary producers, along with bacteria collectively form the uppermost strata of soil known as the biological soil crust, and this intricate ecosystem plays a pivotal role in augmenting soil fertility and ultimately boosting crop productivity (Abinandan et al., 2019; Dineshkumar et al., 2019; Glaser et al., 2022; Ramakrishnan et al., 2023; Vinoth et al., 2020). It enhances crop development and well-being through processes such as nitrogen fixation, the release of trace elements into the soil, nutrient solubilization, production of exopolysaccharides, stress resistance, increasing organic matter, and improved nutrient retention within the plant-soil system, ultimately benefiting plant growth and provide an alternative to chemical fertilizers and pesticides (Alvarez et al., 2021; Berthon et al., 2021; Farhangi-Abriz et al., 2020; Kang et al., 2021b; Lee and Ryu, 2021; Ramakrishnan et al., 2023; Reed and Glick, 2023; Song et al., 2022).

1.2 Symbiosis of microalgae-bacteria association

The unreliability of single-strain inoculations in the rhizosphere can be addressed by using PGPB in multispecies consortia, presenting a promising approach for enhancing plant growth, and offering a novel method to discover complementary PGPB within root and soil communities for the development of advanced biofertilizers (Barua et al., 2023; Khan et al., 2022; Liu et al., 2023). Recently, microalgae-

bacteria interaction has been proposed as a potential strategy to improve crop productivity through the generation of phytohormones such as auxin and cytokinin, the synthesis of polysaccharides, which aid in nutrient absorption, and the regulation of numerous biochemical processes and improve soil health (Fuentes et al., 2016; Gonzalez-Gonzalez and de-Bashan, 2023b; Solomon et al., 2023). A symbiotic partnership between microalgae and bacteria operates through a reciprocal exchange of metabolites. Primarily, bacteria utilize organic carbon released during algal photosynthesis. In return, they facilitate their growth by consuming oxygen, producing carbon dioxide, providing essential nutrients, vitamins, and trace elements to support microalgal growth, and generating growth-promoting substances, chelators, and phytohormones (González-González and de-Bashan, 2021; Solomon et al., 2023). Numerous research investigations indicate that heterotrophic bacteria play a widespread and crucial role in the growth and survival of algae through the provision of hormones and nitrogen sources (Amin et al., 2015; Bunbury et al., 2022; Kim et al., 2014; Smith and Francis, 2016).

1.3 Maize: An important crop worldwide

Over 9,000 years since its initial domestication, maize (*Zea mays* L.), commonly known as corn, has continuously expanded its multifaceted presence within global agricultural and food systems (Kennett et al., 2020). This recent surge in global maize production, driven by growing demand and a meeting of technological advancements, improved

yields, and expanded cultivation areas, positions maize as the current leading cereal by production volume, with the most extensively cultivated and traded crop in the next decade (Erenstein et al., 2022). This adaptable and multi-functional crop serves as a crucial source of feed worldwide while also holding significance as a food source, particularly in sub-Saharan Africa, Asia, and Latin America, in addition to its various non-food applications (FAOStat, 2021). Maize (*Zea mays*) assumes a multifaceted and ever-evolving role within global agricultural and food systems, contributing significantly to food and nutrition security (Grote et al., 2021; Poole et al., 2021; Shiferaw et al., 2011).

The provision of optimal nitrogen plays a pivotal role in shaping plant growth attributes, primarily because it serves as the primary contributor to plant cell components, notably within the photosynthetic apparatus (Luo et al., 2020; Pandey et al., 2000). The utilization of nitrogen (N) fertilizer has a beneficial impact on both the quantity and quality of maize production. This leads to an increase in the number of grains per ear and protein and mineral nutrient levels (Alves et al., 2023; Hammad et al., 2022).

Maize (*Zea mays* L.) exhibits promising responses when treated with soil as a biofertilizer for cyanobacteria, demonstrating enhanced growth, nutrient use efficiency, and increased tolerance to abiotic stress (Chittora et al., 2020; Dineshkumar et al., 2019; Eman et al., 2023; Prasanna et al., 2015; Solomon et al., 2023). Similarly, positive results

were obtained by applying cyanobacterial extracts as foliar biostimulants and seed priming, which further contributed to the overall development and well-being of the plants (Ördög et al., 2021; Santini et al., 2021; Sharma et al., 2020). To ensure the effectiveness of these consortia, it is crucial for their diverse members to maintain positive interactions with each other over an extended period.

1.4 Objective of the study

The literature clearly demonstrated that cyanobacteria and plant growth-promoting bacteria (PGPB) exhibited high efficiency in enhancing plant growth, soil microbial activity, and soil fertility. However, it is still less known how the combined use of different types of strains affects the physiological processes and productivity of crop plants. We also aimed to assess their influence on maize (*Z. mays* L.) growth and soil fertility. Hence, the current research was conducted under field conditions to assess and evaluate the potential of two-member consortia consisting of cyanobacterial biomass and plant growth-promoting bacteria strains on maize. To test this hypothesis, our primary emphasis was on evaluating these strains alone or in combination for different traits like maize physiological and yield attributes, along with the activity of soil microbes and chemical composition of the soil.

2. LITERATURE REVIEW

2.1 Plant-microbe interactions: How well do we understand them?

The interaction between plants and microbes is an intricate, ongoing process that has been in existence since plants first colonized Earth. Over millions of years, this association has led to the emergence of a distinct ecological entity known as the "holobiont," comprised of various host and non-host species (Dolatabadian, 2020; Douglas and Werren, 2016; Rosenberg and Zilber-Rosenberg, 2018). In both natural and agricultural environments, plants routinely encounter a diverse array of microorganisms, predominantly bacteria, fungi, algae, viruses, and protists, encompassing both beneficial and harmful strains (Gupta et al., 2017).

Since the early 1980s, significant molecular research has revealed key principles governing plant-microbe interactions, shedding light on how plants respond to microbial colonization, including pathogens. These fundamental principles involve the detection of microbial signals by precise plant immune receptors, initiating either defensive or symbiotic reactions (Jones et al., 2016), the use of microbial DNA and protein secretion mechanisms to convey effector molecules into plant cells, thereby shaping host cell activities (Büttner and He, 2009; Hwang et al., 2017), the orchestration of microbial and plant developmental processes to promote the creation of specialized structures that exchange or produce nutrients, such as nodules and galls, in the context

of pathogenic and symbiotic interactions (Zipfel and Oldroyd, 2017), and both at the community and binary levels, within plant-microbiota relationships (Hacquard et al., 2017). Nevertheless, there remain unanswered inquiries about how plants distinguish between helpful and harmful microbes, how they differentiate among various pathogenic species, and how gene regulatory networks and signal transduction pathways govern these mechanisms (Cheng et al., 2019).

Furthermore, it has been widely recognized that external environmental conditions significantly influence a vast array of plant-microbe interactions, if not all of them (Cheng et al., 2019). Environmental factors have a direct influence on the composition and function of the plant microbial communities (Andronov et al., 2012; Cheng et al., 2019; Pershina et al., 2016). Plants in their natural environment face a constant array of biotic stress resulting from pest and pathogens, as well as unfavorable environmental factors such as nutrient deficiency, drought, salinity, heavy metal toxicity, high or low light intensities, high and low temperatures, ozone, and UV-B radiation (De Coninck et al., 2015; Flemer et al., 2022; Hacquard et al., 2017; Hasanuzzaman et al., 2012; Smékalová et al., 2014; Zia et al., 2021). Environmental changes that disrupt root-microbe interactions have the potential to modify soil carbon reserves and biogeochemical processes (Moore et al., 2020). High temperatures have detrimental effects on both root architecture and the interactions between roots and surrounding microorganisms (Khan et al., 2021). Conversely, a decrease in temperature in the root zone negatively impacts the nodulation process and the fixation of nitrogen (Grover et al., 2011; Nihorimbere et al.,

2011). Likewise, the existence of heavy metals in the rhizosphere exerts toxic influences on the growth of roots (Pandey et al., 2022). These stresses directly impact the rhizosphere, leading to a significant impact on root development and consequently affecting the overall growth, well-being, and productivity of the plant (Khan et al., 2021).

2.1.1 Microbe-plant signaling

During microbe-plant signaling, microorganisms generate and release signals that initiate symbiotic interactions with the plant. The microorganisms living in close association with plant roots engage in ongoing communication with the plants, and these interactions have a crucial impact on the health and productivity of the crops (Berendsen et al., 2012). For plants to develop well, they must monitor the soil areas around their roots to identify harmful microorganisms while also maximizing the advantages provided by beneficial microorganisms, which aid in nutrient uptake and growth promotion. Typically, three mechanisms are proposed to clarify how microbial activity can enhance plant growth, including the enhancement of the availability of nutrients derived from the soil (van der Heijden et al., 2008), the manipulation of plant hormonal signaling (Verbon and Liberman, 2016), and the repulsion or out-competition of pathogenic microbial strains (Mendes et al., 2013) (Figure 1b & c).

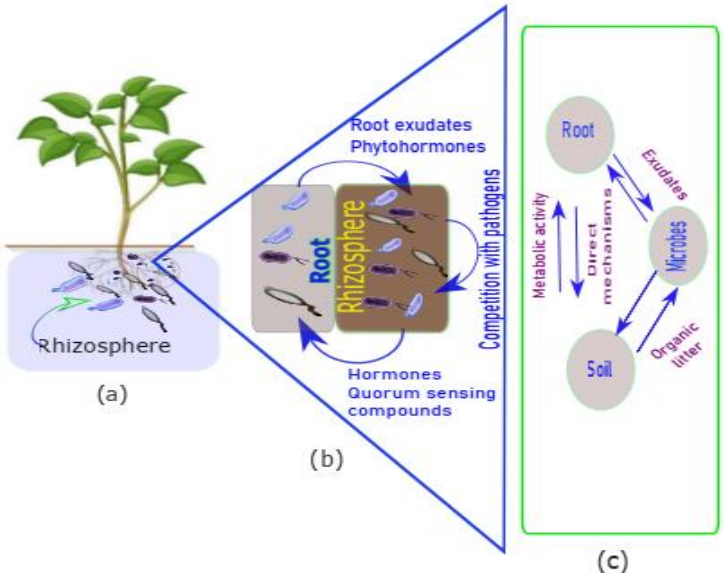


Figure 1: The dynamics rhizosphere structure composition (source: own editing). *(a) A dynamic rhizosphere interplay between roots and microorganisms. (b) Composition of microorganism's community in the rhizosphere and root is influenced by the presence of plants. The microorganism's population in the rhizosphere (narrow soil layer surrounding a plant's root) influenced by root activity and exudates, is more abundant compared to the microbe community found in the rest of soil. Plants have the ability to shape the composition of the rhizosphere microbiome through the release of root exudates. In return, microbes exert an impact on plant growth through various compounds, including hormones and quorum-sensing molecules. Furthermore, microbes indirectly influence plant growth by competing with pathogens for space and nutrients. Microbes play significant roles in both the root zone (rhizosphere) and the soil. Their effects can be both beneficial and detrimental, depending on the types of microbes*

present and their interactions. (c) The relationship between plants, microbiota, and soil involves nutrient exchange, alteration of soil properties by organic matter and microbial activities, direct effects of microorganisms on plants including hormone signaling manipulation and pathogen protection, and plant-microbe communication through root exudates.

2.1.1.1 Improve the availability of nutrients

Within natural ecosystems, a significant proportion of essential nutrients like nitrogen (N), phosphorus (P), and sulfur (S) are predominantly sequestered in organic compounds, resulting in limited bioavailability for plants. However, plants rely on the growth and activities of soil microbes, specifically bacteria, and fungi, that possess the necessary metabolic machinery to break down and convert these organic forms of N, P, and S into mineralized forms, thereby enabling plants to access and utilize these nutrients (Rashid et al., 2016; Singh et al., 2022). Microbes are essential for nutrient cycling in soil, facilitating the availability and uptake of nutrients by plants, and some specific microorganisms can improve soil nutrient supply, reducing the need for chemical fertilizers to support plant growth (Chamkhi et al., 2022; Chen et al., 2023; Grover et al., 2011; Singh et al., 2022). The beneficial microorganisms perform a multitude of plant growth-promoting activities, including nutrient mineralization, fixation, mobilization, and solubilization, as well as the production of growth-promoting substances, siderophores, antagonistic substances, and

antibiotics (Kumar et al., 2022a; Saeed et al., 2021; Suman et al., 2022). Plants attract specific groups of bacteria and fungi in the soil, which are determined by the distinct composition of root exudates released by each plant. As a result, plants tend to attract microorganisms that provide benefits to their growth while repelling potentially harmful pathogens (Glick and Gamalero, 2021). Studies have shown that introducing PGPR and plant-growth promoting bacteria (PGPB), and mycorrhizae of the genera *Pseudomonas*, *Bacillus*, and *Azospirillum*, and plant growth-promoting fungi (PGPF), or utilizing microbe-to-plant signal compounds, can effectively boost nutrient acquisition, nutrient cycling, plant protection, and improve crop growth (Backer et al., 2018; Cameron et al., 2013; Pieterse et al., 2014; Shoresh et al., 2010; Tedersoo et al., 2020; Zamioudis and Pieterse, 2012).

2.1.1.2 Manipulation of plant hormonal signaling

Microbial pathogens or symbionts that are successful in their interactions with plants have evolved strategies to manipulate the signaling pathways of plant hormones, inducing hormonal imbalances that serve their purposes (Gimenez-Ibanez et al., 2016). Recent advancements in the study of plant immunity have revealed valuable discoveries regarding the intricate defense signaling network. Various small-molecule hormones play crucial roles in regulating this network, with their signaling pathways interacting in either opposing or cooperative ways, giving plants the ability to finely control their immune responses (Ding et al., 2022; Pieterse et al., 2009).

Phytohormones play a vital role in regulating diverse physiological processes in plants, including defense responses against both abiotic and biotic stresses, with salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) serving as primary defense hormones, while growth regulators like auxins, brassinosteroids (BRs), cytokinins (CKs), abscisic acid (ABA), and gibberellins (GAs) also contribute to plant immunity (EL Sabagh et al., 2022; Großkinsky et al., 2016; Zheng et al., 2023). However, filamentous pathogens like fungi and oomycetes have evolved diverse strategies, using secreted effectors such as proteins, toxins, polysaccharides, and even phytohormones or their mimics, to interfere with phytohormone pathways. These pathogen effectors manipulate phytohormone pathways by directly modifying hormone levels, disrupting hormone biosynthesis, or interfering with key components of phytohormone signaling pathways (Berger et al., 2020; Han and Kahmann, 2019).

2.1.1.3 Pathogenic microbial strain: Repulsion or competitive exclusion

The rhizosphere communities provide protection against various foliar diseases through the release of antibiotics and the activation of plant defense mechanisms (Hou and Kolodkin-Gal, 2020). Simultaneously, the rhizosphere is also a highly competitive environment, where a multitude of microbial species participating in competitive interactions as they compete for resources and space (Hibbing et al., 2010; Tedersoo et al., 2020). Plant pathogens inhabit the rhizosphere,

intending to penetrate the protective barrier formed by other microorganisms and overcome the natural defense mechanisms of plants, ultimately leading to the onset of disease (Mendes et al., 2013). However, the manipulated beneficial microorganisms serve as an indirect component of the plant immune system, acting as a protective barrier against pathogen infiltration or triggering systemic resistance, whereby plants can selectively modify and attract beneficial microbial communities based on root-specific metabolic properties to positively influence the composition of rhizosphere microorganisms in response to pathogen invasion (Li et al., 2021). The microbial communities in the rhizosphere form a mutually beneficial relationship with plants through the use of quorum sensing signals (Aqsa and Ambreen, 2023). These signals have a strong effect on plants, triggering interkingdom communication and stimulating processes that enhance defense against pathogens and control insect pests (Hartmann et al., 2021; Majdura et al., 2023). In addition, quorum sensing signals have a regulatory function in various microbial activities, including the formation of biofilms, which are complex and structured communities of bacteria in the rhizosphere held together by extracellular matrices. (Aqsa and Ambreen, 2023; Keren-Paz and Kolodkin-Gal, 2020). These biofilms facilitate the coordination of activities among microbial cells, both within and across different species. Biofilms often offer advantages to other organisms, such as biocontrol agents that create biofilms on plant roots, effectively inhibiting the growth of harmful bacteria and fungi (Ajijah et al., 2023; Fessia et al., 2022; Muhammad et al., 2020).

2.1.2 Roles in crop stress tolerance

Crops are stationary organisms that are constantly stressed by biotic and abiotic causes. The influence of abiotic factors such as low and/or high temperatures, salinity, drought, alkalinity, and other factors, can lead to low productivity and yield quality because of the reductions in respiration, photosynthesis, and protein synthesis (Dwivedi et al., 2015; Sharma et al., 2012). The biotic factors caused by pathogenic bacteria, viruses, fungi, weeds, etc., affect the plant's host cell and modify the plant's genetic code, which takes to leads to the death of the plant (Suzuki et al., 2014). Research reports showed that around 30% of world's crop production is lost because of abiotic stress (Goswami et al., 2016). One possible way to reducing the effects of abiotic stress is the application of microalga which can play a substantial role in minimizing this loss by induced systemic tolerance (IST), which is stimulating various types of biochemical and physiological tolerance systems in plants (Sharma et al., 2012). Microbial biostimulants have been used in a sustainable approach for enhancing plant growth, productivity, and nutrition, even in the climate-stress situation (Fadiji et al., 2022). Some bacteria species (*Azospirillum brasilense*, *Pseudomonas* sp., and *Bacillus lentus*) have been used alone or in microbial associations that could minimize drought stress impact in crops (Sangiorgio et al., 2020).

Plant resilience and productivity in the face of global warming are likely influenced by the microbiomes associated with the plants over relatively short to moderate time periods, according to eco-

evolutionary responses (Trivedi et al., 2022). In the face of climatic stress, the intricate interactions between plants and their microbiomes seems to be modulated through chemical conversations. A fascinating phenomenon emerges as plants have developed a mechanism of releasing exudates that acts as a signal for assistance when confronted with challenging environmental circumstances. This signal prompts the recruitment of microbiomes that can help alleviate the stress experienced by the plants (Dubey et al., 2015; Liu et al., 2020; Lugtenberg, 2015; Pantigoso et al., 2022). The interaction between plants and microbes holds significant potential and promise in mitigating diverse forms of stress, including salinity, drought, pathogenic effects, and heavy metal toxicity (Pankaj and Pandey, 2022). Their interaction forms a diverse ecosystem, often characterized by mutualistic relationships between the two partners. The symbiotic relationship between roots and rhizobia also stimulates the plant's defense against root herbivores and provides protection against various diseases that can affect the roots (Maheshwari et al., 2015). The production of mucilage could function as a strategy for plants to uphold swift diffusion of exudates and maintain high microbial activity, even when water availability is restricted (Benard et al., 2018; Holz et al., 2019; Holz et al., 2018). PGPR, endophytes and AMF are among the microorganisms that play a crucial role in alleviating abiotic stresses and, consequently, enhancing plant growth (Khan et al., 2021; Munir et al., 2022).

The root associated microorganisms have the ability to enhance plant growth through various mechanisms such as regulating nutrient and

hormonal balance, producing plant growth regulators, solubilizing nutrients, and inducing resistance against plant stressors (Koza et al., 2022). The activation of defense signaling through the influence of a beneficial rhizomicrobiome, such as PGPR, against phytopathogens and pests, is referred to as induced systemic resistance (ISR). This mechanism operates independently of salicylic acid (SA) and follows a distinct pathway (Pérez-Montaño et al., 2014; Pieterse et al., 2014). The regulation of ISR is governed by the signaling pathways of phytohormones such as ethylene (ET) and jasmonic acid (JA) (Egamberdieva et al., 2017; von Dahl and Baldwin, 2007; Yu et al., 2022). *Pseudomonas fluorescens, Bacillus amyloliquefaciens, B. cereus, B. atrophaeus*, and other similar bacteria have been shown to effectively combat fungal, bacterial, and viral infections by inducing an immune response known as ISR (Wang et al., 2020; Yu et al., 2022). Within the rhizosphere, a dynamic environment, plants engage in continual interactions with a multitude of microorganisms. However, the precise timing and mechanisms by which these intricate interactions between roots, the rhizosphere, and microorganisms take place in the presence of stresses remain somewhat elusive and require further clarification.

One of the promising example observed for mitigating salt stress during seed germination process of bell pepper is application of microalgea extracts from *Phaeodactylum* spp and *Dunaliella* spp (Guzmán-Murillo et al., 2013). According to (Abd El-Baky et al., 2010), it has been advised that the addition of microalgal extracts to wheat (*Triticum aestivum L.*) that are irrigated with seawater could be

beneficial in increasing wheat's resistance to salty environments. Similarly, *Chlorella spp.* and *Spirulina spp.* boosted the antioxidant capacity and protein content of whole grains, as well as improved wheat's resistance to salinity (Abd El-Baky et al., 2010).

2.2 Potential of microalgae in crop production

Algae are photosynthetic organisms that can be found in a different variety of water and soil environments. Algae are generally classified as macroalgae and microalgae, with macroalgae being referred to as seaweeds, which are multicellular large-size algae that can grow up to 65m. However, microalgae are microscopic, single-celled organisms with small size, from 1 to 900 µm. Microalgae are composed of eukaryotic organisms and prokaryotic cyanobacteria (blue-green algae) that have found widespread application as a biological source across a variety of industries, including the agriculture, food, pharmaceutical, and biofuel (Khan et al., 2018; Kusvuran and Kusvuran, 2019; Renuka et al., 2018). In recent years, microalgae have become a sustainable agricultural product due to increasing the availability of nutrients, enhancing plant growth and crop yields, and maintaining the organic carbon and fertility of soil by boosting microbial activity in the soil (Barone et al., 2019; Silva et al., 2023; Youssef et al., 2022). The ability of the photoautotrophic microalgae to produce high-value compounds (like pigments, polyunsaturated fatty acids, and vitamins), alternative energy sources and natural processes for environmental protection (such as CO_2 mitigation, biofuel production, and wastewater treatment)

has led to a large market demand (Prasanna et al., 2016a; Renuka et al., 2018; Touloupakis et al., 2021). Microalgae are potential components of products that are biologically active metabolites such as biofertilizers, biostimulants, and biopesticides, which can be used in crop production, protection, and soil improvement (Gonçalves, 2021; Marks et al., 2019; Pathak et al., 2018; Plaza et al., 2018). The most common species of algae include *Spirulina*, *Chlorella*, *Nostoc spp.*, *Dunaliella*, *Scenedesmus*, *Isochrysis*, *Tetraselmis*, *Skeletonema*, *Pavlova*, *Chaetoceros*, *Phaeodactylum*, *Nitzschia*, and *Thalassiosira* (Beal et al., 2018; Han et al., 2019).

Several studies demonstrate that microalgae containing products can stimulate plant growth and yield either in a single (Table 2) or a consortium with bacteria (Table 3); and have the potential to reduce synthetic fertilizer and can defend against plant pathogens. These are due to a large variety of bioactive compounds producing excellent sources of chemicals such as phytohormones, carotenoids, phycobilins and amino acids. Microalgae enhance crop productivity by promoting plant growth, nutrient availability, and pathogens biocontrol (Michalak and Chojnacka, 2015b; Stirk et al., 2013). These products have diverse functional characteristics in crop production that promote an improvement in soil quality, nutrient uptake, enhancing crop performance, tolerance to biotic and abiotic stress conditions, and plant growth stimulation (Gonçalves, 2021; Kusvuran and Kusvuran, 2019; Renuka et al., 2018).

Table 2**.** Application of microalgae impact on crop production

Microalgae isolates	Tested plant	Effect on crop performance and soil fertility	Reference
Monoraphidium sp.	Tomato *(Solanum lycopersicum)*	Enhance plant biomass by 32% and 12% higher content in chlorophyll a	(Jimenez et al., 2020)
Nostoc piscinale	Maize *(Zea mays)*	Faster vegetative growth and higher chlorophyll content, higher grain yield	(Ördög et al., 2021)
Anabaena spp.	Wheat (*Triticum aestivum L)*, tomato	Enhanced viability and N fixing potential; enhance growth and nutrient uptake, increase yield and fruit quality; exhibited 10-15% lower disease severity	(Chaudhary et al., 2012; Prasanna et al., 2013; Swarnalakshmi et al., 2013)
Chlorella vul. and Spirulina platensis	Maize *(Zea mays)*	Increase plant height, improve yield character, and enhance seed germination.	(Dineshkumar et al., 2017)

2.3 Potential of plant growth promoting bacteria (PGPB)

Plant growth-promoting bacteria (PGPB) are a diverse group of bacteria known for their ability to boost plant growth and safeguard plants against diseases and environmental stresses using a multitude of mechanisms (Souza et al., 2015). Among them, bacteria like endophytes, which form intimate partnerships with plants, may prove particularly effective in promoting plant growth (Souza et al., 2015; Woźniak et al., 2019). Various critical bacterial traits, including but not limited to biological nitrogen fixation, phosphate solubilization, ACC deaminase activity, and the synthesis of siderophores and phytohormones, can be evaluated as indicators of their potential to promote plant growth (PGP) (Souza et al., 2015; Vandana et al., 2021). Moreover, Plant growth-promoting bacteria (PGPB) enhance plant growth and contribute to soil bioremediation by releasing various metabolites and hormones, facilitating nitrogen fixation, and improving the accessibility of other nutrients via mineral solubilization (Poria et al., 2022). The effectiveness and productivity of PGPB as additives for crops depend on several factors, including the bacteria's capacity to establish root colonization, the secretion of substances by plant roots, and the overall condition of the soil (Massa et al., 2022; Souza et al., 2015).

2.4 Mechanisms for plant growth promoting microorganisms

The food production industry is under pressure to maintain productivity and often relies on chemical fertilizers and pesticides, which can have

negative environmental and health effects. Agriculture needs alternative solutions to reduce costs and environmental impact without sacrificing productivity. Microbial agents, particularly microorganisms that possess various abilities related to plant growth, can serve as a beneficial substitute in this regard (Ahluwalia et al., 2021; Khatoon et al., 2020). Rhizospheric microorganisms found in soils possess numerous capabilities that can enhance plant growth, either through direct or indirect mechanisms (Glick, 2012). The direct effects include the production of phytohormones, improvement of nutrient availability, improving the development of root, nitrogen fixation, enhancing the enzymatic activity of plants and solubilization of phosphorus and potassium (Kumar et al., 2022a). The indirect effects on plant growth involve biocontrol, disruption of quorum sensing, and the induction of systemic resistance (Ahluwalia et al., 2021; Bhanse et al., 2022; Rigobelo et al., 2022; Vocciante et al., 2022). By performing these functions, microorganisms play a critical role in ensuring that crops have access to the necessary nutrients and conditions for optimal growth and health. They contribute to the long-term agricultural sustainability, crop growth, and productivity of the soil when used as biofertilizers, biostimulants, and biocontrol agents.

2.4.1 Biofertilizers

Biofertilizers are environmentally friendly containing living microbes or natural materials that improve soil fertility, crop development, and productivity by colonizing the plant's rhizosphere and increasing the

plant ability to absorb nitrogen, phosphorus, potassium, and minerals when applied to soil, plant, or seed (Mahanty et al., 2017; Ronga et al., 2019). Considerable research studies on biofertilizers have demonstrated their ability to supply the required nutrients to the crop in enough amounts that result in the improvement of crop growth and yield. Biofertilizers are living microbes that increase crop productivity by mobilizing or increasing nutrient availability in soil, in an economically feasible and environmentally friendly manner (Singh et al., 2011), and they are an substitute to chemical fertilizers. Biofertilizers are cost-effective; they minimize the side effect of environmental stress to a great extent and enhances soil fertility (Singh et al., 2011). It was testified that the application of biofertilizers improve crop yield by about 10-40% by increasing the contents of amino acids, proteins, nitrogen fixation, and vitamins (Bhardwaj et al., 2014; Prasanna et al., 2017). When microalgae were utilized as a source of biofertilizer, several research found a correlation between increased crop yields, increased nutrient uptake, and increased biomass accumulation (Hajnal-Jafari et al., 2020; Ronga et al., 2019; Shaaban, 2001).

2.4.1.1 Nitrogen fixation

Soil serves as a medium for plant growth and is a crucial resource that must be continually resupplied with nutrients. Among the different features of biofertilizers, formulations based on oxygenic photosynthesis, including cyanobacteria and eukaryotic microalgae,

are of increasing benefit in nutrient cycling, crop productivity, soil fertility and reducing chemical fertilizer application (G et al., 2016; Li et al., 2017). Certain cyanobacteria (free-living blue-green algae) can efficiently transform atmospheric nitrogen (N_2) into organic nitrogen forms, which is one of the vital nutrients for plant growth (Dey et al., 2017; Gonçalves, 2021; Renuka et al., 2018). Cyanobacteria has specialized cells known as heterocysts, that can fix atmospheric nitrogen and, as a result, are able to meet the needs of soil macro and micro fauna as well as flora and plants (Babu et al., 2015; Karthikeyan et al., 2007). Several researchers have investigated that inoculation with cyanobacteria proved to boost the yield and microbial activity by 5%-25%, enhance plant growth, and seed germination in a wide variety of cereal and vegetable crops (Dey et al., 2017; Prasanna et al., 2016b; Prasanna et al., 2017); can contribute to savings of 25%-50% on chemical nitrogen fertilizers (G et al., 2016; Nain et al., 2010; Prasanna et al., 2016a). Due to their abundance in soil and their ability to fix atmospheric N, cyanobacteria like *Nostoc* and *Anabaena* strains are frequently used as biofertilizers (Renuka et al., 2018).

Leaching of biologically fixed N may be an environmental hazard, but the extent may be minimal compared to leaching caused by synthetic fertilizers. Research reports revealed that only 7% of total nitrogen is leached away when microalgae are applied to soil, whereas 50% of total nitrogen is leached when synthetic fertilizer is applied (Jimenez et al., 2020). Exopolysaccharide-producing cyanobacteria generate biological soil crusts and are also said to immobilize access to nitrogen (Mager and Thomas, 2011), which inhabit nitrogen from leaching out

of the soil. Microalgae fertilization increased plant growth rate (shoot + root) by 32% in tomato plants, paralleling an increase in chlorophyll-a content (Jimenez et al., 2020); and it may also enhanced yield and microbial activity by 12-25% (Prasanna et al., 2014).

2.4.1.2 Improve soil structure

As a result of intensive agricultural methods, agricultural land is continuously degraded. Soil erosion, tilling and using heavy equipment too often can affects the soil's structure, water holding capacity, fertility, nutrients movement, and productivity of agricultural soil. For agriculture to be sustainable, it is important to keep the soil's organic matter and structure at the appropriate levels. Numerous varieties of green algae and cyanobacteria that are capable of producing extracellular polymeric substances (EPS) and releasing them into the environment (Xiao and Zheng, 2016). The extracellular polymeric substances (EPS) have adhesive properties that contribute to enhancing soil organic carbon, aggregation of soil particles, enhancing soil structure, and preventing soil erosion to a large degree (Weiss et al., 2012; Xiao and Zheng, 2016). The study found that inoculating cyanobacteria in soil resulted in the formation of organo-mineral soil aggregates composed of filaments and EPS, which increased aggregate stability six weeks after inoculation compared to the uninoculated control (Malam Issa et al., 2007).

In desert and semiarid soils, which are often highly compacted, low in fertility, saline or sodic, poorly aerated, and retain less water,

microalgae make physio-chemical contributions to the health of the soil by supporting to form and stabilize soil aggregates, which increase pore space and continuity (Nichols et al., 2020). This enhances aeration, nutrient cycling, seed germination, water holding capacity, and water infiltration. Following the application of cyanobacteria (*Nostoc* and *Anabaena*) to a loam, silty clay loam, and sandy loam, there was an increase in soil aggregation of 85%, 130%, and 160%, respectively (Kaushik, 2014). Hence, using algal biomass as a biofertilizer could improve the soil's structure, water-holding capacity, and soil aeration.

2.4.2 Biostimulants

Plant biostimulants are derived from microorganisms or organic substances, when applied to the plant in a small quantity, they increase nutrient use efficiency, promotes plant growth, resistance to abiotic and biotic stress, and quality traits, regardless of their essential nutrient content for plant (García-Sánchez et al., 2022). Algae, both macroalgae (seaweeds) and microalgae, have long been viewed as a potentially profitable commercial prospect in the field agronomy and agro-industries due to their high concentrations of plant biostimulants (Kapoore et al., 2021). Hence, microalgal extracts are becoming promising natural resources for plant biostimulation (Romanenko et al., 2016). It is essential to keep in mind that biostimulants are not the same thing as biofertilizers because they do not directly supply the crops with the nutrients that they require; rather, they enhance the uptake of the

nutrient by altering the rhizosphere and the metabolic processes of the plant (Drobek et al., 2019).

The biostimulatory effect of microalgae-based biostimulants under normal and stress situation can modulate microbial community inhabiting in the phyllosphere and rhizosphere areas of plants (Ranjan et al., 2016; Renuka et al., 2018). Recent experimental studies of biostimulatory microalga extracts have been shown to improve vegetative growth, absorption and distribution of nutrients, biomass and yield, resilience to biotic and abiotic stress, and water uptake in many crops under open and greenhouse settings (El Arroussi et al., 2018; Garcia-Gonzalez and Sommerfeld, 2016; García-Sánchez et al., 2022; Prasanna et al., 2017). Biostimulants can also alter root formation, which influences plant health, nutritional composition, and growth by improving water and nutrient uptake (Garcia-Gonzalez and Sommerfeld, 2016). The biostimulant activity of cyanobacteria, *Arthrospira platensis,* root and foliar applications on papaya has been tested. After integrating the findings into a surface model for plant height, stem diameter, leaf number, and leaf area, it was determined that a root treatment of 1.08% (w/v) was ideal for papaya seedling biomass production, whereas foliar spraying had no effect (Guedes et al., 2018). When the cyanobacteria (*Nostoc calcicole or Anabaena vaginicola*) were sprayed on the leaves of tomato, squash, and cucumber plants, compared to the controls, there were substantial increases in fresh weigh, dry weight, hight, root length, and leaf number (Shariatmadari et al., 2013). Therefore, algal biomass can be applied directly to plant leaves or roots to boost plant growth and yield.

The identified potential algal biostimulant metabolites include phytohormones, humic substance, polysaccharides, amino acid, vitamins etc.

2.4.2.1 Phytohormones

The growth and development of plants are influenced to a significant level by phytohormones. In agriculture, the practice of exogenously supplementing plants with plant hormones (either natural or synthetic) has been a common method for increasing crop production and productivity (Aliyu et al., 2011). Extract of microalgal may contain phytohormones like auxin, cytokinins, ethylene, abscisic acid (ABA), and gibberellins, which can be used as biostimulant in agriculture (Stirk et al., 2002; Tarakhovskaya et al., 2007). New evidence reveals that phytohormones in microalgae have similar regulatory actions to those found in higher plants, but their precise role in these organisms remains unclear (Lu and Xu, 2015). The two dominant kinds of auxin such as indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) are regulating growth and development including cell division and expansion (Hashtroudi et al., 2012). Cytokinins influences many traits of plant growth and physiology such as seed germination, shoot, and root development, and leaf senescence (Ha et al., 2012). While gibberellins play an important role mostly involves in elongation and expansion of the cell (Salazar-Cerezo et al., 2018). Ethylene is a gaseous phytohormone that plays an important role in physiological activities of plant, like growth and development, as well as resistance

to biotic and abiotic stressor (Pierik et al., 2006). Abscisic acid is important in regulating several biological processes such as stomatal closure, seed maturation and improves resistance to temperature stress (Sagar and Singh, 2019).

The phytohormones (such as auxin and gibberellins) are found in *Chlorella kessler* when extact, when applied to *Vicia faba*, it increased leaf area, seedling growth parameters, germination, pigment content, and sodium and potassium accumulation in roots and shoots (El-Naggar et al., 2005). (Hussain and Hasnain, 2011) investigated the efficacy of hormone-secreting cyanobacterial strains (cytokinin and auxin) in boosting growth both in axenic and natural environments. As a result, an approach to agronomic techniques that uses prospective phytohormone-excreting cyanobacterial strains as a biostimulant would be an environmentally acceptable way to stimulate plant development. However, research on the evaluation of algal hormone application at a field scale is limited, so this area needs further research.

2.4.2.2 Amino acids and Protein hydrolysates

Amino acids and protein hydrolysates (PHs), together constitute a significant portion of the category within the broader field of plant biostimulants and find widespread application within environmentally responsible agriculture practices (Bulgari et al., 2019) . Amino acids contain a minor amount of lipids, phytohormones, polysaccharides, and elements that are both macro and micronutrients, as well as protein hydrolysates, which may also consist primarily of short peptides

(polypeptides and oligopeptides) (Calvo et al., 2014; Kapoore et al., 2021) . The overall concentration of free amino acid and peptides (including arginine, alanine, proline, glycine, glutamate, valine, leucine and glutamine, among others) can range from 2 to 18% (w/w) to 1 to 85% (w/w), respectively (Calvo et al., 2014). Glycine betaine and proline are known to help to the mobility and uptake of micronutrients, as well as mitigation of environmental stress and antioxidant activity including heat, cold, drought, salinity, oxidative, and heavy metals through chelating actions (du Jardin, 2015; Paul et al., 2019). Bioactive peptides, on the other hand, function in plants similarly to auxin and gibberellin, which improves overall plant growth and productivity (Colla et al., 2017). Certain microalgal strains contain more than 40% dry weight amino acid, including *C. saccharophila*, *Chlorella sp.*, *A. maxima*, and *A. platensis*, and which contains 42.4%, 44.3%, 44.9%, and 46.8% respectively (Hempel et al., 2012), making them appropriate for biostimulants products.

2.4.2.3 Polysaccharides

The polysaccharides found in microalgae have the potential to be used as a bioresource in agriculture, both for the protection and improvement of crops. Polysaccharides are involved in many plants' metabolic pathways and can act as biostimulants to increase crop quality and protect against biotic and abiotic challenges (Rachidi et al., 2020).

The highest concentration of polysaccharides found in the microalgae (*Porphyridium cruentum* and *Chaetoceros gracilis species) and Dunaliella salina* at the range of 40% to 57% and 199.8%, respectively (Kapoore et al., 2021). Studies shows that application of 1 mg/mL microalgae polysaccharides from *D. Salina, Porphorydium sp*., and *A. platensis* on tomato plants significantly improved the shoot dry weight, shoot length, and nodes number , shoot dry weight , and shoot length by 46.6%, 25.26%, and 75 %, respectively, compared to control (Rachidi et al., 2020). Further example, foliar application of microalgae polysaccharide extract from *A. platensis* at concentration of 3 g/L (w/v) increased plant growth and development of leaf area size by 57% and 100%, size of nodes by 33% and 57%, and root weight by 67% and 230%, for pepper and tomato plants, respectively (Elarroussia et al., 2016).

2.4.2.4 Humic substances

Humic compounds are generally included in categories of biostimulants; but their algal origin has not been well studied (Kapoore et al., 2021). Humic substances are naturally occurring components that make up around 60% of the organic matter in the soil. They can be produced either by breaking down microbial, animal, and plant residues or through the metabolism of soil microbes that use these materials (du Jardin, 2015). Humic substance are divided into humic acids, fulvic acids, and humins according to their molecular weights and solubilities (du Jardin, 2015). When applied to crops, humic

material in digestate may bind to algal cells and act as a biostimulant. On the other hand, the biostimulant effect of humic compounds isolated from agro-industrial waste on *S. quadricauda* and *C. vulgaris* showed that there was a significant increase in chlorophyll, lipids, carbohydrate content and biomass (Puglisi et al., 2018).

2.4.3. Biopesticides

Utilizing chemical pesticides for the control of pests and pathogens in agricultural activities poses a threat to the sustainability of agroecosystems. Sustainable crop protection against pests and pathogens by using modern technologies allows to keep plants healthy and achieve stable high yield. Biopesticides are naturally known substances that are obtained from microorganisms, plants, or animals, primarily for insect and plant disease control. These substances or materials, including antioxidant, antimicrobial, antifungal, or antiviral properties, help crop growth by defending plants against harmful effect of pathogens. Some bacteria and fungi are among the most often discovered organisms that can be used for biocontrol (Spadaro and Gullino, 2005). Microalgae, particularly cyanobacteria, have gained attention in recent decades as possible biocontrol agents against pests and diseases (Hernández-Carlos and Gamboa-Angulo, 2011).

Phytohormones are essential for the controlling a growth and development of plants as well as its defense against biotic and abiotic stress via interacting among them (Checker et al., 2018). Many investigations were carried out to evaluate microalgae, as potential

biocontrol substances that have demonstrated antagonistic effects against many plant pathogens, like nematodes, fungi, and bacteria, mainly due to the produce hydrolytic enzymes and biocidal compounds, like benzoic acid and majusculonic acid (Chaudhary et al., 2012; Gupta et al., 2013; Renuka et al., 2018). These antimicrobial substances can suppress microorganisms by either disrupting the cytoplasmic membrane or inhibiting protein synthesis (Swain et al., 2017).

2.5 Impact of microalgae-bacteria interaction on crop production

In nature, not only plant-microbe interactions, but also microbe-microbe associations are vital assemblages affecting plant growth, development, health, and productivity. In natural ecosystems or industrial processes, microalgae and bacteria live together, demonstrating both beneficial and harmful relationships (Unnithan et al., 2014). Under industry settings, bacteria are considered pollutants in algae research, but most recent investigation have demonstrated that most algal symbionts not only stimulate algal growth but also extend benefits in downstream processing (Lian et al., 2018). There have been several studies that have brought attention to the potential of bacteria include nitrogen fixation (*Azotobacter vinelandii, Azospirillum brasilens, Rhizobium etli, and Mesorhizobium loti*), phosphate solubilization (*Azospirillum spp., Pseudomonas spp., Arbuscular mycorrhizal fungi, and Bacillus spp*.), cellulolytic activity (*Bacillus spp., Aspergillus spp., Penicillium spp*., and *Trichoderma spp*.), and the

production of siderophores are recognized as plant growth promoting rhizobacteria (PGPR) (Bhattacharyya and Jha, 2012a; Meena et al., 2020a; Woo and Pepe, 2018). Plant growth promoting bacteria (PGPB) are soil bacteria that can promote plant growth, suppress pathogens, promote nutrient availability to plants, and increase abiotic stress resistance mechanisms (Kumar et al., 2017).

Table 3: Microalga-bacteria interaction effects on crop production

Microalgae	Bacteria	Tested crop	Effect on crop and soil fertility	References
Anabaena cylindrica	*Azospirillum brasilense*	Maize *(Zea mays)*	Increase the initial growth, higher root growth, dry biomass, and yield	(Gavilanes et al., 2020; Matsuo et al., 2022)
Chlorella spp.	*Bacillus megaterium, P. fluorescens*	Maize *(Zea mays)*	Improvement of the stability of soil aggregates and organic carbon in the soil	(Yilmaz and Sönmez, 2017)
Anabaena cylindrica	*Rhizobium + Azospirillum brasilence*	Common bean	Enhanced plant growth, yield and yield component	(Horácio et al., 2020)
Nostocaceae family	*Pseudomonas and Pantoea cypripedii*	Tomato (*Solanum lycopersicum*)	Microbial consortia can have definite synergistic effects on plant growth and seedling	(Toribio et al., 2022)
Anabaena spp.	*Brevundimonas* sp.	Rice *(Oryza sativa)*	Enhanced growth, yield, and improve soil organic carbon and soil health	(Prasanna et al., 2012)

A combined application of the microalgae-bacteria can improve plant growth and control plant disease, which is much more efficient than a sole application (Spadaro and Gullino, 2005). This is due to the microbes in the consortia/combination system having the capability to

improve plant growth and development and/or control pathogens by different mechanisms. (Trivedi et al., 2017) reported that the beneficial microbiome could form relations with other microbiomes, reproduce highly structured systems in the rhizosphere soils, and may have a greater likelihood to assistances the host than a single culture. One of the promising examples showed that the combined application of the cyanobacterial (*Nostoc muscorum and Anabaena flos-aquae*) and bacterial suspensions (*Azotobacter brasilense and Azotobacter chroococcum*) was substantially improved germination rate of Lupinus termis seeds by 53.13%, 211.48%, 129.04%, and 104.1%, respectively, when compared to control (Tantawy and Atef, 2010).

In interaction systems, microalgae and bacteria can be symbiotic to competition (mutualism to antagonism). The relationships between microalgal and bacterial communities are based on signal transduction, gene transfer, and nutrient exchange (Aditya et al., 2022). Microalgae and bacteria engage in a dynamic exchange of carbon, energy, and essential molecules, which is seen in Figure 2. In a synergetic association, microalgae stimulate bacteria grow by supplying oxygen (through photosynthesis) and dissolved organic matter, such as calcium carbonate and organic carbon, that become accessible to bacteria (Cooper and Smith, 2015). On the other side, the bacteria produce carbon dioxide (CO_2) and remineralize nitrogen, phosphorus, and sulphur to maintain further microalgae growth (Yao et al., 2019). Moreover, bacteria supply vitamin B as organic cofactors (Yao et al., 2019), amino acids (Palacios et al., 2016), and hormones (De-Bashan et al., 2008), which become bioavailable for microalgae.

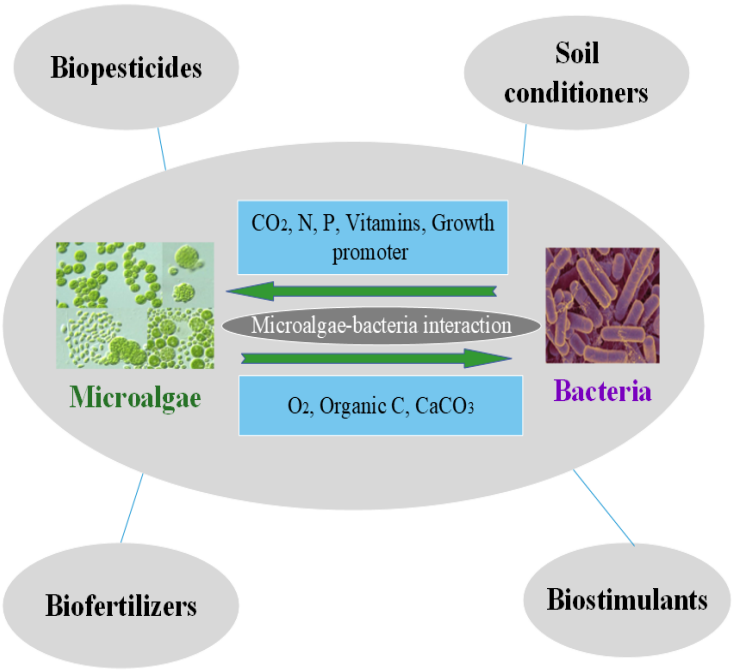


Figure 2: Mechanism of a possible symbiotic interaction of microalgae and bacteria and their potential role in the agricultural production (source: own editing). *In normal interactions, microalgae exude oxygen (O_2), organic carbon, and calcium carbonate ($CaCO_3$), which bacteria can use. In exchange, the bacteria remineralize nitrogen (N) and phosphorus (P), growth promoter and produces carbon dioxide (CO_2) to assist the growth of the microalgae. In specialized interactions, the bacteria supply B vitamins as organic cofactors or create siderophores to bind iron, making it bioavailable to the microalgae. The interaction of microalgae and bacteria offers a unique potential for eco-friendly products such as biofertilizers, biostimulants, biopesticides, and soil conditioners, which reduce reliance on*

agrochemicals and sustainably increase crop production and productivity.

In a pot experiment with rice varieties, the combined treatment of cyanobacteria strains (*Anabaena* sp., *Anabaena oscillarioides,* and *Anabaena laxa*) and bacteria strains (*Brevundimonas* sp., *Ochrobactrum* sp., and *Providencia* sp.,) was examined. In this trial, the authors evaluated that a significantly increase in the growth, grain yield by 19.02%, nitrogen fixing potential of rice, and improving soil fertility by nitrogen savings of 40-80kg/ha , especially with *Ochrobacterium* and *Anabaena* species (Prasanna et al., 2012).

During the spring and summer, the combined biostimulant properties of freshwater algae (*Chlorella vulgaris*) and bacteria (*Azospirillum sp., Azotobacter sp., Herbaspirillum sp., Bacillus licheniformis*, and *Bacillus megatherium*) significantly influenced the weight of the romaine and leaf lettuce crops (Kopta et al., 2018). Moreover, the research also suggested that the photosynthetic substances produced by algae, like carotenoids, could boost the quality and productivity of crops and give support during times of stress. Similarly, the positive result was observed by combined application of algae and bacteria to crops promoted growth, productivity, and quality in common bean, maize, and onion (Gavilanes et al., 2020; Geries and Elsadany, 2021; Horácio et al., 2020). This consortium reduces synthetic pesticide use, making it essential to sustainable agriculture and food safety (Niu et al., 2020). A suitable microalgae-bacteria consortium is necessary to boost the potential of strains to enhance growth and development and

to inhibit pathogen attack (Yanti et al., 2021). Considering these factors, it is feasible to assume that microalgae-bacteria consortium can be successful at increasing soil microbial activities, crop productivity, and plant disease resistance. However, more investigation needs on the molecular mechanisms underlaying the influence of microalgae and bacteria association to help plants development and disease prevention, so that they can be used in agriculture in a safer and more widespread way.

Table 3 highlighted valuable practical reports on the microalgae–bacteria combined treatments in different crop cultivation. Some studies revealed a promise of the microalgae-bacteria association, examined in the field and greenhouse conditions that can promote seedling growth, germination, and biomass in plants (Kang et al., 2021a). An association between microalgae and bacteria can increase plant growth and development by the production of phytohormones (like auxin, cytokinin, and so on) and polysaccharides; it can also stimulate nutrient uptake by regulating a variety of biochemical and physiological processes; and it can reduce the risk of pathogen infection (Fuentes et al., 2016; Michalak and Chojnacka, 2015a).

2.6 Challenges of microalgae-bacteria interactions

The field of microbial consortia is still in its initial stages, and there are still a lot of problems to solve when it comes to how cells communicate to each other and how to make systems that are stable and easy to control. The main challenge of the association of microbes, soil, and

climate in an agricultural setting is to understand their specific structural function activities on plants. A lot of study has demonstrated a promising result in the greenhouse trials, but it fails to confirm in field trials. The microalgae-bacteria interaction can also be affected by environmental factors like pH, temperature, and light intensity (Quijano et al., 2017). It is difficult to depict their ecosystem-wide processes such as metabolic pathways and nutrient cycling because the majority of heterotrophic bacteria and photosynthetic microalgae have not yet been cultured (Zhang et al., 2020). Furthermore, the amount of nutrient present in the growing media has a major impact on the dynamic between microalgae and bacteria (Liu et al., 2012).

The growth phase is another significant aspect that plays a role in the interactions that take place between microalgae and bacteria. Expensive harvesting of biomass, insufficient biomass production, and extraction technologies that need a lot of energy are also main constraints that are preventing their large-scale development. Subsequently, it is challenging to distinguish the individual metabolites that microalgae and bacteria produce in a consortium due to the complexity of their interactions, which are either naturally occurring or artificially engineered for a specific goal (Zhang et al., 2020). The result of microalgae-bacteria interactions is often varied in different studies under different climatic, or soil conditions, which is the main problem in the implementation of the technology. Species and environmental circumstances are the primary variables detrimental to the microalgae-bacteria relationship (Lauritano et al., 2020; Mujtaba and Lee, 2016). Which highlighted the importance of choosing a

suitable combination of microalgae strains versus bacteria strains for the efficient application in the agricultural production. To overcome these restrictions, researchers have focused on enhancing microalgal-bacterial consortia, which offer several economic, energy, and environmental benefits due to their mutual interactions.

2.7 Strategies for implementing: A multitude of approaches

Microbial inoculants are formulations of environmentally friendly microorganisms and serve as a promising alternative to chemical fertilizers and pesticides. They can function as phytostimulants, biofertilizers, or microbial biocontrol agents.

The progress made in rhizosphere research has unquestionably enhance our capacity to translate knowledge into practical technological applications in agriculture, restoration of nature and ecological engineering. Recent research focusing on the rhizosphere ecology of noncultivated plant species has brought about a greater understanding of the potential for ecological engineering of soil biota to reconstruct soil structure. Plant growth-promoting microorganisms, among other microorganisms, have the potential to interact with a variety of crop plants, enhancing their growth and development to resist pathogen attacks and promote healthy growth. Many of the metabolites produced by these microorganisms have been identified as commercially valuable due to their abilities to promote plant growth, facilitate mass production, improve biocontrol efficacy, enhance stress tolerance, remove soil pollutants, and enable proper formulation (de Andrade et

al., 2023; Oleńska et al., 2020; Orozco-Mosqueda et al., 2022; Rosier et al., 2018).

One notable application is rhizoremediation, which offers a cost-effective and environmentally sustainable "Green Technology" for the degradation of petroleum contaminants in soil (Haldar and Sengupta, 2015; Martin et al., 2014). Rhizoremediation is a bioremediation method that involves the enhanced microbial degradation of organic contaminants within the rhizosphere. During the development of microbial inoculants, it is essential to carry out isolation, formulation, and proper application technology (Bashan et al., 2014). These steps play a vital role in ensuring that the necessary quantity of viable and active microbial cells can be applied effectively.

2.7.1 Selection of beneficial strains

The primary goal in the context of plant inoculation with PGPMs, is to identify the most suitable strains or a combination of microorganisms that can achieve the desired impact on the specific crop being targeted (Bashan et al., 2014). To acquire microbial inoculants that can effectively compete with others, it is necessary for the strains employed to possess specific attributes. These include easy to use, high efficacy, beneficial traits for plants, the ability to multiply rapidly and effortlessly, compatibility with native soil microorganisms, extended shelf life beyond a single season, the absence of any adverse effects on non-target organisms and the surrounding natural environment (Bashan et al., 2014).

For any inoculant, there are three key characteristics that are fundamental and essential. Firstly, it should provide a suitable environment for the growth of the intended microorganisms. Secondly, it should maintain a sufficient number of viable microbial cells in a healthy state for a reasonable duration. Lastly, it should deliver an adequate quantity of microorganisms during inoculation to meet the required threshold for a positive plant response (Date, 2001; Stephens and Rask, 2000). In other words, the inoculant must contain enough viable bacteria after the formulation process. To gain a better understanding of the sustainable production potential and feasibility of the microbial products, it is important to assess them under different environmental conditions such as climate, soil type, crop type, and agricultural practices. This evaluation process will help generate a range of potentially beneficial microbial products.

2.7.2 Formulation development: Developing efficient formulation of PGPMs

To enhance the success of a microbial inoculant in soil, it is crucial to ensure both targeted ecological compatibility (occupying a metabolic niche not utilized by the existing microbiota) and protection against unfavorable conditions (e.g., through biofilm formation). The formulation of the inoculant helps shield the microbe from extreme environmental factors, provides an initial food source, and promotes prolonged presence and efficacy (Babalola and Glick, 2012).

The practicality of implementing PGPR in agriculture has progressively grown due to their potential to substitute chemical fertilizers, mineral nutrients and pesticides (Bhattacharyya and Jha, 2012b). *Pseudomonas fluorescens*, *P. putida*, *P. aeruginosa*, *Bacillus subtilis*, and other *Bacillus spp*. are among the PGPR strains that hold significant potential for commercialization. These promising PGPR isolates can be formulated using various organic and inorganic carriers, utilizing either solid or liquid fermentation technologies (Gómez-Godínez et al., 2023; Nakkeeran et al., 2006). Green alternatives to traditional agrochemicals are provided by bioformulations of the products, which promote plant growth, suppress phytopathogens, and enhance soil fertility (Arora et al., 2016). Inoculants can be developed in the form of solid or liquid-based products, with the latter encompassing dry or wet formulations (Bashan, 1998; Berger et al., 2018; Catroux et al., 2001). Hence, the primary objective of inoculation formulation is to enhance the survival rate of PGPR during storage and upon application, ensuring their viability in both appropriate and accessible forms.

Achieving an optimal and compatible association between microbes, carrier materials, and their storage is essential for maximizing the performance of microbial consortia formulations in enhancing crop productivity (Ghosh et al., 2016). The utilization of PGPR formulations containing mixtures of strains has proven to be more effective than using individual strains alone when it comes to managing pest and diseases in crop plants, in addition to promoting plant growth (Nakkeeran et al., 2006). As an example, while two distinct strains of

Escherichia coli can individually metabolize glucose and xylose, their synergistic association enables a more efficient metabolism of these sugars compared to when they are single cultured (Eiteman et al., 2008). It is evident that mixed cultures can effectively carry out complex processes, leading to increased productivity compared to a single culture. The key aspect in the formation of mixed consortia lies in the compatibility among microbial members, as it dictates the longterm stability and suitability for industrial applications. This technology reduces the workload on a single culture, emphasizing the need for in-depth research on the interactions between plants and microbes, as well as microbe-microbe interactions, in order to design, optimize, and develop bioformulations. Bioformulations with PGPMs (plant growth-promoting microorganisms) offer a promising and sustainable approach to address adverse environmental conditions such as excessive use of chemical fertilizers and pesticides, which disrupt biodiversity, pollute the environment, and compromise soil health (Balla et al., 2022). These bioformulations exhibit tolerance to diverse biotic and abiotic stresses, exert beneficial effects on plant growth, protect against pests, aid in bioremediation, and contribute to the restoration of degraded lands (Marcial-Coba et al., 2021; Shanmugam, 2022). Choosing suitable PGPMs, carrier materials, and implementing large-scale preparation and preservation methods are essential steps in developing bioformulations for commercial purposes in the long run. To boost the adhesive capacity of microbes to their hosts and improve the efficiency of bioformulations, a range of supplementary materials like hormones, mineral nutrients, fungicides, and carbon sources are

employed. In recent times, encapsulation technologies have become widely utilized for producing microbial inoculants with diverse compositions and morphologies (Balla et al., 2022; John et al., 2011; Schoebitz et al., 2012).

2.7.3 Application

Using N-fixing bacteria such as *Azospirillum* and *Azobacter* in the process of inoculation enabled the application of only half the recommended amount of nitrogen fertilizer while still resulting in higher sesame seed yield and improved quality of the oil produced (Shakeri et al., 2016). The combination of bacteria consisting of *Bacillus cereus* PX35, *Bacillus subtilis* SM21, and *Serrati asp* XY2 effectively decreased the occurrence of root-knot nematode (*Meloidogyne incognita*) by 63-69% n tomato plants. Additionally, it led to an improvement in fruit yield by 31.5% to 39% and enhanced quality parameters such as soluble sugars, vitamin C, and titratable acids (Niu et al., 2016).

This environmentally friendly approach encounters obstacles and significantly lags its competitors, synthetic fertilizers, and pesticides. It is often observed that bioformulations designed for specific crops do not yield satisfactory results comparable to laboratory conditions (Mishra and Arora, 2016). These limitations and associated restrictions pose significant challenges to this environmentally friendly approach.

3. MATERIALS AND METHODS

3.1 Experimental site description

The field trial was conducted in 2021, 2022, and 2023, employing uniform treatments in three different locations, all featuring Danubian alluvial soil type. Experiments were conducted with the same treatment located at (47°53'32.3"N 17°15'59.0"E), (47°54'26.7"N 17°15'09.3"E), and (47°53'46.7"N 17°15'46.1"E) at Széchenyi István University farm, Mosonmagyaróvár, Hungary. The crop cultivated in the previous year at each experimental field was wheat (*Triticum aestivum* L.), wheat (*Triticum aestivum* L.), and sunflower in 2021, 2022 and 2023, respectively. Daily temperature and rainfall measurements were observed at each location. Figure 3 displays the recorded rainfall, maximum air temperature (T max), and minimum air temperature (T min) throughout the duration of the research.

3.2 Experimental design

The study followed a design of completely randomized block design (CRBD) with four replication and a total of 9 treatments. The experimental design included two main factors, which were: Cyanobacterium (MACC-612, *Nostoc linckia*) biomass and plant growth promoting bacteria (PGPB) (such as *Azospirillum lipoferum (*strain NF5) and *Pseudomonas fluorescens (*strain NCAIM B01666). The three levels of the cyanobacterial biomass (control, 0.3 g/L of MACC-612, and 1.0 g/L of MACC-612) and three levels of bacteria

strains (control, *A. lipoferum (NF5)*, and *P. fluorescens (*NCAIM B01666)) were used for the experiment (Table 4).

Table 4: Treatments combination of the *N. linckia*, MACC-612 and PGPB

Keys assigned to each treatment	Treatments
MACC0 + B0	Control
MACC0 + BA	Untreated with *N. linckia*, MACC-612 + *A. lipoferum*
MACC0 + BP	Untreated with *N. linckia*, MACC-612 + *P. fluorescens*
MACC1 + B0	0.3 g/L of *N. linckia*, MACC-612 + Untreated with bacteria
MACC1 + BA	0.3 g/L of *N. linckia*, MACC-612 + *A. lipoferum*
MACC1 + BP	0.3 g/L of *N. linckia*, MACC-612 + *P. fluorescens*
MACC2 + BA	1.0 g/L of *N. linckia*, MACC-612 + untreated
MACC2 + BA	1.0 g/L of *N. linckia*, MACC-612 + *A. lipoferum*
MACC2 + BP	1.0 g/L of *N. linckia*, MACC-612 + *P. fluorescens*

Cyanobacterium strain (MACC-612, *N. linckia)* was obtained from Mosonmagyaróvár Algal Culture Collection (MACC), Albert Kázmér Faculty of Agricultural and Food Sciences in Mosonmagyaróvár, Széchenyi István University, Hungary. In order to generate the necessary biomass for the experiments, the culture method was used as previously detailed by (Ördög, 1982). The cyanobacterial strain was introduced into the Tamiya nutrient solution after being taken from agar-agar stock cultures (Kuznjecov and Vladimirova, 1964), as cited in (Takács et al., 2019). After a 7-day incubation period, the cultures were transferred into four flasks, each containing 250 mL of Tamiya nutrient solution with an initial concentration of 10 mg/L of algal dry weight (DW). The cultures were then maintained at a temperature of 25 ± 2 °C, under a 14-hour light and 10-hour dark cycle, with a Photosynthetic Photon Flux Density of 130 μmol photons m^{-2} s^{-1} provided from below. During the light period, the cultures received aeration with 1.33 L of 1.5% CO_2-enriched sterile humidified air per minute at a rate of 20 L/h. Following a 7-days period, the four culture suspensions were mixed, their density was measured, and then they were used to inoculate 48 flasks to establish an initial concentration of 10 mg/L of algal dry weight (Takács et al., 2019). The cultures were cultivated under the mentioned conditions for six days, after which they were subjected to a 15-minute centrifugation at room temperature with 2150 *g* employing the Sigma 6 K15. The biomass was then freeze-dried with a Christ Gamma 1-15 machine and kept at -18 °C. The freeze-dried biomass of MACC-612 (*N. linckia)* was reconstituted in distilled water at varying concentrations stated above and then subjected to a 3-

minute sonication process using the VirTis VirSonic 600 Ultrasonic Cell Disruptor just prior to soil application.

A. lipoferum (NF5) and *P. fluorescens (*NCAIM B01666) was obtained liquid-based formulation from Biofil Microbiological, Gene technological and Biochemical LLC, Budapest, Hungary. The bacterial strains were cultured in a liquid medium that was enriched with yeast extract (3 g/L), glucose (5 g/L), sucrose (5 g/L) and then subjected to a 48-hour incubation period in a gyrotary water bath shaker (New Brunswick Scientific CO. INC. EDISON, N.J. U.S.A) set at 120 rotations per minute and maintained at a temperature of 37 degrees Celsius. The cell concentration of *A. lipoferum* and *P. fluorescens* were $7.8*10^8$ CFU/mL and $1.02*10^9$ CFU/mL respectively measured by DEN-1, McFarland Densitometer (suspension turbidity detector).

Each bacteria strain was randomly combined with each of the three levels of microalgae strains with four replications giving a total of 36 (3×3×4) plots. The plants underwent treatment using either tap water as a control or with the cyanobacterium (MACC-612, *N. linckia*) at concentrations of 0.3 or 1.0 g/L dry weight, with the selection of these concentrations being informed by previous research findings on maize (Takács et al., 2019). The solution of the microalgae (*N. linckia)* and PGPB treatments were introduced to the soil using a 15 L manual knapsack sprayer (pump sprayer garden pressure spray) during the sowing process, at an application rate of 300 L/ha. The planting was carried out using a 163-cc mini tractor. For the experimental cultivation, a type of *Zea mays* L. hybrid, obtained from (Saaten Union-Körner kernels Grains), was utilized. Sowing was conducted using a

row spacing of 75 cm, a plant spacing of 20 cm, and a sowing depth of 6 cm. Each plot covered an area of 28.5 m^2 (3 × 9.5 m), allowing for 256.5 m^2 per replication and requiring a total of 1026 m^2 for four replications. Plots were spaced 0.5 m apart, while blocks were kept 1 m apart.

3.3 Data collection and measurements

3.3.1 Plant physiology measurements

The agronomic and physiology measurements (chlorophyll content, NDVI, plant fresh weight, and plant dry weight) were measured.

3.3.1.1 Chlorophyll content

The chlorophyll content of the second youngest leaves was determined with the SPAD-502 Plus Chlorophyll Meter (Toshiba, Japan) portable device which is widely used to estimate foliar chlorophyll content in a non-destructive way (Vesali et al., 2017). Measuring the chlorophyll content in plant leaves involves assessing their green color and providing precise outcomes. This process requires positioning the sample leaves between sensors designed for the measurement. The assessment of chlorophyll content took place on three occasions at 50, 65 and 80 days after sowing (DAS) on 5 randomly selected maize plants from each plot, and the values were averaged. Sampling was conducted on the central portion of the upper leaf surfaces, specifically at a distance from the primary leaf vein. The average SPAD meter

readings obtained at each grid point were utilized for subsequent analysis.

3.3.1.2 Normalized difference vegetation index (NDVI)

The NDVI allows producers to evaluate crop biomass and nutrient content by utilizing indirect reflectance measurements (Farias et al., 2023). Leaf spectral reflectance was assessed on days 50, 65, and 80 after sowing (DAS) with a handheld PolyPen RP 410 device from PSI (Photon Systems Instruments, Drásov, Czech Republic). It measures green vegetation by determining the normalized difference between near-infrared light (reflected by green leaves) and red light (absorbed by vegetation); directly correlates with the photosynthetic capability of the plant. Three readings were obtained from a recently fully developed leaf on the primary stem of each plant, and five plants were evaluated per treatment, and the values were averaged. The NDVI values range from -1 to +1, where positive values signify the crop's vegetative health, and negative values indicate the presence of bare soil or the absence of vegetation.

3.3.1.3 Leaf and root fresh and dry weight

Physiological measurements, specifically the fresh and dry weight of both leaf and root, were taken at two specific time points during the experiment. These measurements were conducted at 50 and 65 days after sowing (DAS). The purpose of this assessment was to track and analyze changes in the plant's weight over this period to gain insights

into its growth and physiological development. The measurement of shoot and root involved a destructive measurement method. Four plants were randomly chosen from the middle rows within each plot.

The process commenced with the measurement of fresh root weight, which was taken immediately after gently rinsing the roots with tap water to eliminate any contaminants, including soil residues and dust. Following the removal of excess moisture from the roots using absorbent paper, the fresh weight of the roots was recorded. Subsequently, the dry weight was determined after the shoots and roots were subjected to two days of oven drying at a temperature of 70°C. The heated aluminum foil packets were used to cool at room temperature for 5 minutes. Then the plants were individually measured using a digital balance and, then the mean values were calculated.

3.3.2 Plant nitrogen determination

Following the maize's maturity phase, leaves, stalks, cobs, and seeds were gathered from every plot. Then plant samples, which had been dried in an oven and, then underwent grinding using a grinder prior to their analysis for total nitrogen content. Nitrogen analysis was performed using the Rapid N cube (manufactured by Elementar Analysensysteme GmbH, Langenselbold, Germany), employing the Dumas method, a dry combustion approach. The process involves subjecting the sample to quantitative combustion at around 960°C in an oxygen-rich environment. Tinted palettes were prepared by dispensing 150 mg of powdered sample onto tin foil, which was then

folded into palettes. These palettes were subsequently positioned within the combustion chamber for analysis.

3.3.3 Plant yield attributes

The research encompassed a comprehensive assessment of various parameters following the maturation of maize. Four plants were selected from each central plot for detailed measurements, including plant height, the count of grains per ear, thousand grains weight, and grain yield. Subsequently, the total yield in tons per hectare was calculated, considering the harvest data from each specific plot.

3.3.4 Soil parameters analyses

Soil samples were extracted from three consecutive years of drilling within the experimental field. Field sampling procedures adhered to regulatory guidelines, specifically targeting the uppermost 0-20 cm layer of the productive soil stratum. The sampling technique employed was the diagonal method, ensuring a comprehensive and representative collection of soil samples across the designated area. The soil collected underwent a sieving process using a 2 mm mesh sieve, followed by thorough mixing to ensure uniformity. Prior to initiating the experiment, soil samples were gathered for chemical analysis (Table 5). The soil samples underwent comprehensive analysis at the Beta Research Institute Nonprofit Limited Company (Beta Kutató Intézet Nonprofit Kft), where a thorough examination and evaluation were

conducted to assess the various components and characteristics of the soil.

3.3.4.1 Soil pH analysis

The pH of the soil's particles smaller than 2 mm was assessed using a solution of 1 M KCl following the procedures outlined in accordance with the MSZ-08-0206-2:1978 2.1. Hungarian standard.

3.3.4.2 Humus analysis

The determination of humus content was meticulously executed by following the procedures outlined in accordance with the MSZ-08-0452:1980 Hungarian standard.

3.3.4.3 Nitrate and nitrite analysis

Flow Injection Analysis (FIA) was utilized to determine the concentrations of nitrate and nitrite nitrogen in the samples as outlined in the procedure specified by (ISO, 1996). Samples were mixed with imidazole buffer and treated with copperized cadmium to convert nitrate to nitrite. The analytical procedure involved a color-developing reagent, namely Griess Reagent, which consisted of sulfanilamide (SA) and N-(1-naphthyl) ethylenediamine dihydrochloride (NED). The nitrite reacted with sulfanilamide, forming a red azo dye with N-(1-Naphthyl)-ethylenediamine (NED), which was detected spectrophotometrically at a specific wavelength (546 nm) using a flow cell or a reaction coil within the FIA system.

3.3.4.4 Soil phosphorus analysis

The determination of the available phosphate in soil was carried out using a sodium bicarbonate solution (Olsen, 1954). The amount of available phosphorus (P) content was extracted and quantified employing according the outlined in the MSZ 20135:1999 5.4.2.2 Hungarian standard, with the analysis performed using inductively coupled plasma-optical emission spectroscopy (ICP-OES).

3.3.4.5 Soil potassium analysis

The soil analysis procedures were systematically executed in accordance with the Hungarian specifications outlined in the MSZ 20135:1999 5.3 standard. This involved precise sample preparation, acid digestion, calibration with known standards, and subsequent analysis with the ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy) instrument, ensuring reliable and reproducible results for the quantitative assessment of potassium concentration in the samples.

3.3.4.6 Soil total nitrogen analysis

The total nitrogen content analysis was carried out using the Dumas method, following the AACC 46-30.01 procedure (ACC, 2023). The Elementar Rapid N III Analyzer, located in Langenselbold, Hesse, Germany, was employed for the precise determination of nitrogen levels in accordance with the specified methodology.

3.3.5 **Soil microbial biomass analysis**

The microorganisms were evaluated using the agar-plate method according to (Clark, 1965), which is the predominant cultural approach for assessing soil microbial populations, aiding in their identification and quantification. In direct approaches, microorganisms in the soil are quantified by counting the number of colonies forming units (CFU) through a soil dilution series using most probable number (MPN). The MPN method involves dispersing soil samples in a sequence of dilutions to estimate the density of the population by observing the presence or absence of microbial cells (Alexander, 1965). Therefore, if microbial growth is detected in the 10^{-4} dilution but not in the 10^{-5} dilution, the estimated number of cells falls within the range of 10^{4} to 10^{5}.

To assess soil bacteria population, we tested various dilution factors; no more than 250 colonies should be on any Petri plate. To inoculate, 1 mL of extract was added to sterilized solid agar, then incubated at room temperature for 24 hours before bacterial assessment.

To cultivate actinomycetes, we utilized Dextrose Nitrate Agar as described by (Williams et al., 1983). Serial dilution was employed to achieve dilution factors ranging from 10^{-4} to 10^{-8}, with 1 mL of each dilution then introduced to the agar medium. These dilutions were then introduced to the agar medium, with 1 mL of each dilution applied. Subsequently, the plates were incubated at 28 °C for a period of 5 to 25 days.

Utilizing a logarithmic scale, like log CFU, facilitates the representation of these counts in a more practical and informative fashion.

$$\log \text{CFU} = \log10(\text{CFU})$$

In this equation, CFU denotes the precise tally of colonies, while log CFU signifies the base 10 logarithm of the colony count. Through this logarithmic transformation, values are condensed, facilitating the comparison and visualization of variations in microbial populations.

3.4 Statistical Analysis

All statistical computations and the creation of visual representations were conducted using R studio (version 4.3.1) (R Core Team, 2013), utilizing the software package known as "agricolae." (version 1.3-6) (Mendiburu, 2023). The outcomes from all the experiments, which exhibited a normal distribution pattern, underwent two-way analysis variance (ANOVA) used to test yield attributes, soil chemical analysis and microbial abundance. The result has displayed the average values of the treatments ± standard deviation within each treatment. Following this, Tukey's HSD post-hoc analysis was applied at a significance level of $P \leq 0.05$. To streamline data interpretation and statistical analysis of the microbial biomass, we applied a logarithmic transformation, which resulted in condensed values. This approach simplifies the process of comparing and visualizing fluctuations within microbial populations.

4. RESULT

4.1. Experimental field description

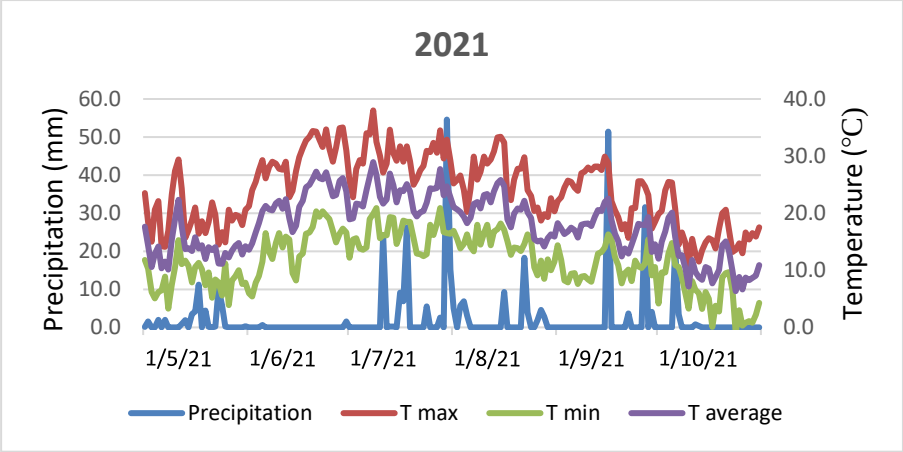
The experimental area featured Danubian alluvial soil. As depicted in Table 5, the soil pH values across the experimental fields remained relatively consistent over the years, displaying a marginal rise from 2021 to 2023, suggesting a potential progression toward a slightly more alkaline state. While humus content varied across the three locations, notably, a rise in humus content was observed in 2023 compared to preceding years in those locations. Furthermore, a marginal increase in nitrate and nitrite nitrogen levels was observed in the 2022 locations compared to both the 2021 and 2023 locations. The phosphorus (P) and potassium (K) levels peaked in 2022, undergoing a slight decrease in both 2021 and 2023 compared to the levels observed in 2022 (Table 5). The fluctuations in soil properties across different locations indicated variations in soil nutrient content over the three-year period. These changes could be influenced by factors such as agricultural practices, environmental conditions, or natural soil processes.

Table 5: The soil chemical characteristics collected from the experimental field prior to sowing.

Soil parameters	**2021**	**2022**	**2023**
pH (KCl)	7.29±0.01	7.33±0.02	7.44±0.13
Humus (m/m%)	2.06±0.47	1.91±0.23	2.72±0.62
$(NO_3^-+NO_2^-)$-N (mg/kg)	9.81±1.12	10.24±0.89	9.51±1.56
Total Nitrogen (%)	0.26	0.29	0.27
P (mg/kg)	158±24.6	187±17.55	182±20.11
K (mg/kg)	176±10.89	193±16.7	178±14.52

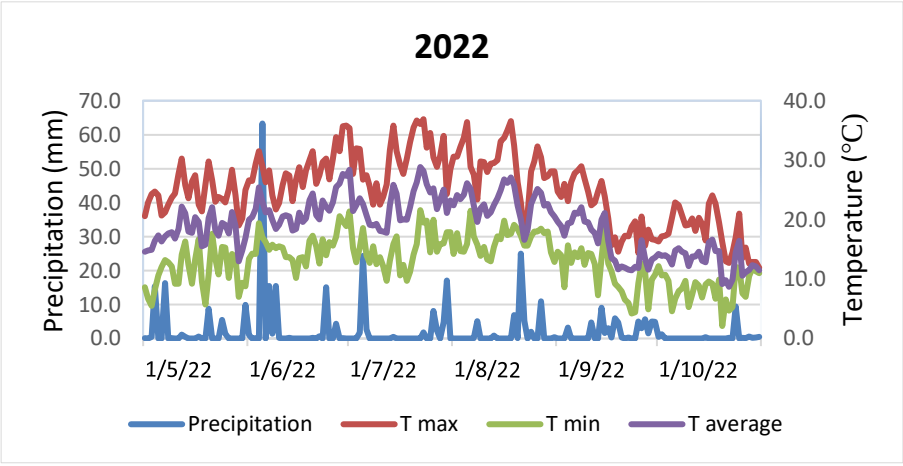
The mean of triplicates, with a standard deviation as denoted ±

Figure 3, 4 and 5 displays meteorological data for the three years. An amount of 373.5, 369.5, and 403.9 mm rainfall was recorded during the crop season of 2021, 2022, and 2023 field trail, respectively at the Széchenyi István University farm in Mosonmagyaróvár. In the 2022 and 2023 field trial, there was higher precipitation, particularly during the vegetative growth phase, compared to the 2021 production year. However, the precipitation during the reproductive stage was relatively consistent in 2021 and 2023 compared to 2022. The field's temperature remained conducive during the sowing across all seasons; however, during the reproductive stage in 2021, it exhibited higher temperatures compared to the following year, 2022 and 2023.



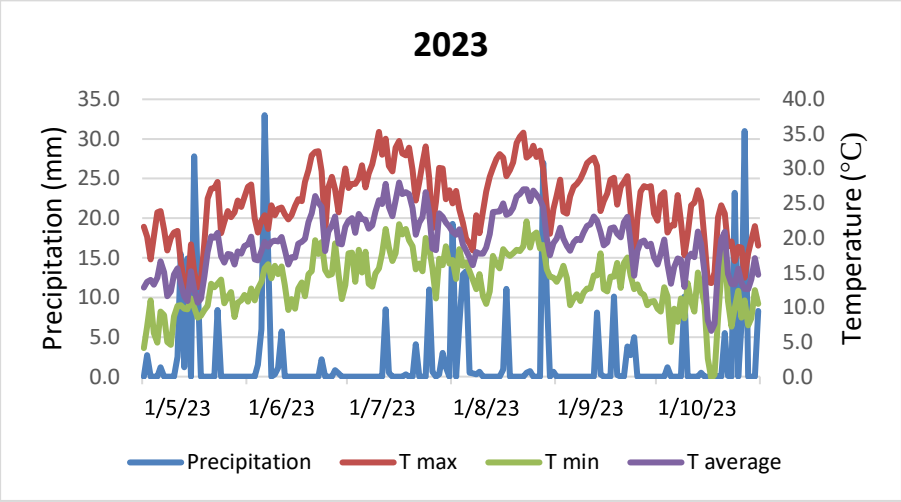
Where: T min= Temperature minimum, T average= Temperature average, T max= Temperature maximum

Figure 3: Daily precipitation and temperature data recorded in the experimental field during the interval across from sowing to harvest in the production years of 2021.



Where: T min= Temperature minimum, T average= Temperature average, T max= Temperature maximum

Figure 4: Daily precipitation and temperature data recorded in the experimental field during the interval across from sowing to harvest in the production years of 2022.



Where: T min= Temperature minimum, T average= Temperature average, T max= Temperature maximum

Figure 5: Daily precipitation and temperature data recorded in the experimental field during the interval across from sowing to harvest in the production years of 2023.

4.2 Plant physiological parameters

4.2.1 Chlorophyll content

Chlorophyll plays a crucial role as a photosynthetic pigment in plants, significantly impacting their ability to photosynthesize and consequently influencing their growth. Large variation in chlorophyll

content was observed among different treatments throughout the experimental years. Over a span of three years, statistical significance ($P \leq 0.05$) was noted in the chlorophyll content specifically at 65 DAS (as shown in Fig. 6, 7, 8). However, except for the data from 2022, the chlorophyll content didn't show significance difference ($P \leq 0.05$) at 50 DAS. Across the entire experimental duration, the peak chlorophyll content was consistently registered at 65 DAS, while the lowest levels were consistently observed at 50 DAS. As shown in figure 6, 7, and 8, the control level exhibited the lowest chlorophyll content, whereas the combined application of *N. linckia* at 3 g/L and 1 g/L along with *A. lipoferum* and *P. florescence* showed higher chlorophyll content.

In 2021, apart from the data noted at 65 DAS, there were no statistically significant variations observed in chlorophyll content (Fig. 6). Nonetheless, notably elevated chlorophyll levels were documented upon the combined application of *N. linckia* at 3 g/L and 1 g/L alongside *A. lipoferum* and *P. florescence* whereas the lower chlorophyll content was recorded at control group.

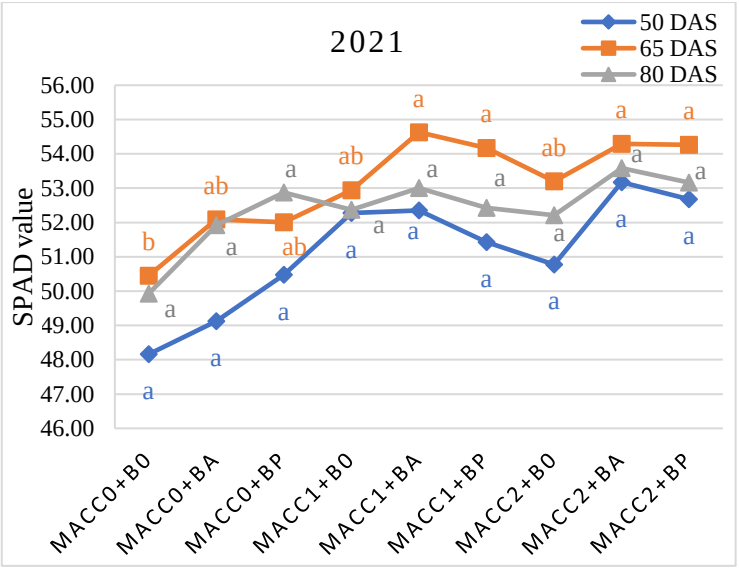


Figure 6: Chlorophyll content of maize (Zea mays L.) leaf assessed at different growth stage from SPAD reading in 2021. Where: MACC0, MACC1, and MACC2, which are levels of concentration of the N. *linckia* at 0, 0.3 g/L, and 1 g/L, respectively. BA and BP are *A. lipoferum* and *P. florescence*, respectively.

In 2022, statistical significance in chlorophyll content was evident across all stages of plant growth (Fig. 7). The peak chlorophyll content was observed upon the joint application of *N. linckia* at 0.3 g/L alongside *A. lipoferum*, while the lowest levels were noted in the control group. However, at 65 DAS, the chlorophyll content showed no statistical difference between the combined applications of *N. linckia* at 0.3 g/L and 1 g/L alongside *A. lipoferum*.

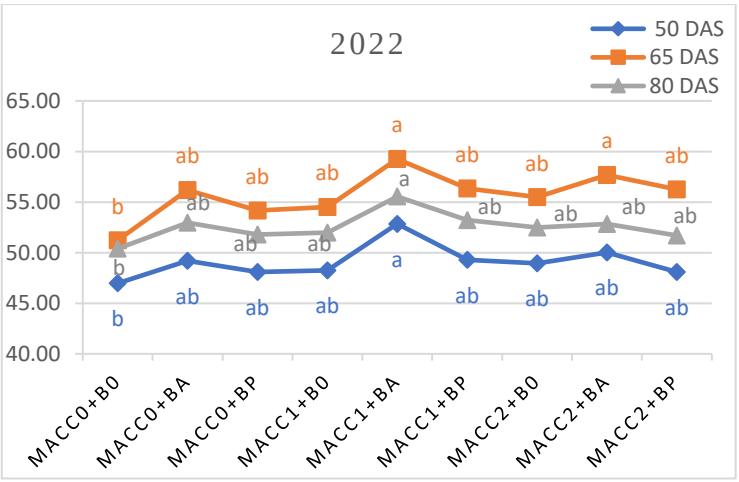


Figure 7: Chlorophyll content of maize (Zea mays L.) leaf assessed at different growth stage from SPAD reading in 2022. Where: MACC0, MACC1, and MACC2, which are levels of concentration of the N. *linckia* at 0, 0.3 g/L, and 1 g/L, respectively. BA and BP are *A. lipoferum* and *P. florescence*, respectively.

In 2023, the results revealed no statistically significant difference in chlorophyll content at 65 DAS between the control level and the sole application of *N. linckia* at 0.3 g/L. However, notably elevated chlorophyll levels were observed upon the application of *N. linckia* at 0.3 g/L and 1 g/L in combination with *A. lipoferum* and *P. florescence* (Fig. 8).

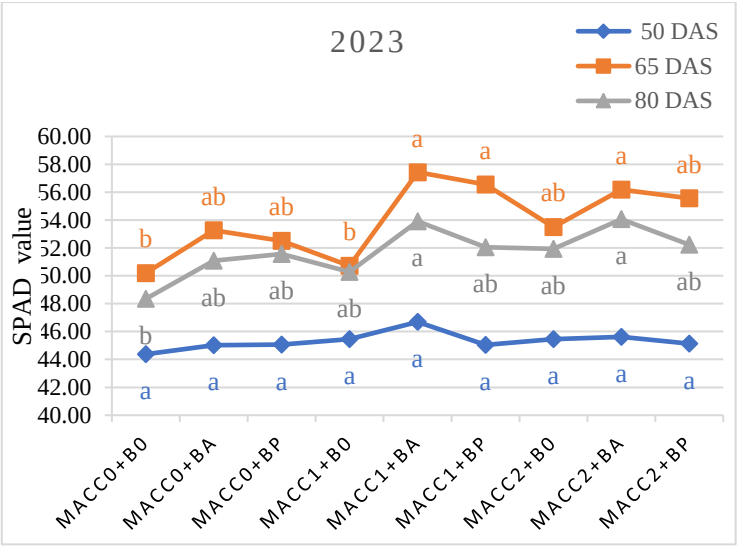


Figure 8: Chlorophyll content of maize (Zea mays L.) leaf assessed at different growth stage from SPAD reading in 2023. Where: MACC0, MACC1, and MACC2, which are levels of concentration of the N. *linckia* at 0, 0.3 g/L, and 1 g/L, respectively. BA and BP are *A. lipoferum* and *P. florescence*, respectively.

4.2.2 The Normalized Difference Vegetation Index (NDVI)

NDVI serves as a tool for gauging vegetation's greenness, aiding in assessing vegetation density and identifying alterations in plant health. These indirect measurements of reflectance have been employed for the estimation of both plant biomass and yield. The NDVI values exhibited significant differences ($P \leq 0.05$) across different measurement times. Generally, the highest average measurement was observed consistently on the 65 DAS across different experimental periods (Fig. 9, 10, 11). The differences in NDVI values were significant ($P \leq 0.05$) at 65 days after sowing, except in the year 2023.

In 2021, NDVI measurements demonstrated statistical significance ($P \leq 0.05$) except those taken at 50 days after sowing (Fig. 9). At 65 days after sowing (DAS), the most elevated mean value was documented in the combined treatment of *N. linckia* at 0.3 g/L and 1 g/L in conjunction with *A. lipoferum* and *P. florescence*, whereas the lowest value was observed in the control group (Fig 9). However, by 80 days after sowing (DAS), the peak NDVI value was documented in the combined treatment involving *N. linckia* at 0.3 g/L with *P. florescence*, as well as *N. linckia* at 1 g/L alongside *A. lipoferum*.

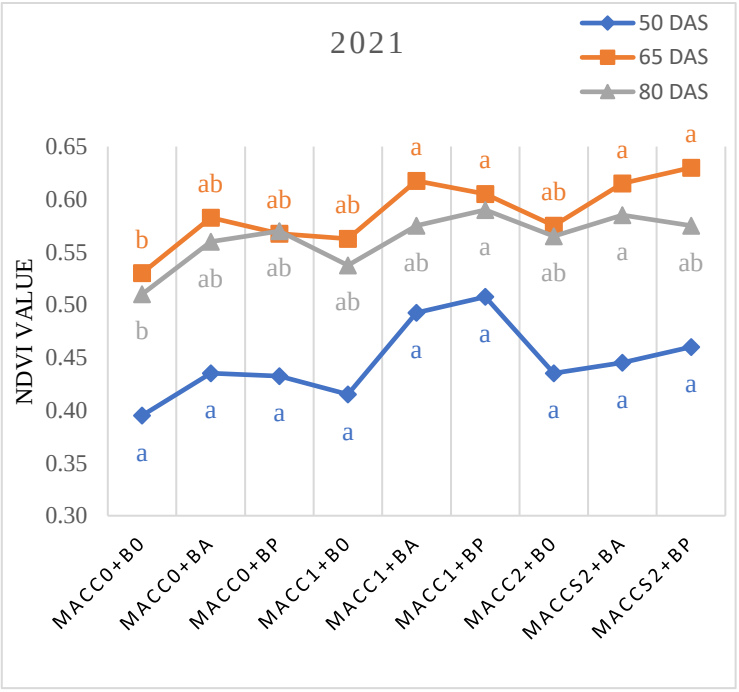


Figure 9: The NDVI value of maize (Zea mays L.) assessed at different days after sowing (DAS) in 2021. Where: MACC0, MACC1, and MACC2, which are levels of concentration of the N. *linckia* at 0, 0.3 g/L, and 1 g/L, respectively. BA and BP are *A. lipoferum* and *P. florescence*, respectively.

In 2022, except for 65 days after sowing, the NDVI values displayed statistical insignificance ($P \leq 0.05$) (Fig. 10). The highest value was observed when *N. linckia* at 0.3 g/L was combined with *A. lipoferum*, whereas the lowest values were noted in both the control group and when *N. linckia* was applied alone at 0.3 g/L.

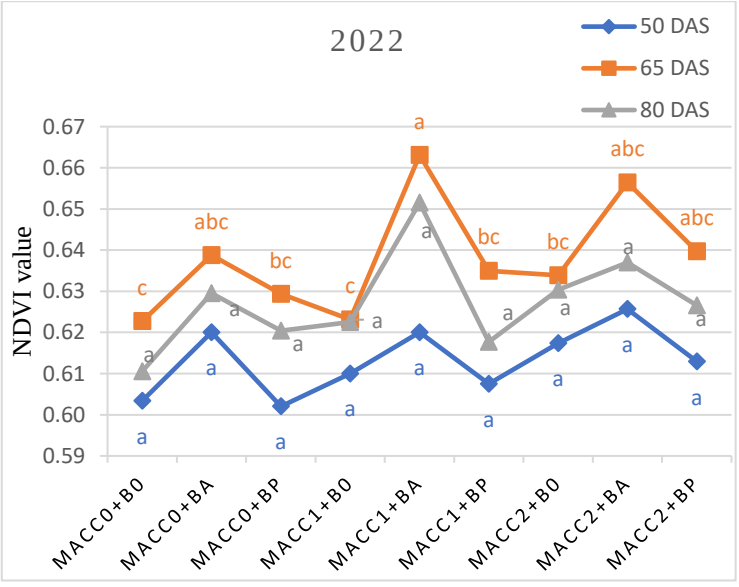


Figure 10: The NDVI value of maize (Zea mays L.) assessed at different days after sowing (DAS) in 2022. Where: MACC0, MACC1, and MACC2, which are levels of concentration of the N. *linckia* at 0, 0.3 g/L, and 1 g/L, respectively. BA and BP are *A. lipoferum* and *P. florescence*, respectively.

In 2023, apart from the 50 DAS measurement, the other treatment showed no statistical significance ($P \leq 0.05$) (Fig 11). The highest

NDVI value was documented upon the combined application of *N. linckia* at both 0.3 g/L and 1 g/L, accompanied by *A. lipoferum* and *P. florescence.*

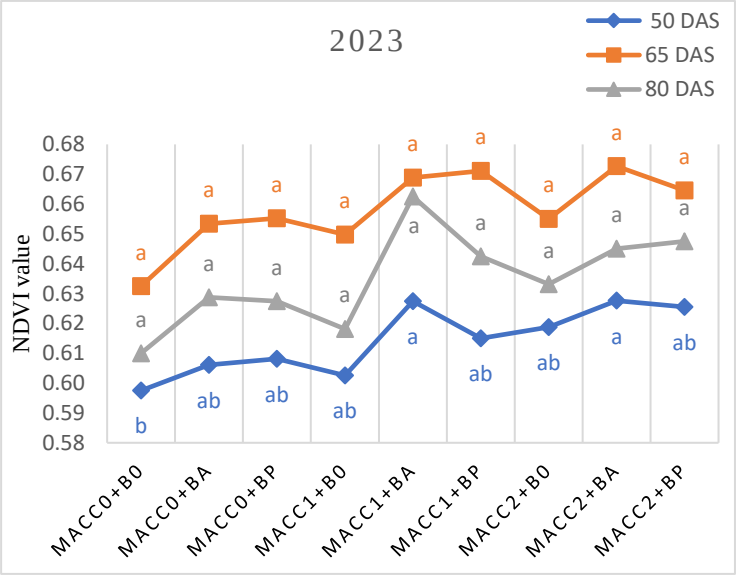


Figure 11: The NDVI value of maize (Zea mays L.) assessed at different days after sowing (DAS) in 2023. Where: MACC0, MACC1, and MACC2, which are levels of concentration of the N. *linckia* at 0, 0.3 g/L, and 1 g/L, respectively. BA and BP are *A. lipoferum* and *P. florescence*, respectively.

4.2.3 Leaf and root fresh and dry weight

The findings indicated that both the application of *N. linckia* and the presence of PGPB significantly ($P \leq 0.05$) impacted the growth characteristics of the plants, notably affecting the fresh and dry weights

of both above and below-ground parts when compared to the control group.

In 2021, except dry root weight, the interaction effect of *N. linckia* and PGPB was statistically insignificant ($P \leq 0.05$) on the fresh and dry plant biomass at 50 DAS (Table 6). However, the maximum values of both fresh and dry plant biomass were observed in the combined application of the *N. linckia* and PGPB. Conversely, the control group exhibited the lowest values (Table 6 and 7). The main effect of *N. linckia* and PGPB was shown significantly affect the plant fresh and dry biomass at 50 and 65 DAS (Table 6 and 7).

Table 6: Fresh and dry leaf and root weights (grams per plant) at 50 days after sowing in 2021.

Treatment	2021-50 DAS			
	Fresh leaf (g)	Dry leaf (g)	Fresh root (g)	Dry root (g)
MACC0+B0	25.07±1.20^{d}	3.33±0.30^{b}	8.89±0.47^{e}	0.93±0.25^{b}
MACC0+BA	29.30±1.42^{cd}	3.99±0.30^{ab}	11.81±0.73^{bcd}	1.78±0.28^{a}
MACC0+BP	31.44±2.13^{abcd}	4.74±0.78^{a}	11.61±1.68^{cd}	1.96±0.27^{a}
MACC1+B0	30.73±3.20^{bcd}	4.77±0.89^{a}	10.79±1.88^{de}	1.88±0.27^{a}
MACC1+BA	36.88±3.22^{ab}	5.04±0.58^{a}	15.12±0.66^{a}	2.17±0.21^{a}
MACC1+BP	37.26±1.75^{ab}	5.22±0.25^{a}	14.62±0.92^{a}	2.06±0.26^{a}
MACC2+B0	36.14±6.43^{abc}	4.71±0.87^{a}	11.98±1.06^{bcd}	1.95±0.17^{a}
MACC2+BA	37.23±2.57^{ab}	4.77±0.25^{a}	14.24±1.23^{ab}	2.11±0.24^{a}
MACC2+BP	38.29±3.76^{a}	5.09±0.45^{a}	13.81±1.12^{abc}	2.16±0.18^{a}
MACC	***	***	***	***
B	***	**	***	***
MACC*B	ns	ns	ns	**

Values represent means ± standard deviation (n=4), and different superscript letters denote significant differences based on pairwise comparisons conducted by Tukey's test at $P \leq 0.05$. Where: ns indicates non-significant, * significant at $P \leq 0.05$, ** significant at $P \leq 0.01$, *** significant at $P \leq 0.001$. MACC0, MACC1, and MACC2, which are levels of concentration of the N. *linckia* at 0, 0.3 g/L, and 1 g/L, respectively. BA and BP are *A. lipoferum* and *P. florescence*, respectively.

Table 7: Fresh and dry leaf and root weights (grams per plant) at 65 days after sowing in 2021.

Treatment	2021-65 DAS			
	Fresh leaf (g)	Dry leaf (g)	Fresh root (g)	Dry root (g)
MACC0+B0	119.36±3.93^{c}	14.69±1.82^{d}	27.19±2.08^{b}	3.72±0.46^{d}
MACC0+BA	134.21±4.81^{bc}	17.89±1.75^{cd}	30.80±1.65^{b}	4.76±1.42^{cd}
MACC0+BP	135.56±4.37^{bc}	18.51±2.65^{bcd}	30.86±1.82^{b}	4.38±0.74^{d}
MACC1+B0	130.26±7.17^{c}	18.22±1.91^{bcd}	29.93±1.86^{b}	4.63±0.51^{cd}
MACC1+BA	159.78±14.2^{a}	22.43±2.97^{abc}	40.19±2.69^{a}	7.72±0.88^{a}
MACC1+BP	161.66±15.2^{a}	23.51±1.41^{a}	38.62±3.49^{a}	7.37±0.48^{ab}
MACC2+B0	151.56±10.4^{ab}	21.39±2.06^{abc}	36.90±3.57^{a}	6.02±0.54^{bc}
MACC2+BA	160.61±7.36^{a}	22.68±2.61^{abc}	38.03±1.78^{a}	7.03±0.47^{ab}
MACC2+BP	165.68±14.6^{a}	23.09±1.73^{ab}	37.29±1.96^{a}	6.98±0.84^{ab}
MACC	***	***	***	***
B	***	***	***	***
MACC*B	ns	*	**	**

Values represent means ± standard deviation (n=4), and different superscript letters denote significant differences based on pairwise comparisons conducted by Tukey's test at $P \leq 0.05$. Where: ns indicate non-significant, * significant at $P \leq 0.05$, ** significant at $P \leq 0.01$, *** significant at $P \leq 0.001$. MACC0, MACC1, and MACC2, which are levels of concentration of the N. *linckia* at 0, 0.3 g/L, and 1 g/L, respectively. BA and BP are *A. lipoferum* and *P. florescence*, respectively.

Table 8 highlights the combined application of *N. linckia* and PGPB was significantly affect the dry leaf and root plant biomass, while the effect on the fresh leaf and root plant biomass was statistically insignificant at 50 DAS in 2022. Except fresh leaf weight, the

interaction effect of *N. linckia* and PGPB on the fresh and dry plant weight was recorded statistically insignificant at 65 DAS in 2022 (Table 9). However, the combined utilization of *N. linckia* and PGPB resulted in the highest recorded values for both fresh and dry plant weight, while the control levels showcased the lowest values, as depicted in Tables 8 and 9.

Table 8: Fresh and dry leaf and root weights (grams per plant) at 50 days after sowing in 2022.

Treatment	2022-50 DAS			
	Fresh leaf (g)	Dry leaf (g)	Fresh root (g)	Dry root (g)
MACC0+B0	33.89±3.13^{b}	6.19±1.37^{b}	13.97±0.79^{a}	1.06±0.25^{d}
MACC0+BA	42.50±8.21^{ab}	9.50±2.49^{b}	16.30±3.33^{a}	2.17±0.68^{cd}
MACC0+BP	41.37±2.83^{ab}	8.97±1.63^{b}	14.81±1.16^{a}	2.28±0.43^{cd}
MACC1+B0	39.04±3.69^{ab}	8.25±2.23^{b}	14.10±1.61^{a}	1.91±0.42^{cd}
MACC1+BA	56.32±5.41^{a}	16.81±3.31^{a}	18.99±1.02^{a}	5.17±0.91^{ab}
MACC1+BP	57.90±6.62^{a}	17.48±1.73^{a}	17.12±1.38^{a}	4.88±0.84^{ab}
MACC2+B0	54.55±9.75^{a}	15.82±1.78^{a}	16.00±6.55^{a}	3.41±1.70^{bc}
MACC2+BA	58.39±5.33^{a}	17.76±2.75^{a}	19.20±5.12^{a}	5.80±0.59^{a}
MACC2+BP	57.78±2.59^{a}	18.68±1.15^{a}	18.61±0.91^{a}	4.84±0.51^{ab}
MACC	***	***	ns	***
B	*	***	*	***
MACC*B	ns	*	ns	*

Values represent means ± standard deviation (n=4), and different superscript letters denote significant differences based on pairwise comparisons conducted by Tukey's

test at $P \leq 0.05$. Where: ns indicate non-significant, * significant at $P \leq 0.05$, ** significant at $P \leq 0.01$, *** significant at $P \leq 0.001$. MACC0, MACC1, and MACC2, which are levels of concentration of the N. *linckia* at 0, 0.3 g/L, and 1 g/L, respectively. BA and BP are *A. lipoferum* and *P. florescence*, respectively.

Table 9: Fresh and dry leaf and root weights (grams per plant) at 65 days after sowing in 2022.

Treatment	2022-65 DAS			
	Fresh leaf (g)	Dry leaf (g)	Fresh root (g)	Dry root (g)
MACC0+B0	136.14±3.17^{c}	18.78±2.43^{d}	35.07±3.71^{c}	5.21±1.13^{c}
MACC0+BA	143.47±4.71^{bc}	22.17±3.09^{abcd}	42.17±1.82^{abc}	6.29±0.78^{bc}
MACC0+BP	142.57±5.09^{c}	21.61±3.17^{bcd}	41.12±2.65^{abc}	6.04±1.02^{bc}
MACC1+B0	140.60±4.11^{c}	19.98±2.19^{cd}	38.34±2.42^{bc}	6.02±1.19^{bc}
MACC1+BA	169.33±3.58^{a}	27.92±1.81^{a}	49.20±5.58^{a}	9.27±4.47^{a}
MACC1+BP	167.51±3.78^{a}	26.48±3.89^{ab}	47.07±3.13^{ab}	9.06±2.07^{a}
MACC2+B0	157.73±7.22^{ab}	24.56±1.25^{abcd}	44.97±6.49^{ab}	6.90±1.20^{ab}
MACC2+BA	164.67±7.04^{a}	26.45±2.72^{ab}	47.55±1.79^{ab}	9.55±1.32^{a}
MACC2+BP	165.30±9.04^{a}	25.30±2.69^{abc}	45.70±4.23^{ab}	8.10±0.93^{a}
MACC	***	***	**	**
B	***	**	***	*
MACC*B	**	ns	ns	ns

Values represent means ± standard deviation (n=4), and different superscript letters denote significant differences based on pairwise comparisons conducted by Tukey's test at $P \leq 0.05$. Where: ns indicate non-significant, * significant at $P \leq 0.05$, ** significant at $P \leq 0.01$, *** significant at $P \leq 0.001$. MACC0, MACC1, and MACC2, which are levels of concentration of the N. *linckia* at 0, 0.3 g/L, and 1 g/L, respectively. BA and BP are *A. lipoferum* and *P. florescence*, respectively.

In 2023, except from fresh root weight at 50 and 56 DAS and dry root at 65 DAS, the interaction impact between *N. linckia* and PGPB showed statistical significance across other plant biomass measurements at 50 and 65 days after sowing, as outlined in Tables 10 and 11. Notably, the combined application of *N. linckia* and PGPB had

a highly significant impact ($P \leq 0.001$) on the dry leaf weight at both 50 and 65 days after sowing. Moreover, the combined application of *N. linckia* at 0.3 g/L and 1 g/L alongside *A. lipoferum* and *P. florescence*, as well as the alone application of *N. linckia* at 1 g/L, exhibited statistically at par on the fresh and dry leaf weights at both 50 and 65 days after sowing (DAS) (Table 10 and 11). The dry root weight observed from the sole application of *N. linckia* at 0.3 g/L, as well as the alone application of *P. florescence* was statistically at par to that of the control group at 50 and 65 DAS.

Table 10: Fresh and dry leaf and root weights (grams per plant) at 50 days after sowing in 2023.

Treatment	2023-50 DAS			
	Fresh leaf (g)	Dry leaf (g)	Fresh root (g)	Dry root (g)
MACC0+B0	31.41±3.03^{b}	5.25±2.13^{c}	14.15±1.39^{d}	1.52±0.25^{c}
MACC0+BA	41.59±3.33^{b}	9.18±1.45^{b}	16.99±2.65^{bc}	3.03±1.11^{bc}
MACC0+BP	40.76±3.63^{b}	8.96±1.98^{b}	15.51±1.79^{cd}	2.78±0.59^{c}
MACC1+B0	38.61±2.54^{b}	8.29±2.07^{b}	16.14±0.16^{cd}	2.47±1.03^{c}
MACC1+BA	57.93±3.42^{a}	17.41±2.32^{a}	20.45±2.18^{a}	5.88±1.59^{a}
MACC1+BP	56.86±8.09^{a}	16.98±1.41^{a}	19.43±3.02^{ab}	5.77±0.93^{a}
MACC2+B0	53.94±6.48^{a}	15.61±2.52^{a}	19.19±1.14^{ab}	4.79±1.39^{ab}
MACC2+BA	57.36±5.68^{a}	16.22±1.33^{a}	20.99±1.22^{a}	6.37±1.16^{a}
MACC2+BP	56.22±2.76^{a}	16.59±1.78^{a}	19.63±1.82^{a}	6.02±1.76^{a}
MACC	***	***	***	***
B	***	***	**	***
MACC*B	*	***	ns	*

Values represent means ± standard deviation (n=4), and different superscript letters denote significant differences based on pairwise comparisons conducted by Tukey's test at $P \leq 0.05$. Where: ns indicate non-significant, * significant at $P \leq 0.05$, ** significant at $P \leq 0.01$, *** significant at $P \leq 0.001$. MACC0, MACC1, and MACC2, which are levels of concentration of the N. *linckia* at 0, 0.3 g/L, and 1 g/L, respectively. BA and BP are *A. lipoferum* and *P. florescence*, respectively.

Table 11: Fresh and dry leaf and root weights (grams per plant) at 65 days after sowing in 2023.

Treatment	2023- 65 DAS			
	Fresh leaf (g)	Dry leaf (g)	Fresh root (g)	Dry root (g)
MACC0+B0	134.38±4.20^{c}	17.72±2.13^{c}	36.42±3.96^{d}	5.82±2.22^{c}
MACC0+BA	145.16±4.91^{b}	21.85±1.45^{b}	41.71±3.09^{bcd}	7.24±0.45^{bc}
MACC0+BP	145.50±5.06^{b}	22.04±1.98^{b}	42.21±3.63^{bcd}	6.88±0.74^{c}
MACC1+B0	144.75±3.45^{b}	20.40±2.07^{b}	40.92±4.97^{cd}	6.20±0.96^{c}
MACC1+BA	171.18±2.93^{a}	29.46±2.32^{a}	51.41±3.76^{a}	11.89±3.99^{a}
MACC1+BP	167.57±4.75^{a}	29.03±1.41^{a}	49.19±2.03^{ab}	11.66±3.17^{a}
MACC2+B0	161.57±5.24^{a}	25.34±2.52^{a}	45.93±3.81^{abc}	9.53±2.39^{abc}
MACC2+BA	170.06±4.35^{a}	29.06±1.33^{a}	51.70±3.76^{a}	11.82±2.63^{a}
MACC2+BP	169.10±2.35^{a}	29.32±1.78^{a}	49.30±2.13^{ab}	11.56±3.28^{ab}
MACC	***	***	***	***
B	***	***	***	***
MACC*B	**	***	ns	ns

Values represent means ± standard deviation (n=4), and different superscript letters denote significant differences based on pairwise comparisons conducted by Tukey's test at $P \leq 0.05$. Where: ns indicate non-significant, * significant at $P \leq 0.05$, ** significant at $P \leq 0.01$, *** significant at $P \leq 0.001$. MACC0, MACC1, and MACC2, which are levels of concentration of the N. *linckia* at 0, 0.3 g/L, and 1 g/L, respectively. BA and BP are *A. lipoferum* and *P. florescence*, respectively.

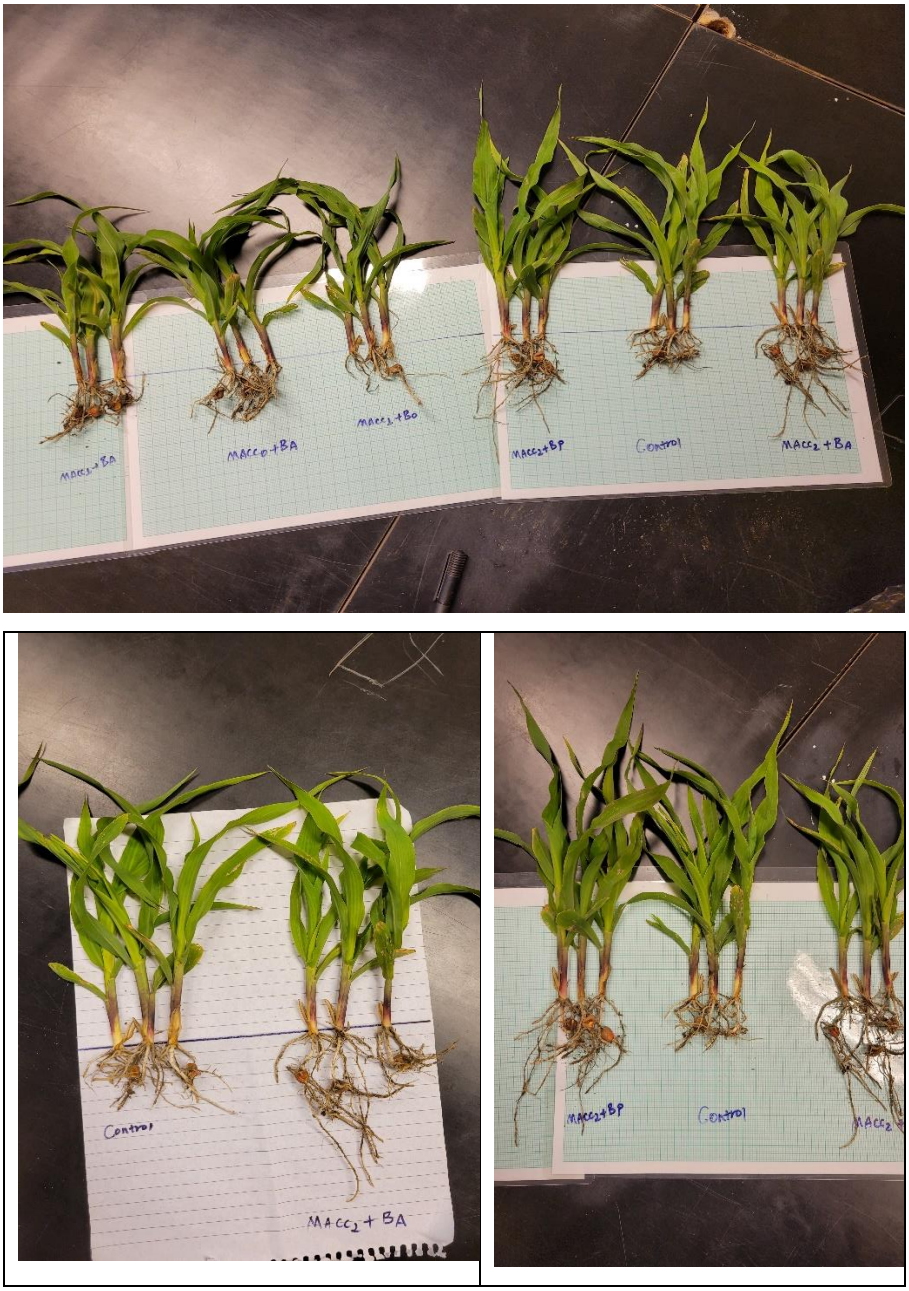


Figure 12: The image of the impacts of different treatment of *N. linckia* and PGPB on the growth of maize seedlings on the 40th days.

4.3 Nitrogen content of plant biomass

Table 12 indicated that the combined impact of *N. linckia* and PGPB did not yield statistically significant ($P \leq 0.05$) changes in plant biomass nitrogen content across all observed years. Furthermore, the main and combined effects of *N. linckia* and PGPB demonstrated statistical insignificance on seed and leaf nitrogen content in 2021 and 2022, respectively (Table 12). In 2023, the nitrogen content in both leaf and seed, following the alone use of *N. linckia* at 0.3 g/L 1 g/L, as well as the sole application of *A. lipoferum* and *P. florescence*, demonstrated statistical similarity to the that control group.

Table 12: Nitrogen content of plant biomass

Treatment	2021		2022		2023	
	Leaf N (%)	Seed N (%)	Leaf N (%)	Seed N (%)	Leaf N (%)	Seed N (%)
MACC0+B0	1.11±0.11^{c}	1.05±0.04	1.18±0.08	1.22±0.13^{b}	1.13±0.09^{c}	1.07±0.14b
MACC0+BA	1.31±0.15^{abc}	1.29±0.44	1.32±0.20	1.43±0.06^{ab}	1.20±0.08^{c}	1.16±0.02^{ab}
MACC0+BP	1.32±0.13^{abc}	1.16±0.06	1.24±0.03	1.39±0.07^{ab}	1.19±0.07^{c}	1.15±0.05^{ab}
MACC1+B0	1.19±0.06^{bc}	1.11±0.11	1.21±0.13	1.35±0.12^{ab}	1.18±0.05^{c}	1.13±0.02^{ab}
MACC1+BA	1.44±0.16^{a}	1.33±0.05	1.43±0.20	1.44±0.04^{a}	1.42±0.16^{ab}	1.37±0.32^{a}
MACC1+BP	1.33±0.07^{ab}	1.42±0.47	1.46±0.33	1.43±0.11^{ab}	1.31±0.16^{abc}	1.31±0.06^{a}
MACC2+B0	1.32±0.07^{abc}	1.31±0.51	1.31±0.18	1.40±0.09^{ab}	1.28±0.11^{bc}	1.19±0.04^{ab}
MACC2+BA	1.42±0.10^{a}	1.37±0.33	1.43±0.18	1.44±0.11^{a}	1.40±0.15^{ab}	1.39±0.17^{a}
MACC2+BP	1.40±0.03^{ab}	1.34±0.26	1.40±0.20	1.43±0.08^{ab}	1.49±0.11^{a}	1.39±0.22^{a}
MACC	**	ns	ns	ns	***	**
B	***	ns	ns	*	***	**
MACC*B	ns	ns	ns	ns	ns	ns

Values represent means ± standard deviation (n=4), and different superscript letters denote significant differences based on pairwise comparisons conducted by Tukey's test at $P \leq 0.05$. Where: ns indicate non-significant, * significant at $P \leq 0.05$, ** significant at $P \leq 0.01$, *** significant at $P \leq 0.001$. MACC: *N. linckia*; B: *A. lipoferum* and *P. florescence.*

4.4 Plant yield parameters

Table 13 provides data on how the plant growth parameters of maize were affected by different concentrations of *N. linckia* and PGPB strains. The yield and its component traits were influenced by *N. linckia*, PGPB, and their interaction. The statistical analysis showed a significant ($P \leq 0.05$) increase in the number of seeds per ear, thousand-grain weight, and yield when *N. linckia* and PGPB were applied alone or jointly in both production years. However, the main and interaction impact of *N. linckia* and PGPB on the plant height was statistically insignificant across the experimental period, except main effect of the PGPB in 2023 (Table 13).

The highest number of seeds per ear was documented when using *N. linckia* at a concentration of 0.3 g/L in combination with *A. lipoferum* in all the experimental periods, whereas the lowest number of seeds per ear was observed in the untreated trials. However, the combined application of *N. linckia* at concentrations of 0.3 g/L with *A. lipoferum* and *P. fluorescens*, as well as *N. linckia* at 1 g/L with *P. fluorescens*, demonstrated statistically similar results in terms of the number of seeds per ears in 2021. The statistical significance of the interaction between *N. linckia* and PGPB varied across years, displaying significance at $p \leq 0.5$ in 2021 and 2023, while reaching a higher significance level of $p \leq 0.001$ in 2022 (Table 13).

Table 13: Impact of single and combined application microalgae and PGPB on maize yield components

Treatment	Plant height (cm)			Number of seeds/ears		
	2021	2022	2023	2021	2022	2023
MACC0+B0	213.15±5.96	171.75±8.22	206.38±7.52	385.10±35.05^{b}	400.34±14.91^{d}	421.00±38.17^{d}
MACC0+BA	214.40±6.56	178.98±5.08	215.33±10.86	473.47±54.57^{a}	476.50±15.73^{bc}	503.88±35.74^{abcd}
MACC0+BP	216.84±4.17	180.03±8.15	211.47±7.03	472.68±23.62^{a}	441.33±49.72^{cd}	491.63±40.95^{bcd}
MACC1+B0	217.00±5.50	182.03±9.03	210.67±11.67	472.68±25.27^{a}	435.80±20.30^{cd}	487.38±30.81^{bcd}
MACC1+BA	217.95±3.55	184.69±9.79	223.12±2.45	517.05±19.33^{a}	548.60±6.00^{a}	589.00±48.34^{a}
MACC1+BP	218.03±5.63	181.75±2.74	215.98±7.29	514.88±20.90^{a}	510.20±11.25^{ab}	548.90±33.61^{abc}
MACC2+B0	214.40±5.84	182.81±9.67	213.25±6.17	482.53±46.09^{a}	504.67±24.65^{ab}	463.63±46.11^{cd}
MACC2+BA	215.70±8.99	183.63±5.58	219.42±4.33	461.90±28.02^{ab}	508.50±6.31^{ab}	577.25±26.59^{ab}
MACC2+BP	218.10±7.24	180.31±9.10	217.97±7.34	475.08±19.46^{a}	472.50±14.92^{bc}	551.88±42.65^{abc}
MACC	ns	ns	ns	**	***	***
B	ns	ns	*	*	***	***
MACC*B	ns	ns	ns	*	***	*

Values represent means ± standard deviation (n=4), and different superscript letters denote significant differences based on pairwise comparisons conducted by Tukey's test at $P \leq 0.05$. Where: ns indicate non-significant, * significant at $P \leq 0.05$, ** significant at $P \leq 0.01$, *** significant at $P \leq 0.001$. MACC: *N. linckia*; B: *A. lipoferum* and *P. florescence.*

Table 14 highlighted that the interaction between *N. linckia* and PGPB showed statistical insignificance on the thousand seed weight, except for 2022, where significance was observed at $P \leq 0.001$. In 2021 and 2023, the highest thousand seed weight was achieved through the combined use of *N. linckia* at 0.3 g/L and 1 g/L with *A. lipoferum* and *P. fluorescens*. In 2022, the highest thousand seed weight was observed with *N. linckia* at a concentration of 0.3 g/L combined with *A. lipoferum*, while the lowest weight was seen in the control. The application of *N. linckia* at 0.3 g/L alongside *A. lipoferum* notably increased the thousand grain weight, showing impressive enhancements of 99.02%, 83.33%, and 90.9% in 2021, 2022, and 2023, respectively, compared to untreated plots.

The influence of *N. linckia* and PGPB on maize grain yield was statistically significant ($P \leq 0.05$) in all years except 2022, indicating their substantial impact on the crop's productivity. The utilization of *N. linckia* at a concentration of 0.3 g/L in combination with *A. lipoferum* resulted in a significant upsurge in grain yield, demonstrating a remarkable 33.20% enhancement, with a significance of $P \leq 0.05$, in the initial year and a substantial 31.53 and 32.34% increase in 2022 and 2023, respectively when compared with untreated plots (Table 14). In general, the grain yield demonstrated a high performance in the third year as opposed to the other years.

Table 14: Impact of single and combined application microalgae and PGPB on maize yield components

Treatment	Thousand seed weight (kg)			Yield (ton/ha)		
	2021	2022	2023	2021	2022	2023
MACC0+B0	0.26 ± 0.02^{b}	0.35 ± 0.01^{h}	0.30 ± 0.03^{b}	5.20 ± 0.29^{d}	5.93 ± 1.21^{b}	6.22 ± 0.47^{c}
MACC0+BA	0.43 ± 0.08^{ab}	0.57 ± 0.01^{f}	0.59 ± 0.16^{ab}	5.97 ± 0.42^{bcd}	7.16 ± 0.73^{ab}	6.81 ± 1.22^{abc}
MACC0+BP	0.41 ± 0.05^{ab}	0.52±0.004g	0.56 ± 0.33^{ab}	5.78 ± 0.54^{cd}	6.85 ± 1.07^{ab}	6.65 ± 1.16^{bc}
MACC1+B0	0.38 ± 0.03^{ab}	0.61 ± 0.01^{e}	0.52 ± 0.19^{ab}	6.03 ± 0.41^{bcd}	7.13 ± 0.55^{ab}	6.37 ± 1.38^{c}
MACC1+BA	0.77 ± 0.41^{a}	0.85 ± 0.02^{a}	0.80 ± 0.16^{a}	7.27 ± 0.45^{a}	8.15 ± 0.41^{a}	8.62 ± 1.23^{a}
MACC1+BP	0.72 ± 0.23^{a}	0.72 ± 0.02^{b}	0.71 ± 0.22^{a}	7.09 ± 0.69^{ab}	7.71 ± 0.70^{a}	7.99 ± 1.17^{abc}
MACC2+B0	0.47 ± 0.09^{ab}	0.68 ± 0.01^{c}	0.67 ± 0.25^{ab}	6.02 ± 0.43^{bcd}	7.33 ± 0.32^{ab}	7.04 ± 1.22^{abc}
MACC2+BA	0.67 ± 0.15^{a}	0.73 ± 0.01^{b}	0.81 ± 0.06^{a}	7.07 ± 0.67^{ab}	7.96 ± 0.22^{a}	8.31 ± 1.57^{ab}
MACC2+BP	0.64 ± 0.06^{ab}	0.64 ± 0.01^{d}	0.73 ± 0.37^{a}	6.75 ± 0.41^{abc}	7.03 ± 0.25^{ab}	7.89 ± 1.50^{abc}
MACC	**	***	**	***	**	**
B	**	***	**	***	**	***
MACC*B	ns	***	ns	*	ns	*

Values represent means ± standard deviation (n=4), and different superscript letters denote significant differences based on pairwise comparisons conducted by Tukey's test at $P \leq 0.05$. Where: ns indicate non-significant, * significant at $P \leq 0.05$, ** significant at $P \leq 0.01$, *** significant at $P \leq 0.001$. MACC: *N. linckia*; B: *A. lipoferum* and *P. florescence.*

4.4 Soil chemical properties

Following the maize plant harvest from each treatment, the soil was gathered and subjected to analysis to assess the post-treatment impacts on soil properties, including pH, organic matter content, (NO_3^-+ NO_2^-)-nitrogen, total nitrogen, phosphorus (P), and potassium (K) levels. Overall, whether applied individually or in combination, different concentrations of *N. linckia* and PGPB strains noticeably elevated soil pH, humus, (NO_3^-+ NO_2^-)-nitrogen and total nitrogen, whereas P and K were statistically insignificant during the experimental periods, as illustrated in Figure 13, 14, 15, 16, and 17. In 2021, statistical analysis revealed that, except for soil humus and total nitrogen content, all other examined soil parameters demonstrated no significant differences at the level of $P \leq 0.05$. In contrast to the findings in the initial year, the results of the soil analysis in 2022 showed a statistically significant effect between *N. linckia* and PGPB with respect to pH, humus, and (NO_3^-+ NO_2^-)-N and total nitrogen content, indicating that the combined application of *N. linckia* and PGPB increases soil fertility. Conversely, in 2023, excluding (NO3-+ NO2-)-nitrogen and total nitrogen content, the remaining measured soil parameters were statistically insignificant.

Figure 13 highlighted that except for the year 2022, the statistical significance of pH values in the remaining seasons was not observed. In 2022, the highest pH value (7.42) was observed in the control group, signifying slightly alkaline soil conditions. In contrast, lower pH values

were recorded in the treatments involving *N. linckia* at a concentration of 0.3 g/L in combination with *A. lipoferum* (7.24) and *P. fluorescens* (7.23), as well as *N. linckia at* a concentration of 1 g/L with *A. lipoferum* (7.26), indicating that the combined application of *N. linckia and* PGPB slightly lowers the alkalinity level (Fig. 13).

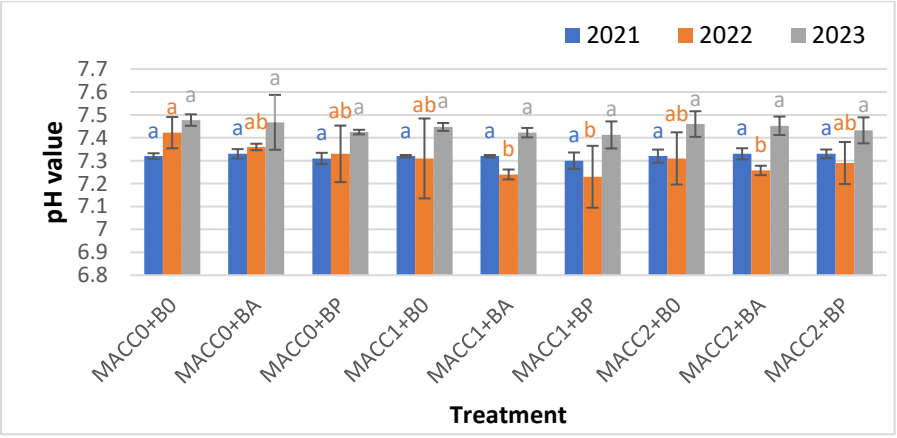


Figure 13: Effect of different application of N. linckia and PGPB on soil pH. Mean values are presented as mean ± standard deviation. Error bars indicate standard deviation. The bars with different lowercase letters are significantly different at $p \leq 0.05$ by Tukey's test. Where: MACC0, MACC1, and MACC2, which are levels of concentration of the N. *linckia* at 0, 0.3 g/L, and 1 g/L, respectively. BA and BP are *A. lipoferum* and *P. florescence*, respectively.

Figure 14 reveals that, except for 2023, the humus content exhibited statistically significant variations across all other seasons. In 2021, the highest humus content was obtained through the combined application of *N. linckia* at a concentration of 0.3 g/L along with *A. lipoferum*,

which resulted in 31.67% increases in humus content compared to untreated group. Conversely, lower soil humus content was observed in a sole application of *P. fluorescens* and *N. linckia* at 0.3 g/L, and the combined application of *N. linckia* at 0.3 g/L and 1 g/L with *P. fluorescens* (Fig 14).

Furthermore, in 2022, the combined use of *N. linckia* at a concentration of 0.3 g/L in combination with *A. lipoferum* resulted in 20.25% increase in humus content compared with the control trails (Fig. 14). Similarly, in 2023, the application of *N. linckia* at a concentration of 0.3 g/L along with treatments of A. *lipoferum* resulted in humus levels that were 15.71% higher compared with the untreated trails (Fig. 14).

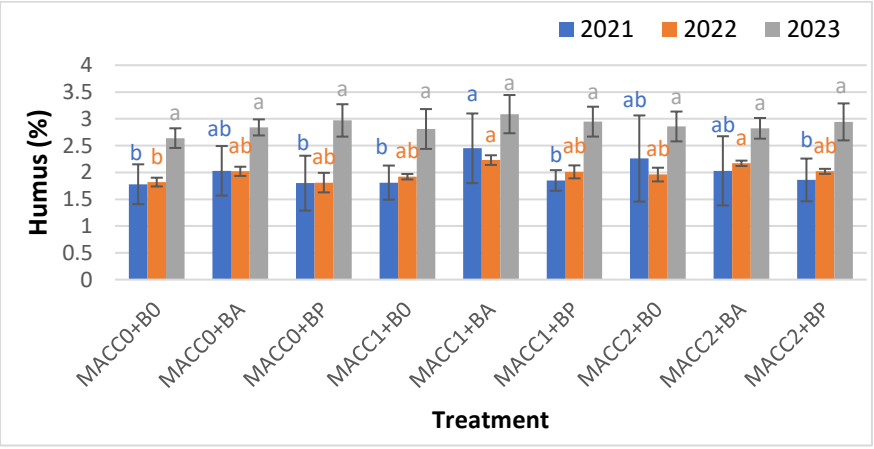


Figure 14: Effect of different application of *N. linckia* and PGPB on soil humus. Mean values are presented as mean ± standard deviation. Error bars indicate standard deviation. The bars with different lowercase letters are significantly different at $p \leq 0.05$ by Tukey's test. Where: MACC0, MACC1, and MACC2, which are levels of concentration of the *N. linckia* at 0, 0.3 g/L, and 1 g/L, respectively. BA and BP are *A. lipoferum* and *P. florescence*, respectively.

Figure 15 reveals that, excluding the year 2021, the treatment of soil during sowing with *N. linckia* and PGPB showed statistical significance in terms of soil (NO_3^-+ NO_2^-)- nitrogen content. During 2022, the analysis of bulk soil showed a noteworthy rise in (NO_3^-+ NO_2^-)-N content within the treatments subjected to *N. linckia* at a concentration of 0.3 g/L with *A. lipoferum* treatment (a notable increase of 59.2%), *N. linckia* at a concentration of 1 g/L along with treatments of *A. lipoferum* treatment (an increase of 43.2%), and *N. linckia* at a concentration of 1 g/L along with *P. fluorescens* treatment (an increases of 21.82%), in comparison to the control group (Fig. 15).

In 2023, the highest soil (NO_3^-+ NO_2^-)-N content was observed with the combined application of *N. linckia* at a concentration of 1 g/L along with *A. lipoferum*, while the lowest content was noted at control levels (Fig. 15). The data indicates a notable 51.54% increase in (NO_3^-+ NO_2^-)-N content with the combined application of *N. linckia* at a concentration of 1 g/L along with *A. lipoferum* compared to the control group, as depicted in figure 15.

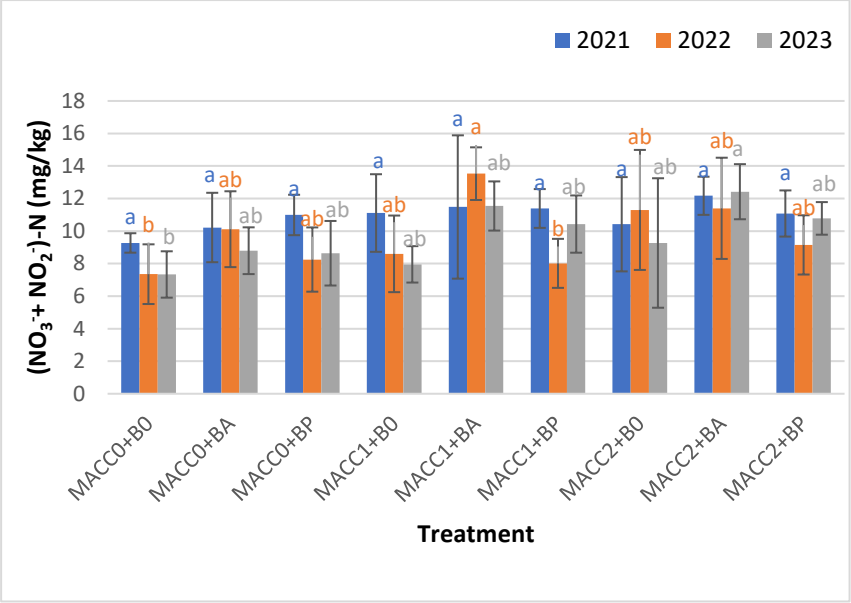


Figure 15: Effect of different application of *N. linckia* and PGPB on soil nitrate-nitrite-nitrogen. Mean values are presented as mean ± standard deviation. Error bars indicate standard deviation. Error bars indicate standard deviation. The bars with different lowercase letters are significantly different at $p \leq 0.05$ by Tukey's test. Where: MACC0, MACC1, and MACC2, which are levels of concentration of the *N. linckia* at 0, 0.3 g/L, and 1 g/L, respectively. BA and BP are *A. lipoferum* and *P. florescence*, respectively.

Figure 16 highlighted that the application of *N. linckia* and PGPB, either alone or in combination exhibited statistical significance on the total nitrogen content. However, the interaction effect of *N. linckia* and PGPB on total nitrogen revealed statistical insignificant in 2023.

In 2021, the highest nitrogen content occurred at the combined application of *N. linckia* at the concentration of 0.3 g/L and 1 g/L along with *A. lipoferum,* which resulted 40% increases in nitrogen content

compared to untreated trails. Conversely, the lowest content was recoded at control levels.

In 2022 and 2023, the highest total nitrogen content was recorded at combined applications of *N. linckia* at the concentration of 0.3 g/L along with *A. lipoferum,* while conversely, the lowest was noted at control levels (Fig. 16). The combined application of *N. linckia* at the concentration of 0.3 g/L with *A. lipoferum* resulted in a 20.69% and 27.59% increase in total nitrogen content in 2022 and 2023, respectively, compared to untreated plots (Fig. 16).

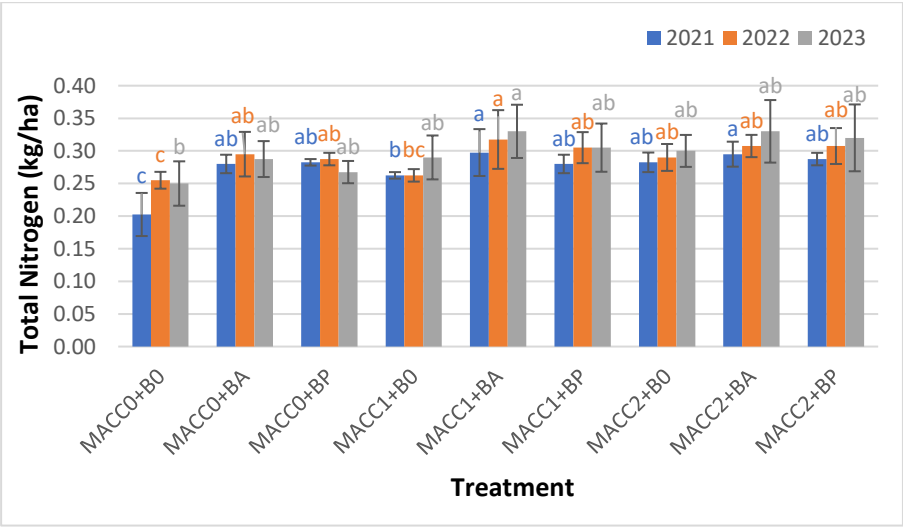


Figure 16: Effect of different application of *N. linckia* and PGPB on soil total nitrogen. Mean values are presented as mean ± standard deviation. Error bars indicate standard deviation. The bars with different lowercase letters are significantly different at $p \leq 0.05$ by Tukey's test. Where: MACC0, MACC1, and MACC2, which are levels of concentration of the *N. linckia* at 0, 0.3 g/L, and 1 g/L, respectively. BA and BP are *A. lipoferum* and *P. florescence*, respectively.

Throughout the experimental period, the data revealed no statistically significant differences in the soil phosphorus (P) and potassium (K) content (Fig 17and 18).

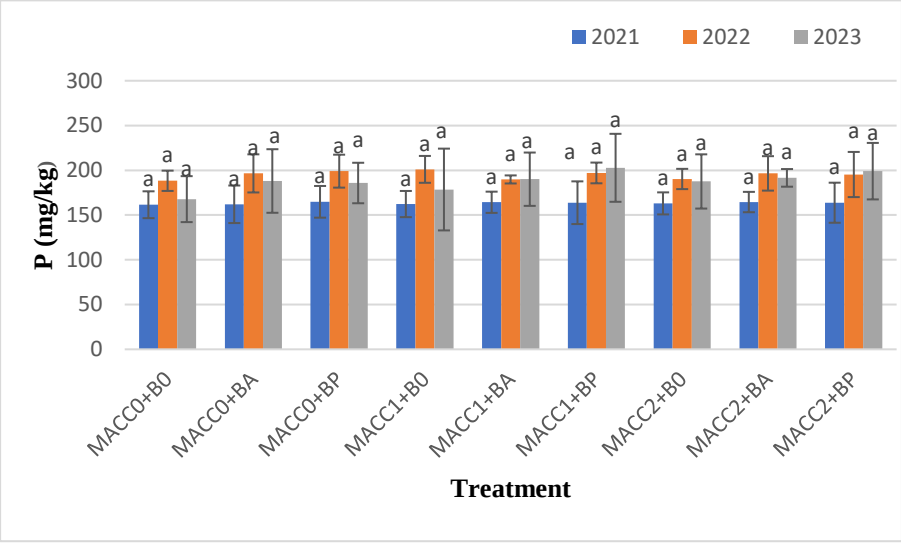


Figure 17: Effect of different application of *N. linckia* and PGPB on soil phosphorus. Mean values are presented as mean ± standard deviation. Error bars indicate standard deviation. The bars with different lowercase letters are significantly different at $p \leq 0.05$ by Tukey's test. Where: MACC0, MACC1, and MACC2, which are levels of concentration of the *N. linckia* at 0, 0.3 g/L, and 1 g/L, respectively. BA and BP are *A. lipoferum* and *P. florescence*, respectively.

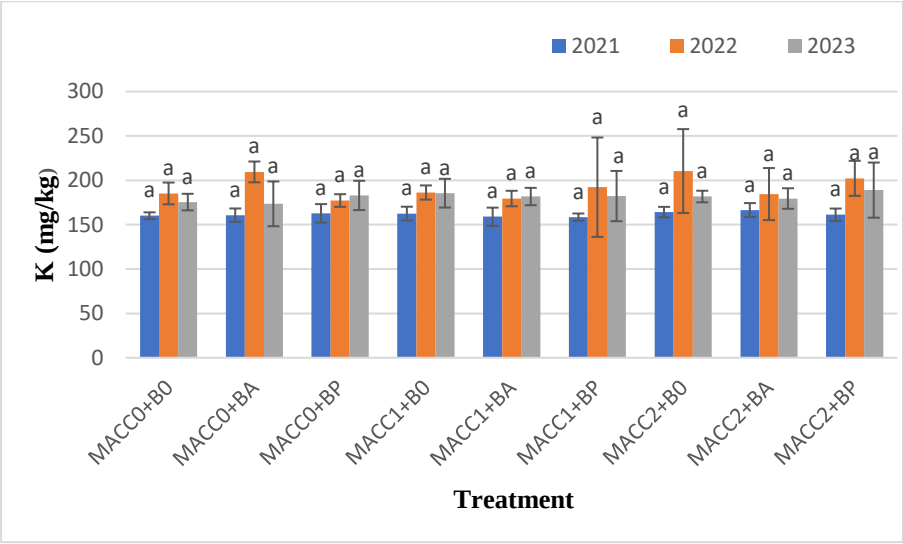


Figure 18: Effect of different application of *N. linckia* and PGPB on soil potassium. Mean values are presented as mean ± standard deviation. Error bars indicate standard deviation. The bars with different lowercase letters are significantly different at $p \leq 0.05$ by Tukey's test. Where: MACC0, MACC1, and MACC2, which are levels of concentration of the *N. linckia* at 0, 0.3 g/L, and 1 g/L, respectively. BA and BP are *A. lipoferum* and *P. florescence*, respectively.

4.5 Microbial activity of the soil

Table 15 show that, the activity of the bacteria and actinomycete population exhibited statistically significant differences in 2021 and 2022. However, the statistical insignificance of the interaction effect between *N. linckia* and PGPB was observed, except for the bacterial

population in 2021. Throughout the course of this study, the lowest levels of bacterial and actinomycete populations were consistently observed in the control groups (Table 15).

The findings revealed that the bacterial biomass reached its peak in the 2021 production season when *N. linckia* was applied at a concentration rate of 1 g/L in combination with *A. lipoferum* and *P. fluorescens*. Similarly, during the 2022 season, the highest bacterial biomass was observed with the combined application of *N. linckia* at a concentration rate of 0.3 g/L with *A. lipoferum*.

Moreover, an elevated count of actinomycetes was noted during the 2021 season, particularly in the treatment involving *N. linckia* at a concentration rate of 1g/L combined with P. fluorescens, as well as in the sole application of *N. linckia* at the concentration of 1 g/L. Additionally, in the 2022 season, the highest actinomycete count was observed in the treatment where *N. linckia* at a concentration rate of 1 g/L was combined with *A. lipoferum* and *P. fluorescens*.

Table 15: Effect of the *N. linckia* and PGPB on activity of soil bacteria and actinomycete

Treatment	Bacteria		Actinomycete	
	2021	2022	2021	2022
MACC0+B0	6.06±0.42^{d}	6.15±0.29^{d}	5.27±0.29^{b}	5.68±0.30^{bc}
MACC0+BA	6.64±0.22^{cd}	7.51±0.28^{bc}	5.51±0.28^{ab}	6.18±0.43^{abc}
MACC0+BP	6.54±0.38^{cd}	7.16±0.77^{bcd}	5.46±0.45^{ab}	5.97±0.62^{abc}
MACC1+B0	6.22 ±0.21^{d}	6.73±0.76^{cd}	5.63±0.07^{ab}	6.26±0.42^{ab}
MACC1+BA	7.05±0.05^{bc}	8.93±0.32^{a}	5.71±0.04^{ab}	6.52±0.03^{ab}
MACC1+BP	7.55±0.35^{ab}	7.74±0.78^{abc}	5.73±0.04^{ab}	6.51±0.05^{ab}
MACC2+B0	7.30±0.05^{ab}	7.27±0.34^{bcd}	5.93±0.15^{a}	6.21±0.75^{ab}
MACC2+BA	7.84±0.13^{a}	8.21±0.25^{ab}	5.76±0.13^{ab}	6.56±0.06^{a}
MACC2+BP	7.77±0.04^{a}	8.21±0.64^{ab}	5.84±0.03^{a}	6.57±0.03^{a}
MACC	***	***	***	**
B	***	***	ns	ns
MACC*B	*	ns	ns	ns

Values represent means ± standard deviation (n=4), and different superscript letters denote significant differences based on pairwise comparisons conducted by Tukey's test at $P \leq 0.05$ Where: ns indicate non-significant, * significant at $P \leq 0.05$, ** significant at $P \leq 0.01$, *** significant at $P \leq 0.001$. Where: MACC0, MACC1, and MACC2, which are levels of concentration of the N. *linckia* at 0, 0.3 g/L, and 1 g/L, respectively. BA and BP are *A. lipoferum* and *P. florescence*, respectively.

5. DISCUSSION

Maize (*Zea mays* L.) holds a prominent position as one of the most frequently cultivated field crops on a global scale, being grown extensively across various regions of the world (FAO, 2022). It is a globally cultivated essential crop used for food, animal feed, and as a raw material for diverse industries. Maize requires a significant amount of nitrogen fertilizer, making it important to explore alternative fertilizer sources that offer benefits in agronomy, environmental sustainability, and economics (Alves et al., 2023; Hungria et al., 2022).

The utilization of beneficial microorganisms in plants contributes to the improvement of soil health and increased crop yields in diverse agricultural systems. The study was conducted to investigate how the combined impact of cyanobacteria (*N. linckia)* and PGPR influences the growth of maize plants and soil fertility under different concentrations of cyanobacteria and bacterial strains. The impact of *N. linckia* and PGPR, both separately and in combination, yielded diverse outcomes when subjected to analysis. The utilization of both cyanobacteria and PGPB in soil treatment resulted in a significant enhancement in the chlorophyll, vegetation index, yield components, soil microbial population and soil fertility. However, the effectiveness of biofertilizer treatment on both plant growth and soil fertility is dependent on the concentration rate of *N. linckia* and the PGPB strains used.

5.1 Plant physiological parameters

Chlorophyll serves as the primary light-absorbing pigment for plant photosynthesis, and while evolution favors high chlorophyll content in leaves (Cho et al., 2024). The amount of chlorophyll in leaves is crucial for signaling both plant stress and its nutrient status (Liang et al., 2017; Zhang et al., 2022). We found that the joint usage of *N. linckia* and the PGPB strains had a beneficial impact on the chlorophyll and green health vegetation of maize crops. Our investigation revealed that the peak chlorophyll content throughout the experiment period, was achieved through the joint use of *N. linckia* at a concentration of 0.3 g/L in conjunction with *A. lipoferum*, while the minimum chlorophyll content was noted in the control group. Similarly, (Prasanna et al., 2012) has observed that cyanobacteria inoculants demonstrated a substantial superiority over the uninoculated control, manifesting a noteworthy improvement in Soil Plant Analysis Development (SPAD) values, specifically achieving an enhancement ranging from 10 to 15% in maize crop. Moreover, the biostimulatory impact of cyanobacteria on chlorophyll content, exhibited a notably higher significance in the treated maize and wheat during the reproductive stages (Ördög et al., 2021; Takács et al., 2019).

We observed decline in chlorophyll concentration after 65 days from planting was noted, potentially attributable to the plant entering its flowering stages. Study also revealed that chlorophyll concentration showed elevated levels in the early phases of growth, spanning the vegetative and early reproductive stages, but a noticeable decline was

observed as the plants advanced into the flowering and subsequent maturity stages (Ciganda et al., 2008; Mushongi et al., 2013).
In the year 2021, at 50 days after sowing (DAS), the chlorophyll content exhibited a diminished level relative to the preceding consecutive years (Fig. 6). This disparity can be attributed to reduced precipitation during the seedling and vegetative stages of maize development (Fig. 3). Similarly, extended and severe water stress experienced by maize plants during the seedling stage can lead to structural damage in the photosynthetic membrane, consequently causing a reduction in chlorophyll content and inevitable yield losses (Song et al., 2019; Zhang et al., 2018).

Leaf spectral values, influenced by plant biomass, developmental stage, and responses to environmental and various stress factors, serve as robust indicators, offering valuable insights into the plant's present condition. Our findings suggest that the joint application of *N. linckia* and PGPB had a significant impact on the NDVI values throughout the entire experimental duration. At 50 days after sowing (DAS) in 2021, the NDVI value exhibited a decrement compared to the subsequent consecutive year, attributable to the imposition of stress on the plants during the vegetative phase. The highest NDVI value was recorded at combined application of *N. linckia* at the concentration of 0.3 g/L and 1 g/L along A. *lipoferum.*

Our observations revealed that the joint application of *N. linckia* and PGPB resulted in elevated chlorophyll content, plant biomass, and

NDVI values. Conversely, lower values were recorded at the control levels. This underscores a direct correlation between chlorophyll levels, NDVI, and maize biomass. The studies also revealed a compelling relationship between NDVI values and the accumulation of biomass in both maize and wheat crops (Verhulst et al., 2011). The NDVI allows producers to assess crop biomass and yield by extensively using indirect reflectance measurements for estimation purposes (El-Hendawy et al., 2022). Reflectance indices are connected to the biomass of vegetation, specific physiological processes, and the biochemical compositions present in plants. These indices serve as valuable tools for monitoring plants over both short-term and long-term periods (Kior et al., 2021). Hence, our research showcases how the combined application of *N. linckia* and PGPB effectively enhances both the vegetative growth and development of maize, ultimately boosting its productivity and final yield.

5.2 Fresh and dry weight of plant biomass

The main application of *N. linckia* and PGPB, whether employed individually or in combination, results in a notable augmentation in both the fresh and dry weight of leaf and root biomass. The combined inoculation of *N. linckia* and PGPB exhibited a significant enhancement in fresh leaf weight, ranging between 38.49-59.37% and 21.73-32.5% at 50 and 65 DAS, respectively compared to control levels. Furthermore, the combined use of *N. linckia* and PGPB results

in an improvement of dry leaf weight by 35.56% to 107.32% at 50 DAS and 29.58% to 49.77% at 65 DAS, in comparison to the control group. In prior studies conducted by (Adesemoye et al., 2009; Mäder et al., 2011), it was observed that the inoculation of cereal plants with Plant Growth-Promoting Rhizobacteria (PGPR) yielded consistently favorable outcomes, eliciting significant enhancements in root length (54%), root weight (74%), root area (75%), and shoot weight (23%). The study similarly demonstrates that the concurrent utilization of cyanobacteria and plant growth-promoting rhizobacteria elicited a substantial increase in dry shoot mass, registering a notable augmentation ranging from 76% to 80% (Kholssi et al., 2021). However, our study shows that the combined influence of *N. linckia* and PGBP on both fresh and dry leaf weight was found to be statistically insignificant at 50 DAS in the year 2021. Overall, the lowest fresh and dry leaf weight was observed in 2021, while the highest was recorded in 2023. The diminution in both fresh and dry leaf weight observed in 2021 can be attributed to the inadequate precipitation levels during the vegetative stage of maize growth. According to (Wang and Frei, 2011; Yang et al., 2023), inadequate soil moisture availability compromises the metabolic processes of maize, diminishes its biomass growth, and attenuates its photosynthetic efficiency by diminishing chlorophyll concentrations in foliage. Consequently, this culminates in a reduction in maize yield.

According to (Li et al., 2019), the synergy between cyanobacteria and PGPB emerges as a promising alternative for augmenting crop growth

and yield in major crops like maize, owing to their inherent capacity to stimulate the expansion of root systems. Likewise, our result revealed that the combined application of *N. linckia* and PGPB resulted in a significant increase in fresh root weight, with enhancements of 20.27-51.89% observed at 50 DAS and 26.31-38.59% at 65 DAS compared to the control group. Moreover, the combined application of *N. linckia* and PGPB led to a notable rise in dry root weight, exhibiting increases of 75.59-130.19% at 50 DAS and 56.08-69.93% at 65 DAS compared to the control group. The pervious study confirmed that microbes within the rhizosphere play a crucial role in nutrient cycling, promoting improved nutrient mobilization and facilitating uptake, ultimately resulting in heightened root growth, biomass, and plant yield (Manjunath et al., 2016). In this study, the highest value of dry root weight was recorded at combined application of *N. linckia* at a concentration of 1 g/L along with *A. lipoferum*. Plants coexist in intimate proximity with countless microorganisms in their surroundings, on their surfaces, and within their structures. As stated by (Harman et al., 2021), when specific symbiotic strains of bacteria and fungi colonize plant roots, these plants exhibit enhanced performance compared to those whose roots are only inhabited by wild microbial populations. The present study could confirm this symbiosis. Hence, the most effective approach was the joint use of *N. linckia* and PGPB, followed by the alone application of *N. linckia* or PGPB, which also showed notable improvements compared with the control group.

5.3 Nitrogen content of plant biomass

The statistical insignificance of the interaction effects between *N. linckia* and PGPB on the nitrogen content of plant biomass persisted consistently across the entire duration of the study. Except for the year 2022, the main effects of *N. linckia* and PGPB on leaf nitrogen content were statistically significant ($p<0.05$). In contrast to (Rana et al., 2015), the introduction of plant growth-promoting bacteria (PGPB) and cyanobacteria through inoculation emerges as a potent strategy, yielding substantial enhancements in the nitrogen, phosphorus, and potassium content in wheat-rice cropping system. Numerous studies consistently demonstrate that the application of Plant Growth-Promoting Bacteria (PGPB) has led to a discernible augmentation in the nutrient profile of plants, encompassing elevated levels of nitrogen, phosphorus, potassium, and iron (Abadi et al., 2020; Ambrosini and Passaglia, 2017; Reed and Glick, 2023).

5.4 Yield of attributes of maize

The data clearly showed that using *N. linckia* and PGPB alone/combination in the soil significantly boosted maize growth, resulting in more seeds per ear, higher thousand seed weight, and increased yield compared with the respective control. The improvement in various plant growth factors is likely a consequence of plants being better able to absorb essential nutrients from the soil. This enhanced nutrient uptake process makes vital nutrients more readily available to plants, supporting their overall growth and development.

Furthermore, microbes have been reported to facilitate nutrient movement toward plant roots, and a substantial portion of soil microorganisms possess the capacity to improve plant nutrient uptake, offering eco-friendly strategies to address plant nutritional needs (Saia et al., 2015; Singh et al., 2022).

Our observations revealed that the application of *N. linckia* and PGPB, either individually or in combination, yielded statistically insignificant (p<0.05) effects on plant height over studied period. In contrast, the utilization of cyanobacteria and plant growth promoting rhizobacteria on the maize and wheat crop demonstrated a notably superior performance in terms of plant height compared to the uninoculated control, underscoring the efficacy of the cyanobacterial application in influencing the vertical growth of the maize plants (Kholssi et al., 2021; Manjunath et al., 2011; Prasanna et al., 2015; Prasanna et al., 2016b). During studied period, the statistical significance (p < 0.05) of the number of seeds per ear was evident when employing *N. linckia* and PGPB, whether applied individually or in combination. The highest number of seeds per ear was achieved at combined application of *N. linckia* at concentrations of 0.3 g/L along with A. *lipoferum* while the lowest number of seeds per ears was noted in the control level throughout the entire studied periods. Furthermore, with the exception of the year 2022, the statistical analysis revealed that the interaction effect of *N. linckia* and PGPB on the thousand seed weight was deemed statistically insignificant. However, the main effect of *N. linckia* and PGPB remained statistically significant across the entire experimental

duration. The maximum thousand seed weight was attained through the combined application of *N. linckia* at concentrations of 0.3 and 1 g/L, in conjunction with *A. lipoferum*.

Our study revealed that the inoculation of *N. linckia* at the concentration of 0.3 g/L along with A. *lipoferum* positively influenced yield of maize, leading to a significant enhancement in grain yield by 7.09 $tonha^{-1}$ (33.20%) during 2021, 7.71 $tonha^{-1}$ (31.53 %) in season 2022, and 8.62 $tonha^{-1}$ (32.34%) in season 2023, as compared to the control. In earlier research, it has been demonstrated that applying cyanobacteria and PGPB, whether separately or together, leads to enhanced maize growth and yield by either directly improving resource utilization and adjusting plant hormone levels or indirectly reducing the negative impact of various harmful agents (Di Benedetto et al., 2017; Gavilanes et al., 2020; Reed and Glick, 2023). In the present study, it was observed that the highest result in terms of the number of seeds per ear, seed weight and yield was recorded at combined application *N. linckia* at a concentration of 0.3 g/L with A. *lipoferum* (Table 2). The *Azospirillum* genus, consisting of free-living diazotrophs found in plant rhizospheres, is esteemed as a prime example of Plant Growth-Promoting Bacteria (PGPB) as a biofertilizers due to their beneficial influence on plant growth, crop yields, and nitrogen content (Vuolo et al., 2022). Utilizing cyanobacteria and PGPB could serve as a viable alternative to enhance crop growth and yield in significant crops such as maize, enhance nutrient use efficiency (NUE) (Nilde Antonella Di et al., 2017; Pandey et al., 2021), and enhance the uptake of essential

nutrients, including nitrogen (N) (Múnera-Porras et al., 2020; Sharma et al., 2020).

5.5 Soil properties

The use of either *N. linckia* or PGPB, as well as their combined application, resulted in a notable improvement in soil pH, humus content, (NO_3^-+ NO_2^-)-N, and total nitrogen levels. Microbial technologies provide environmentally friendly and cost-effective methods for promoting sustainable soil health and crop production (Manjunath et al., 2016).

Apart from 2022, it is evident from the data that the joint utilization of *N. linckia* and PGPB did not result in statistically significant (p<0.05) changes in pH values throughout the remaining seasons. In the year 2022, the combined application of *N. linckia* and PGPB resulted in a moderate reduction of soil pH. Moreover, the combined use f *N. linckia* at a concentration of 0.3 g/L, in along with *A. lipoferum*, yielded noteworthy enhancements in humus content, with increments of 31.67%, 20.24%, and 15.71% observed for the respective years 2021, 2022, and 2023, against untreated control trials, as illustrated in Figure 13. It may be due to the improvement of organic matter in the soil has had a positive impact on the soil's physicochemical and biological properties. This enhancement of organic matter has been facilitated by beneficial microorganisms, including microalgae and bacteria, known for their ability to promote soil health and fertility (Gonzalez-Gonzalez and de-Bashan, 2023a; Kumar et al., 2022b; Mutum et al., 2022;

Ramakrishnan et al., 2023; Singh et al., 2016). Further, the research findings indicated a substantial augmentation in organic carbon levels across all microbial-inoculated treatments, following the co-inoculation of bacteria-cyanobacteria, a trend that exhibited a discernible correlation with microbial biomass carbon values (Prasanna et al., 2012).

The study shows that the synergistic alliance between PGPR and cyanobacteria not only optimizes soil fertility and nutrient utilization to augment plant growth but also fortifies plant resilience to environmental adversities like drought and salinity (Pathak et al., 2018; Prasanna et al., 2012). Our study revealed that the combined application of *N. linckia* at the concentration of 1 g/L with *A. lipoferum* resulted increases the (NO_3^-+ NO_2^-)-N content by 27.05% in 2021 and 51.54% in 2023 compared to the control levels. Moreover in 2022, the combined application of *N. linckia* at 0.3 g/L along with *A. lipoferum* resulted in the highest soil (NO_3^-+ NO_2^-)-N content, showcasing a significant 59.20% increase compared to the control levels (Fig 15).

Numerous studies revealed that application of cyanobacteria improves soil properties, particularly when combined with PGPB, leading to enhanced plant growth, organic matter, improved soil fertility, nutrient utilization, and increased plant stress tolerance (Eman et al., 2023; Gonzalez-Gonzalez and de-Bashan, 2023a; Mutale-Joan et al., 2023; Prasanna et al., 2021; Singh, 2014; Singh et al., 2016). Our studies shows that the synergistic application of *N. linckia* at a concentration of 0.3 g/L, in conjunction with *A. lipoferum*, led to significant

improvements in total nitrogen levels, registering increments of 40%, 20.69%, and 27.59% for the years 2021, 2022, and 2023, respectively, when compared to untreated control trials. Cyanobacteria, serving as eco-friendly inputs, not only enhance plant growth and soil fertility but also outperformed the uninoculated control by augmenting available nitrogen (N) in the soil, consequently resulting in a substantial nitrogen fertilizer saving of 40-50 kg N ha^{-1} (Prasanna et al., 2015).

The increase in soil nitrate-nitrite nitrogen content with the combined application of *N. linckia* and PGPB can be attributed to the synergistic interactions between *N. linckia* and the plant growth-promoting bacteria (PGPB). Further, *N. linckia* may contribute to enhanced nutrient availability and uptake by the plants, promoting nitrogen assimilation. Additionally, PGPB can facilitate nitrogen fixation or enhance nutrient mobilization in the soil, leading to increased nitrate-nitrite nitrogen levels. The combined effect of these factors results in a higher concentration of soil nitrate-nitrite nitrogen (Aquino et al., 2021; Calvo et al., 2019). In the current study, applying *N. linckia* and PGPB either alone or in combination had no statistically significant impact on phosphorus and potassium levels. In contrast to this study, the research findings demonstrated that PGPB enhanced soil characteristics, elevating the availability of phosphorus and potassium content by a significant margin 100% and 70%, respectively compared to the untreated soil (Schoebitz et al., 2014).

5.6 Soil microbial populations

In our current research, whether we applied *N. linckia* or PGPB alone or in combination to the soil, we observed significant differences in bacterial and actinomycete populations in 2021 and 2022 production years. Notably, the control group consistently exhibited the lowest levels of these populations throughout the study period. Cyanobacteria and Plant Growth-Promoting Bacteria (PGPB) play a pivotal role in regulating the abundance and functions of diverse soil microbial communities and enhance plant growth (Ranjan et al., 2016; Sharma et al., 2020). The soil's microbial communities in the rhizosphere play a crucial role in fostering eco-friendly agricultural practices, promoting sustainability, soil fertility, and ensuring agricultural productivity (Uzoh and Babalola, 2018). The incorporation of microbial biomass in the soil resulted in enhanced microbial diversity, changes in the abundance of organic matter-decomposing microorganisms, improved soil health, and the promotion of greater microbial variety (Alobwede et al., 2022; Ranjan et al., 2016).

6. CONCLUSION

Maize, a globally important crop, often relies heavily on nitrogen fertilizers, prompting the need for sustainable alternatives. This comprehensive study highlights the potential of beneficial *N. linckia* biomass and PGPB as microbial inoculants, with the aim of enhancing maize the growth, yields, and soil fertility. The application of *N. linckia* biomass and PGPB, either individually or in combination, led to a substantial augmentation in physiological parameters and plant biomass. The study underscored the positive correlation between the microbial application and enhanced biomass, emphasizing the potential for improved crop productivity.

The data demonstrated that the joint application of *N. linckia* biomass and PGPB significantly enhanced maize growth, resulting in increased seeds per ear, higher thousand seed weight, and elevated overall yield. This outcome highlighted the practical implications of employing these microbial agents in agriculture, as they positively impacted various aspects of maize growth and productivity. Furthermore, the application of *N. linckia* biomass and PGPB, either individually or combined, positively influenced soil properties, including pH, humus content, (NO_3^-+ NO_2)-N, and total nitrogen contents. The positive effects extended to soil properties and microbial populations, showcasing the potential for sustainable and eco-friendly agricultural practices through the strategic use of beneficial microorganisms.

The study underscores that the combined use of *N. linckia* biomass and PGPB was the most effective strategy. In this work, all strains

displayed a high degree of compatibility for co-growth, however, the most optimal synergistic grouping were established by integrating both *N. linckia* biomass at a concentration of 0.3 g/L along with *A. lipoferum*, resulting enhancing maize growth, yield, soil fertility and microbial populations. The formulation of biofertilizers through synergistic combinations of two or more microorganisms, such as algae-bacteria, holds promise for enhancing crop productivity.

7. NOVEL SCIENTIFIC RESULTS OF DOCTORAL REASERCH

- Present results support the view that the formulation of biofertilizers through well-chosen combination and concentration of two or more microorganisms, such as algae-bacteria, may have synergistic effects, and it holds promise for enhancing crop productivity.
- The study reveals a nuanced impact of *N. linckia* biomass and PGPB on chlorophyll and green vegetation content in maize, highlighting the potential for enhancing plant photosynthesis through the joint application of these microorganisms.
- We found that that the joint application of *N. linckia* biomass and PGPB significantly enhanced maize growth, resulting in increased seeds per ear, higher thousand seed weight, and elevated overall yield.
- The research shows the synergistic effects of *N. linckia* biomass and PGPB improved soil properties such as pH, humus, nitrate-nitrite-nitrogen, total nitrogen, and microbial populations, providing a foundation for sustainable and eco-friendly agricultural practices.
- Optimal synergistic groupings were identified by combining *N. linckia* biomass at a concentration of 0.3 g/L with *A. lipoferum*, leading to enhanced maize growth, increased yield, improved soil fertility, and increased microbial populations.

- Since biological agents are increasingly favored over chemical fertilizers for their eco-friendly and cost-effective nature and for upkeeping of soil biodiversity and health, our results may contribute to develop suitable combinations of microbes in order to achieve this goal.

8. PUBLICATIONS

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9. CONFERENCE PRESENTATION AND PARTICIPATION:

- Wogene Solomon, Tibor Janda, Molnar Zoltan (2024). Effect of Microalgae-Bacteria Synergy on Maize (*Zea mays* L.) Growth and Soil Fertility. Student conference on plant biology, Gregor Mendel Institute of Molecular Plant Biology GmbH, Vienna, Austria, February 22-23, 2024.
- Wogene Solomon, Mutum Lamnganbi, Tibor Janda, Molnar Zoltan (2022). Promising effect of the microalgae-bacteria interaction on maize growth performance, poster presentation. Plant-microbe interaction conference, Novo Nordisk foundation science cluster, Copenhagen, Denmark, November 13-17, 2022.
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10.DEDICATION

I wish to pay tribute to my father, Solomon Kabato (Abebo), who passed away during my Ph.D. studies, by dedicating this dissertation to his memory.

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