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Cyanobacteria-microalgae consortia as bio-inoculants for enhancing soil fertility and plant growth

Submitted in fulfilment of the requirements of the degree of Doctor of Philosophy: Biotechnology in the Faculty of Applied Sciences at Durban University of Technology

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DECLARATION

Cyanobacteria-microalgae consortia as bio-inoculants for enhancing soil fertility and plant growth

Shisy Jose

2024

I declare that my thesis for the degree of Doctor of Philosophy: Biotechnology at the Durban University of technology is the result of my own research and has not been previously submitted to any other university

Shisy Jose **Date: 27 March 2024**

APPROVAL

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This 27 day of March 2024, at the Durban University of Technology.

ABSTRACT

Modern agriculture that heavily utilizes synthetic fertilizers has raised significant environmental concerns worldwide. Microalgae-based bio-inoculants have become a more viable and eco-friendly option to reduce the reliance on chemical fertilizers for improving soil fertility and plant growth in agronomic practices. Indigenous microalgal species can colonize and thrive well under local conditions, making them well-suited for use as bio-inoculants. Additionally, it appears that using microalgal consortia of green microalgae and nitrogen (N)-fixing cyanobacteria could be beneficial because the green microalgae can supply carbon (C), the cyanobacteria can fix C and N in the soil. They both can produce agriculturally beneficial metabolites and improve soil nutrient availability. This synergistic relationship could enhance the overall effectiveness of bio-inoculants and promote sustainable agriculture practices. In this study, thirteen microalgal strains were isolated from agricultural fields of Durban, KwaZulu Natal, South Africa. Two N-fixing cyanobacteria (*Nostoc* sp. and *Calothrix* sp.) and two green microalgae (*Desmodesmus armatus* and *Chlorella* sp.) strains were selected and analyzed for metabolites of agricultural significance for the development of suitable microalgal consortia under N-deficient conditions. The amount of indole acetic acid (IAA) in biomass extracts from cyanobacteria (*Calothrix* sp. (2.54 ng g^{-1}) and *Nostoc* sp. (1.52 ng g^{-1})) was significantly higher than in extracts from green microalgae (*Chlorella* sp. (0.32 ng g^{-1}) and *Desmodesmus armatus* (0.20 ng g^{-1})). A completely randomized design was used to develop and evaluate eight microalgal consortia on a N-deficient medium with the selected microalgal strains. A significant improvement in biomass productivity, indole acetic acid production, nutrients *viz* C, N, phosphorus (P), potassium (K), calcium (Ca), copper (Cu), iron (Fe), and manganese (Mn) was observed in the selected consortium compared to the individual isolates. The microalgal consortium was further analyzed for biostimulant properties using seed germination assay in chili seeds. A significant increase in seedling length and leaf number was observed in seeds treated with biomass extracts of consortium compared to the control. The pot culture study also supported the effect of microalgal bio-inoculant on soil fertility, chili plant growth and native soil microbiomes. Soil enzyme activity increased significantly ($p < 0.05$) with microalgal treatments, with soil dehydrogenase activity (DHA), organic carbon (OC), soil chlorophyll (Chl), total polysaccharides (TP) and nutrients such as C,

N, P, K and Mn being more enriched at 100 % and 50 % treatment applications in comparison to control (Cnt). Growth responses in terms of shoot and root (fresh and dry weight), root length and leaf number were significantly high in 50 % microalgal treatments (Al(50 %)+CF(50 %)) when compared to Cnt. With different soil nutrient parameters and microbiome (bacterial and fungal) indicators, we could successfully predict higher soil fertility and plant growth responses to microalgal inoculations. Results using 16SrRNA and ITS amplicon sequencing suggested that microalgal bio-inoculation improved the diversity and composition of native soil microbiome, leading to an increase in soil fertility, plant growth and yield. Further, shotgun metagenomics has confirmed a higher enzymatic activity involved in C, N, and P metabolisms in 50 % and 100 % microalgal treatment compared to the control. Potential shifts in microbial taxa and functional genes indicated that microalgal bio-inoculant was a major driver of microbial metabolic change. The findings from the study suggested that microalgal bio-inoculation improved the diversity and composition of native soil microbiomes, leading to an increase in soil fertility, plant growth, and yield in chili plants.

DEDICATION

This work is dedicated to my family, who I love so much, and to all the blessings in my life.

My father, **Jose M.U.**

who taught me that perseverance, hard work and being kind is the best medicine for success

My loving Mother **Sherly Jose**

for her faith, advice, love, care, and prayers

My in laws **M.T. Mathew** and **Rachel Mathew**

for their encouragement, support and prayers

My husband, **Renoy Mathew**

for his unfailing love, patience, support, and encouragement

My darlings, **Nathan** and **Nithya**

for their overwhelming love and care

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LIST OF ABBREVIATIONS

ANOVA- Analysis of variance

Al (50 %)+CF (50 %)- 50 % microalgal consortia + 50 % chemical fertilizer

Al (100 %)- 100 % microalgal consortia

C- Carbon

Ca- Calcium

CF- Chemical fertilizer

Chl- Soil chlorophyll

Cnt-Control

Cu- Copper

Cr- Carrier

DHA- Dehydrogenase activity

DT- Doubling time

D.W- Distilled water

EPS- Exopolymeric substances

Fe- Iron

GP- Germination percentage

IAA- Indole acetic acid

ICP-OES- inductively coupled plasma-optical spectroscopy

K- Potassium

LDA- Linear discriminant analysis

Lefse- Linear discriminant analysis effect size

MAA- Mycosporine aminoacid-like substances

MP-AES- Microwave plasma- atomic emission spectrometer

Mg- Magnesium

Mn- Manganese

N- Nitrogen

NPQ- Non photochemical quenching

OC- Organic carbon

PCoA- Principal coordinates analysis

P- Phosphorus

PLFA- Phospholipid fatty acid

PCR- Polymerase chain reaction

PGPR- Plant growth promoting rhizobacteria

RDA- Redundancy analysis

rETR – relative electron transport rate

SOC- Soil organic carbon

TP- Total polysaccharides

UHPLC LCMS/MS - High-throughput ultra-performance liquid chromatography- Liquid chromatography mass spectrometry

Zn- Zinc

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PREFACE

Publications

Journal articles

Shisy Jose, Nirmal Renuka, Sheena Kumari, Sachitra Kumar Ratha, Faizal Bux. 2023. Microalgal bio-inoculants for sustainable agriculture and their interaction with biotic and abiotic components of the soil. Pedosphere. https://doi.org./10.1016/j.pedsph.2023.12.002

Shisy Jose, Nirmal Renuka, Sheena Kumari, Sachitra Kumar Ratha, Faizal Bux. 2023. Bioprospecting of microalgae from agricultural fields and developing consortia for sustainable agriculture. Algal Research. https://doi.org/10.1016/j.algal.2024.103428

Shisy Jose, Muneer Malla, Nirmal Renuka, Faizal Bux, Sheena Kumari. 2023. Microalgal bio-inoculation enhances plant growth and soil fertility in chili crop by shaping the native soil microbiomes. (Under review)

Papers In-Preparation

Shisy Jose, Muneer Malla, Nirmal Renuka, Faizal Bux, Sheena Kumari. 2024. Integrating metagenomics and metabolomics to reveal the impact of microalgal bio-inoculants in chili rhizosphere

Book Chapter

Karen Reddy, **Shisy Jose**, Nirmal Renuka, Sheena Kumari, Sachitra Kumar Ratha, Faizal Bux. 2022. Microbe-assisted bioremediation of pesticides from contaminated habitats: Current status and prospects. Bioremediation for Sustainable Environmental Clean-up. CRC press, Taylor & Francis 109-124.

Conference

Developing indigenous cyanobacteria and microalgae consortia for their biostimulants properties and agricultural applications. **Shisy Jose**, Nirmal Renuka, Sheena Kumari, Sachitra Kumar

Ratha, Faizal Bux. Accepted for poster presentation at AlgaEurope in Prague from 12 – 15 December.

CHAPTER ONE

1.1 General Introduction

Modern agriculture practices heavily rely on chemical fertilizers to increase the overall productivity of crops since the beginning of the green revolution (Chittora et al., 2020). The continuous large scale use of chemical fertilizers with intensive agriculture practices helped to achieve the food production target in order to meet growing population needs; however, it also generated a huge shortfall in agro-diversity around the world (Singh et al., 2016, Osorio-Reyes et al., 2023). A lack of proper control over the use and application of synthetic fertilizers can have a negative impact on beneficial soil microorganisms, which are responsible for maintaining the fertility of the soil (Renuka et al., 2018). This could also lead to soil degradation, water pollution, and greenhouse gas emissions, posing a serious threat to the environment (Chittapun et al., 2017, Ammar et al., 2022, Osorio-Reyes et al., 2023).

Synthetic fertilizers have a long shelf life in the soil and atmosphere and have a negative impact on the environment (Dar et al., 2021). The contamination of soil due to the persistence of chemical residues is responsible for decreasing soil biodiversity and fertility (Dilnashin et al., 2020, Do Nascimento et al., 2019). The persistent soil contaminants decrease soil health by obstructing the breakdown of soil organic matter and altering nutrient cycling, reducing crop yield and affecting food safety, revealing the importance of maintaining soil health in the agriculture ecosystem. Thus, it is vital to shift from intensive agriculture practices based on fertilizers and pesticides to natural, renewable, resource-based, environmentally friendly and sustainable agriculture (Osorio-Reyes et al., 2023).

Bio-inoculants have an impact on the native microbial communities in the soil, depending on the chemical and physical properties of the soil as well as the environmental conditions (Manfredini et al., 2021). The use of inoculum based on live microalgae and cyanobacteria is beneficial because it offers benefits beyond preventing soil erosion and nutrient leaching, such as continuous nutrient sequestration during plant growth phases and soil structure and fertility maintenance (Cao et al., 2023). According to reports, microalgae have a major impact on resolving this challenging agricultural scenario and maintaining sustainability in agricultural

practices (Ammar et al., 2022, Cao et al., 2023, Solomon et al., 2023). Microalgae include both prokaryotic cyanobacteria and eukaryotic green microalgae with similar morphologies and are composed of unicellular, multicellular, aggregated, colonial to filamentous unbranched and branched forms (Alvarez et al., 2021a). Based on their ability to fix N, cyanobacteria are further classified into heterocystous (containing cells with specific N-fixing functions) and non-heterocystous forms (Li et al., 2017). Additionally, both cyanobacteria and green microalgae are able to mobilize organic and inorganic nutrients, produce metabolites of agronomic significance such as exopolymers and plant growth promoters, and significantly help in maintaining the soil microbial community, fertility and enhancing crop yield (Renuka et al., 2018).

The most significant contributions that microalgae make to soil fertility are generally acknowledged to be the assimilation of organic C via photosynthesis and organic N via N-fixation to form nutrient rich biomass (Cao et al., 2023). The use of microalgae-based biofertilizers has been shown to increase the organic C content of soil (Osorio-Reyes et al., 2023). Similarly, Yilmaz and Sönmez (2017) demonstrated increased soil organic C content by using microalgae as a biofertilizer through a pot experiment. Moreover, the amount of organic C in the soil can be greatly increased via the algal assimilation of carbon dioxide (CO_2) (Hou et al., 2022).

It has also been demonstrated that cyanobacterial and microalgal inoculation leads to the release of exopolymers (EPS) into the environment, which is one of the main strategies for modulating and improving soil microbial activity (Marks et al., 2019). EPS not only act as a source and sink of C for the development of microbes that are useful for agriculture but also supports aggregate soil particles, improves soil stabilization and may be a promising biotechnology tool to generate sustainable agriculture (Costa et al., 2018). Microalgae have also been reported to produce endogenous phytohormones, including auxins, gibberellins, cytokinins, brassinosteroids, abscisic acid and ethylene (Do et al., 2020). Recent research suggests that phytohormones in microalgae exhibit regulatory functions comparable to those found in higher plants, but their specific role in these organisms is unknown (Lu and Xu, 2015). Hussain et al. (2010) evaluated the effectiveness of hormone-secreting cyanobacterial strains (cytokinin and auxin) in promoting plant growth in both axenic and natural environments. Moreover, extracts of *Chlorella* sp., *Coenochloris* sp.,

Tetracystis sp., *Chlamydomonas* sp., *Scenedesmus quadricauda, Chlorella minutissima*, and *Protococcus viridis* showed auxin and cytokinin-like activity that contributed to the increase in cucumber cotyledon weight and root number (Stirk et al., 2002, Ördög et al., 2004). Apart from encouraging plant growth, these phytohormones are known to offer the possibility of modulating microalgal growth and enhancing biomass yields in large-scale microalgal cultivation for agricultural or other commercial purposes (Liu et al., 2017, Novoveska et al., 2023).

The abundance of indigenous cyanobacteria populations is seen as a significant factor that affects outcomes in agricultural fields (Hashem, 2001). Non-indigenous cyanobacteria, for example, do not become dominant in soils containing high concentrations of indigenous cyanobacteria, indicating that native cyanobacteria have a competitive advantage in such soils (Mishra and Pabbi, 2004). Interestingly, a study found that after non-indigenous cyanobacterial strains were introduced into 102 rice soil samples from different countries (Philippines, India, Malaysia, and Portugal), the abundance of indigenous cyanobacteria was increased (Roger et al., 1987). This implies that the introduction of non-indigenous strains did not suppress the native populations but, instead, possibly stimulated their growth (Roger et al., 1987). Thus, Roger et al. (1987) recommended that research should focus on agricultural approaches that encourage the establishment of indigenous strains of cyanobacteria.

The use of microbial consortia, indeed offers several advantages compared to inoculations with single isolates (Bharti et al., 2017, Alvarez et al., 2021a). Silambarasan et al. (2021) have shown that the microalgal consortium of *Chlorella* sp. and *Scenedesmus* sp. in tomato cultivation stimulated plant growth and improved nutrient composition as well as productivity by 174 % compared to the control. Thompson et al. (2012) reported a symbiotic association between cyanobacteria and microalgae, which involved the sharing of N, fixed by a cyanobacterium and C, fixed by a unicellular alga. Thus, it has been postulated that the co-cultivation of green microalgae with N-fixing cyanobacteria and/or their consortia will be an advantageous approach to overcome the challenges associated with the large scale microalgal bio-inoculant production. Such consortia could be a promising bio-inoculant option to enhance soil fertility and plant growth since the cyanobacterial partner could fulfill the N demand, while green microalgae could provide C in the soil environment, apart from the production of exopolymeric substances

(Renuka et al., 2018). Cyanobacterial inoculation in the soil, seed dressing or seed priming are reported to increase seed germination rate, plant growth and yield in a variety of cereals, horticultural and vegetable crops (Prasanna et al., 2016b, Rupawalla et al., 2022).

Furthermore, Manjunath et al. (2016) observed that the cyanobacterial inoculation leads to modulation of the rhizosphere microbiome, leading to alterations in the structure and abundance of microbial communities. Similarly, a recent study by Wei et al. (2023) reported a significant increase in the rhizosphere microbial diversity and soil fertility after the biofertilizer (*Chlorella vulgaris* co-cultivated with *Mesorhizobium sangaii*) application. A recent study using shotgun metagenomics demonstrated that the bacterial communities were positively influenced by the organic matter, total N and pH (Nwachukwu et al., 2022). The impact of microalgae-based bio-inoculants on native microbial communities, however, has not yet been investigated in depth, and relatively little is known about how abundant and rare taxa respond to microalgal bio-inoculants.

Considering the observed gaps in research and technological advancements, this work aimed to create consortia of native microalgae, mainly N-fixing cyanobacteria and non-N-fixing green microalgae for bio-inoculant application. The research was focused on developing microalgal consortia under an N-free medium and further assessing their potential as biostimulants and bio-inoculants in soil microbiomes and chili plants. In-depth studies on the evaluation of the compositional changes in the soil microbial community using shotgun metagenomics would be helpful in determining the role and competency of microalgal bio-inoculants in the rhizosphere. Such studies may aid in understanding the impact of microalgal consortia on soil and inquire into potential relationships between microbial communities, soil and plant parameters. The metagenomic sequencing of the soil microbiome can provide important insights into community structure and metabolic potential and a better understanding of the dynamic reciprocity between microalgal bio-inoculants and native microbial communities. Based on these important research aspects, the following aims and objectives were undertaken:

1.2 Aim

To evaluate the efficacy of cyanobacteria and green microalgae consortia as bio-inoculants for improving plant growth, nutrition, and photosynthetic activity and to assess their effect on soil fertility and native soil microbial community through metagenomic analysis

1.3 Objectives

- To isolate and characterize native cyanobacteria and green microalgae from agricultural fields
- To develop cyanobacteria and green microalgae consortia based on growth and compatibility
- To assess the efficacy of selected bio-inoculants in enhancing the plant growth, nutrition and photosynthetic activity
- To evaluate the effect of bio-inoculants on soil fertility through nutrient analysis and dehydrogenase activity
- To evaluate the effect of bio-inoculant amendment on the native soil microbial community structure using metagenomic analysis

1.4 Thesis outline

This thesis is divided into 6 major chapters as listed below:

Chapter 1 provides an introduction to the research conducted in this thesis. A general description of the significance of using green microalgae and cyanobacteria as bio-inoculants to enhance soil fertility and plant growth. Additionally, it outlines the particular objectives that must be met in order to meet the aim of the research project. An overview of the information in each chapter is then given.

Chapter 2 presents the background/literature review of microalgal bio-inoculants for sustainable agriculture. It also focuses on the influence of microalgae and their consortia on soil microbial community and on physicochemical factors that are essential for the improvement of soil,

background information about metagenomic studies, biocontrol agents, bioremediation and genetically modified microalgae as bio-inoculants are reviewed.

Chapter 3 addresses the first two objectives of the thesis, focusing on an in-depth analysis of the isolation, screening, and characterization of cyanobacteria and green microalgae from different agricultural fields in Durban, KwaZulu Natal, South Africa. This chapter also discusses the consortia development of N-fixing cyanobacteria and non-N-fixing green microalgae, their screening, and characterization of selected consortia that were further used as bio-inoculants to improve soil fertility and plant growth.

Chapter 4 directly focuses on the thesis's third and fourth objectives, which evaluate the efficacy of selected bio-inoculants in enhancing plant growth and soil fertility based on the in vivo pot culture studies of chilli plants. Focuses on metagenomics investigations based on 16S and ITS amplicon sequencing, as well as the effect of bio-inoculant amendment on the structure of native soil microbial communities using shotgun metagenomics. The alpha diversity, beta diversity and taxonomic profiling of microbial communities and their potential relationships between microbial communities and soil parameters have also been discussed.

Chapter 5 focuses on the functional profiling and abundance of rhizosphere microorganisms involved in the C, N and P metabolism following the application of microalgal bio-inoculants using shotgun metagenomic analysis.

Chapter 6 addresses conclusions and recommendations.

CHAPTER TWO

The major portion of the chapter has been published in *Pedosphere* journal with the title "**Microalgal bio-inoculants for sustainable agriculture and their interaction with biotic and abiotic components of the soil"**

Shisy Jose, Nirmal Renuka, Sachitra Kumar Ratha, Sheena Kumari, Faizal Bux

Literature review

2.1 Microalgal bio-inoculants for sustainable agriculture

Soil is a complex ecosystem containing a wide range of microbes that play a critical role in the decomposition of organic matter and the biogeochemical cycling of nutrients (Xun et al., 2015). Modern agriculture, however, has been adversely affected by the extensive use of synthetic agrochemicals to improve agricultural production (Alori and Babalola, 2018, Osorio-Reyes et al., 2023). A sustainable alternative to synthetic agrochemicals may be ecofriendly bio-inoculants such as symbiotic N-fixers, rhizobacteria, mycorrhizae, and microalgae that provide multiple benefits to plants, thereby reducing the need for synthetic agrochemicals (Singh et al., 2016, Li et al., 2017, Song et al., 2022, Alvarez et al., 2021a).

As bio-inoculants, microalgae (prokaryotic cyanobacteria and eukaryotic green microalgae) are gaining importance; because of their advantages such as high photosynthetic productivity, ability to produce agriculturally important metabolites such as EPS, plant growth promoters, mycosporine-like amino acids (MAAs), and provide significant contributions to the soil structure, soil microbial activity and crop yield (Pérez et al., 2016, Li et al., 2016, Malik et al., 2018, Cao et al., 2023) (Fig. 1.1). For example, inoculation of *Aphanothece* sp. and *Gloeotrichia* sp. into the soil during pot cultivation of rice in the presence or absence of urea caused significant increases in plant growth and yields (65.5 % during the wet season and 40.5 % during the dry season) (Dash et al., 2016). Similarly, the combination of *Nostoc carneum* and *N. commune* with a half dose of chemical fertilizer significantly increased rice production *i.e.,* increases in spike number and total grain weight compared to the control supplemented

with the standard quantity of fertilizer (Maqubela et al., 2009). Microalgae are also regarded as biocontrol agents or biopesticides due to their ability to control or limit the growth of pathogens such as fungi, bacteria, and nematodes *via* the generation of biocidal chemicals (Osorio-Reyes et al., 2023). Furthermore, studies have shown that cyanobacteria inoculation can improve the availability of various micronutrients *e.g.,* Zinc (Zn), Copper (Cu), Iron (Fe) and macronutrients *e.g.*,C, N, P, K in soil and their translocation into plants, even up to grains (Rana et al., 2015, Coppens, 2016, Grzesik et al., 2017).

Microalgal bio-inoculants have the potential to influence or alter the soil microbiome and interact with higher plants, thereby enhancing soil functionality and changing soil physical and chemical properties (Ramakrishnan et al., 2023). Molecular methods such as metagenomic approaches have allowed a better understanding of the key functional microbial community structure of the soil that can be used as 'biomarkers' (plant growth promoting rhizobacteria, diazotrophs, nitrifiers, denitrifiers, phosphate solubilizing bacteria, etc.) to determine the soil health (Reza et al., 2018, Cania et al., 2019). The inoculation of microalgal inoculants has been shown to impact soil microbial abundance (both bacteria and fungi) (Lv et al., 2020; Wei et al., 2023). A study by Lv et al. (2020) demonstrated the positive effects of microalgal biofertilizers (*Anabaena circinalis* and *Scenedesmus quadricauda*) on cucumber plant growth and microbial biodiversity in the rhizosphere.

In recent reviews on microalgal bio-inoculants (cyanobacteria and green microalgae), the production of microalgae-based fertilizers, plant growth promoting metabolites produced by microalgae, and the circular bio-economy perspective have been highlighted (Cao et al., 2023, Osorio-Reyes et al., 2023, Parmar et al., 2023). However, the extensive application of microalgae-based bio-inoculants in agricultural contexts is still constrained due to the lack of understanding and fragmented knowledge regarding the interactions of microalgal bio-inoculants with biotic and abiotic soil factors. In this review, the interaction between microalgal inoculants and soil biotic and abiotic components is examined in order to gain a deeper understanding of the potential of microalgae for enhancing rhizosphere microbial communities and soil physicochemical properties. It also highlights the potential of cyanobacteria and green microalgae as plant growth promoters, biocontrol agents, and producers of photoprotective compounds, as

well as their role in land reclamation and desertification reversal. In addition, future perspectives regarding the use of microalgal bio-inoculants to develop sustainable agricultural management systems are discussed.

2.2 Interaction of microalgal bio-inoculants with soil biotic and abiotic components

Microorganisms and selected microbial enzymes in soil are considered important indicators of soil quality (Kour et al., 2020, Rana et al., 2020). The use of microalgal bio-inoculants (cyanobacteria and green microalgae) has been shown to contribute to the development of key soil microbial communities that influence both soil and plant health (Chatterjee et al., 2017, Alvarez et al., 2021a, Cordeiro et al., 2022, Osorio-Reyes et al., 2023).

2.2.1 Interaction with rhizosphere microbial communities, soil enzyme, and plant pathogenic microbes

Rhizosphere microbial communities are essential for soil sustainability and play an important role in nutrient transformation and availability for plants (Kallenbach and Grandy, 2011, Prasanna et al., 2017, Glick, 2018, Cao et al., 2023). The major microbial communities include rhizobacteria that promote plant growth, mycorrhizae, cyanobacteria, green microalgae, phosphate solubilizers, diazotrophs, biocontrol agents, and epiphytes (Thapa et al., 2017). It has been shown that microalgal bio-inoculants can interact with rhizosphere microbiome and improve the diversity of the native microbial community, which is beneficial to soil and plants. In many cases, these microorganisms are involved in mutualism, commensalism, or parasitic relationships, which are considered to be species-specific (Sapp et al., 2007, Cho et al., 2015). A description of the benefits of the interactions of microalgal bio-inoculants (cyanobacteria and green microalgae) and selected microbial communities (bacteria and fungi) in soil is presented in Fig. 2.1

Microalgae can colonize plant surfaces and influence the microbial community in the rhizosphere (Lee and Ryu, 2021). However, the mechanism of interaction between microalgae and other

microorganisms in soil has received limited attention (Lv et al., 2020). Thus far, no systematic research has been conducted to assess the roles of several microorganisms detected in the phycosphere, a microecosystem surrounding microalgal cell walls (Cho et al., 2015). The application of *Nostoc muscorum* in a poorly structured silt loam soil in a greenhouse experimentshowed increases in indigenous soil microbial populations such as bacteria (500 times), fungi (16 times) and actinomycetes (48 times) compared to the control (Rogers, 1994). Based on phospholipid fatty acid (PLFA)-based community profiles, Ranjan et al. (2016) found that cyanobacterial (*Calothrix elenkenii*) inoculation can influence the microbiome of rice rhizosphere, resulting in an improvement in soil nutrient availability and plant growth.

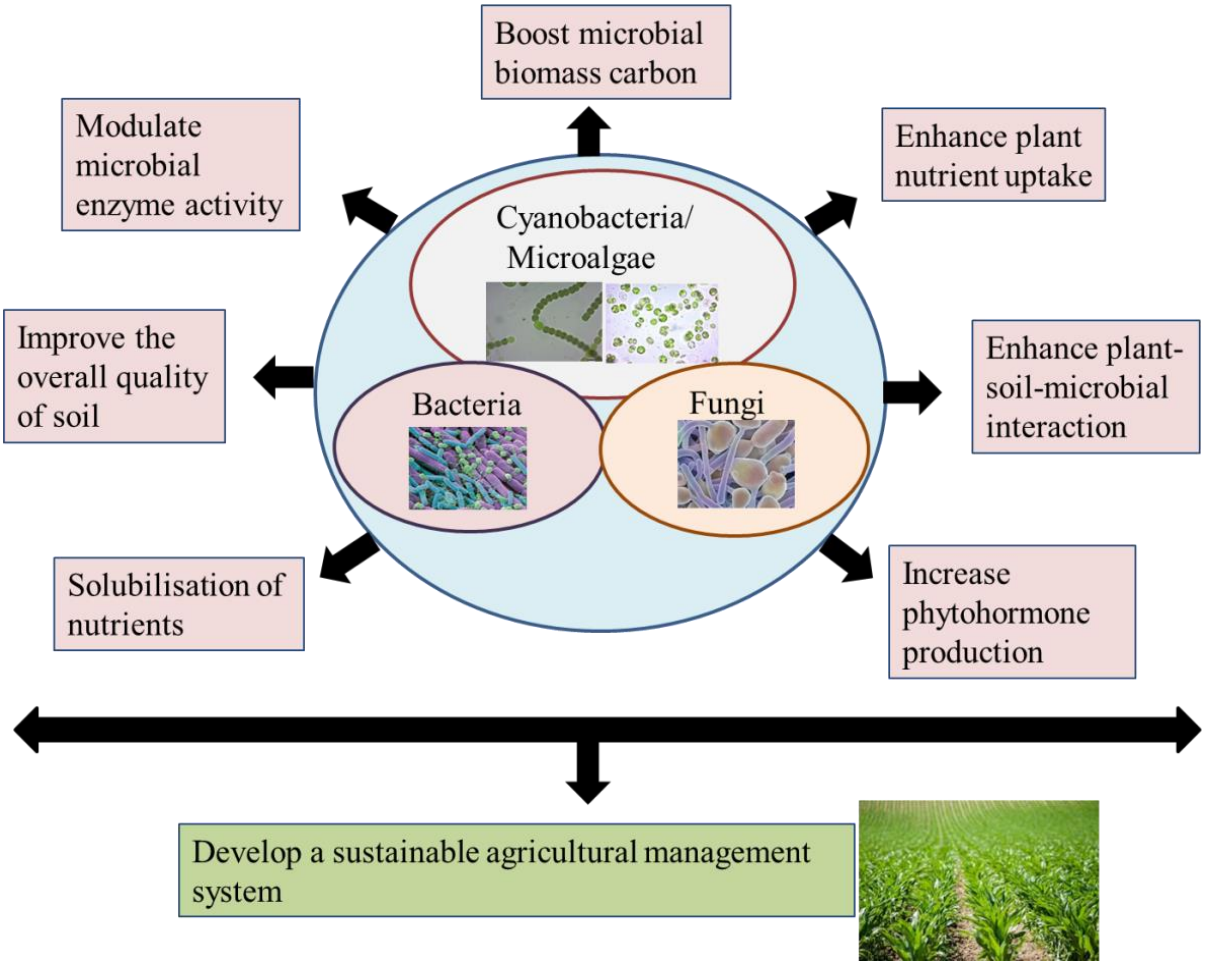

Figure 2.1 The potential benefits of the interaction of microalgae (cyanobacteria and green microalgae) with other microbes (bacteria and fungi) in soil.

In soil, enzymatic activity and soil fertility are closely related and mediate a variety of transformation processes, leading to changes in soil properties and differences in crop yields (Prasanna et al., 2016a). Most studies have demonstrated that cyanobacterial inoculation increases microbial enzyme activities in soil, including dehydrogenase, fluorescein diacetate (FDA) hydrolase, invertase, alkaline phosphatase, and phosphatase (Table 2.1). For example, dehydrogenase activity (DHA) is a biomarker of microbial oxidative activity and occurs in all viable microbial cells that function as a measurement of the active metabolic state of the microorganisms (Dotaniya et al., 2019, Alvarez et al., 2021b). Similarly, FDA and invertase activities are measures of microbial hydrolysis in soil (Dick et al., 1996, Nisha et al., 2017).

Alkaline phosphatase catalyzes the conversion of organic phosphate compounds to inorganic forms that can be used by plants and soil microbes (Manjunath et al., 2016). Several studies have used the microbial enzyme activities as a measure to assess the impact of microalgal bio-inoculants in soil, specifically with native soil microbial communities (Prasanna et al., 2016b, Grzesik et al., 2017, Alvarez et al., 2021a). Using enzymatic assays, Nain et al. (2010) demonstrated for the first time the positive interactions between cyanobacteria and bacteria and their potential in integrated nutrient management of wheat crop. Substantial improvements in the activities of dehydrogenase (51.21 %), FDA hydrolase (7.26 %), and alkaline phosphatase (51.11 %) were observed, along with an increase in soil microbial biomass (0.013 %) and a significant increase in crop yield of up to 48.76 % (Nain et al. (2010). Similarly, the inoculation of cyanobacterium *Calothrix elenkinii* in rice plants led to a 10-fold increase in soil bacterial population diversity as demonstrated by increases in nitrogenase and hydrolytic activities, indole acetic acid (IAA) production, and plant defense enzyme activity (Prasanna et al., 2016b).

Compared to the fertilization control, FDA hydrolysis and dehydrogenase activities were significantly increased in rice and wheat after treatments with cyanobacteria, bacteria, and their combinations (Rana et al., 2015). In general, inoculation of cyanobacteria and green microalgae has shown to improve soil microbial activity and health by interacting with bacterial communities (Dineshkumar et al., 2021). However, despite the advancements made in this field, little is known about the biochemical and molecular mechanisms underlying these interactions.

In natural environments, microalgae interact with other microorganisms, exchange different metabolites for mutualistic support, and modulate enzyme activities that enhance the growth of beneficial microorganisms (Ranjan et al., 2016). The role of microalgal bio-inoculants in the reclamation of degraded soils has also been explored (Couradeau et al., 2019, Lan et al., 2022). For example, using high-throughput 16S rRNA gene amplicon sequencing, Couradeau et al. (2019) assessed the microbiome of soil sample bundles associated with *Microcoleus vaginatus* in two different locations (warm Chihuahuan Desert and cold Great Basin Desert). By comparing the microbial communities closely associated with these bundles (cyanosphere) with those of the entire biocrust community, the researchers discovered that the cyanosphere of *M. vaginatus* bundle contained a 100-fold higher concentration of the *nif* H gene than the bulk soil

crust, and that it contained a cyanosphere that was compositionally differentiated and concentrated on N-fixation. Furthermore, they discovered that *M. vaginatus* acted as a significant spatial organizer of the biocrust microbiome (Couradeau et al., 2019).

Further, Lan et al. (2021) demonstrated that cyanobacterial community shift is correlated with the succession of biocrusts in the Gurbantunggut desert in China by analyzing the 16S rRNA and *nif* H genes. According to their findings, cyanobacterial abundance decreased as the succession process progressed, and N-fixing cyanobacteria were gradually replaced by C-fixing cyanobacteria. Cyanobacteria inhabiting successional biocrusts exhibit different sensitivities to moisture, temperature, and disturbance, making them useful environmental indicators (Lan et al., 2021). Under controlled conditions, Jiménez-González et al. (2022) used indigenous biocrust cyanobacteria to perform a microcosm experiment and studied their potential for soil restoration. They demonstrated that these cyanobacteria are capable of surviving the extreme harshness of Australian arid environments. As a result of inoculation, cyanobacteria produced gypsum on the soil surface, indicating that they have the potential to actively alter the upper layers of the soil profile and enhance its habitability.

Microalgae-based consortia have been demonstrated to be effective at modulating the rhizosphere microbial community and influencing soil fertility in various case studies (Subashchandrabose et al., 2011, Fuentes et al., 2016, Prasanna et al., 2017, Xue et al., 2017, Al-Maliki and Ebreesum, 2020). Using PLFA profiles, Prasanna et al. (2016b) investigated the interaction between 10 maize hybrids and five cyanobacteria-based inoculations, demonstrating the importance of specificity and selectivity in the interactions between microbes and genotypes. Several microbial groups were found to be abundant in the rhizosphere of maize hybrids as a result of the interaction between maize genotypes and bio-inoculants, with the lowest concentration of PLFA in control and the highest concentration in hybrids with *Anabaena* and *Nostoc* consortium (Prasanna et al., 2016b). An additional study conducted by Prasanna et al. (2017) analyzed the effect of two plant growth promoting cyanobacterial formulations (*Anabaena-Mesorhizobium ciceri* biofilm and *Anabaena laxa*), in conjunction with *Mesorhizobium ciceri*, on the symbiotic performance of desi- and kabuli chickpea cultivars using a hybrid polymerase chain reaction (PCR)- denaturing gradient gel

electrophoresis (DGGE) technique. Nodules from both desi- and kabuli cultivars showed highly diverse microbial communities, with 23 archaeal, nine bacterial, and 13 cyanobacterial predominant phylotypes, which were strongly influenced by the inoculation of cyanobacteria. A comparison of the inoculants, particularly *Calothrix* and the consortium of *Anabaena* and *Providencia*, based on their microbial activity and soil nutrient dynamics, as well as the generation of distinct PCR-DGGE profiles, indicated their superiority as well as the significant increase in nutrients in soil and plants as compared to control (Manjunath et al., 2016). In addition to improving the enzymatic activity of soil microbes, the interaction between microalgae and fungi in the rhizosphere can also promote nutrient solubilization and mobilization. A study conducted by Al-Maliki and Ebreesum (2020), found that mycorrhizal fungi and microalgae in soil can interact with each other to increase the amount of C contributed by other native bacteria. Even though mycorrhizal fungi alone did not induce soil decomposition, microalgal treatment alone resulted in the highest rates of soil C deposit, proving that microalgae may benefit mycorrhizal fungi by increasing root density, which can result in higher root exudates that can stimulate soil microbiota, increase microbial biomass C, improve soil aggregate stability and enhance soil quality (Al-Maliki and Ebreesum, 2020).

Table 2.1 Effects of microalgal bio-inoculants (microalga only or microalga consortia with other microorganisms) on soil enzyme activities and other properties, crop growth and crop yield

Microalgal bio-inoculant	Crop	Enzymes with increased activities	Other effects	Reference
Consortia of cyanobacteria and bacteria	Carrot	Dehydrogenase, fluorescein diacetate (FDA) hydrolase, and alkaline phosphatase	Increased microbial activity	Nain *et al.*, 2010

Consortia of cyanobacteria and bacteria	Rice	Dehydrogenase, FDA hydrolase, and alkaline phosphatase	Improved soil health, rice growth, and rice grain yield	Prasanna *et al.*, 2012
Consortia of cyanobacteria and bacteria	Rice, wheat	Dehydrogenase, FDA hydrolase, and alkaline phosphatase	Increased micronutrient contents and yield of rice	Rana *et al.*, 2015
Consortia of cyanobacteria and bacteria	Wheat	Dehydrogenase and phosphatase	Increased rhizosphere microbial activity and wheat yield	El-Gamal *et al.*, 2015
Consortia of cyanobacteria and bacteria	Okra	Dehydrogenase and alkaline phosphatase	Altered soil microbiome and increase crop yield	Manjunath *et al.*, 2016
Consortia of cyanobacteria and fungus	Wheat	Dehydrogenase	Increased abundances and activities of various soil microbial communities	Sharma *et al.*, 2020
Consortia of *Anabaena* sp. and *Providencia* sp.	Maize	Dehydrogenase	Altered rhizosphere bacterial community	Prasanna *et al.*, 2016b
Anabaena doliolum,	Millet and	Dehydrogenase, invertase, and	Improved soil structure stability and	Nisha *et al.*, 2007

Cylindrospermum sphaerica, and *Nostoc calcicola*	wheat	phosphomonoesterase	productivity under a limited water regime	
Nostoc ellipsosporum and *N. punctiforme*	Pearl millet and wheat	Dehydrogenase, invertase, and phosphomonoesterase	Improved saline soil productivity and increased crop growth and yield,	Nisha *et al.*, 2018
Microcystis aeruginosa, *Anabaena* sp., and *Chlorella* sp.	Willow	Dehydrogenase and alkaline phosphatase	Increased enzyme activities and improved willow plant growth	Grzesik *et al.*, 2017
Spirulina platensis	Wheat	Dehydrogenase and phosphatase	Improved soil structure and enhanced plant growth	Mahmoud *et al.*, 2007

DHA- Dehydrogenase; FDA- Fluorescein diacetate

It has been demonstrated that microalgal bio-inoculants can interact with plant pathogenic microorganisms and act as biocontrol agents for pathogenic fungi and soil-borne diseases in plants (Costa et al., 2019). Studies have shown that cyanobacteria and green microalgae produce hydrolytic enzymes (*e.g.,* chitosanases, phenylalanine hydroxylase, xylanases, and endoglucanases), antibacterial compounds, antifungal compounds and secondary metabolites that act as biocontrol agents on various crops such as rice, cotton, tomato, legumes and increase the defense mechanism in plants (Kulik, 1995, Prasanna et al., 2016b, Brasil et al., 2017). Prasanna et al. (2014) reported the establishment of cyanobacterial inoculants in the rhizosphere of crops and their role in preventing the growth of fungal pathogens. Plant growth promotion and the role of cyanobacteria in improving nutrient availability have also been demonstrated. A research study

by Kim et al. (2018) reported that *Chlorella fusca* reduced gray mold (*Botrytis squamosal*) disease severity in Chinese chives by more than 24.2 % compared to the control and improved the overall plant growth.

2.2.1.1 Comparison of microalgal bio-inoculants with plant growth promoting rhizobacteria (PGPR)

Rhizobacteria, which promote plant growth, are a group of bacteria that colonize plant roots and have a positive effect on plant development (Dutta and Podile, 2010, Beneduzi et al., 2012, Nosheen et al., 2021). On a wide range of agricultural crops, PGPRs have been employed to improve plant growth, and crop yield and modulate bacterial activity in the rhizosphere (Mohanty et al., 2021). The main mechanism through which they interact with soil microbes is the production of phytohormones (Khan et al., 2014, Romanenko et al., 2015, Wang et al., 2020). It is well established that phytohormones play an important role in the growth and development of plants (Lu and Xu, 2015, Chu et al., 2017). The production of IAA has been reported in many PGPR isolates, including *Enterobacter, Pseudomonas, Azospirillum, Gluconacetobacter, Pseudomonas,* and *Rhizobium* spp. (Khan et al., 2014, Mohanty et al., 2021). Several other strains including *Bacillus*, *Azotobacter, Frankia*, *Methylobacterium*, have also been reported to produce cytokinins and gibberellins (Mohanty et al., 2021). Kalam et al. (2020) reported the production of IAA by *Bacillus* isolates from tomato rhizosphere, which improved tomato plant growth and productivity.

Cyanobacteria and green microalgae have also been reported to produce phytohormones similar to those of higher plants (Kapoore et al., 2021, Suchithra et al., 2022). Lu and Xu (2015) suggested that the hormone system in higher plants may have evolved from the primary metabolic system in microalgae. Species of *Anabaena, Nostoc, Cylindrospermum, Plectonema, Chlorella, Scenedesmus, Acutodesmus, Chlorococcum, Gyoerffyana, Nautococcus, Raphidocelis, Klebsormidium, Calothrix, Phormidium, Coenochloris, Chlamydomonas, Arthrospira, Synechococcus*, and many more also reported to produce phytohormones such as IAA (Sergeeva et al., 2002), cytokinins (Hussain et al., 2010, Romanenko et al., 2016),

gibberellins (Stirk et al., 2013), abscisic acid (Stirk et al., 2009), salicyclic acid (Wu et al., 2016), jasmonic acid (Wu et al., 2016), brassinosteroids (Stirk et al., 2013), and ethylene (Romanenko et al., 2015). Stirk et al. (2013) reported endogenous IAA, cytokinins, and gibberellins from 24 axenic microalgae strains from the families Chlorophyceae, Trebouxiophyceae, Ulvophyceae, and Charophyceae, with ranges of 0.50 -71.49 nmol g^{-1}, 0.29 - 21.40 nmol g^{-1}, and 342.7 - 4746.1pg mg^{-1} respectively, on dry weight basis.

In the process of nutrient uptake, phytohormones released by green microalgae and cyanobacteria can be transferred to plants with other nutrients from the soil (Mazhar et al., 2013, Abinandan et al., 2019, Alvarez et al., 2021a). It has also been demonstrated that microalgal metabolites can act as elicitors of plant growth and modulate phytochemical composition (Singh et al., 2016). As reported by Zarezadeh et al. (2020), cyanobacterial suspensions (*Nostoc carneum, Wollea vaginicola, and Nostoc punctiforme*) combined with plant growth-promoting hormones IAA, indole 3-propionic acid, and indole 3-butyric acid were effective in increasing the growth rate of *Matricaria chamomilla* and altering the quality and quantity of essential oil. Gitau et al. (2022) reported the production of IAA and EPS by *Chlamydomonas reinhardtii* cc 124 and *Chlorella* sp. MACC-360. The application of microalgal bio-inoculants in soil and microalgal extracts to *Solanum lycopersicum* L. plants exerted a biostimulatory effect and resulted in earlier and more intense flowering, and increases in fruit diameter and weight (Mutale-Joan et al., 2022). Rupawalla et al. (2022) quantified 15 known phytohormones from the fast-growing species of *Chlorococcum, Micractinium, Scenedesmus* and *Chlorella* using high-throughput ultra-performance liquid chromatography-tandem mass spectrometry. They reported that the application of extracts from phytohormone producing microalgae improved seed germination of spinach by 1.7-fold on day 5.

Studies using the combination of PGPRs and cyanobacterial strains as soil amendments have demonstrated significant improvements in soil fertility and plant growth (Manjunath et al., 2011). A study conducted by Manjunath et al. (2011) reported that plant growth can be enhanced by combining two PGPR proteobacteria (*Providencia* sp WRB4 and *Alcaligenes* sp WRB10) with two cyanobacteria (*Anabaena oscillarioides* sp WRC3 and *Anabaena torulosa* WRC4). As compared to chemical fertilizer control, they observed improvements in plant

growth parameters, including shoot length, root length, shoot weight, root weight, and crop biomass, with a positive interaction between proteobacteria and cyanobacterial strains.

The characteristics that PGPR and microalgae have in common include biological N fixation, P, K, and Zn solubilization, and siderophore production. Both PGPR and microalgae, in particular heterocystous cyanobacteria, are capable of fixing atmospheric N through nitrogenase and are therefore being investigated as N biofertilizers for a variety of agriculturally important crops (Masson-Boivin and Sachs, 2018, Cao et al., 2023). Microalgae and PGPR have been shown to be effective in making P available to plants by mineralizing biomass and solubilizing complex soil components (Kafle et al., 2019, Alvarez et al., 2021a). Microalgal bio-inoculants increase the K concentration in soil by releasing K slowly into the soil and making it available to plants (Slinksienė et al., 2022). For example, *Bacillus* species produce carboxylic acids that largely solubilize K complexes in the soil and help in increasing soil fertility and crop productivity (Khatoon et al., 2020). Using soil drenching techniques, Mutale-Joan et al. (2022) found that biomass extracts from combined green microalgae-cyanobacteria (*Dunaliella salina*, *Chlorella ellipsoidea*, *Aphanothece* sp, and *Arthrospira maxima*) stimulated tomato plant growth. They further demonstrated that the biomass extracts improved the growth of tomato plants and their composition, as measured by chlorophyll content and essential nutrients such as N, P, and K. Both PGPR and microalgal bio-inoculants have been reported to solubilize Zn in soil and improve its availability to the plants (Lefèvre et al., 2014, Adak et al., 2016). According to Adak et al. (2016), microalgal bio-inoculants increased Zn concentrations in rice grains by 15-41 % compared to uninoculated controls. Similarly, certain strains of PGPR, such as *Bacillus*, *Pseudomonas*, and *Serratia*, have been reported to improve Zn mobilization in wheat (7-12 %) and soybean crops (Lefèvre et al., 2014). Both microalgal bio-inoculants and PGPR have the ability to form siderophores that can chelate Fe under Fe deficient conditions and make it available to microbes and plants (Rana et al., 2012, Sinha and Parli, 2020).

In addition to improving the availability of specific nutrients, PGPR can also influence soil fertility and plant growth by solubilizing macro- and micronutrients, promoting nutrient uptake, producing phytohormones, and reducing the negative effects of phytopathogenic microorganisms in the rhizosphere (Gupta et al., 2015). Additionally, microalgal bio-inoculants

and their consortia with PGPR have been shown to modulate soil microbial activity, enhance plant nutrient uptake, and increase crop yield (Manjunath et al., 2011, Prasanna et al., 2016b). In recent years, however, studies have demonstrated that using a single strain of green microalgae or cyanobacteria can produce all of the desired effects more effectively than using a group of bacteria for the same effects and enhanced SOC levels thereby reducing greenhouse gas emissions (Osorio-Reyes et al., 2023).

2.2.1.2 The interaction of microalgae bio-inoculants with soil microbial community in the plant rhizosphere

Rhizosphere soil microorganisms serve an important role in agricultural systems, as they are directly involved in controlling plant nutrient acquisition and soil nutrient cycles (Lin et al., 2022). As a result, it is critical to uncover potential alterations in the soil microbial community in order to investigate the principle of interactions (Quince et al., 2017). Shotgun sequencing can be used to characterize the taxonomic composition and functional potential of microbial communities, as well as to recover entire genome sequences (Molefe et al., 2021). Frey et al. (2022) used shotgun metagenomics to compare microbes in deepsoil and topsoil, which provided insights into the biogeochemical processes that may change with different soil characteristics, the functional diversity of diverse microbial assemblages, and their metabolic capacity. The functioning of the bacterial communities in soil samples from the two farms was determined further by measuring their capacity to utilize different C sources (Nwachukwu et al., 2022). High-throughput sequencing and other developments in molecular biology technology have improved our capacity to identify alterations in microbial populations at high resolution. Nevertheless, few researchers have used this technique to apply microalgal modifications.

The 16S and ITS rRNA high throughput Illumina sequencing is used to investigate the effects of microalgal biomass as a biofertilizer on cucumber growth and microbial (bacterial and fungal) populations in the cucumber rhizosphere (Lv et al., 2020). The findings indicated that microalgal biofertilizers exhibited significant effects on cucumber growth and bacterial and fungal richness in the cucumber rhizosphere. Moreover, most members of the microbial community are dormant at any given time, it is important to keep in mind that DNA-based methods may not accurately reflect the active microbial community (Jones and Lennon, 2010). As a result, it may be difficult

to separate the detection of changes in community diversity from changes in function, as revealed by enzyme assays. On the other hand, combining high-throughput metagenomics with enzyme assays may be able to fully grasp the responses and possibilities of the dynamic microbial population. The research in this area can provide vital information on microalgal biofertilizer interaction with plant, soil microbes and their surroundings to unravel the responses that will pave the way for sustainable agriculture. In-depth investigations on the compositional and functional changes in the soil microbial community would be beneficial in determining the role and competency of microalgal bio-inoculants in the rhizosphere.

2.2.2 Interaction of microalgal bio-inoculants with soil abiotic components

The inoculation of cyanobacteria and green microalgae or in combination with other microorganisms can enhance soil physical properties such as soil stability, and water retention capacity, as well as chemical properties such as nutrient profile, and organic content (Table 2.2) (Uysal et al., 2015, Grzesik et al., 2017, Kumar et al., 2018, Al-Maliki and Ebreesum, 2020).

Table 2.2 The effect of microalgal bio-inoculants on soil physicochemical properties.

Microalgal bio-inoculant	Effect	Mechanism	Reference
Phormidium ambiguum and *Scenedesmus javanicum*	Enhanced soil aggregate stability	Filaments form a network in the upper soil layer, and sticky polysaccharides bind soil particles	Chamizo *et al.*, 2018a
Nostoc commune, *Scytonema hyalinum*, and	Improved soil fertility with higher organic C and N	Form a stable organo-mineral layer in soil	Román *et al.*, 2018

Tolypothrix distorta			
Microcoleus vaginatus and *S. javanicum*	Improved microenvironment under water-limited conditions	Trap and retain water in sandy soil, and increase water availability in soils	Colica *et al.*, 2014
Phormidium ambiguum and *Scenedesmusjavanicum*	Improved C source for microbial activity	Change soil hydrology and restore soil moisture	Chamizo *et al.*, 2018a
Leptolyngbya boryana, *Leptolyngbya tenerrima*, *Oscillatoria splendida*, *Oscillatoria tenuis*, *Microcoleus vaginatus*, *Phormidium tergestinum* and *Nostoc commune*	Improved the soil aggregate stability	Reduce clay dispersion, and increase dehydration tolerance	Sepehr *et al.*, 2019
Nostoc kihlmani and *Anabaena cylindrica*	Improve the aggregate stability and quality of the soil	--[a)]	Gheda and Ahmed, 2015

Nostoc muscorum	Improved soil aggregate stability	Increase soil organic C content	Rogers and Burns, 1994
Oscillatoria sp., *Nostoc* sp., and *Scytonema* sp.	Increased soil organic matter and nutrients	Promote microbiotic crust formation and enhance C/N-cycling microorganisms	Acea *et al.*, 2003
Chlorella vulgaris	Increased soil organic matter and improved soil quality	Accelerate soil organic matter decomposition	Gougoulias *et al.*, 2018
Nostoc commune, *Scenedesmus hyalinum*, and *Tolypothrix distorta*	Increased soil fertility	Improved soil functions and act as natural bio-inoculants	Román *et al.*, 2018
Consortium of *Anabaena torulosa*, *Nostoc carneum*, *Nostoc piscinale*, *Anabaena dolilolum*, *Anabaena laxa*, *Anabaena azollae*, and *Anabaena oscillarioides*	Increased availabilities of micro- and macronutrients	--[a)]	Rana *et al.*, 2015, Prasanna *et al.*, 2016a
Arthrospira	Restored soil	Increased soil total P, N,	Alobwede *et al.*,

platensis and *Chlorella* sp.		physicochemical properties	and C	2019

[a)] Not available. SOC- Soil organic carbon, N- Nitrogen, C- Carbon, P- Phosphorus, K- Potassium

2.2.2.1 Effect on soil texture

The heterogeneity of soil texture correlates positively with the diversity of the soil bacterial community. It provides a unique niche for the diverse microbial communities (Seaton et al., 2020). Green microalgae and cyanobacteria have been reported to alter the micromorphological characteristics and stability of soil by producing EPS and mucilaginous exudates (Rossi et al., 2022) (Fig. 2.2). EPS acts as a cementing agent in soil and increases the size of soil aggregates by entrapping soil particles together and forming biological soil crusts or microbial mats that are resistant to wind and water erosion (Rawls et al., 2003, Abdel-Raouf, 2012, Williams et al., 2013, Zhang et al., 2018). In several studies, cyanobacteria and green microalgae have been shown to initiate biocrust formation, influence the formation of heterotrophic microbial communities, and deposition of organic matter, and changes in topsoil texture (Rozenstein et al., 2014, Chamizo et al., 2016, Maier et al., 2018). According to Rozenstein et al. (2014), the size of soil particles plays an important role in biocrust formation after inoculation with cyanobacteria. In their study, it was found that inoculating fine sand particles with *Microcoleus vaginatus* resulted in a higher rate of biocrust formation. Mugnai et al. (2020) also demonstrated that *Leptolyngbya ohadii* inoculated in sand formed biocrusts within 15 d of inoculation. Furthermore, stable biocrust formation has been observed in fine particles with higher tensile strength and aggregate stability. Their results indicated that by increasing the size of cyanobacterial inoculums, stable biocrusts may form on coarse sand particles. *Microcoleus vaginatus* is considered one of the most important microalgal bio-inoculants for arid and semi-arid sandy soils, as demonstrated in various studies (Belnap, 2006, Lan et al., 2014, Rozenstein et al., 2014, Román et al., 2018). As reported by Zaady et al. (2010), the polysaccharide content of a biocrust dominated by *Microcoleus vaginatus* was positively correlated with the PLFA-based soil microbial biomass. Further, cyanobacteria and microalgae have been shown to

increase the aggregate stability of sandy (Xie et al., 2007), silty (Maqubela et al., 2009), and clayey soils (Dineshkumar et al., 2018).

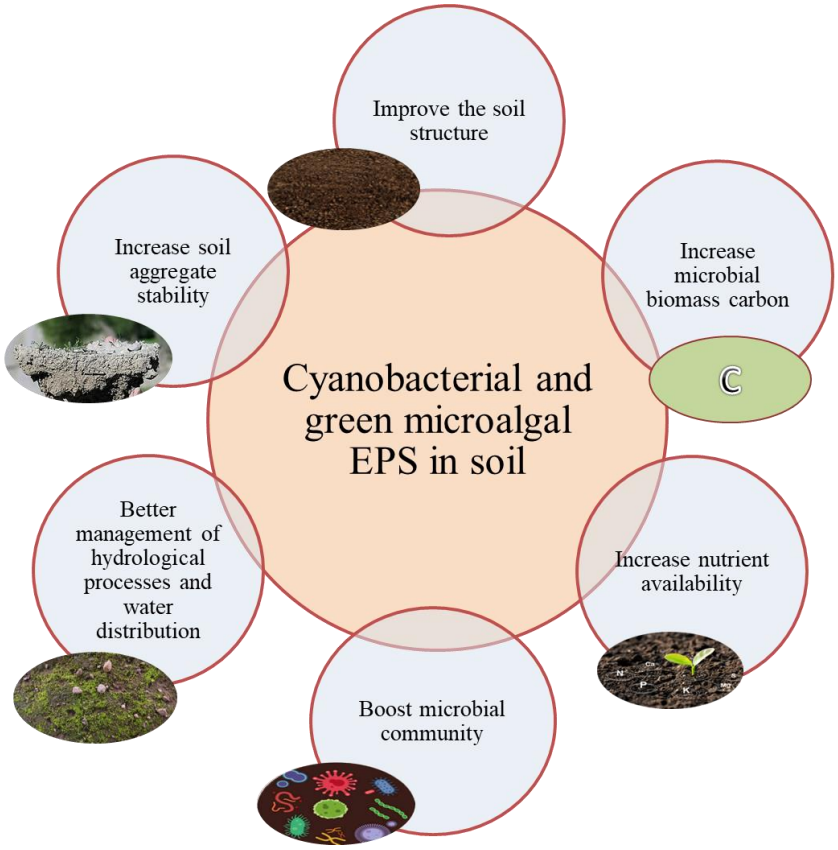

Figure 2.2 Schematic representation of the influence of cyanobacterial and green microalgal exopolymeric Substances (EPS) on soil physicochemical and biological characteristics.

Cyanobacteria produce EPS, which aids in the binding of soil particles, helps soil retain water, and helps microbial communities adapt to desiccated conditions (Djemiel et al., 2022). These substances can stimulate the activity of nutrient-mobilizing microbes in biocrusts (Mugnai et al., 2018, Wu et al., 2022, Karimi et al., 2023) and agricultural soils (Prasanna et al., 2016a, Mutum et al., 2022, Velmourougane et al., 2023), thereby enhancing the nutrient availability to the plant (Fig.2.3). A study conducted by Karimi et al. (2022) investigated the effects of inoculating soil with *Microcoleus vaginatus* ATHK43 on biocrust development, and soil physicochemical and biological properties. Significant increases in organic C, N content, and microbial activity were observed after 90 d of inoculation. In addition, Chamizo et al. (2018) examined how

cyanobacterial biocrusts developed and evolved over time in various textured soils (silty loam, sandy loams, loamy sand, and sandy soil) using two species of cyanobacteria, *Phormidium ambiguum* (non-fixing) and *Scytonema javanicum* (N-fixing). Compared to the control soil, the soil inoculated with *S javanicum* showed an average increase in EPS content of 27 %, 22 %, 24 %, and 355 % in the silty loam, sandy loam, loamy sand, and sandy soil after 90 d, whereas the soil with *P. ambiguum* showed an average increase of 44 %, 39 %, 22 %, and 195 %, respectively. These bio-inoculants have also been shown to have a positive effect on soil surface stability; however, depending on soil characteristics and cyanobacterial species, thin and fragile biocrusts develop in sandy soil, whereas thick and resistant biocrusts develop in fine soil (Chamizo et al., 2018a). Furthermore, these organisms can also live in extremely dry environments, and are the primary colonizers of bare land (Lan et al., 2014). Additionally, green microalgae such as *Chlorella* sp. can serve as a biofertilizer, stabilizing soil aggregates, reducing soil water loss, and improving soil health (Solé-Bundó et al., 2017, Yilmaz and Sönmez, 2017). In general, it has been demonstrated that the microalgal inoculants can function as a pioneer agent in soil stabilization, microbial community stimulation, and biocrust formation, which makes them promising bio-options in sustainable agriculture.

2.2.2.2 Effect on soil moisture

Soil moisture plays a major role in soil hydrological, biological, and biogeochemical processes, as well as providing nutrients to plants and other organisms (Chamizo et al., 2016). Water deficiency is one of the most significant constraints on plant growth and agricultural production in the world, which directly affects soil microbial community and organic matter, as well as soil texture and structure (Belnap, 2006). By forming biocrust communities, cyanobacteria and green microalgae can enhance soil moisture availability and reduce evaporation, infiltration, and runoff (Bu et al., 2014, Adessi et al., 2018). Their EPS is composed of polysaccharides, proteins, and other compounds with a variety of chemical characteristics that help to absorb a large amount of water (Pereira et al., 2009). Therefore, by secreting EPS, biocrust can improve the water retention capability of soil, increasing water availability and maintaining surface moisture (Chamizo et al., 2016).

Evaluation and quantification of the influence of cyanobacterial crust on water potential under controlled conditions has been conducted by Adessi et al. (2018). The authors concluded that crustal organisms are more likely to survive under water-limited conditions when EPS is present (Adessi et al., 2018). In a recent study, Kidron et al. (2021) have demonstrated that with the assistance of multilayered green microalgae and cyanobacterial filaments and swelling polysaccharide sheaths, soil moisture in the Western Negev Desert can be preserved by preventing pore clogging, and reducing evaporation, which helps organisms resist water stress and drought (Kidron, 2014). Similarly, Sepehr et al. (2019), reported an increase in soil water retention (2.69 %) in cyanobacteria-dominated areas and the highest fraction (0.99mm) of coarser soil aggregates, which clearly shows that EPS produced by cyanobacteria bind soil particles together, increasing soil stability and improving the microbial survival under drought conditions. Additionally, the microalgal bio-inoculants cover the soil surface with EPS secretion, and the filament forms microstructures that increase infiltration, crust resistance, and soil stability against erosive forces and run-off (Malam Issa et al., 2009, Rossi et al., 2017a, Kheirfam, 2020). Chamizo et al. (2016) demonstrated that soil moisture content decreased and evaporation increased after the removal of cyanobacterial biocrust, showing 2.9 times higher moisture loss than soils covered with biocrust. Microalgae such as chlorophyte can stabilize soils and alter the hydrological properties (such as water retention) of crust-covered soils in arid and semi-arid climates (Mutum et al., 2022). Thus, microalgal bio-inoculants may improve moisture content by improving soil water retention and infiltration and reducing evaporation, leading to better water stress management (Kheirfam et al., 2020).

2.2.2.3 Microalgal strategies to survive high temperature and ultraviolet (UV) radiation

Soil temperature has an important influence on biogeochemical cycling, hydraulic activity, microbial biomass, and plant growth (Onwuka, 2018). According to Wang et al. (2015), UV radiation can alter soil microbial community, destroy proteins, inactivate hormones, and decrease microbial respiration by 44.9 %. Among the most versatile microorganisms, cyanobacteria have the ability to hold up under adverse environmental conditions, such as low and high temperatures (Wang et al., 2013, Jasser et al., 2022) as well as elevated UN radiation (Kidron et al., 2009)

(Table 2.3). The primary colonizers of soil in the aftermath of volcanic eruptions are identified as cyanobacteria. It is believed that they initiate the formation of biocrust by producing a mucilaginous gelatinous sheath in such harsh environments (Abinandan et al., 2019). A number of microalgae (such as *Scenedesmus platydiscus*, *Chlamydopodium* sp., and *Chlorella* sp.) are reported to recover their photosynthetic ability after desiccation by inhabiting microsites within soil crust organisms, thereby changing the soil quality and nutrient availability for other organisms (Gray et al., 2007). While high temperatures damage the soil organically and inhibit the growth of microorganisms, cyanobacteria are able to survive by maintaining soil moisture and enhancing nutrient levels (Singh et al., 2016). Cyanobacteria and green microalgae are known to survive strong UV radiation by producing pigments such as MAAs and scytonemin (Table 2.3) (Holzinger and Lutz, 2006). The UV-protectant pigments in microalgae have absorption maxima ranging from 310 to 362 nm, are resistant to several abiotic stresses, and act as UV-B photoprotective filters (Volkmann and Gorbushina, 2006). These pigments have absorption maxima ranging from 310 to 362 nm, and are resistant to various abiotic stresses, and also function as UV-B photoprotective filters. In general, MAAs are small, intracellular, colorless, and composed of hydrophilic compounds; scytonemin, on the other hand, is yellow-brown, occurs in extracellular polysaccharide sheath, and is lipid-soluble with a maximum absorption at 384 nm (Rastogi and Incharoensakdi, 2014). These pigments can absorb both UV-A and UV-B radiation and protect other agriculturally important organisms in the soil from high levels of UV radiation. This photoprotective property of cyanobacteria and green microalgae make them suitable as a potential biofertilizers for areas with high UV-B radiation (Singh et al., 2013). It has been shown that the photoprotective compounds present in *Nostoc commune* can effectively break down the absorbed radiation in the form of heat without producing reactive oxygen species (ROS) (Singh et al., 2017). Additionally Singh et al. (2013) demonstrated that *Anabaena doliolum* and *Anabaena* strain L31, two common strains of soil cyanobacteria, are capable of protecting themselves from UV-B radiation by employing both enzymatic and non-enzymatic defense mechanisms According to the study, *Anabaena doliolum* synthesized three types of MAAs under UV-B radiation and exhibited a five-fold increase in MAA production, whereas *Anabaena* strain L31 exhibited a high antioxidant enzyme activity and was capable of surviving UV radiation better. High concentrations of UV-absorbing pigments were found in the upper layer of the

biological soil crust, along with green microalgae and cyanobacteria, demonstrating their role in photoprotection (Bowker et al., 2002). Moreover, cyanobacterial strains are capable of withstanding prolonged UV exposure, making them suitable for use as soil restoration bioengineering tools (Román et al., 2018). In addition, they can safeguard other beneficial microbes from extreme conditions by protecting them from harsh environments such as UV radiation (Singh et al., 2013). Consequently, this can be considered one of the most important characteristics when selecting cyanobacteria and green microalgae for soil use. In order to understand how these factors interact with the soil microbial community and other physicochemical factors, even under extreme environmental conditions, such as high light intensity, high temperature, and high UV radiation, further studies are required.

Table 2.3 Strategies taken by microalgae (cyanobacteria and green microalgae) to survive ultraviolet (UV) radiation and high temperature**.**

Cyanobacteria/Microalgae	Strategy	Reference
Gloeocapsa sp.	Increase the resistance to UV radiation	Garcia-Pichel (1992)
Nostoc sphaeroides	Promote antioxidant activity to protect photosystem II	Wang et al. (2007)
Microcoleus vaginatus, Nostoc sp., Scytonema javanicum, and Chlorella vulgaris	Develop tolerance and repair mechanisms to counteract the damaging effect of UV-B radiation	Chen et al. (2012)
Anabaena dolilolum, and *Anabaena strain L31*	Increase antioxidant enzymes to scavenge damaging ROS	Singh et al. (2013)
Microcoleus vaginatus, and *Scytonema*	Protects colonies by gliding	Rossi et al.

javanicum	up and down within the topsoil, and protect cyanobacterial crust by stratifying at soil surface	(2017b)
Nostoc commune	Break down the absorbed solar radiation in the form of heat	Singh et al. (2017)
Chlorella vulgaris	Enhance photosynthetic pigment contents, secondary metabolite production, antioxidant enzyme activities and drought resistance	Kusvuran (2021)
Thermoleptolyngbya	Possess heat shock proteins to with stand high temperatures	Jasser et al. (2022)

ROS- Reactive oxygen species

2.2.2.3 Effect on soil pH

Soil pH is a measure of soil acidity and alkalinity, and it is an important indicator of soil health (Al-Maliki and Ebreesum, 2020). As well as shaping and regulating soil microbial communities, it is also used as a master soil variable in order to improve soil properties and processes such as enzyme activity, nutrient conversion, and biochemical cycling (Wang et al., 2017b, Wang et al., 2019, Al-Maliki and Ebreesum, 2020, Tao et al., 2020). Soil pH is highly influenced by climatic conditions, mineral content, land use, and management practices (Singh and Dhar, 2010). In a study by Ren et al. (2018), the influence of soil pH on the composition

and diversity of microbial communities was examined using a shotgun metagenomics sequencing approach. The authors concluded that soil pH is a significant driver of microbial diversity, organic matter decomposition, and soil nutrient supply in agricultural systems.

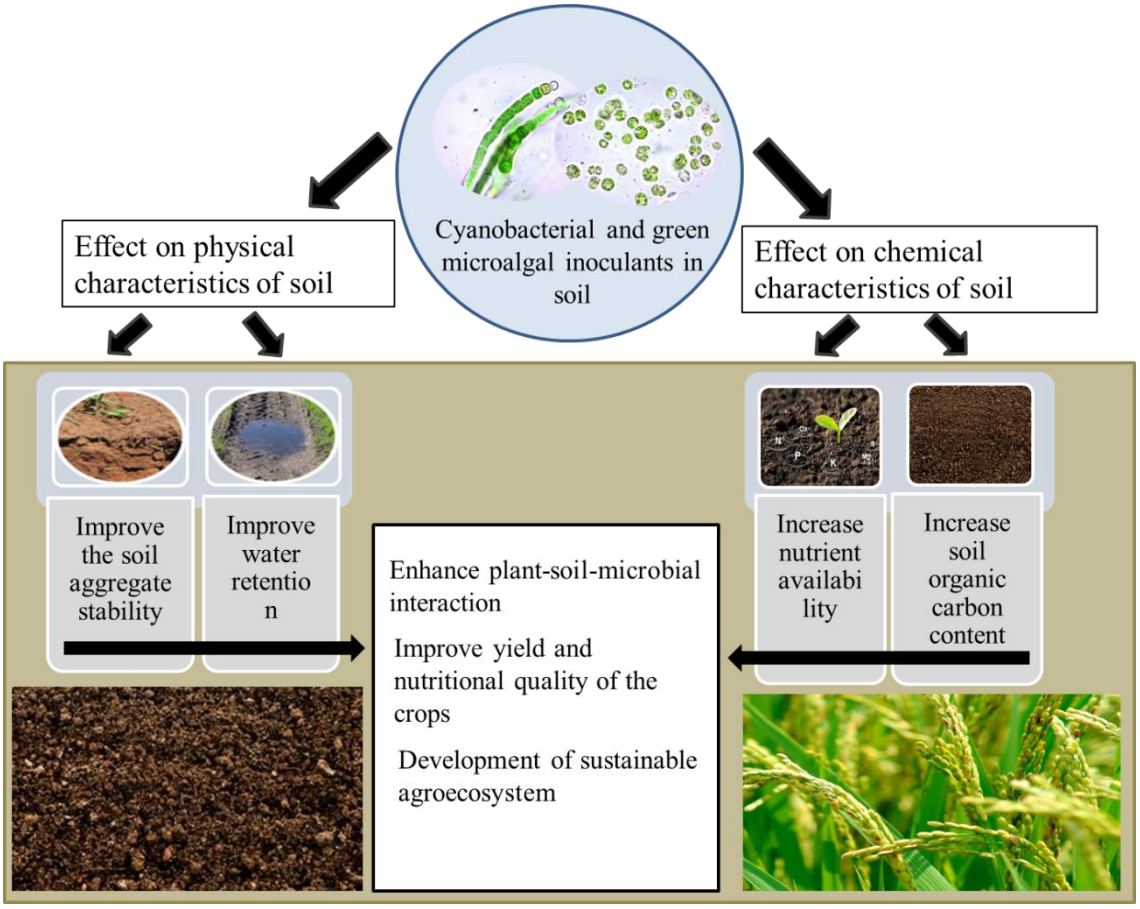

Figure 2.3 An overview of the effects of cyanobacterial and green microalgal inoculants on soil properties, crop production, and agroecosystem.

Both green microalgae and cyanobacteria are ubiquitous organisms that can grow in wide pH ranges, acclimatize to extreme environments, and withstand highly acidic and alkaline soils (Pereira et al., 2009, Chatterjee et al., 2017). Several studies have demonstrated that under natural conditions, green microalgae can reduce alkaline soil pH (*e.g.*, from 10.2 to 8.2) with an increase in organic C, N, and P content as well as binding soil particles to enhance soil permeability and airflow (Pandey et al., 2005, Al-Maliki and Ebreesum, 2020). Al-Maliki and Ebreesum (2020), revealed that application of mycorrhizae and green microalgae to soil

resulted in a significant decrease in soil pH from 7.7 to 6.96. In another study, native soil microbial activity was significantly increased when the pH decreased from 7.23 to 6.96, along with a significant change in soil physicochemical characteristics (Gheda and Ahmed, 2014). This suggests that microalgal inoculants have potential applications in the reclamation of alkaline soils, where they act as soil conditioners to enhance soil fertility and boost microbial communities. According to Singh and Dhar (2010), cyanobacteria and green microalgae can survive in an alkaline environment by synthesizing sucrose, osmotic stress proteins, and specific fatty acids. Many cyanobacteria and green microalgae such as *Anabaena*, *Nostoc*, *Westelliopsis*, *Calothrix*, *Aulosira*, *Hapalosiphon*, *Cylindrospermum*, *Scytonema*, *Phormidium*, *Oscillatoria*, *Lyngbya*, *Aphanocapsa*, *Chlamydomonas*, and *Chlorella vulgaris* have been reported to proliferate and colonize soils of diverse pH levels which can be exploited for soil applications (Nayak, 2007, Gougoulias et al., 2018, Al-Maliki and Ebreesum, 2020).

2.2.2.4 Effect on soil organic carbon

As a measurable component of soil organic matter, organic C improves soil chemical composition, microorganism biological activity, nutrient holding capacity, and overall productivity in agroecosystems and natural environments (Jensen et al., 2019, Ramesh et al., 2019, Tao et al., 2020). In the natural ecosystem, cyanobacterial biocrust contributes significantly to soil C accumulation and improves soil fertility and stability in degraded soils (Chamizo et al., 2013). Cyanobacteria and green microalgae are identified as the primary producers of organic compounds in soil by fixing carbon dioxide through photosynthesis and contribute significantly to soil organic matter accumulation and C content increase (Yan-Gui et al., 2013, Rossi et al., 2017a).

Nostoc muscorum, when grown under laboratory conditions, increased SOC content by 50 % - 63 % (equivalent to 670 kg/ha) within six months (Kaushik, 1994, Rogers, 1994) and significantly improve N and other nutrients in the soil (Nisha et al., 2007). Other studies have also shown that soil samples inoculated with cyanobacterial species increased more than those applied with chemical or organic fertilizers (Ibraheem, 2007, Mager and Thomas, 2011). It was

also found that the C level in sodic soil increased dramatically (5.3-7.6 t C ha^{-1} cropping $season^{-1}$) after the application of microalgal bio-inoculants (Lange, 2003). Similarly, inoculating cyanobacteria to heated soil restored the C and organic matter (Acea, 2003). A study conducted by Román et al. (2018) examined soil restoration using selected strains of cyanobacteria (*Nostoc commune*, *Scytonema hyalinum*, and *Tolypothrix distorta*) in three semiarid ecosystems. In all experimental combinations, the authors observed an improvement in soil function as a result of inreased total organic C (6 g kg^{-1}) and total N (0.60 g kg^{-1}). Additionally, green microalgae have also been reported to increase soil C mineralization. Al-Maliki and Ebreesum (2020) documented a progressive increase in C mineralization after the application of green microalgae *Chlamydomonas* sp. Furthermore, the microalga inoculation led to a greater decomposition of C with the release of available nutrients, which promoted plant growth. Gougoulias et al. (2018) reported that C mineralization was maximized following the combination of microalgal dried biomass with soil. Therefore, the application of cyanobacteria and green microalgae could be a potential practice for increasing soil organic matter, as well as one of the most promising biotechnological strategies to recover degraded soil ecosystems and maintain the sustainability of soil and plant productivity in the agriculture.

2.2.2.5 Effect on soil nutrient availability

In a healthy soil ecosystem, nutrient levels are maintained and stabilized *via* nutrient cycling. Agricultural soils, however, are generally deficient in N, P, K, and other trace elements due to improper land management and synthetic fertilizer application (Manjunath et al., 2016). As a consequence, an alternative source of biofertilizers promoting macro- and micro-nutrient availability by recycling organic nutrients, reducing ecological risk, and enhancing soil physicochemical properties would be highly beneficial for the agricultural industry in order to reduce its reliance on synthetic fertilizers (Alobwede et al., 2019). It has been demonstrated that cyanobacteria and green microalgae improve the availability of macro- and micronutrients, as well as plant growth-promoting substances, which stimulate soil microbial activity, plant growth, and crop yield (Coppens, 2016). Heterocystous cyanobacterial strains can fix atmospheric N and have the potential to achieve nutrient balance in soil and boost biological

activity (Mahanty et al., 2017, Renuka et al., 2018). They are extensively used as biofertilizers to improve soil fertility and have been reported to stimulate the activity of nutrient solubilizing microbes and increase soil N, P and organic matter contents (Subashchandrabose et al., 2011).

Nitrogen is the most widely used and vital nutrient for crop production (Prasanna et al., 2016a, Sharma et al., 2020). Microalgae (cyanobacteria and green microalgae) play a major role in macro- and micro nutrient cycling, N-fixation, P mobilization, trace element release from insoluble minerals, soil fertility improvement, and plant productivity enhancement (Singh et al., 2016, Chamizo et al., 2018). Prasanna et al. (2016a) showed that the cyanobacterial inoculation increased soil N content and saved nearly 40 % of chemical N fertilizers. Similarly, Grzesik et al. (2017) reported that the applied monocultures of *Microcystis aeruginosa*, *Anabaena* sp., and *Chlorella* sp. increased N, P, and K contents in willow plants and enzyme activities in soil such as dehydrogenases, RNase, acid and alkaline phosphatases, and nitrate reductase. A few other studies also reported that inoculation of cyanobacteria increased the N content in plants, particularly in rice (Priya et al., 2015), wheat (Swarnalakshmi et al., 2013), corn and cucumber (Mulbry et al., 2005), pea (Osman et al., 2010), and cotton and maize (Prasanna et al., 2014).

Additionally, P plays an important role in soil and is the second most crucial nutrient for microorganisms and plants after N. In soil, the majority of phosphates are insoluble (*e.g.,* ferric orthophosphate, tricalcium diphosphate, aluminium phosphate, and hydroxyapatite), which are soluble by microorganisms, including microalgae. To cope with unfavorable conditions, microalgae accumulate polyphosphate (poly-P) granules in their cells and release them slowly or moderately to the soil as soluble P (Mukherjee et al., 2015). There is evidence that *Calothrix elenkinii* has the metabolic capacity to solubilized P in soils (Priya et al., 2015). Moreover, cyanobacteria act as an efficient sink for P and improve the nutrition of plants by activating unavailable soil P besides enriching the soil with N and organic matter (Mandal et al., 1992). Different cyanobacterial and rhizobacterial inoculations have shown an increase in P content in wheat grains of up to 54 % (Rana et al., 2012).

The element K is also important for agricultural production, as it enhances the structural stability of soils and aids in water retention. Deficiency of this nutrient in soils is associated with unbalanced fertilization, leading to the depletion of soil K reserves, whereas its deficiency in

plants can restrict the utilization of N, thereby resulting in nitrate leaching (ZÖrb et al., 2014). The study by Renuka et al. (2017b) reported that microalgal consortium can increase residual levels of K at harvest stage and enhance crop productivity.

A number of studies have reported a significant increase in the bioavailability of micronutrients when using microalgal bio-inoculants (Renuka et al., 2016, Alobwede et al., 2019) (Fig. 2.4). A study by Rana et al. (2015) found that cyanobacteria and bacteria significantly increased the concentrations of Zn, Fe, Cu, and manganese (Mn) in rice grains, and 44--46 % increase in Fe and Cu concentrations in wheat grains. Moreover, it has been reported that microalgal bio-inoculants enhance Zn, Fe, Mn, Cu, and selenium (Se) concentrations in wheat grains, thus providing a viable solution to trace element deficiencies (Wuang et al., 2016). The evidence suggests that microalgal bio-inoculants can be used to improve soil parameters that benefit microbial diversity, productivity, and crop yields in a broad range of soil conditions. Although cyanobacteria are the most researched potential bio-inoculants (Prasanna et al., 2017), recent studies have shown that green microalgae can also enhance soil fertility, mineralization of nutrients, and plant productivity (Alvarez et al., 2021a). Therefore, understanding the metabolic potential of the different microalgal strains will enable us to assess their contribution to nutrient cycling and enhance microbial activity while increasing soil nutrient availability.

2.2.3 Bioremediation of contaminated soil

In recent years, industrialization as well as improper agricultural practices has resulted in an increase in the level of heavy metals in the terrestrial environment (He et al., 2017, Mahanty et al., 2017). Increased level of heavy metals in the soil ecosystem decreases soil fertility, quality, microbial metabolic activities, and even plant growth (Wang et al., 2017b). Microalgae, such as cyanobacteria and green microalgae, have been demonstrated to survive under metal contamination (Trejo et al., 2012, Subramaniyam et al., 2016, Kheirfam et al., 2020). Several studies have shown that microalgal inoculations can be helpful in the remediation of heavy metals such as Cu, Zn, Ni, cadmium (Cd), lead (Pb), and chromium (Cr) (Machado and Soares, 2016, Hamed et al., 2017, Kaur and Goyal, 2018) (Table 2.4). Cyanobacteria are capable of detoxifying heavy metals by producing a metal-binding protein called metallothionein to form

phytochelatin-metal complexes (Thajuddin and Subramanian, 2005). In as recent study, Hamed et al. (2017) examined the response of microalgae to Cu stress, using *Chlorella sorokiniana* and *Scenesdesmus acuminates*. Compared to *S. acuminates*, *C. sorokiniana* displayed high sensitivity to metal stress and high Cu binding capacity. In another study, broad beans grown in heavy metal-contaminated soil treated with *Nostoc minutum* and *Anabaena spiroides* in combination with organic fertilizer had marked decreases in Pb, Ni, and Cd levels in both shoots and seeds (Al-Sherif et al., 2015). These studies have demonstrated that cyanobacteria have the natural ability to survive in high concentrations of heavy metals and to improve soil quality through their remediation.

The green microalgae (*Chlorococcum* sp.) and cyanobacteria (*Anabaena* sp., *Nostoc* sp.) isolated from dichlorodiphenyltrichloroethane (DDT) contaminated soil have demonstrated a high level of efficiency in catabolizing DDT into dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE). The diazotrophic cyanobacteria metabolized DDT into DDD, while the green alga metabolized it into DDE (Megharaj et al., 2000). Similarly, according to Zhang et al. (2012), N-fixing cyanobacterium *Anabaena azotica* are able to degrade lindane at an initial concentration of 0.2 mg L^{-1} in a Chinese paddy soil. Nevertheless, selecting the strain or consortium with the best remediation capability is of crucial importance. Developing sustainable soil bioremediation technologies, therefore, requires additional research in order to understand their diversity and adaptability to metal-contaminated environments.

Table 2.4 Remediation of heavy metal and oil/petroleum using microalgal bio-inoculants.

Heavy metals/ Oil	Cyanobacteria/ Microalgae	Mechanism	Reference
Pb	*Microchaete tenera*	Metal biosorption	Zaccaro, (2001)
Cu, Zn, Ni	*Cyanospira capsulata* and *Nostoc* PCC7936	Metal biosorption	De Philippis et al. (2007)

Al, Ca, Fe, Mg, Mn	*Chlorella* sp.	Metal bioadsorption of heavy metals	Wang et al. (2010)
Cd, Pb, Cu, Zn	*Nostoc muscorum*	Metal biosorption and bioaccumulation	Hazarika et al. (2015); Goswami et al. (2015)
Crude oil	*Anabaena oryzae, Chlorella kessleri*	Degradation of alkanes and polycyclic aromatic hydrocarbon	Hamouda et al. (2016)
Cd, Cr, Cu, Zn	*Pseudokirchneriella subcapitata*	Intracellular reactive oxygen species accumulation and glutathione reduction	Machado and Soares, (2016)
Cu	*Chlorella sorokiniana* and *Scenedesmus acuminatus*	Antioxidant defense system to neutralize oxidative damage	Hamed et al. (2017)
Cu, Zn, Cr, Pb	*Nostoc muscorum, Anabaena variabilis, Tolypothrix tenuis* and *Aulosira Fertilissimia*	Increased accumulation of metals	Kaur and Goyal, (2018)
Cr	*Limnococcus* sp.	Biosorption and bioaccumulation	Sen et al. (2018)

Al- Aluminium, Ca- Calcium, Cd- cadmium, Cr- Chromium, Cu- Copper, Mg- Magnesium, Mn- Manganese, Ni- Nickel, Pb- lead, Zn- Zinc

2.2.4 Genetically modified microalgae as bio-inoculants

Genetically modified microalgae with desired characteristics may prove to be a promising option for bio-inoculant applications (Kselikova et al., 2022). Genome editing technologies, including Zn finger nucleases, transcription activator-like effector nucleases (TALENs), clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 systems, Cre/loxP recombination systems, and RNA interference (RNAi) have been employed in microalgal bioengineering (Osakabe and Osakabe, 2015; Tanwar et al., 2018; Kselikova et al., 2022). Gene stacking toolkits such as Golden Gate modular cloning (MoClo) have also been developed for *Nannochloropsis* and *Chlamydomonas* strains, providing a library of molecular building blocks for genetic engineering (Ranjbar and Malcata, 2022). Attempts have also been made to manipulate the N-fixing mechanism in heterocysts in order to enhance their ability to fix N (Chaurasia and Apte, 2011). In cyanobacteria, the *het*R gene regulates heterocyst differentiation. Using a recombinant *Anabaena* strain, Chaurasia and Apte, (2011) found that overexpression of the *het*R gene increased heterocyst production and nitrogenase activity by 1.5–2-fold.

To date, genetic engineering studies on green microalgae and cyanobacteria have focused primarily on improving biomass productivity, biofuel production, and the production of high-value-added compounds (Kselikova et al., 2022). The large-scale production of microalgal EPS by genetic engineering has also gained attention in recent years due to the potential use of industrial EPS in products such as gums, bio-flocculants, soil conditioners, and bio-sorbents (Bhunia et al., 2018). There is, however, no research being conducted on microalgal genetic engineering for agricultural purposes.

In recent years, advances in full genome sequencing have enabled the identification of key genes involved in various metabolic pathways in green microalgae and cyanobacteria, which could lay the foundation for future genetic engineering applications (Ortiz-Marquez et al., 2013). The release of genetically modified microalgae, especially in agriculture, however, requires extreme caution and comprehensive investigation since microalgae are the primary

producers in ecological systems. Genetically modified microalgae could cause detrimental ecological effects such as altering food webs, reducing native microbial communities, causing hazardous algal blooms, and displacing native phytoplankton (Campbell, 2011). Therefore, a comprehensive ecological risk assessment of genetically modified microalgae is necessary to ensure the safety of the environment.

2.3. Conclusion and future perspectives

Cyanobacteria and green microalgae have been proven promising in improving soil fertility and maintaining plant growth through the production of metabolites and interaction with native soil flora. The use of cyanobacteria and green microalgae for soil applications has been documented in previous research, but there is still a need to popularize their use and better understand their interaction with biotic and abiotic factors. There are several most relevant knowledge gaps that still need to be filled.

The selection of microalga strains suitable for agricultural purposes requires the development of a database containing relevant information about their most appropriate characteristics, including N-fixation, EPS production, UV protection pigments, phytohormones, metal/stress tolerance, and other relevant characteristics. The selection of microalgal strains, and their combination, their application in diverse soil types, and the evaluation of their performance to predict soil stability and plant growth are challenging. In order to develop a sustainable microalgae-based agricultural system, it is therefore essential to evaluate their potential as bio-inoculants in various climatic conditions and soil types. Microalgae and rhizosphere communities could be explored in greater depth to discover new chemical signals that could improve plant productivity through symbiotic interaction, tolerance to environmental stress, and immunity against phytopathogens. Microalgae, particularly cyanobacteria, are also known to remove organic pollutants and heavy metals, as well as prevent the bio-accumulation of these pollutants. Despite the fact that soil reclamation is an emerging field in research, it has yet to be thoroughly researched at field scale and needs to be expanded for application in the field. Further research is required to determine the metabolic pathways by which microalgae respond to stress conditions and to investigate the mechanisms by which microalgae enhance soil

fertility, plant growth, and their ability to adapt to different environmental conditions. A deeper understanding of the interaction of soil microorganisms and microalgae may be possible with the use of advanced molecular tools, such as OMICS. For microalgae-based commercial products to be economically viable, it is necessary to develop the most efficient and economically feasible mass cultivation system using photobioreactors, open mixing raceways, or high-rate microalgal ponds. A technoeconomic evaluation and life cycle analysis at the laboratory and field scale are necessary to establish the commercial feasibility of bio-inoculants derived from cyanobacteria and green microalgae. The use of OMICS approaches, which encompass genome, transcriptome, proteins, metabolites, etc., may provide researchers with an opportunity to design new engineered strains with desired traits for soil and agricultural purposes.

CHAPTER THREE

This chapter has fully published in *Algal Research* journal with the title **Bioprospecting of indigenous microalgae isolated from agricultural fields and developing consortia for sustainable agriculture**

Shisy Jose, Nirmal Renuka, Sachitra Kumar Ratha, Faizal Bux, Sheena Kumari

3.1 Introduction

Microalgae-based biofertilizers, mainly cyanobacteria and green microalgae, have made great strides worldwide in recent years (Alvarez et al., 2021). The characteristics of microalgae-based bio-inoculants, such as their ability to fix CO_2 and N, increase nutrient availability, produce bioactive compounds, and act as biocontrol agents, make them promising environmentally friendly and sustainable alternatives to synthetic fertilizers (Priyanka et al., 2020, Alvarez et al., 2021a). Cyanobacteria and green microalgae are capable of producing a diverse range of organic compounds, including carbohydrates, lipids, proteins, pigments, and secondary metabolites of agricultural significance, such as exopolymers EPS and phytohormones (Grzesik et al., 2017). The production of EPS is one of the most important mechanisms for modulating and improving soil microbial activity (Renuka et al., 2018). Besides creating a hydrated matrix, they also serve as a source of organic C that supports the growth of beneficial soil microbes (Xiao and Zheng, 2016).

Cyanobacteria and microalgae can produce endogenous growth hormones such as auxins, gibberellins, cytokinins, brassinosteroids, and abscisic acid that can be used as biostimulants in agriculture (Do et al., 2020, Duong et al., 2021). It has been demonstrated in previous studies that cellular extracts and growth medium obtained from numerous types of microalgae have a positive impact on plant growth and development (Garcia-Gonzalez and Sommerfeld, 2015). Rupawalla et al. (2022) reported that the application of microalgae such as *Chlorococcum*, *Micractinium*, *Scenedesmus* and *Chlorella*, in an in-vivo seed germination assay improved the

spinach seed germination rate by 1.7-fold, green cotyledon emergence up to 2-fold, and seedling biomass output upto 2.1 compared to the control. In another study the rice seeds soaked with *Anabaena* spp. (*A. spiroides*, *A. variabilis*, *A. torulosa* and *A. osillarioides*) showed faster seed germination, doubled the seedlings height and root length when compared to the control (Saadatnia and Riahi, 2009).

Numerous studies have been conducted for individual isolates in order to assess their potential as bio-inoculants (Maqubela et al., 2008, Garcia-Gonzalez and Sommerfeld, 2015, Prasanna et al., 2015). While N-fixing cyanobacteria have been widely explored for their agricultural uses, the use of green microalgae has been relatively underexplored (Alvarez et al., 2021). The requirement of a large quantity of N fertilizer for the growth of green microalgae poses a significant economic challenge for the bio-inoculant industry (Renuka et al., 2018). Thompson et al. (2012) described a symbiotic relationship in which N was fixed by a cyanobacterium and C was fixed by a unicellular microalga. Thus, co-cultivation of green microalgae with N-fixing cyanobacteria and their consortia may prove to be beneficial since less N is required for the biomass production of microalgal bio-inoculants. Such consortia could be a promising bio-inoculant option to enhance soil fertility and plant growth since the cyanobacterial partner could fulfill the N demand, while green microalgae could provide C, apart from the production of exopolymeric substances (Alvarez et al., 2021a).

Co-cultivation studies have also been performed between cyanobacteria and green microalgae, emphasizing the synergistic relationship between the two organisms (Gautam et al., 2019). Several studies conducted over the last two decades have demonstrated that heterocystous and non-heterocystous forms of microalgae, bacteria, and fungi can act as effective biofertilizers for crops (Osorio-Reyes et al., 2023). Moreover, the use of microalgal consortia has shown better potential than microalgal monocultures to produce metabolites and their use as biofertilizers in agriculture. As a result, co-cultivating different N-fixing cyanobacteria and microalgae may be a beneficial strategy to reduce the N requirement during cultivation. Yet, the development of effective bio-inoculants for local applications requires the selection of the most compatible indigenous strains. By choosing strains that are well-adapted to the local environment, we can maximize the effectiveness of bio-inoculants and promote sustainable agricultural practices.

Thus, this study was focused on the isolation, screening and characterization of indigenous green microalgae and cyanobacteria from different agricultural fields in Durban, KwaZulu Natal, South Africa, for agricultural applications. Different microalgal consortia were developed from the selected cyanobacteria and microalgae and subsequently screened for higher biomass productivity and selected metabolites of agricultural significance. Additionally, seed germination assays were conducted using a commercially important plant *Capsicum annuum* (chili plant) to assess the biostimulant properties of the selected microalgal consortium.

3.2 Materials and methods

3.2.1 Isolation and purification of indigenous microalgal isolates

For the isolation of indigenous microalgae, random soil samples were collected from different agricultural fields in Durban, KwaZulu Natal, South Africa (-29.57902.31.119090). The composite soil samples were transferred to sterile plastic containers and transported to the laboratory for further processing. 10 mg of soil samples were then enriched in standard BG-11 medium with and without N to establish the growth of non-N-fixing (green microalgae) and N-fixing (cyanobacteria) microalgal isolates respectively. In the subsequent steps, microalgal isolates were obtained from the enriched cultures using the standard protocol for isolation, viz., serial dilution and repeated streak plate method. To maintain the stock cultures, sub-culturing was performed monthly on BG-11 media agar slants and the purity was determined by microscopy. Preliminary identification of the isolates was done using the light microscopy (Axiolab, Zeiss, Germany) based on morphotaxonomic characteristics.

3.2.2 Microalgal cultivation conditions

The microalgal cultures were grown in 1 L conical flasks with a working volume of 500 mL in BG-11 medium, with or without N, at 25 °C, 120 μmol m^{-2} s^{-1} photon flux, and 16:08 daylight/dark cycle (Renuka et al., 2018). The cultivation conditions were kept constant

throughout the experiment. The experimental flasks were inoculated with 10 % fresh microalgal inoculum of the total working volume. All the experiments were performed in triplicate.

3.2.3 Molecular identification and phylogenetic analysis of the isolates

In order to confirm the purity and identify the isolates, 16S rRNA and 18S rRNA gene sequencing were carried out for the chosen isolates at a commercial laboratory (Inqaba Biotechnical Industries (Pty) Ltd. in South Africa) using certain primer sets. In order to determine the closest similarity, the sequences were edited using Finch TV (v1_4_0) and analyzed using the BLAST Program (NCBI BLAST, USA). The sequences were thereafter aligned in CLUSTAL X and exported to MEGA11 for evolutionary analysis.

3.2.4 Screening of isolates for biomass productivity and metabolites production

3.2.4.1 Growth, biomass and chlorophyll estimation

The growth of green microalgae and cyanobacteria was monitored spectrophotometrically using a UV/VIS–spectrophotometer (Spectroquant R Pharo 300, Merck Germany) at 680 nm and 750 nm, respectively. The measurements were conducted on alternate days until the cultures reached the stationary phase (Sutherland et al., 2021, Reddy et al., 2023). The biomass concentration was calculated at the late log phase using gravimetric analysis. The chlorophyll concentration was determined spectrophotometrically by methanol extraction method (Lichtenthaler, 1987). The total chlorophyll was calculated as equation 1

$$\text{Total chlorophyll; } Chl_{a+b}\ (\mu g\ mL^{-1}) = 1.44A_{665.2} + 24.93A_{652.4} \qquad (1)$$

3.2.4.2 Metabolite analyses

3.2.4.2.1 Exopolymers

In order to analyze exopolymer production, 10 mL of microalgae culture was centrifuged at 1107 g for 15 min, 10 °C. The recovered supernatant was incubated at 40 °C overnight and was

analyzed for carbohydrates and proteins using the phenol sulfuric acid and Bradford methods, respectively (Chamizo et al., 2020, Zhong et al., 2021).

Briefly, to the supernatant, 1 mL of phenol solution (5 % w/v) was added and thoroughly mixed, followed by 5 mL of sulfuric acid. After 10 min of incubation at room temperature, the mixture was centrifuged at 1107*g* for 10 min. Thereafter, the absorbance of the supernatant was measured at 485 nm using a spectrophotometer (Spectroquant Pharo300, Merck). The carbohydrate concentrations were calculated using a calibration curve of glucose concentrations ranging from 10 to 150 μg mL^{-1} (Chia et al., 2015).

The protein content of the supernatant was determined using the Bradford method with bovine serum albumin as standard (Bradford, 1976). For this, 1.5 mL of 0.5 M NaOH was added to the supernatant and extraction was carried out for 120 min at 100 °C in an oven. Four mL of Bradford reagent was added per mL of the supernatant and allowed to stand for 5 min at room temperature. The absorbance of the solution was then read at 595 nm. Total protein concentration was determined using BSA calibration curves ranging from 10 to 150 μg mL^{-1} (Chia et al., 2015).

3.2.4.2.2 Indole acetic acid analysis

To analyze the amount of IAA produced by each microalgal isolate, a colorimetric assay was performed with salkowski reagent using salkowski's method (Mohite, 2013). To 1 mL of each microalgal culture supernatant, one drop of orthophosphoric acid and 2 mL of salkowski reagent was added. Absorbance was measured at 535 nm using a spectrophotometer (Spectroquant Pharo300, Merck) (Mohite, 2013).

3.2.5 Consortia development

Microalgal consortia were developed with a mixture of N-fixing cyanobacteria and non-N-fixing green microalgae isolates, which were selected according to their biomass productivity, phytohormone levels, and EPS production. To prepare the inocula for the consortia development, the cyanobacterial and green microalgal inocula were initially grown in standard BG-11 medium without-N and with-N, respectively. Before combining the inoculums, the green microalgal

inoculum was rinsed with N-free medium to remove any residual N. An equal quantity of selected microalgal strains on the basis of biomass content (0.1 – 0.2 µg mL^{-1}) were combined and grown in N-free BG-11 medium. Eight combinations of microalgal consortia, based on a completely randomized design (CRD) were developed using green microalgae such as *Desmodesmus armatus* (A), *Chlorella* sp., (B) and filamentous N-fixing cyanobacteria, *Calothrix* sp. (C), *Nostoc* sp. (D). The different treatments include T1 (*Desmodesmus armatus* and *Calothrix* sp.), T2 (*Chlorella* sp., and *Calothrix* sp.), T3 (*Desmodesmus armatus* and *Nostoc* sp.), T4 (*Chlorella* sp., and *Nostoc* sp.), T5 (*Desmodesmus armatus*, *Chlorella* sp., and *Calothrix* sp.), T6 (*Desmodesmus armatus*, *Chlorella* sp., and *Nostoc* sp.), T7 (*Desmodesmus armatus*, *Calothrix* sp., and *Nostoc* sp.), and T8 (*Chlorella* sp., *Calothrix* sp., and *Nostoc* sp.).

An inoculum of exponentially growing cells was utilized to inoculate the media for the consortium cultures and control cultures of *Desmodesmus armatus*, *Chlorella* sp., *Calothrix* sp., and *Nostoc* sp. The harvesting time for each microalgal consortia was optimized based on their maximal growth rate, i.e., the late log phase. The individual microalgal and consortia cultures were cultivated and harvested at their respective late log phases.

3.2.5.1 Growth profile analysis of consortia

The specific growth rate (µ) and the divisions per day ($Div.day^{-1}$) of the different consortia and controls were determined using Equations (2) and (3), respectively (Gumbi et al., 2017).

$$\text{Specific growth rate } \mu\ (day^{-1}) = \frac{\ln N_2 - \ln N_1}{t_2 - t_1} \quad (2)$$

where N_1 is the optical density on the initial day t_1 and N_2 is the optical density measured on day t_2.

$$\text{Divisons per day } (Div.day^{-1}) = \frac{\mu}{ln2} \quad (3)$$

The estimation of other parameters such as biomass and chlorophyll were done following the protocols described in section 3.2.4.1. The microalgae and consortia biomass were harvested at late log phase by centrifugation, washed twice using distilled water and dried in an oven at 60 °C and used for further analysis.

3.2.5.2 Characterization of selected microalgal consortia

The microalgal consortium revealing maximum growth and biomass productivity was selected and further characterized for phytohormones and macro-and micronutrients. The detailed procedures are as follows

3.2.5.2.1 Phytohormones using Ultra high performance liquid chromatography UHPLC MS/MS system

The phytohormone analysis was done using Sciex Exion LC AD series liquid chromatography system coupled to a Sciex 5500+ Triple Quadrupole mass spectrometer (Promolab T/A separations, South Africa) (UHPLC MS/MS) for the selected individual isolates of cyanobacteria and green microalgae as well as the consortium. The standards of phytohormones such as indole acetic acid (IAA: $C_{10}H_9NO_2$ MW 175.18) and zeatin (Z:$C_{10}H_{13}N_5O$ MW 219.24) were purchased from Sigma-Aldrich (USA). Separate stock solutions were prepared and diluted in methanol (LC-MS CHROMASOLVTM ≥99.9 %) to plot the calibration graph. The microalgal phytohormones were extracted based on methods described by Yalçın et al. (2019) with slight modifications. The wet microalgal biomass was ground in liquid N and the phytohormones were extracted with methanol/ water (3: 1 v/v) in ultrasonic bath. After extraction, the mixture was centrifuged and the residue was re-extracted with additional solvent for 1 h (Yalçın et al., 2019). The combined extract was filtered and evaporated to dryness using inert N. The dried microalgal extract was redissolved in 20 % methanol and the final extract was purified and enriched by solid-phase extraction processes (Supelco HLB cartridges) (Appendix 2). The final extract was further analyzed using UHPLC MS/MS. The mobile phase solvent A consisted of LCMS water and 0.1 % formic acid (LCMS Lichropur™, 97.5-98.5 %) and solvent B with LCMS methanol and 0.1 % formic acid (LCMS Lichropur™, 97.5-98.5 %). The phytohormones were separated on a Shimpack GISS Reverse phase C18 column and column temperature maintained at 30 ºC.

MS generated data were then processed using the Sciex OS data managing software and calibration curves were also plotted using standards. The supernatant of consortium culture was also analyzed for IAA using the colorimetric assay as described in section 3.2.4.2.2 (Mohite et al., 2013).

3.2.5.3 Macro-and micro nutrient analysis

The dried microalgal biomass was digested in a microwave (Milestone S.R.L., 1200 W; Italy) at 1000 W and 100 °C for 10 min for the nutrient analysis. Micronutrients such as Ca, Fe, Cu, Mn and Zn, in the samples were analyzed by Agilent 4200 Microwave plasma- atomic emission spectrometer (MP-AES) (Ramsundar et al., 2017). Macronutrients such as P, K were quantified using inductively coupled plasma-optical spectroscopy (ICP-OES) (ICPE-9000, Shimadzu, Japan) (Rao et al., 2011). The C and N content in the microalgal biomass was quantified using CHNS analyzer (Vario EL Cube Elemental Analyzer, Frankfurt Germany).

3.2.6 Biostimulant potential of the selected microalgal consortium

A seed germination assay was performed to study the potential of selected microalgal consortium as biostimulant on chili. The biomass extract of the microalgal consortium, supernatant, and consortium culture were evaluated to determine the biostimulant potential using a seed germination assay. The extracts were prepared using fresh biomass of the consortium (500 mg) and homogenized with 100 mL of distilled water (D.W.) at pH 7. Seeds were treated with different concentrations of the biomass extracts of the consortium. Different treatments were as follows:

1. S1, Control, 0 % extract (10 mL D.W.)
2. S2, 50 % concentration (5 mL extract in 5 mL D.W.)
3. S3, 100 % concentration (10 mL extract)
4. S4, Consortium culture supernatant (10 mL)
5. S5, Consortium culture (10 mL)

The chili seeds were surface sterilized in 70 % ethanol for 1 min followed by 0.1 % $HgCl_2$ for 30 seconds then further washed four times with sterile distilled water (Priya et al., 2015). The pre-treated seeds were transferred to sterile Petri plates and soaked in 10 mL of respective treatment solutions for 24 h for priming. Following the 24 h priming period, the seeds were placed on moist 90 mm Whatman no 1 filter paper placed in Petri plates and incubated in dark under standard laboratory conditions. The germinated seeds were incubated in a culture room under standard laboratory conditions with a 16 h light/8 h dark cycle. The moisture of the plates were maintained using distilled water and the seeds were monitored every 24 h for 10 days and were considered germinated if at least 2 mm of the radicle emerged (Garcia-Gonzalez and Sommerfeld, 2015). The morphometric parameters of seeds, such as germination percentage (GP), shoot length, root length, fresh weight, dry weight and number of leaves, were recorded (Garcia-Gonzalez and Sommerfeld, 2015). GP was calculated using equation 4.

$$GP = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} * 100 \quad (4)$$

3.2.7 Statistical analysis

Statistical analysis was done using SPSS 29.0 software. All experiments were carried out in triplicate and the results are expressed as the average value ± standard deviation. One way ANOVA was used to determine whether there were significant differences between the mean values of treatments and their respective controls. A post hoc (Tukey's HSD) test using Duncan's test algorithms was carried out at the level of $p < 0.05$.

3.3 Results and discussion

3.3.1 Isolation and screening of cyanobacteria and microalgae

A key component of microalgal research is the search for indigenous microalgae in unexplored biodiverse terrestrial habitats that are capable of exhibiting bio-inoculant properties. A variety of isolates from natural environments could easily be adapted, propagated, and maintained in artificial environments (Gumbi et al., 2017). In the present study, thirteen microalgal strains (cyanobacteria and green microalgae) were isolated from various soil environments around

Durban, Kwa Zulu Natal, South Africa (Fig. 3.1). Different isolates were categorized based on the microscopic morphometric analysis. A total of six green-microalgal strains *viz*. *Chlorella sorokiniana, Chlorella* sp. (two strains), *Desmodesmus armatus, Graesiella emersonii, Chlamydomonas* sp., and seven cyanobacterial strains *viz*. *Anabaena* sp., *Nostoc* sp. (three strains), *Desmonostoc magnisporum, Nostoc linckia, Calothrix* sp. were identified (Fig. 3.1). It was observed that the cyanobacteria that were isolated were able to fix N due to the presence of heterocysts within the cells. The molecular identification using 16S rRNA and 18S rRNA gene sequencing further confirmed the identity of isolated cyanobacteria and green microalgal strains, respectively (Table 3.1). The phylogenetic tree was constructed from the alignment and bootstrap analysis using 1000 replicates by neighbour-joining method (Appendix 1). The sequences for all the isolates were submitted to GenBank, and the obtained accession number is listed in Table 3.1.

Table 3.1 Taxonomic identity of microalgae isolates from native agricultural fields, Durban, KwaZulu Natal.

Strain name	Genus	Identity (%)	Query cover (%)	Genbank number
1_Alg-AB1	*Chlorella sorokiniana*	99.87	98	LC505550.1
2_Alg-AB1	*Chlorella* sp.	99.47	99	KJ654314.1
3_Alg-AB1	*Desmodesmus armatus*	99.85	99	MN817678.1
4_Alg-AB1	*Chlorella* sp.	99.7	100	MN906179.1
5_Alg-AB1	*Graesiella emersonii*	100	100	MK541794.1
6_Alg-AB1	*Chlamydomonas* sp.	98.84	100	FR865553.1
9_907-R	*Nostoc muscorum*	92.63	100	AY218828.2

10_907-R	*Nostoc* sp.	98.86	99	MK247970.1
11_907-R	*Nostoc* sp.	98.4	100	MK247969.1
12_907-R	*Desmonostoc magnisporum*	99.5	100	MH497066.1
13_907-R	*Nostoc linckia*	94.49	100	AP018222.1
14_907-R	*Nostoc* sp.	98.99	100	KY283062.1
15_907-R	*Calothrix* sp.	98.84	100	LC455619.1

3.3.2 Preliminary characterization of microalgal isolates

The preliminary characterization of different microalgal strains was carried out based on their growth, biomass, and chlorophyll concentration. As shown in the figures (Fig. 3.1a, b, c and d), cyanobacteria and green microalgae exhibited different growth profiles. According to their growth profiles, all strains were harvested at the late log phase of their growth cycle. Biomass and chlorophyll concentration were higher ($p < 0.001$) in *Nostoc* sp. (1.2 g L^{-1} and 7.1 µg L^{-1}), *Desmonostoc magnisporum* (1.0 g L^{-1} and 7.7 µg L^{-1}), *Chlorella* sp. (1.0 g L^{-1} and 22.5 µg L^{-1}) and *Desmodesmus armatus* (0.93 g L^{-1} and 28 µg L^{-1}) as compared to other strains (Fig. 3.1a-d). Among the cyanobacteria, the highest productivity was observed in *Nostoc* sp. (41.0 mg L^{-1} d^{-1}) followed by *Desmonostoc magnisporum* (36.49 mg L^{-1} d^{-1}). While among green microalgae, the highest biomass productivity was observed in *Chlorella* sp. (65 mg L^{-1} d^{-1}) followed by *Desmodesmus armatus* (58.13 mg L^{-1} d^{-1}). Also, the lowest biomass productivity was observed in one of the *Nostoc* sp. (20.00 mg L^{-1} d^{-1}) and one of the *Chlorella* sp. (23.75 mg L^{-1} d^{-1}). Thus, out of thirteen strains, four strains of N-fixing cyanobacteria (*Desmonostoc magnisporum*, *Nostoc linckia*, *Nostoc* sp., and *Calothrix* sp.) and four strains of non-N-fixing green microalgae

(*Desmodesmus armatus, Graesiella emersonii, Chlamydomonas* sp., and *Chlorella* sp.) were selected for further screening (Fig. 3.1a-m & 3.2a-d).

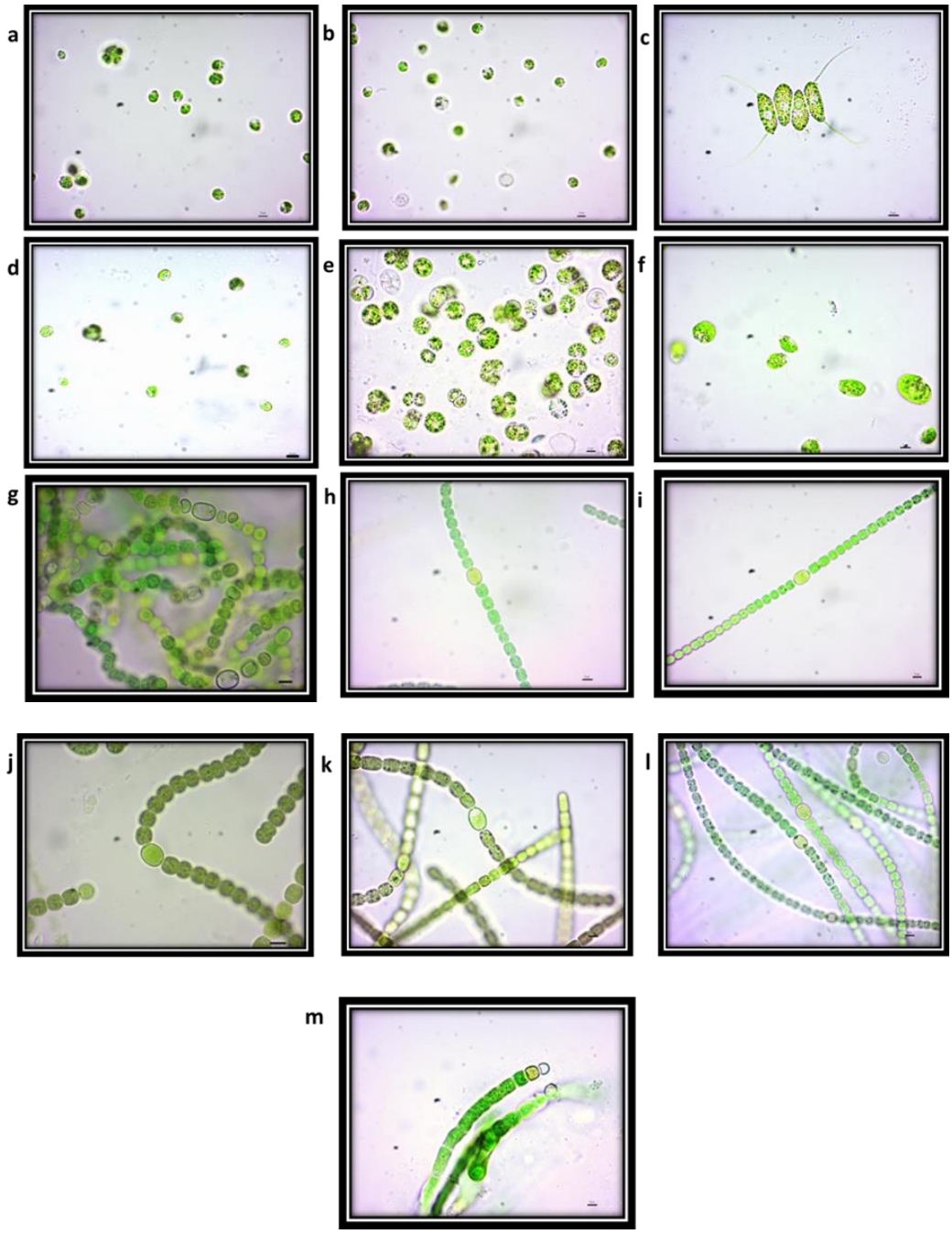

Figure 3.1 Photomicrograph of isolated indigenous green microalgae and cyanobacteria from the agricultural fields. (a) 1_Alg-AB1, *Chlorella sorokiniana* (b) 2_Alg-AB1, *Chlorella* sp. (c) 3_Alg-AB1, *Desmodesmus armatus* (d) 4_Alg-AB1, *Chlorella* sp. (e) 5_Alg-AB1, *Graesiella armatus* (f) 6_Alg-AB1, *Chlamydomonas* sp. (g) 9_907-R, *Nostoc muscorum* (h) 10 _907-R,

Nostoc sp. (i) 11 _907-R, *Nostoc* sp. (j) 12_907-R, *Desmonostoc magnisporum* (k) 13_907-R, *Nostoc linkia* (l) 14 _907-R, *Nostoc* sp. (m) 15 _907-R, *Calothrix* sp Scale bar 10µm.

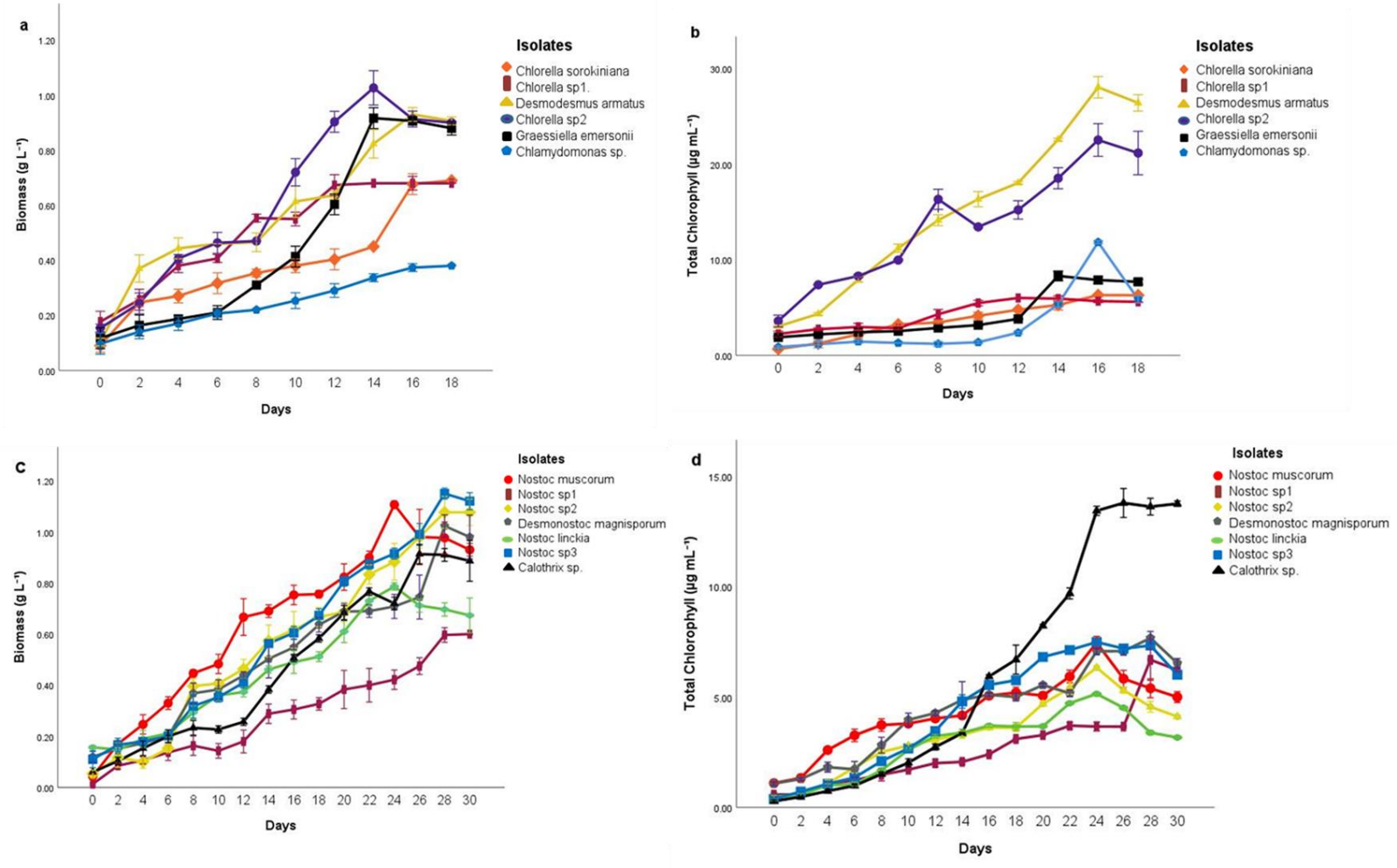

Figure 3.2 Biomass and chlorophyll content of green microalgal isolates (a, b) and cyanobacterial isolates (c, d).

3.3.3 Characterization and selection of microalgal isolates for consortia development

3.3.3.1 Exopolymers

Many cyanobacteria and green microalgae have a mucilaginous coating composed primarily of polysaccharides (carbohydrates) and are classified as exopolymers (EPS) based on their chemical composition (Rawls et al., 2003). It has been demonstrated that microalgal EPS can be used in agriculture to prevent soil erosion by binding the soil particles together and increasing the size of soil aggregates (Alvarez et al., 2021b). In the present study, the selected cyanobacterial and green microalgal isolates were analyzed for exopolymer production at the late log phase of growth. Among the tested cyanobacterial and green microalgal strains, the carbohydrate EPS content was significantly high in *Nostoc* sp. (78.8 mg L^{-1} ($p < 0.001$)), followed by *Calothrix* sp. (64.26 mg L^{-1} ($p < 0.001$)). The highest protein EPS content of 109.9 mg L^{-1} and 108.7 mg L^{-1} ($p < 0.001$) was observed in *Desmonostoc magnisporum* and *Nostoc* sp. (Fig. 3.3a). Heterocystous filamentous N-fixing cyanobacterial strains showed higher production of the carbohydrate and protein EPS compared to the green microalgal isolates. In our study, the carbohydrate EPS content of *Nostoc* sp. and *Calothrix* sp. was 2.5 and 2.6 times higher than the EPS content of *Nostoc* sp. (31 mg L^{-1}) and *Calothrix* sp. (24 mg L^{-1}) reported in a previous study, respectively (Singh et al., 2011). However, Pippo et al. (2013) reported 5.5 % and 7.3 % higher carbohydrate EPS content in *Nostoc* sp. and *Calothrix* sp. as compared to the strains in our study, respectively. The EPS content in *Nostoc* sp. was 2.07 times higher than the released polysaccharide content of *Anabaena cylindrica* and *Nostoc* sp. reported in a previous study (Xue et al., 2017). Thus, *Nostoc* sp., and *Calothrix* sp. were identified as potential exopolymer producing strains from the agricultural soil of the KwaZulu Natal region, South Africa.

3.3.3.2 Phytohormones

The selected isolates were further analyzed for the plant growth promoting substances. Initially, colorimetric analyzes were done for the eight strains. The colorimetric results have shown that, each of the eight strains released IAA into the culture medium. A significantly higher IAA concentration of 1.7 mg L^{-1} ($p < 0.001$) was observed in *Calothrix* sp. and *Nostoc* sp., followed by *Desmonostoc magnisporum* (1.4 mg L^{-1}) and *N. linckia* (1.3 mg L^{-1}) (Fig. 3.3b). Among the green microalgae, *Chlamydomonas* sp. showed the highest IAA

concentration of 1.5 mg L^{-1} followed by *Chlorella* sp. of 1.4 mg L^{-1}, *D. armatus* and *Graessiella emersonii* of 1.3 mg L^{-1}.

Studies have shown that microalgal extracts may contain phytohormones such as auxins, cytokinins, ethylene, abscisic acid and gibberellins, which can be used as biostimulants in agriculture (Stirk, 2013, Stirk et al., 2013, Parmar et al., 2023). Lu and Xu (2015) revealed the phytohormones in microalgae showed similar regulatory actions as in higher plants, but the detailed studies are still lacking. Microalgae such as species of *Chlorella*, *Coenochloris*, *Acutodesmus*, *Scenedesmus*, and *Chlorococcum* were reported to contain auxin concentrations ranging from 0.18 to 99.83 nmol g^{-1} dry weight (Stirk, 2013). As shown in our study, the production of IAA varied considerably among the isolated cyanobacteria and microalgae. Similarly, *Nostoc* sp., *Calothrix* sp., *Chlorella* sp., and *D. armatus* showed comparatively higher concentrations of biomass, exopolymers and phytohormones; thus, these strains were selected for consortia development (Fig. 3.1a-d).

3.3.4 Development and screening of microalgal consortia, and their comparison with individual isolates

In total, eight consortia were developed *viz*. T1, T2, T3, T4, T5, T6, T7, T8 using the selected cyanobacterial and green microalgal strains in N free BG-11 medium (Fig. 3.5 a-h).

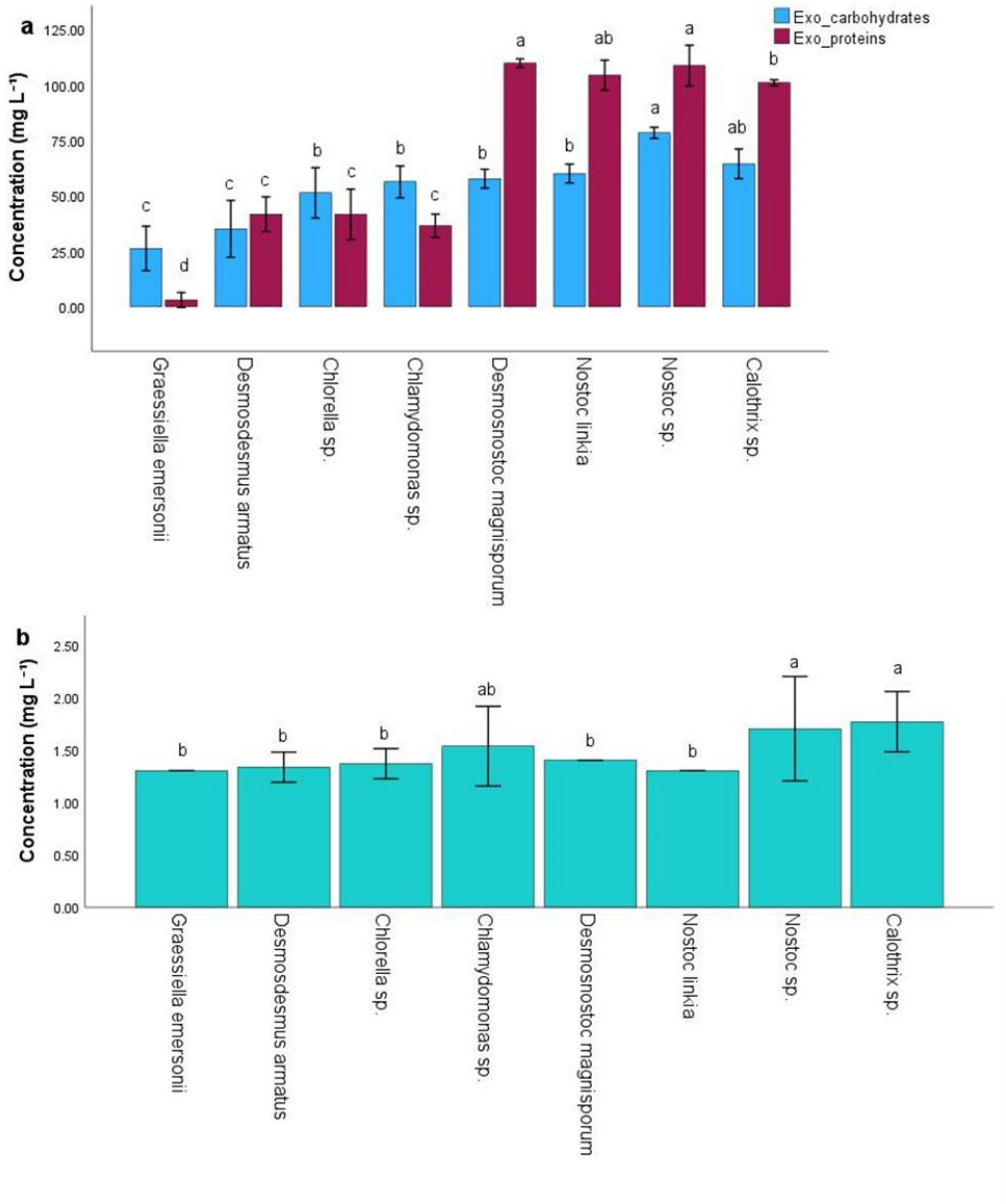

Figure 3.3 (a) Exopolymers (Carbohydrates and proteins) and (b) the indole acetic acid concentration in the culture supernatant of selected cyanobacterial and green microalgal strains. Bars represented by different letters are significantly different at $p < 0.05$. The values indicate the average (n=3), while the bars represent the standard deviation.

The developed consortia exhibited an S-shaped growth curve pattern, and microscopic analysis provided a visual confirmation of the growth of each microalgal strain within the consortium (Fig. 3.4). The maximum biomass and chlorophyll concentration was observed in the respective consortia compared to the individual microalgae cultures. All the 8 consortia were analyzed for growth parameters periodically and harvested at the late log phase (Fig. 3.5 a-h). Among the 8 different combinations of consortia, T1, T3, T7, and T8 were growing together with high biomass production compared to other consortia i.e., T2, T4, T5, and T6.

Fig. 3.4, illustrates the biomass production of the eight microalgal consortia during the two-week period

Studies on green microalgal-cyanobacterial interactions have not been well documented (Manjunath et al., 2016). In the present study, the average specific growth rate, divisions per day and biomass productivity were also analyzed for different consortia at the late log phase and compared with the individual strains (Table 3.2). The consortium, T7 (*Desmodesmus armatus, Calothrix sp., and Nostoc* sp.) revealed the highest specific growth rate (0.25 d^{-1}), with divisions per day (0.36 $Div.day^{-1}$) and biomass productivity of 91.3 mg L^{-1} d^{-1}, which were significantly higher ($p < 0.001$) than all other consortia combinations and individual isolates. Consortium T7 showed 54.5 %, 53.7 %, 54.1 % and 50.2 % increase in the biomass production when compared to the individual isolates of *Desmodesmus armatus, Chlorella* sp., *Calothrix* sp., and *Nostoc* sp., respectively. Thus, results indicated that the selected microalgal consortium can significantly enhance the production of biomass. Gautam et al. (2019) reported that the co-cultivation of *Scenedesmus obliquus* with *Anabaena variabilis*, *Nostoc muscorum* and *Westiellopsis prolifica* produced 52.4 %, 135 % and 50 % increase increase in biomass production than the individual *Scenedesmus obliquus* culture. These findings support our observations that the N-fixing cyanobacteria and green microalgae in the consortia could grow without the addition of N to the growth media, indicating that N fixed by cyanobacteria supported the growth of the green microalgae. A previous study reported that biomass productivity increased remarkably when *Chlorella vulgaris* was co-cultured with *Mesorhizobium sangaii* in N-deficient conditions (Wei et al., 2019). While there is considerable literature on microalgal and N-fixing bacterial interaction showing enhanced biomass, further studies are required to confirm the mutual growth of non-N fixing green microalgae and N-fixing cyanobacteria (Fuentes et al., 2016).

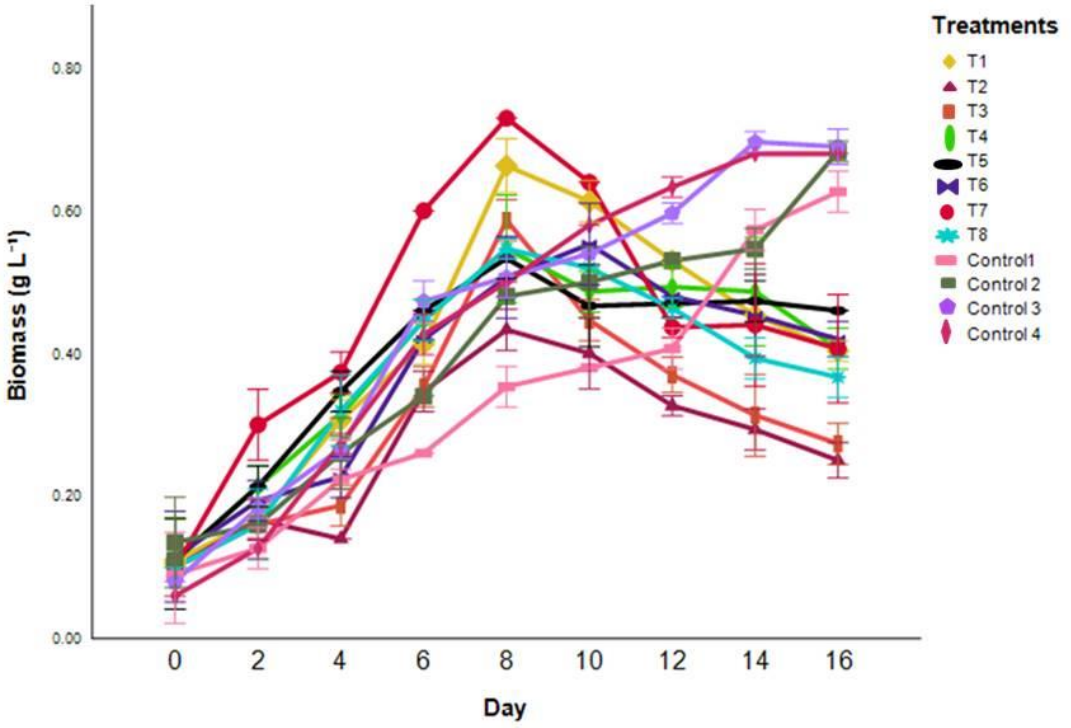

Figure 3.4 Growth profile of the eight different microalgal consortia and individual isolates. Control 1 (*Calothrix*), Control 2 (*Nostoc*), Control 3 (*Desmodesmus armatus*), Control 4 (*Chlorella*), T1 (*Desmodesmus armatus* and *Calothrix* sp.), T2 (*Chlorella* sp., and *Calothrix* sp.), T3 (*Desmodesmus armatus* and *Nostoc* sp.), T4 (*Chlorella* sp., and *Nostoc* sp.), T5 (*Desmodesmus armatus*, *Chlorella* sp., and *Calothrix* sp.), T6 (*Desmodesmus armatus*, *Chlorella* sp., and *Nostoc* sp.), T7 (*Desmodesmus armatus*, *Calothrix* sp., and *Nostoc* sp.), and T8 (*Chlorella* sp., *Calothrix* sp., and *Nostoc* sp.). The bars represent standard deviation.

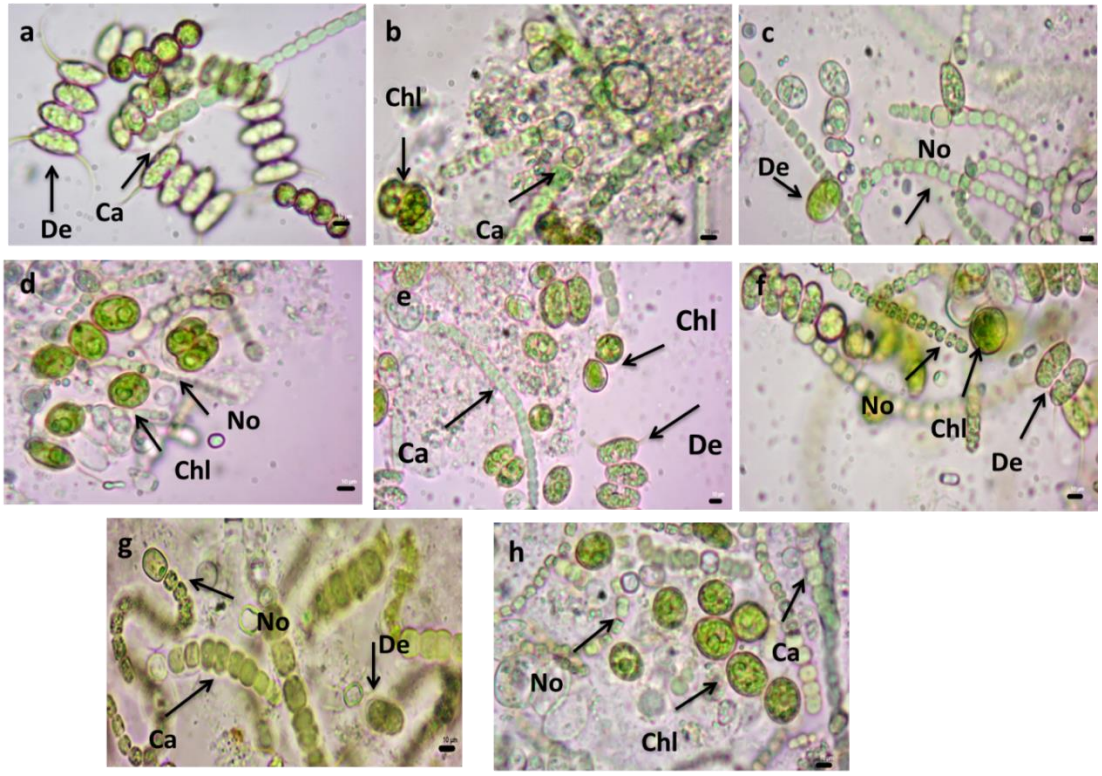

Figure 3.5 Photomicrographs illustrating the different microalgal consortia grown in nitrogen-deficient BG-11 medium at their late-log phase of growth. a, *Desmodesmus armatus* and *Calothrix* sp.; b, *Chlorella* sp., and *Calothrix* sp.; c, *Desmodesmus armatus* and *Nostoc* sp.; d, *Chlorella* sp., and *Nostoc* sp.; e, *Desmodesmus armatus*, *Chlorella* sp., and *Calothrix* sp.; f, *Desmodesmus armatus*, *Chlorella* sp., and *Nostoc* sp.; g, *Desmodesmus armatus*, *Calothrix* sp., and *Nostoc* sp.; h, *Chlorella* sp., *Calothrix* sp., and *Nostoc* sp. De, *Desmodesmus armatus*, Chl, *Chlorella* sp., Ca, *Calothrix* sp., No, *Nostoc* sp. Scale bar 10µm

3.3.5 Characterization of the selected microalgal consortium and its comparison with individual strains

3.3.5.1 Exopolymers

A comparison of the exopolymer content of the different consortium treatments and that of the individual microalgal strains was also conducted (Fig. 3.6a). The carbohydrate EPS content in consortium T7 was found to be 5.03 mg L^{-1}, which was significantly lower ($p < 0.001$) than *Desmodesmus armatus* (23.12 mg L^{-1}). While the protein EPS content was significantly higher ($p < 0.001$) in consortium T7 (41.9 mg L^{-1}) than *Calothrix* sp. (30.14 mg L^{-1}) and *Desmodesmus armatus* (26.02 mg L^{-1}), however, there was no significant difference in the protein EPS content of T7 and *Nostoc* sp. (41.31 mg L^{-1}).

Table 3.2 Specific growth rate, divisons per day and biomass productivity of the selected individual microalgal strains (control) and different consortia at their respective late log phases.

Microalgal strains	Specific growth rate (per day)	Divisions per day (per day)	Biomass productivity (mg L^{-1} d^{-1})
Desmodesmus armatus	0.15 ± 0.00^{de}	0.22 ± 0.00^{de}	49.8^{i}
Chlorella sp.	0.18 ± 0.00^{cd}	0.26 ± 0.01^{cd}	49.1^{k}
Nostoc sp.	0.12 ± 0.01^{e}	0.18 ± 0.01^{e}	49.4^{j}
Calothrix sp.	0.13 ± 0.03^{e}	0.19 ± 0.02^{e}	45.9^{l}
T1	0.18 ± 0.02^{cd}	0.26 ± 0.02^{cd}	82.9^{b}
T2	0.21 ± 0.03^{bc}	0.30 ± 0.02^{bc}	54.2^{h}
T3	0.22 ± 0.01^{ab}	0.32 ± 0.01^{ab}	73.3^{c}
T4	0.21 ± 0.02^{abc}	0.30 ± 0.02^{bc}	68.3^{e}
T5	0.21 ± 0.01^{bc}	0.30 ± 0.02^{bc}	66.7^{f}
T6	0.14 ± 0.01^{e}	0.21 ± 0.01^{de}	55.5^{g}
T7	0.25 ± 0.02^{a}	0.36 ± 0.02^{a}	91.3^{a}
T8	0.16 ± 0.01^{abc}	0.24 ± 0.02^{de}	69.1^{d}

Values in the columns denoted by different letters are significantly different at $p < 0.05$.

In a previous study, Xue et al. (2017) studied the growth profile and exopolymer production in *Anabaena cylindrica, Nostoc* sp. during their co-cultivation. Their results indicated that the protein EPS content in co-cultivated *Anabaena cylindrica* and *Nostoc* sp. was significantly higher than in pure cultures, in agreement with our findings. In another study, after 10 days of cultivation, the protein EPS content of *Chlorella vulgaris* and *Mesorhizobium sangaii* co-

cultures varied between 2.04 and 11.94 mg L^{-1}, which was higher than the control and the polysaccharide concentration was lower than the control (Wei et al., 2019). Carbohydrates may serve as a source of energy for mutual growth in consortium cultures and could reduce the carbohydrate concentration in the supernatant (Wei et al., 2019), possibly explaining decreased carbohydrate EPS in consortium T7.

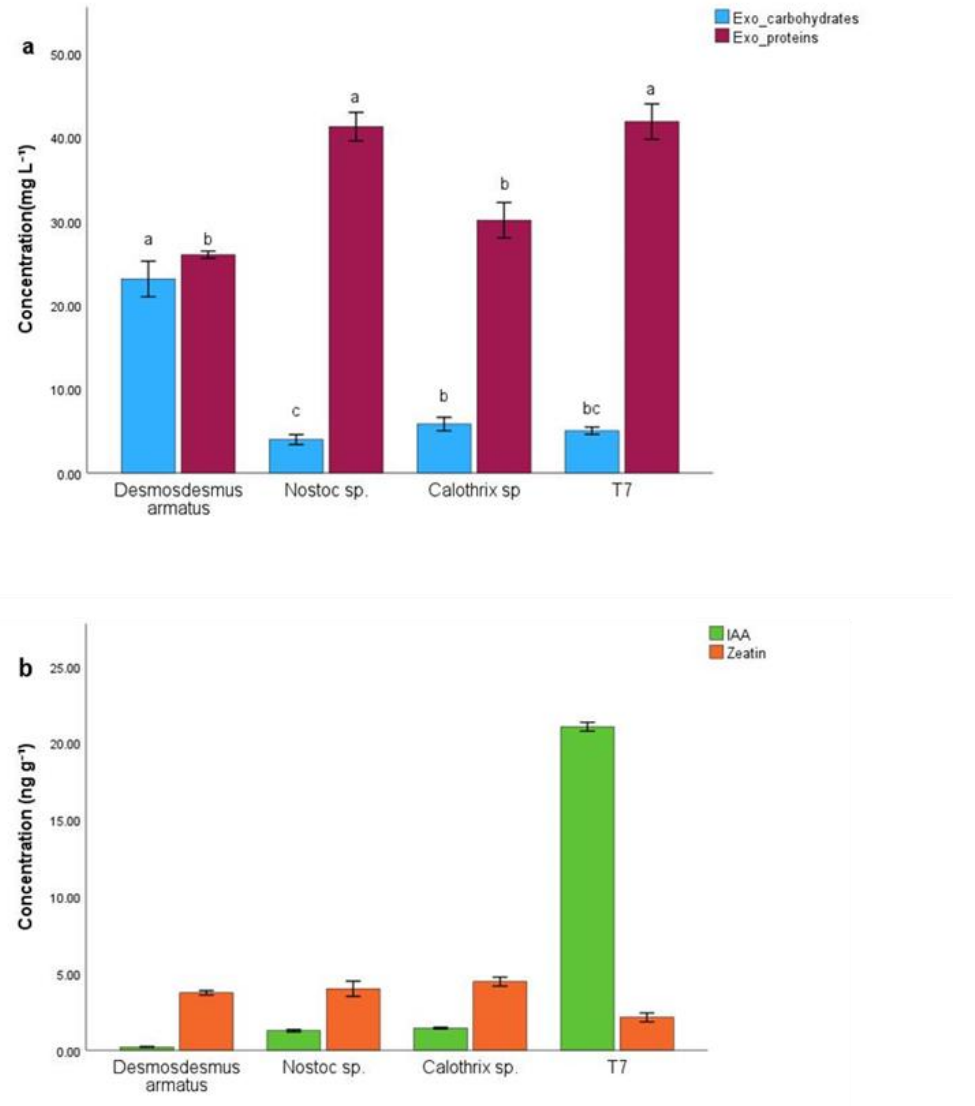

Figure 3.6 Comparison of individual strains such as *Desmodesmus armatus, Nostoc* sp., *Calothrix* sp. and selected consortium, T7 (*Desmodesmus armatus, Nostoc* sp. and *Calothrix* sp.) a) exopolymers b) phytohormones. Bars represented by different letters are significantly different at $p < 0.05$. The values indicate the average (n=3), while the bars represent the standard deviation.

3.3.5.2 Phytohormones

Phytohormones are organic substances produced in trace amounts by plants and microorganisms which regulate physiological processes in plants and function in harmony with one another to aid the growth, development, and stress response of plants (Patel et al., 2015). Among the major phytohormones, auxins and cytokinins play a central role in plant development and physiology, including root and shoot growth (Woodward and Bartel, 2005, Duran-Medina et al., 2017). In the present study, the best performing consortium T7 was evaluated for phytohormone production potential. IAA and Zeatin concentrations were estimated in T7 using UHPLC LCMS/MS system and compared with the individual strains (Fig. 3.6b). At the late log phase, T7 showed significantly higher phytohormone concentration of IAA (21.06 ng g^{-1}) ($p < 0.001$) compared to the *Desmodesmus armatus* (0.2 ng g^{-1}), *Nostoc* sp. (1.52 ng g^{-1}) and *Calothrix* sp., (2.54 ng g^{-1}) (Fig. 8b). However, zeatin concentration in the consortium (2.2 ng g^{-1}) was observed to be less than the individual strains. The highest zeatin concentration of 4.4 ng g^{-1} was found in *Calothrix* sp., followed by *Nostoc* sp. (4.0 ng g^{-1}), *Desmodesmus armatus* (3.8 ng g^{-1}). Moreover, in the colorimetric assay, the concentration of IAA in the consortium supernatant was higher (2.4 mg L^{-1}) as compared to the individual strains of *Calothrix* sp. (1.7 mg L^{-1}), *Nostoc* sp. (1.7 mg L^{-1}) and *Desmodesmus armatus* (1.3 mg L^{-1}).

Hashtroudi et al. (2012) quantified three auxins (IAA, indole-3-propionic acid and indole-3-butyric acid) in three Iranian isolates belonging to heterocystous cyanobacteria *Anabaena* and *Nostoc* and reported phytohormone concentrations ranging from 2.19 to 9.93 ng g^{-1}. These concentrations were slightly higher than the microalgal strains in our study except *Calothrix* sp. A study using *Wollea vaginicola*, *Nostoc carneum* and *N.punctiforme* found IAA levels of 73 , 35 and 37 ng g^{-1}, respectively, which were significantly higher than those in this study (Zarezadeh et al., 2020). In the present study, the consortium of cyanobacteria and green microalgae produced significantly more IAA than individual strains. However, studies on the effect of co-culturing of green microalgae and cyanobacteria are lacking to support the hypothesis that IAA concentration may be enhanced in the microalgal consortia as compared to the monocultures.

3.3.5.3 Macro- and micronutrients

As shown in Table 3.3, the selected consortium and individual isolates were also tested for macro- and micronutrients. The macro- and micronutrient content were comparatively higher in the selected consortium than the individual strains. There was a significant increase in the total C content in the consortium at 46.54 % ($p=0.001$), compared to the individual strains. The N content also increased by 17 % in the consortium than the individual strains. A 2.45 and 1.8-fold ($p < 0.01$) increase in P and K was also observed in the consortium in comparison with the individual isolates. A similar trend was also observed for the micronutrient content such as Ca, Fe, Mn (Table 3.3). Yandigeri et al. (2011) reported that cyanobacterial species like *Westelliopsis* spp. and *Anabaena variabilis* release phthalic acid to solubilize P from phosphate rock and tricalcium phosphate. Few studies also reported the ability of cyanobacteria and microalgae to produce siderophores, which help to chelate ferric iron under Fe deficient conditions and make it available to microbes and plants (Goldman, 1983, Benderliev, 1999). In another study, it was reported the cyanobacteria-bacteria consortium enriched the micronutrients, in particular Fe, Mn, Cu, and Zn, in plant parts, including grains (Manjunath et al., 2016).

3.3.6 Biostimulant potential of selected microalgal consortium

The culture, culture supernatant and biomass extracts (50 % and 100 %) of the selected consortium T7 (*Desmodesmus armatus*, *Calothrix* sp., and *Nostoc* sp.) were evaluated for their biostimulant potential based on seed germination assay in *Capsicum annum*. Approximately a seed germination percentage of 50 % was achieved on the second day of incubation. After the fifth day of incubation, the majority of treatments reached 100 % seed germination percentage. On day 6, the seed germination rate was observed to be significantly lower in the treatment with 100 % consortium biomass extracts (S3) than the control and 50 % consortium biomass extracts (S2) (Fig. 3.7a). Similarly, Hernández-Herrera et al. (2013) reported that higher concentrations of seaweed extracts exhibited a negative effect on the seed germination of tomato. Furthermore, the 50% biomass extract treatment significantly increased shoot length by 46.6% as compared to the control. The 100% biomass extract increased seedling fresh biomass by 27% and seedling dry biomass by 40% on the 10^{th} day of incubation. However, the differences were non-significant (Table 3.4). When compared to all other treatments, the leaves appeared in the majority of the treatments i.e., 50 % biomass

extract, 100 % biomass extract and supernatant treated seeds on the 10th day of analysis (Fig. 3.7b). The 50 % and 100 % biomass extract treatment showed the highest number of leaves (p = 0.02), which were significantly higher than the control (Fig. 3.8).

Previous studies have shown that microalgal extracts enhance the growth, health and nutritional composition of agricultural crops (Garcia-Gonzalez and Sommerfeld, 2015, Priya et al., 2015). Moreover, the GP of tomato and barley seeds treated with *Chlorella vulgaris*, in particular intact cells or supernatant, was up to 25 % higher after two days, and the germination time was significantly shorter (germinated on average 0.5 to 1 day sooner) than when treated with *Scenedesmus obliquus* or the water control (Alling et al., 2023). Similarly, Sugar beet seeds were hydrated with extracts from *Chlorella vulgaris* and *Scenedesmus quadricauda*, which boosted the germination rate, seedling vigor, root length, diameter, and root surface area, as well as the number of root tips (Puglisi et al., 2022).

There is inadequate data in support of using live microalgal cultures to better understand how microalgae are used as biostimulants. In this study, chili seeds treatment with biomass extracts of consortium (*Desmodesmus armatus, Calothrix* sp., and *Nostoc* sp.) and supernatant triggered seedling growth and leaf development. The seed treatment with 100 % microalgal extract showed an increase in seedling biomass, while 50 % microalgal extract enhanced the shoot length significantly. The seed treatment with 100 % microalgal extract showed an increase in seedling biomass, while 50 % microalgal extract enhanced the shoot length significantly. Both the 50 % and 100 % microalgal extracts showed a significant increase in the number of leaves when compared to the control. This indicated that microalgal cells could be used as a biostimulant and the biomass extracts can be directly applied for seed priming to improve chili seedling growth parameters.

Table 3.3 The comparison of macronutrients and micronutrients present in the selected consortium and the individual microalgae cultures

Parameters	**Microalgae**	**Cyanobacteria**		**Consortium**
	Desmodesmus armatus	*Nostoc* sp.	*Calothrix* sp.	*T7*
Carbon (%)	38.14 ± 0.2^{c}	39.41 ± 0.2^{bc}	42.02 ± 0.4^{b}	46.54 ± 0.3^{a}
Nitrogen (%)	7.17 ± 0.02^{b}	5.31 ± 0.1^{c}	5.44 ± 0.1^{c}	8.44 ± 0.1^{a}
Phosphorus (mg/kg)	21.1 ± 0.7^{c}	22.31 ± 0.1^{b}	24.32 ± 0.04^{b}	51.9 ± 0.1^{a}
Potassium (mg/kg)	5.72 ± 0.4^{b}	5.39 ± 0.2^{b}	4.27 ± 0.2^{b}	10.4 ± 0.4^{a}
Calcium (mg/kg)	22.4 ± 0.7^{c}	23.1 ± 0.4^{b}	23.6 ± 0.4^{b}	49.03 ± 0.6^{a}
Copper (mg/kg)	0.20 ± 0.04^{a}	0.12 ± 0.03^{a}	0.14 ± 0.02^{a}	0.17 ± 0.00^{a}
Iron (mg/kg)	3.6 ± 0.3^{bc}	4.42 ± 0.1^{bc}	6.2 ± 0.07^{b}	10.28 ± 0.01^{a}
Manganese (mg/kg)	1.26 ± 0.06^{b}	0.85 ± 0.02^{b}	1.2 ± 0.05^{b}	4.29 ± 0.02^{a}
Zinc (mg/kg)	12.2 ± 0.1^{a}	3.6 ± 0.1^{c}	8.3 ± 0.1^{b}	8.42 ± 0^{b}

*T7 (*Consortium of *Desmdesmus armatus*, *Nostoc* sp., and *Calothrix* sp.) The values in the rows represented by different letters are significantly different at $p < 0.05$. The values indicate the average (n=3).

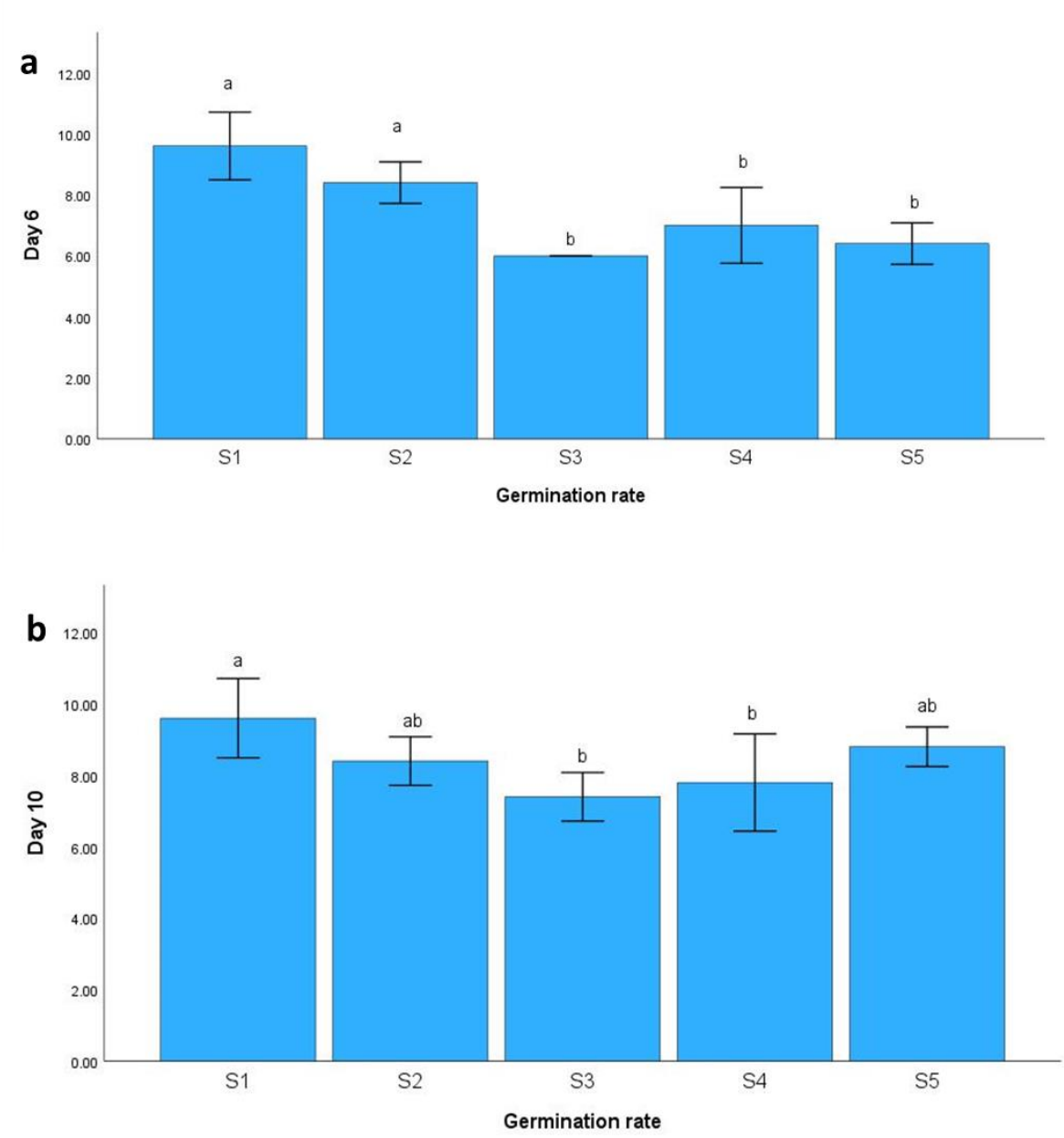

Figure 3.7 The effect of different seed treatments with microalgal consortia (*D. armatus*, *Calothrix* sp., and *Nostoc* sp.) on the germination of chili seeds on Day 6 (a) and Day 10 (b). S1, control; S2, 50 % biomass extract; S3, 100 % biomass extract; S4, consortium culture supernatant; and S5, consortium culture. Columns represented by different letters are significantly different at $p < 0.05$. The values indicate the average (n=30); bars represent standard deviation.

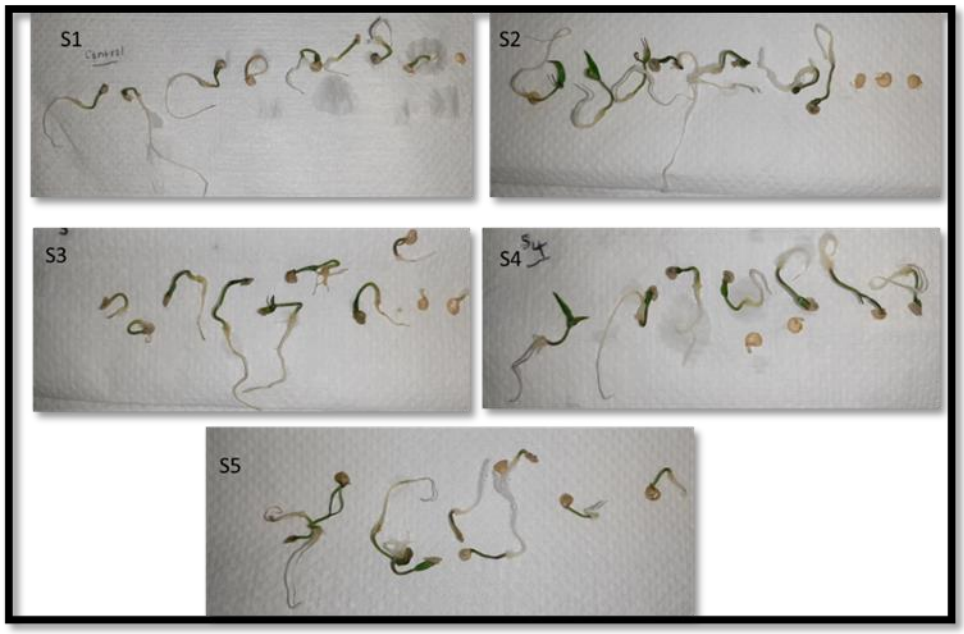

Figure 3.8 Seed germination analysis on the 10th day using different seed treatments with the biomass of microalgal consortia T7 (*D. armatus, Calothrix* sp., and *Nostoc* sp.). S1, control; S2, 50 % biomass extract; S3, 100 % biomass extract; S4, consortium culture supernatant; and S5, consortium culture.

Table 3.4 Influence of the microalgal consortium treatment on chili seeds on different plant parameters on 10th day of incubation

Treatment	Shoot length	Root length	Fresh biomass (g)	Dry biomass (g)	Number of leaves
S1	1.5 ± 0^{bc}	4.6 ± 0.7^{a}	0.022 ± 0^{ab}	0.005 ± 0^{a}	0 ± 0^{b}
S2	2.2 ± 0.2^{a}	5.2 ± 0.7^{a}	0.018 ± 0^{b}	0.003 ± 0^{a}	1.6 ± 0.9^{a}
S3	1.9 ± 0.2^{ab}	2.7 ± 0.4^{c}	0.028 ± 0^{a}	0.007 ± 0^{a}	1.6 ± 0.5^{a}
S4	1.2 ± 0.4^{c}	4.2 ± 0.7^{ab}	0.023 ± 0^{a}	0.006 ± 0^{a}	1.4 ± 0.9^{a}
S5	2.0 ± 0.5^{ab}	3.2 ± 0.4^{bc}	0.027 ± 0^{a}	0.005 ± 0^{a}	1 ± 0.8^{ab}

S1, Control; S2, 50 % concentration; S3, 100 % concentration; S4, Consortium culture supernatant; S5, Consortium culture. Values in the columns represented by different letters are significantly different at $p < 0.05$. The values indicate the average (n=30).

3.4 Conclusion

This study demonstrated the possibility of co-cultivating N-fixing cyanobacteria and green microalgae for developing consortia and biomass production in the N-deficient growth medium. Among the thirteen strains tested, the consortium of *Desmodesmus armatus*, *Nostoc* sp., and *Calothrix* sp. showed the highest growth and biomass production. The study suggested that N fixed by the cyanobacteria could support the synergistic growth of the green microalgae in the consortia. The consortium also exhibited a higher nutrient and metabolite content, such as exopolymers and phytohormones, compared to the individual strains. The 50 % extract of the selected consortium significantly increased the shoot length compared to the control. The 50 % and 100 % consortial extract-treated seeds showed a higher number of leaves on the 10^{th} day of germination, which was significantly higher than the control, thus showing the biostimulant property. Further research should focus on the interaction of microalgal consortia and the signalling and regulatory gene networks using a model system to elucidate the mechanisms of growth promotion. Techno-economic studies are required to validate the economic viability of the developed microalgal consortia for their use as bio-inoculants. Furthermore, more studies in microcosms, greenhouses, field-scale trials, and other plant species are needed to prove the potential of developed consortia as bio-inoculants.

CHAPTER FOUR

This chapter has been fully submitted to publish in journal with the title **Cyanobacteria-green microalgae consortia enhance soil fertility and plant growth by shaping the native soil microbiome of *Capsicum annuum***

Shisy Jose, Muneer Malla, Nirmal Renuka, Faizal Bux, Sheena Kumari

4.1 Introduction

Soil fertility is a key determinant of plant growth and crop productivity (Ramakrishnan et al., 2023). Soil fertility in turn is influenced by the diversity and quality of the soil microbiome, which plays an important role in the decomposition of organic matter and nutrient recycling (Alvarez et al., 2021a). Thus, soil microorganisms are highly important for preserving soil functions and are considered indicators of soil quality and fertility (Velmourougane et al., 2023). In the recent past, the extensive and indiscriminate use of chemical fertilizers in agronomic practices has resulted in environmental pollution, biodiversity loss, disturbances in the native microbiome and a reduction in crop yields (Malla et al., 2023).

The use of microalgal bio-inoculants has emerged as a potential solution to enhance soil fertility through the release of nutrients, stimulation of native microbiota, and improved plant growth and crop yields (Chittapun et al., 2017, Ammar et al., 2022). Microalgal bio-inoculants are known to contain high levels of nutrients, including macro- and micronutrients that are essential for plant growth (Osorio-Reyes et al., 2023). The application of microalgal bio-inoculants can modulate the soil microbial community and stimulate dehydrogenase activity leading to increased mineralization of organic matter and increased organic C content, which may contribute towards improved soil fertility and plant growth (Prasanna et al., 2017, Cao et al., 2023).

In agricultural soils, ecosystem function depends on the maintenance of dynamic microbial communities, and knowledge of how these microbes react to organic matter, inorganic fertilizers and bio-inoculants and sustainable soil management can help to enhance soil health and plant productivity (Tautges et al., 2016). When microalgal bio-inoculants are applied to soil, high

throughput analysis of bacterial and fungal communities can reveal taxonomic changes that influence the patterns of ecological interactions that determine the structure, function, and resilience of these communities (Wei et al., 2023). Using polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) analysis, soil inoculations with *Calothrix* sp. and a consortium of *Anabaena* sp. and *Providencia* sp. revealed changes in soil bacterial populations in comparison to non-inoculated controls in an okra crop (Manjunath et al., 2016). In addition, Priya et al. (2015) found 10-fold increase in bacterial populations (represented in species of *Bacillus*) in the roots and shoots of rice seedlings upon cyanobacterial inoculations. Renuka et al. (2016) also reported the wastewater grown green microalgal consortia (species of *Chlorella, Scenedesmus, Chlorococcum, Chroococcus*) as biofertilizer improved yields of wheat crop. After soil inoculation with microalgal biomass grown on a main effluent from a meat processing factory, a biofilm dominating *Chlorella vulgaris* boosted shoot and leaf dry mass in 60-day-old millet plants (Castro et al., 2017).

Studies have shown that root colonization by cyanobacteria and green microalgae improves plant growth and development, crop productivity, nutrient uptake and resistance to stress (Dagnaisser et al., 2022, Kholssi et al., 2022). Furthermore, it has been demonstrated that soil sampled from the rhizosphere has a stronger influence on microbial populations than bulk soil (Liu et al., 2023). As a result, it is critical to uncover the potential alterations in microbial communities in order to investigate the principle of interactions. The beneficial effects of cyanobacteria and green microalgae inoculation on plants are largely attributable to direct interactions between these organisms and their host plants; however, the microbial ecological mechanisms underpinning these effects remain largely unknown. The application of microalgal inoculants has been widely recognized as improving and enhancing soil quality and plant performance (Garcia-Gonzalez and Sommerfeld, 2015), little is known about the exact mechanism and the extent to which bio-inoculants stimulate beneficial effects.

Thus, in the present study, a chili plant-based pot experiment was established for 180 days to investigate the impacts of microalgal bio-inoculants on chili plant growth, soil physicochemical properties and native soil microbiome. The findings of this study will help in deciphering the

linkage between microalgal bio-inoculants and soil microbiome manipulations aimed at promoting sustainable plant growth and soil quality.

4.2 Materials and methods

4.2.1 Microalgal bio-inoculants and its cultivation

The microalgal isolation, cultivation and selection strategies were described in the previous section (3.2.1, 3.2.2, and 3.2.5). In brief, two native N-fixing cyanobacteria *viz*. *Nostoc* sp. and *Calothrix* sp., and a non-N-fixing green microalga *Desmodesmus armatus* were isolated from different agricultural fields of Durban, KwaZulu Natal, South Africa. The isolated species were used to develop microalgal consortia and analyzed for their potential as bio-inoculants. The developed consortia were grown in N free BG 11 media, harvested at their late log phase (Renuka et al., 2017a) and used for the preparation of formulations.

4.2.2 Experimental design

A pot-experiment was conducted in a greenhouse at Botanic gardens, Durban, South Africa - (29^0.85′ S and 31^0 14 ′ E). The average temperature in the greenhouse was 28 ± 2 °C and non-sterile topsoil was used for the experiment. A completely randomized experiment was conducted in five replicates with five treatments including (1) control (Cnt) with no treatment; carrier (Cr) control containing vermiculite: compost (1:1); (3) chemical fertilizer (Full dose) (CF) control; (4) 50 % microalgal consortium biomass + 50 % chemical fertilizer (Half dose) (Al(50 %)+CF(50 %)); (5) 100 % microalgal consortium biomass (Al(100 %)).

The microalgal formulations for Al(100 %) were prepared by mixing 1g of the wet biomass of the consortium with 1 kg of the carrier composed of vermiculite and compost (1:1) in polyethylene bags (Appendix 11). While the treatment for Al(50 %)+CF(50 %) was prepared by mixing 0.5 g of the wet biomass of the consortium with 1 kg of the carrier composed of vermiculite and compost (1:1) in polyethylene bags, and later, half dose of the chemical fertilizer was supplied (Appendix 11). The water holding capacity (WHC) of the carrier and soil was

maintained at 60 % to keep the microbes in a proper metabolic state (Prasanna et al., 2014). The developed formulations were thoroughly mixed with 2 kg of soil in the respective pots. Prior to inoculation, the chili seeds were soaked in distilled water overnight. Equal numbers of five seeds were sown in each treatment, and the seeds were germinated for 14 days in a climate-controlled culture bed maintained at 22 ± 2 °C. The fresh and dry weight and other morphological parameters, soil nutrient profile and enzymes activity were recorded at the beginning, and mid of the experiment (at 50 d) and chili crops were harvested (at 180 d), and fruits were further analyzed based on their morphological parameters (Appendix 12 and 13).

4.2.3 Plant analysis and yield analysis

The fresh weight (g) of the plants was recorded at their harvesting stage (180 d) (Appendix 13). For dry weight (g) estimation, the plants were air dried and kept in oven for 24 hours at 80 °C. Total length of the plants (cm), total number of leaves per plant, number of branches arising on the main stem in the plant was measured. The total chlorophyll (µg cm^{-2}) of the leaves at mid-crop stage was also recorded. The photosynthetic performance was recorded at mid-crop (50 d) stage of chili plant by using a DUAL-PAM 100 Chlorophyll Fluorometer (Heinz Walz Gmbh, Effeltrich, Germany). Maximal quantum yield of PSII photochemistry (Fv/Fm), and relative electron transport rate (rETR) were also calculated as described by White et al. (2011). The number of fruits harvested from plants in each treatment was counted and expressed as number of fruits per plant. The fruits were picked from plants collected and fresh weight (g) was measured. The fruits were air dried and kept in oven for 24 hours at 80 °C and dry weight (g) was determined. The length of the fruits was also measured and reported as cm (Singh, 2014). The dried plant biomass was digested in a microwave (Milestone S.R.L., 1200W; Italy) at 1000 W and 100 °C for 10 min for the macro and micronutrient analysis.

4.2.4 Soil analysis

Soil samples (15-20cm deep) were collected at the initial and mid of the experiment and subjected to different physico-chemical analysis. For dehydrogenase activity 6 gm of soil were

mixed with 0.1g calcium carbonate and 3 % Triphenyl tetrazolium chloride and incubated for 24 hrs at 30 °C. The tubes were vortexed after the addition of methanol and the absorbance was measured at 485nm with Triphenyl formazon as standard (Prasanna et al., 2015, Bharti et al., 2020). Organic C was estimated by following the standard protocol as described by (Olsen, 1954). Briefly, 1 gm of soil in 250 mL Erlenmeyer flask was oxidised by 10 mL of 1N potassium dichromate. The reaction is assisted by 20 mL of Sulphuric acid (H_2SO_4), allowed to cool for 20 - 30 min and the mixture was diluted to 200 mL using distilled water. The mixture was titrated with ferrous sulphate using ferroin indicator until the reddish colour appeared (Bartlett et al., 1994). For analysis of the total polysaccharides, 0.5 g of soil sample was taken in an Erlenmeyer flask, and 4 mL of 12 M Sulphuric acid (H_2SO_4) was added to the flask and incubated for 2 h. After incubation, the solution was diluted to 0.5 M by adding 92 mL of distilled water, and the flask was autoclaved for 1 h (103 kPa, 121 °C). After cooling, the polysaccharides in the flask were filtered, and the volume was made up to 100 mL by distilled water. For analysis, 1 mL of the filtrate was taken in a test tube, to which 1 mL of 5 % phenol solution and 5 mL of concentrated H_2SO_4 were added. After incubating at room temperature for 10 min the absorbance was measured at 490 nm using glucose as standard (Liu et al., 2005, Bharti et al., 2020). The enumeration of the total bacteria and fungus in soil samples was analysed by serial dilution plate technique using nutrient agar medium (NAM) for bacterial and potato dextrose agar (PDA) medium for fungi. The population of microbes was examined in different treatments and expressed as CFU/ml. 1g of soil samples were dissolved in 10ml of distilled water taken in test tube and its dilution factor is 10^{-1}. Similarly samples were diluted in remaining test tubes and dilution factor obtained as 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9}. From these diluted samples 0.1 mL of sample was taken and transferred to NAM plates and PDA plates and incubated at room temperature until the colony appeared (Tamizhazhagan et al., 2016).

Macronutrients such as K were quantified using Inductively coupled plasma-optical spectroscopy (ICP-OES) (Rao et al., 2011). The C and N content in the soil sample was quantified using CHNS analyzer (Vario EL Cube Elemental Analyzer, Frankfurt Germany). The dried soil sample was digested in a microwave (Milestone S.R.L., 1200 W; Italy) at 1000 W and 100 °C for 10 min for the nutrient analysis. And the micronutrients such as Ca, Fe, Cu, Mn and Zn were then

analyzed by Agilent 4200 Microwave plasma- atomic emission spectrometer (MP-AES) (Kompala-Baba et al., 2021). Soil chlorophyll was measured as an index of photosynthetic biomass using 4mL dimethyl sulphoxide (DMSO) and acetone (1:1) as extractant per gram soil. The absorbance was recorded at 663, 645 and 630 nm (Nayak et al., 2004, Bharti et al., 2020, Kokila et al., 2022). The concentrations were calculated by the formula described in Equation 4.

$$\text{Soil chlorophyll (mg g}^{-1}\text{soil)} = (11.64 * A_{663}) - (2.16 * A_{645}) + (10.10 * A_{630}) \quad (4)$$

4.2.5 Soil DNA extraction and amplicon sequencing

Soil samples from the rhizosphere were collected at their mid-crop stage (50 days after planting) as significant changes in yield between different treatments were observed. Composite soil samples were collected from each treatment pot and filtered through a 2-mm sieve to achieve uniform samples. In total, 20 samples (5 treatments x 5 replicas) were collected and stored at -80 ^{0}C for DNA extraction. Immediately, the soil DNA was extracted from 0.25g of soil (wet weight) using DNeasy Power Soil Pro Kit (Qiagen, Hilden, Germany), following the manufacture's protocol. The quality and quantity of the extracted DNA were determined by 1 % agarose gel electrophoresis and Nano-Drop 1000 spectrophotometer (Coleman Technologies Inc., USA). The general universal bacterial primers 515F (5' GTGYCAGCMGCCGCGGTAA 3') and 806R (5' GGACTACNVGGGTWTCTAAT 3') (Wasimuddin et al., 2020) and fungal primers 18S-566F 5'- CAGCAGCCGCGGTAATTCC-3' and 18S- 1200R 5'- CCCGTGTTGAGTCAAATTAAGC-3' (Hadziavdic et al., 2014) were used to amplify the 16S and ITS regions of the DNA. Resulting amplicons were purified, end repaired, and illumina specific adapter sequences were ligated to each amplicon (NEBNext Ultra II DNA library prep kit). Following quantification, the samples were individually indexed (NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1), and another AMPure XP bead-based purification step was performed. The constructed libraries were then sequenced on Illumina's NextSeq500 platform, Inqaba Biotechnical Industries, Durban South Africa).

4.2.6 Bioinformatic data processing

The Illumina generated raw reads were demultiplexed and quality filtered using Fast QC v0.11.9 (Andrews, 2010). The quality filtered reads were then imported QIIME2 v2022.2.0 (Bolyen et al., 2019), denoised using DADA 2 v1.26.0 (Callahan et al., 2016). OTU (Operational taxonomic unit) tables were created using VSEARCH global alignment algorithm (Rognes et al., 2016), and the reads were allocated to 975 and 1608 OTUs, respectively, for bacterial and fungal groups, at 97 % similarity threshold. The representative sequences of bacterial and fungal OTUs were classified against SILVA 132 database (Quast et al., 2013, Haro et al., 2021) and UNITE database (Nilsson, 2019).

4.2.7 Statistical analysis

Statistical analysis was performed by using R software (R Core, 2020) and SPSSv29.0.0.0 (Wang et al., 2019), and all the tests were deemed significant at 95 % significance level ($P \leq 0.05$). In the pot experiment, we used One-way analysis of variance (ANOVA) to test the significance between control and treatment groups, and applied Tukey's post-hoc test to determine the significance among the groups. For both bacterial and fungal communities, the alpha diversity indices and richness, including Shannon, Simpson, Chao1, ACE, Observed were calculated by using R Vegan package (function: diversity) (Liu et al., 2021). Linear discriminant analysis (LDA) effect size (LEfSe) was used to identify the differentially abundant groups (Cuartero et al., 2021). Additionally, PCoA analysis with PERMANOVA were conducted based on the Bray-Curtis distance, using the "betadisper" and "adonis" functions to test the relationship between different treatment groups (Chen et al., 2022). Venn diagram was created to identify the shared taxa between different treatments (Malviya et al., 2022). Then Redundancy analysis, RDA and Spearman's rank-order correlation were applied to test the relationships between the soil properties and soil microbial community using "envfit" function available in the vegan package in R. All the tests were performed using R microeco package and plots were made using the ggplot2 package v 3.4.2.

4.3 Results and Discussion

4.3.1 Effect of microalgal bio-inoculation on soil parameters

Microalgal inoculation significantly increased the soil dehydrogenase activity ($p < 0.05$; Fig. 4.1a). Both treatments Al(50 %)+CF(50 %) and Al(100 %) significantly increased the DHA, with Al(100 %) showing the highest increase (26.27 µg TPF $g^{-1}d^{-1}$), followed by Al(50 %)+CF(50 %) (18.98 µg TPF $g^{-1}d^{-1}$), CF (15.6 µg TPF $g^{-1}d^{-1}$), Cr (12.9 µg TPF $g^{-1}d^{-1}$) and Cnt (13.3 µg TPF $g^{-1}d^{-1}$) ($p < 0.05$; Fig. 4.1a). The soil DHA was 2-folds higher in Al(100 %) and 1.4-folds higher in Al(50 %)+CF(50 %) compared to Cnt; however, the differences were statistically significant ($p < 0.05$). In previous studies, the cyanobacterial formulations indicated 10–20 % increase in DHA activity compared to the control (Rana et al., 2015, Prasanna et al., 2016a). In this study, the application of Al(100 %) showed best performance with the enhancement of 97 % in the DHA activity compared to Cnt. This increased DHA activity in treatments may be due to the effect of N availability in soil (Storni de Cano et al., 2002, Prasanna et al., 2016b).

The microalgal treatments increased the soil organic C compared to the Cnt. The soil organic C was significantly higher in Al(100 %) (1.22 %) followed by Al(50 %)+CF(50 %) (1.1 %), Cr (0.62 %) CF (0.53 %), and Cnt (0.59 %) ($p < 0.05$; Fig. 4.1b). According to Yilmaz and Sönmez (2017) study, the photosynthetic activities of specific microalgae species may be related to a significant increase in organic C in the soil upon microalgal application. Microalgae incorporate organic C into their biomass through photosynthesis and influence the soil microbial biomass functioning (Mfundo, 2012, Osorio-Reyes et al., 2023). Microalgal bio-inoculant treatments significantly increased the soil chlorophyll ($p < 0.01$; Fig. 4.1c). The soil chlorophyll in Al(100 %) treatment was significantly higher (3-folds) compared to Al(50 %)+CF(50 %) (2.2-folds) treatments ($p < 0.05$; Fig. 4.1c). Furthermore, a significantly higher soil chlorophyll was observed in the groups of bio-inoculants compared to the Cnt ($p < 0.05$, Fig. 4.1c). Similarly, Total polysaccharides in the rhizosphere soil varied from 36.32 – 55.23 µg g^{-1}, respectively (Fig. 4.1d). When compared to Cnt, Al(100 %) showed a significant 1.5-fold increase ($p < 0.003$) among the various treatments, followed by Al(50 %)+CF(50 %) with a 1.3-fold increase ($p < 0.02$) (Fig. 4.1d). Similar results were also reported by Bharti et al. (2020), where the authors

reported that *Anabaena laxa*-primed treatments resulted in one-fold increase in soil chlorophyll and polysaccharides.

In both microalgal treatments, bacterial and fungal colony count significantly increased when compared to Cnt ($p < 0.05$; Fig. 4.1e and f). The bacterial colony count in the Al(50 %)+CF(50 %) was higher (43.2×10^{-5}) followed by Al(100 %) compared to CF, Cnt and Cr ($p < 0.05$; Fig. 4.1e). While, the fungal colony count was significantly higher (31.4×10^{-4}) Al(100 %), followed by Al(50 %)+CF(50 %) treatments compared to Cr, Cnt and CF ($p < 0.01$; Fig. 4.1f). Additionally, the treatment groups (both Al(50 %)+CF(50 %) and Al(100 %) substantially increased the soil nutrients compared to the control treatments ($p < 0.05$; Table 4.1). Nutrients such as C, P, K, and Mn showed a significant increase after the application of Al(50 %)+CF(50 %) ($p < 0.05$; Table 4.1), while, Cu and Zn were slightly decreased in Al(50 %)+CF(50 %). Similarly, soils treated with Al(100 %) showed significant increase in C ($p = 0.006$), N ($p = 0.02$), Fe ($p = 0.005$) Mn ($p = 0.009$) and Cu contents, whereas Zn decreased (Table 4.1). Microalgae are a reasonable source of C, N, and other micronutrients for soil, so their addition will affect the activity of the soil microbial populations. Moreover, fungus and microalgae are considered as mutually symbiotic organisms and Du et al. (2019) reported that fungi obtained C from living algae not from the dead algal cells. Thus, the fungal colony count was high in Al (100 %) treatments. Our findings also support the earlier work, which found that soil inoculations with formulations based on cyanobacteria boosted the soil's concentration of Fe, Zn, Mn, and Cu (Manjunath et al., 2016). In addition, it is also reported the importance of Fe, Cu, Mn and Zn in shaping the structure of microbial communities (bacteria, fungi) in soil (Peng et al., 2022). However, the timing of these effects will probably depend on the environment and the establishment of the inoculum (Cao et al., 2023). Overall, the present study demonstrated the multiple benefits of microalgal bio-inoculation on soil nutrient and enzymatic characteristics.

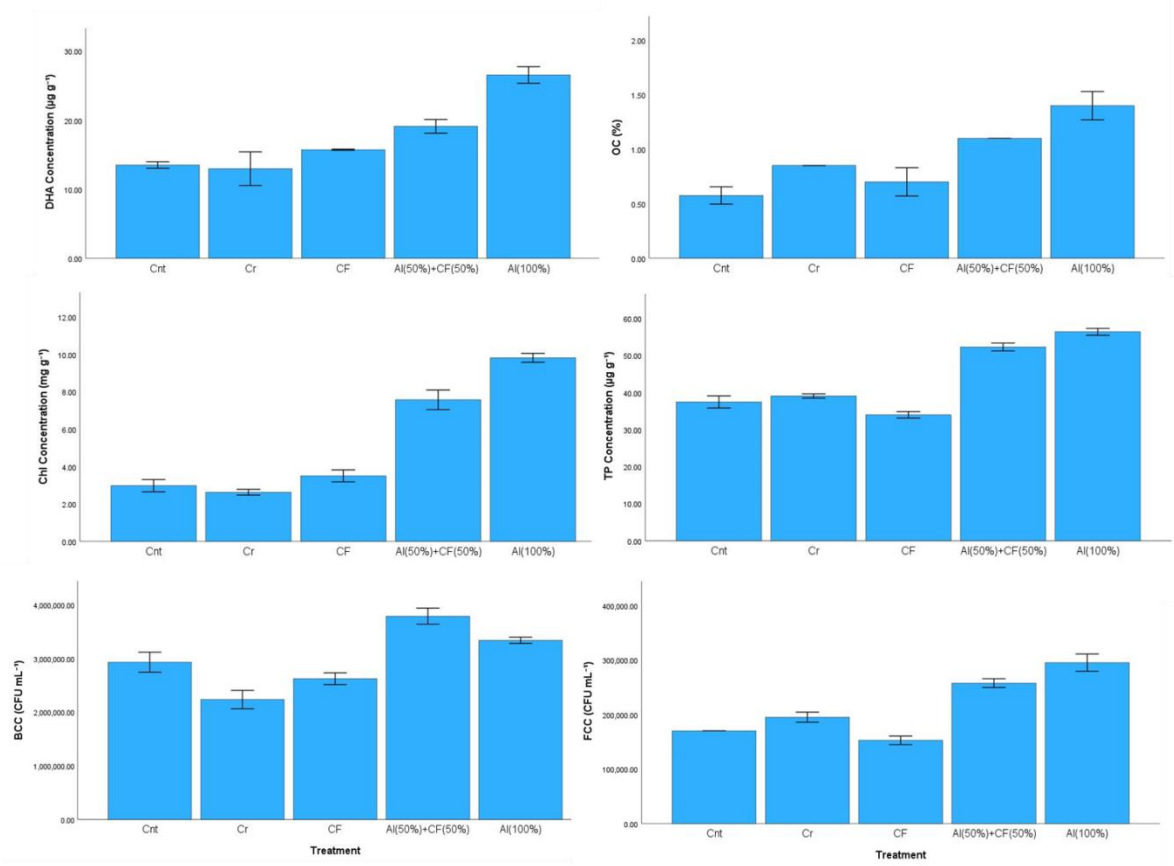

Figure 4.1 Effect of microalgal bio-inoculants on different soil parameters

Cnt, Control; Cr, Carrier; CF, Chemical fertilizer; Al(50 %)+CF(50 %), Combination of 50 % microalgal consortia and 50 % chemical fertilizer; Al(100 %), 100 % microalgal consortia. DHA, Dehydrogenase activity; OC, Organic carbon; TP, Total polysaccharides; Chl, Soil chlorophyll; BCC, Bacterial colony count; FCC, Fungal colony count. ***$p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Table 4.1 The soil macro- and micronutrient content in different treatments after the microalgal bio-inoculant application

Treatments	Carbon (%)	Nitrogen (%)	Potassium ($mgkg^{-1}$)	Phosphorus ($mgkg^{-1}$)	Calcium ($mgkg^{-1}$)	Copper ($mgkg^{-1}$)	Iron ($mgkg^{-1}$)	Manganese ($mgkg^{-1}$)	Zinc ($mgkg^{-1}$)
Cnt	4.2 ± 0.3	0.35± 0.0	10.5 ± 0.7	5.12± 0.1	6.4 ± 0.4	0.06 ± 0.0	22.7 ± 0.5	0.5 ± 0.0	1.1 ± 0.0
Cr	4.0 ± 0.1	0.32 ± 0.0	10.7 ± 0.2	5.11± 0.3	6.6 ± 0.2	0.06 ± 0.0	23.2 ± 0.1	0.4 ± 0.0	1.1 ± 0.0
CF	4.3 ± 0.2	0.35 ± 0.0	11.4 ± 0.7	11.2± 0.4*	6.7 ± 0.0	0.05 ± 0.0	23.3 ± 0.0	0.4 ± 0.0	1.0 ± 0.0
Al(50 %)+CF(50 %)	5.0 ± 0.5**	0.41 ± 0.0	22.7 ± 0.3 **	13.2± 0.03**	7.1 ± 0.1	0.05 ± 0.0	23.8 ± 0.1 **	0.5 ± 0.0	1.0 ± 0.0
Al(100 %)	5.3 ± 0.1 ***	0.42 ± 0.0*	19.1 ± 0.2	6.7± 0.2	7.1 ± 0.1	0.05 ± 0.0	23.8 ± 0.1	0.5 ± 0.0	1.0 ± 0.0

Cnt, Control; Cr, Carrier; CF, Chemical fertilizer; Al(50 %)+CF(50 %), Combination of 50 % microalgal consortia and 50 % chemical fertilizer; Al(100 %), 100 % microalgal consortia.***$p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

4.3.2 Correlation between soil parameters across different treatments

Correlation analysis showed that DHA across different treatments was significantly positively associated with Ca (r^2= 0.855, $p < 0.001$), Chl (r^2= 0.799, $p < 0.001$), Fe (r^2 = 0.786, $p < 0.001$), OC (r^2 = 0.777, $p < 0.001$), Mn (r^2 = 0.691, $p < 0.001$), Zn (r^2 = 0.674, $p < 0.01$), TP (r^2 = 0.671, $p < 0.01$) (Appendix 4). In microalgal treatments, a significantly positive correlation was observed between DHA, OC, Chl, TP, Ca, Cu, Fe, Mn, Zn (Appendix 4). The correlation between DHA and Ca, in Al(50 %)+CF(50 %) (r^2 = 1.000, $p < 0.001$) treatment showed a higher coefficient than other treatments. Similarly, in the correlation between DHA and OC, TP, Zn, Cu, the higher coefficients were recorded in the treatment with Al(50 %)+CF(50 %) and Al(100 %) than in other treatments. These findings supported by Mia et al. (2019) that OC and total N content were significantly correlated with DHA. Manjunath et al. (2016) also illustrated a significant effect of DHA, Zn and Fe concentration in soil, showing a positive correlation of microbial activity with enhanced bioavailability of nutrients. In another study, two species of acid-tolerant microalgae such as *Desmodesmus* spp. and *Heterochlorella* spp., noted an increase in C content (up to 57 %), and an increase in DHA activity (more than 500 %), which have the ability to enhance the fertility and health of soil (Shanthakumar et al., 2021). Thus, microalgae play a crucial role in soils for improving DHA, organic C, TP, microbial activity, soil fertility and plant productivity (Abinandan et al., 2019).

4.3.3 Effect of microalgal bio-inoculation in soil on plant growth and yield

The microalgal treatment significantly increased the growth and yield of chili plants compared to the Cnt ($p < 0.05$; Table 4.2 and Fig.4.2). Significant differences were observed within different treatments, shoot fresh weight (40.7 %) ($p = 0.004$), shoot dry weight (50 %) ($p < 0.001$), root fresh weight (14 %), root dry weight (11 %), root length (54 %) ($p = 0.006$) and the number of leaves (65 %) ($p = 0.03$) were high in Al(50 %)+CF(50 %) treatment compared to Cnt. The shoot length increased by 25 % and 14 % in treatments Al(100 %) and Al(50 %)+CF(50 %) compared to the Cnt ($p < 0.05$; Table 4.2). Microalgal bio-inoculants revealed the biofertilizing

properties and biostimulant properties, especially enhancement in soil quality, improvement in plant fresh weight and plant length.

The photosynthetic efficiency of chili plant leaves was recorded at the mid-crop stage. PSII quantum yield (Fv/Fm) of the leaves showed 14 % increase, and the rETR showed 2 % increase in Al(50 %)+CF(50 %) compared to Cnt, but the results were statistically insignificant ($p > 0.05$). Photosynthetic performance indicates overall health, productivity, and underlying physiological conditions (Kromdijk et al., 2016). An increase in the shoot and root (fresh and dry) weight, root length and number of leaves was concomitant with increased photosynthetic performance (Table 4.2). The macronutrients such as N and K showed 1.1 and 2.4-fold increase ($p < 0.05$) in Al(50 %)+CF(50 %) followed by Al(100 %) showing 1.04 and 1.85-fold increase, while the micronutrients such as Ca, Cu, Fe Mn and Zn were 1.3, 1.4, 1.4 and 2.8-fold higher in the Al(50 %)+CF(50 %), followed by Al(100 %), showing 1.03, 1.1, 2, 1.4 and 2.7-fold when compared to Cnt. The P content was significantly high, 2.3-fold in Al(100 %) and 2.2-fold in Al(50 %)+CF(50 %) than the Cnt ($p < 0.001$) (Appendix 8). The observed increase in macro- and micronutrients on microalgal inoculation corroborates with previous studies (Manjunath et al., 2016, Alvarez et al., 2021a, Suchithra et al., 2022). Grzesik et al. (2017) found that monocultures of *Microcystis aeruginosa, Anabaena* sp., and *Chlorella* sp. increased N, P, and K levels in willow plants. Soil inoculation using cyanobacteria-based formulations enhanced micronutrient concentrations in plant parts such as Zn in maize leaves and Ca, Fe, Zn, Mn, and Cu in wheat grains (Prasanna et al., 2015, Manjunath et al., 2016, Wuang et al., 2016).

The chili fruit parameters were analyzed at the harvest stage. Al(50 %)+CF(50 %) showed a significant increase in plant growth and yield compared to Cnt. The length of chili fruit (11 % and 10 %; $p > 0.05$), fresh weight of chili (64.5 % and 51.6 %; $p < 0.001$), dry weight of chili (50 % and 25 %), and number of fruits per plant (28 % and 132 %; $p = 0.04$) were higher in Al(50 %)+CF(50 %) and Al(100 %) than the Cnt (Fig. 4.3). Previous studies exploring the use of cyanobacteria-based inoculants or biofilmed formulations in wheat and leguminous crops showed a significant increase in plant growth (Prasanna et al., 2013, Swarnalakshmi et al., 2013, Suchithra et al., 2022). In another study, Chittapun et al. (2017) also reported the usage of half dose (50 %) of chemical fertilizer with cyanobacteria on rice plants showed significant

enhancement of the growth and yield in the rice plants. Similarly, Dash et al. (2016) reported that the application of native cyanobacterial strains in a pot experiment resulted in a significant increase in the grain yield. Thus, indigenous species grow and adapt to the local environment, enabling them to function in changing climatic conditions. Additionally, cyanobacteria and microalgae produce auxins, cytokinins and gibberellins, the bioactive chemicals that help in plant growth regulation and their metabolism (Hashtroudi et al., 2012). In this study also, the selected microalgae produce auxin and cytokinins that are considered as plant growth promoting substances enhancing plant weight, length and yield. This supports the present observations regarding the potential of microalgal consortia to improve the overall productivity of the chili crop.

Table 4.2 Effect of microalgal bio-inoculants on chili plant parameters

Treatments	Shoot fresh weight	Shoot dry weight	Shoot length	Root fresh weight	Root dry weight	Root length	Number of leaves per plant
Cnt	5.2±0.3	1.4±0.1	24.8±0.5	1.6±0.1	1.0±0.0	7.6±0.4	32.0±0.9
Cr	4.6±0.5	1.7±0.1	26.9±0.9	1.5±0.1	0.9±0.0	7.8±0.6	34.0±0.4
CF	5.3±0.3	1.5±0.1	28.0±0.6	0.8±0.1	0.4±0.1	10.6±0.9	36.0±2.2
Al(50 %)+ CF(50 %)	7.4±0.3***	2.1±0.0***	28.3±0.6	3.8±0.1	2.1±0.0	11.7±0.6**	53±1.2**
Al(100 %)	6.8±0.2	1.9±0.1	31.0±0.9	3.2±0.0	1.5±0.0	10.9±0.7	45.3±0.5

Cnt, Control; Cr, Carrier; CF, Chemical fertilizer; Al(50 %)+CF(50 %), Combination of 50 % microalgal consortia and 50 % Chemical fertilizer; Al(100 %), 100 % microalgal consortia. p value: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

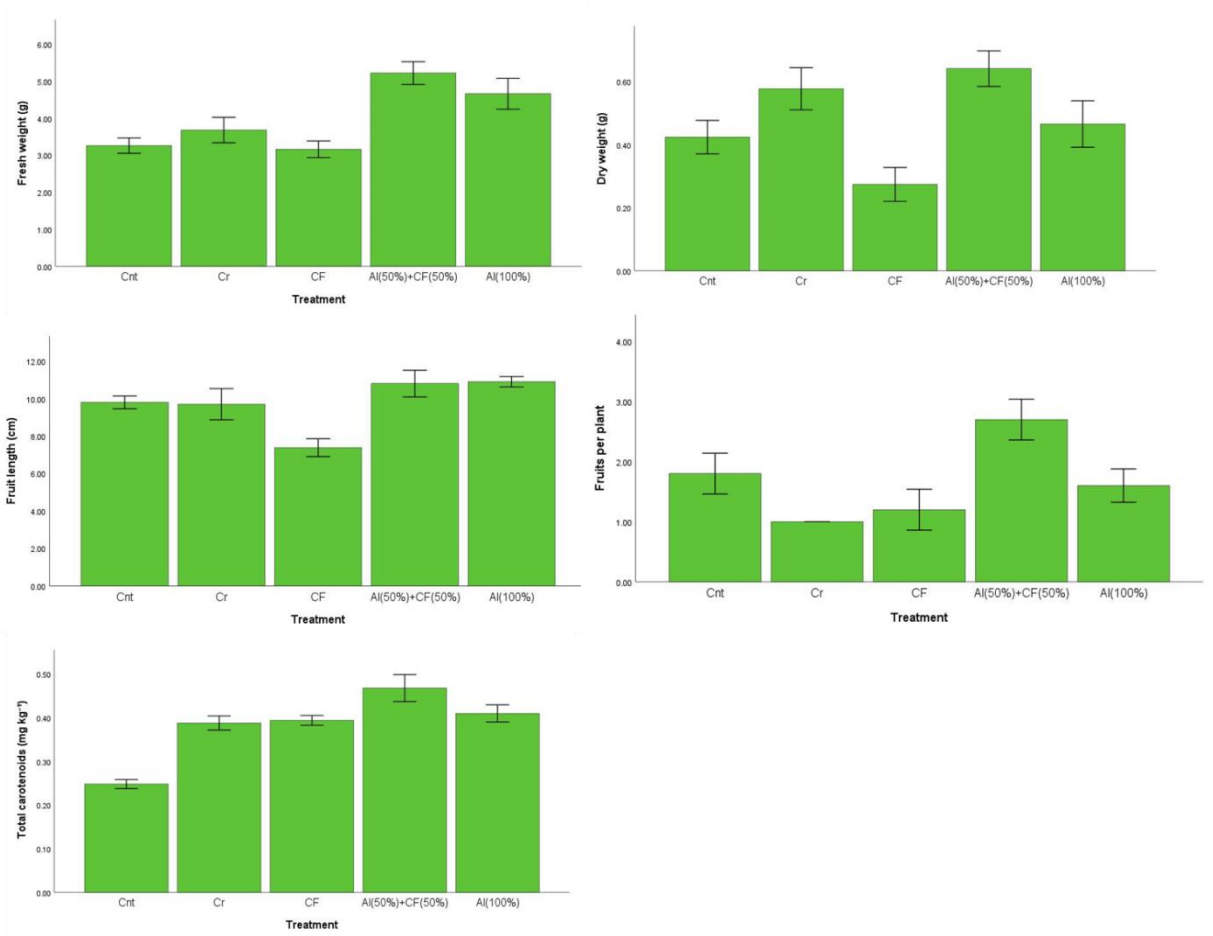

Figure 4.2 Effect of microalgal bio-inoculants on chili fruit parameters

Table 4.3 Photosynthetic efficiency of chili leaves at mid-crop stage (50 d)

Chili leaves			
Treatment	Total chlorophyll (µg cm^{-2})	Fv/Fm	ETR
Cnt	4.3 ± 0.4	0.7 ± 0.01	15 ± 0.6
Cr	4.7 ± 0.3	0.8 ± 0.01	14.3 ± 0.3
CF	3.7 ± 0.2	0.8 ± 0.01	15 ± 0.2
Al(50 %)+CF(50 %)	5.7 ± 0.6	0.8 ± 0.0	15.3 ± 0.1
Al(100 %)	3.7 ± 0.3	0.7 ± 0.02	13.8 ± 0.8

Cnt, Control; Cr, Carrier; CF, Chemical fertilizer; Al(50 %) +CF(50 %), Combination of 50 % microalgal consortia and 50 % Chemical fertilizer; Al(100 %), 100 % microalgal consortia.

4.3.4 Correlation between soil parameters and plant parameters

Correlations between different soil parameters and plant parameters were also analyzed to evaluate the influence of these parameters on plant growth. Correlation analysis showed a significant positive correlation of DHA, OC, Chl and TP with plant fresh weight ($r^2 = 0.629$, $p < 0.01$; $r^2 = 0.629$, $p < 0.01$; $r^2 = 0.467$, $p < 0.05$; $r^2 = 0.620$, $p < 0.01$), DHA and TP with shoot dry weight ($r^2 = 0.493$, $p < 0.05$; $r^2 = 0.451$, $p < 0.05$), and DHA, OC, Chl and TP with leaf number ($r^2 = 0.575$, $p < 0.001$; $r^2 = 0.527$, $p < 0.05$; $r^2 = 0.724$, $p < 0.001$; $r^2 = 0.760$, $p < 0.001$) respectively (Appendix 5). In Al(50 %)+CF(50 %) treatments, DHA and OC were significantly positively correlated with plant fresh weight ($r^2 = 0.997$, $p < 0.01$;$r^2 = 0.999$, $p < 0.01$). Al(100 %) also showed a positive correlation of DHA, OC and TP with plant fresh weight ($r^2 = 0.697$; $r^2 = 0.733$; $r^2 = 0.682$), DHA, OC, Chl and TP with root length ($r^2 = 0.866$; $r^2 = 0.871$; $r^2 = 0.319$; $r^2 = 0.824$), Chl and TP with root dry weight ($r^2 = 0.729$; $r^2 = 0.144$) and Chl with shoot length ($r^2 = 0.647$). Thus, the increase in DHA, OC, Chl and TP influenced the growth of chili plant and can be considered as important soil parameters. Our findings are in line with a previous study by Bharti et al. (2021), where they reported a positive association between soil polysaccharides and plant growth using *Anabaena torulosa* as potting medium. In another study, the treatment with *Anabaena* biofilm showed a significant positive correlation between soil nutrients (OC, N, P, Zn, Cu and Mn) and plant yield parameters (Kanchan et al., 2018). Overall, the positive impacts of microalgal bio-inoculants were mediated via rhizosphere microbial community modification and included higher enzyme activities, organic C, soil chlorophyll, total polysaccharides, and nutrient availability in soils, as well as improved growth and yield of chili plants. These results also helped to highlight the important roles that soil nutrient availability and microbiological activity play in improving plant growth and yield.

4.3.5 Effect of microalgal bio-inoculation in soil on bacterial diversity and community composition

The bacterial richness and diversity indices such as Observed OTUs, ACE, Simpson, Shannon and Chao1 showed no significant differences among the control and treatment groups ($p > 0.05$;

Fig. 4.3 a-e). Microalgal inoculation (both 50 % and 100 % treatments) increased the bacterial richness and diversity compared to the control. Furthermore, the richness and diversity indices in Al(50 %)+CF(50 %) were higher than Al(100 %), but the differences were not significant ($p > 0.05$, Fig. 4.3 a-e). The PCoA (principal coordinate analysis) based on Bray-Curtis and Jaccard distance revealed differences in the bacterial communities across different treatments (Fig. 4.3a and b). Further, the PCoA analysis, revealed the two components (PCoA1 and PCoA2) together explaining 71 % (Bray-Curtis) (Fig. 4.4a) and 25.4 % (Jaccard distance) (Fig. 3.4b) of the total variations between the bacterial community structures. No significant differences were observed between the control and the treatment groups ($P > 0.05$; Table 4.4).

Venn diagram showed that 561 responsive bacterial OTUs were shared between the different treatments (Appendix 6). The bacterial taxonomic identification, were done at the phylum and class level and their relative abundance in different treatment groups. All the potential beneficial bacteria were classified to 29 phyla (Fig. 4.5a) and the dominant class across the treatments were *Actinobacteria* (59-63 %), *Bacilli* (4.5-5.3 %), *Thermomicrobia* (4.5-5 %), *Plantomycetacia* (3.7-4.8 %), *Cytophagia* (3-5 %), *Clostridia* (2.9-3.2 %), *Gammaproteobacteria* (1.4-2.1 %), *Alphaproteobacteria* (0.9-1.2 %), *Chloroflexi* (0.7-0.9 %), *Deltaproteobacteria* (0.7-0.9 %), *Sphingobacteria* (0.6-0.9 %) etc (Fig. 4.5b). Among the treatments such as Al(50 %)+CF(50 %) and Al(100 %), a higher relative abundance of *Bacilli* (5 %), *Thermomicrobia* (5 %), *Plantomycetacia* (4.7 %), *Cytophagia* (5 %), *Clostridia* (3 %), *Gammaproteobacteria* (2 %), *Alphaproteobacteria* (1 %), and *Deltaproteobacteria* (0.9 %) bacterial classes in Al(50 %)+CF(50 %) and a substantially higher abundance of *Plantomycetacia* (4.8 %), *Clostridia* (3 %), *Chloroflexi* (0.9 %), *Cyanobacteria* (0.8 %), *Bacteroidia* (0.5 %), *Ktedonobacteria* (0.2 %), *Acidobacteria* (0.1 %) in Al(100 %) (Fig. 4.5b) were observed. Among all these responsive bacterial taxa, the Spearman's rank-order correlation analysis found 41 bacterial classes linked to soil parameters ($p < 0.05$) (Fig. 4.9a). The higher relative abundance of these potentially beneficial bacterial taxa in the treatment groups compared to Cnt was significantly positively correlated to soil fertility parameters and hence can be associated with plant growth and performance (Fig. 4.9a).

In this experiment, microalgal bio-inoculant increased the abundance of *Bacilli, Thermomicrobia, Planctomycetacia, Clostridia, Chloroflexi, Gammaproteobacteria, Alphaproteobacteria, Deltaproteobacteria, Cyanobacteria, Bacteroidia, Ktedonobacteria,* and *Acidobacteria*. Previous study has reported that *Bacilli* suppress pathogens, which are considered plant growth-promoting rhizobacteria (PGPR) with biological promotion of different characteristics of plant growth (Sansinenea, 2019, Yang et al., 2021). According to a previous report, *Planctomycetacia* are involved in biological activities in soil, including ammoxidation and the metabolism of carbohydrates and polysaccharides (Wang et al., 2018). This implies that *Planctomycetacia* improves plant production by enhancing soil fertility. Moreover, *Chloroflexi* plays a key role in manipulating soil microbiome and in the breakdown of organic molecules in the soil environment (Wang et al., 2018). *Alphaproteobacteria* plays a significant role in the degradation of inorganic compounds and important role in N-fixation. *Deltaproteobacteria* also considered as an important soil organic degraders and has ecosystem function (Mhete et al., 2020). In addition, *Proteobacteria and Acidobacteria* serve as bacterial indicators for changes in land usage (Kim et al., 2021). The beneficial bacteria isolated from the rhizosphere as PGPR can directly and indirectly promote plant growth (Yang et al., 2021). This shows that microalgal bio-inoculants improved PGPR abundance and rhizosphere bacterial response to microalgal bio-inoculants.

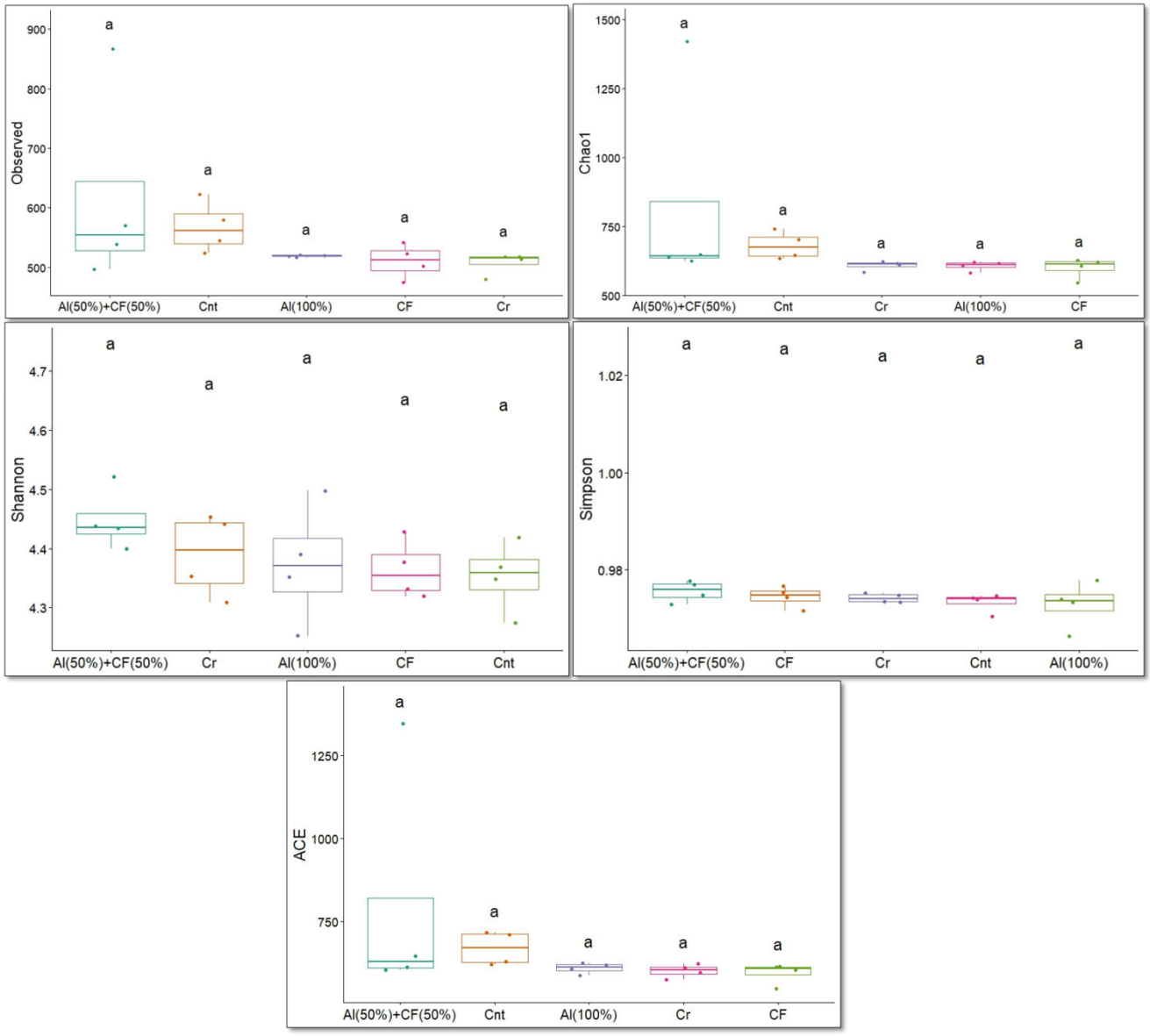

Figure 4.3 The boxplot of a) Observed index, b) Chao1 index, c) Shannon index, d) Simpson diversity index and e) ACE index of bacterial community between different treatments. The dot and line inside the boxplot respectively represent the mean and median. Cnt, Control; Cr, Carrier; CF, Chemical fertilizer; Al(50 %)+CF(50 %), Combination of 50 % microalgal consortia and 50 % Chemical fertilizer; Al(100 %), 100 % microalgal consortia.

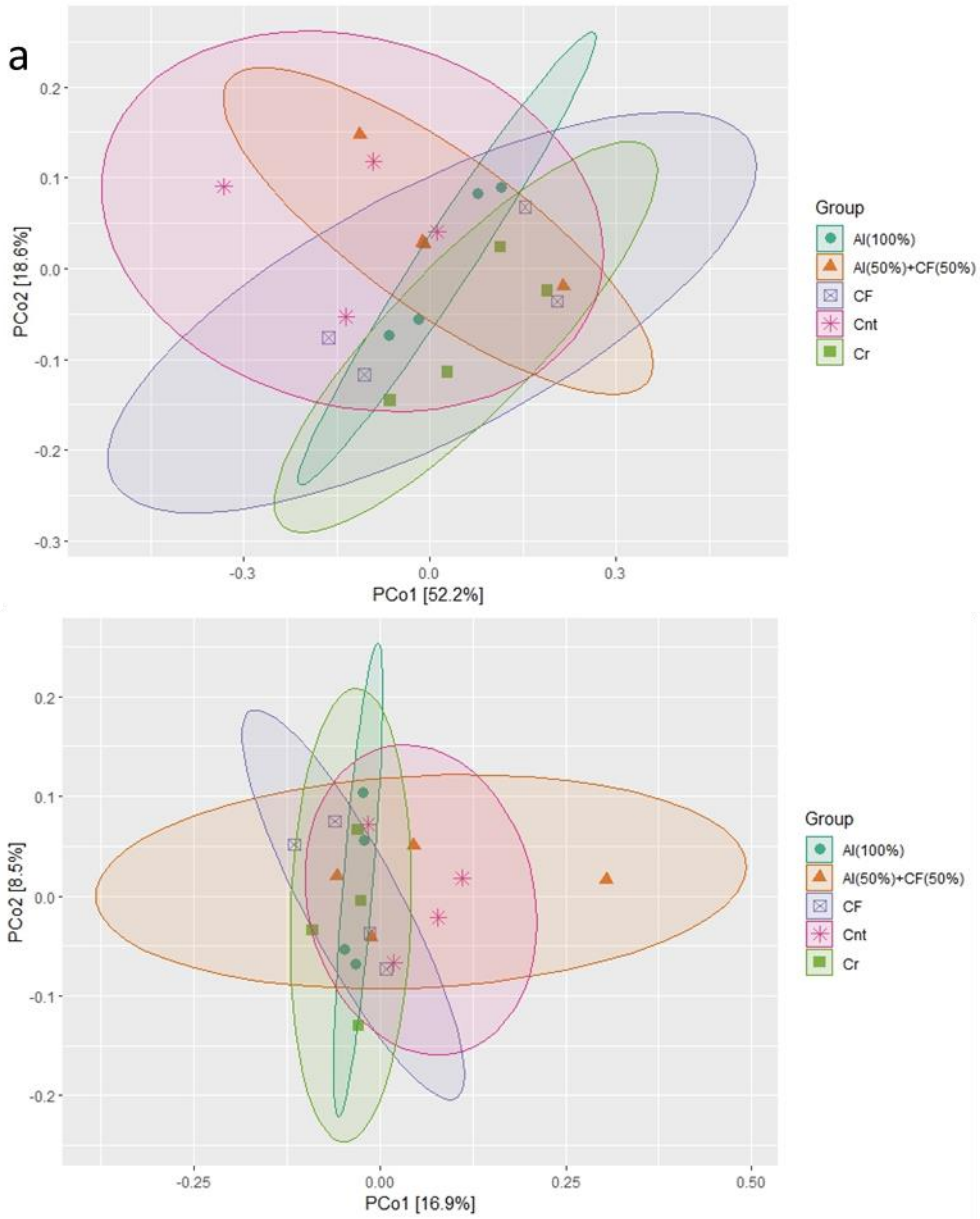

Figure 4.4 Principal coordinate analysis (PCoA) of five treatments based on the (a) composition of bacterial community using Bray-curtis distance (b) composition of bacterial community using Jaccard distance. Cnt, Control; Cr, Carrier; CF, Chemical fertilizer; Al(50 %)+CF(50 %), Combination of 50 % microalgal consortia and 50 % Chemical fertilizer; Al(100 %), 100 % microalgal consortia.

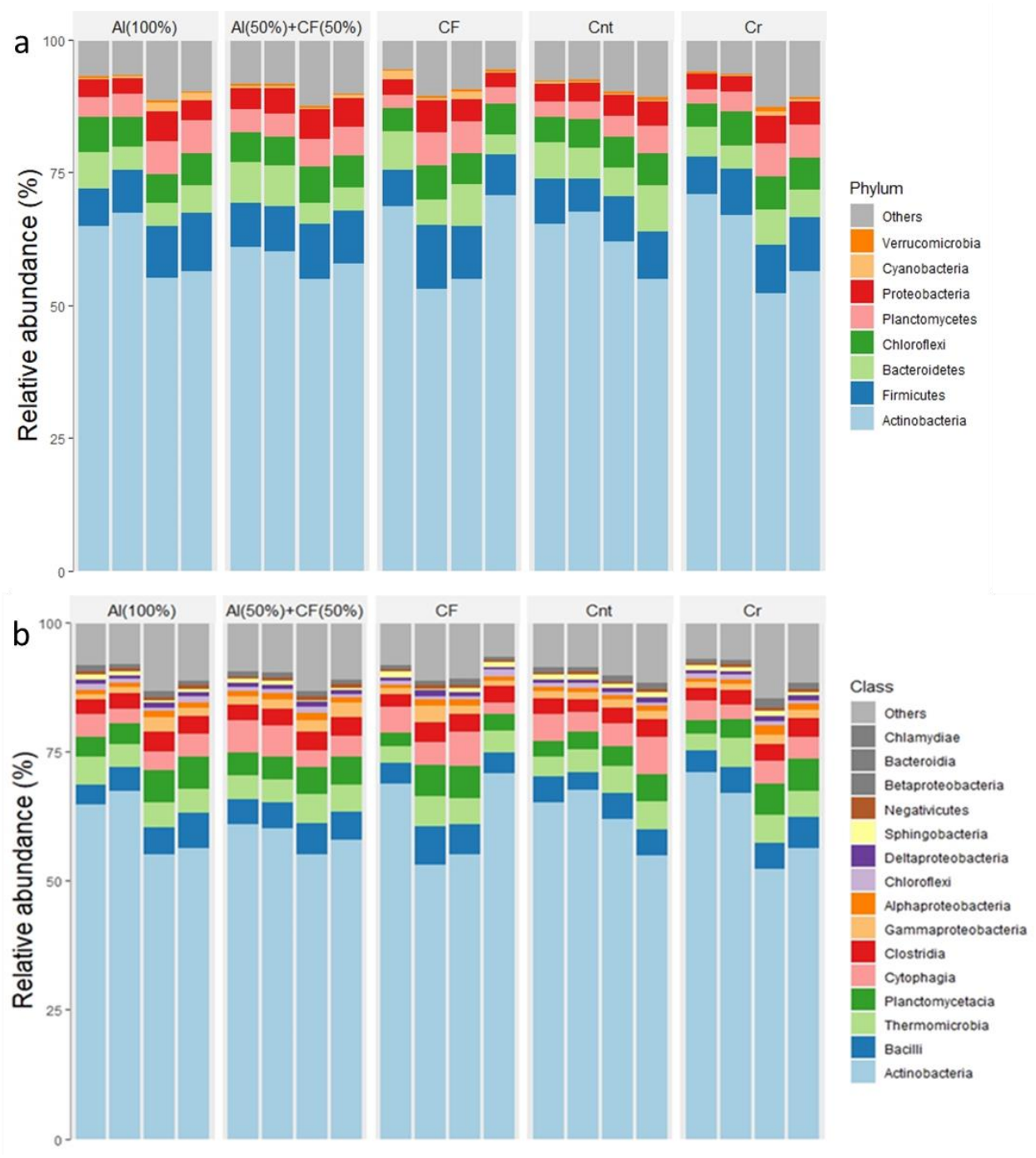

Figure 4.5 Relative abundance of dominant (a) phylum (b) class in the rhizosphere bacterial community under control and treatments. Cnt, Control; Cr, Carrier; CF, Chemical fertilizer; Al(50 %)+CF(50 %), Combination of 50 % microalgal consortia and 50 % Chemical fertilizer; Al(100 %), 100 % microalgal consortia.

4.3.6 Effect of microalgal bio-inoculation in soil on fungal diversity and community composition

The fungal richness and diversity were calculated among the different treatments. It showed a lower fungal abundance among the control groups than the treatments; however, the differences were not significant ($p > 0.05$, Fig. 4.6a-e). Among the microalgal treatments, the richness and diversity indices in Al(50 %)+CF(50 %) were lower than Al(100 %) ($p > 0.05$, Fig. 4.6a-e). The beta diversity observed through PCoA revealed significant ordinations for fungal communities, with PCo1 (26.3 %) and PCo2 (14.8 %) differentiating the fungal communities in terms of Bray-Curtis dissimilarity matrix (Fig. 4.7a). Similarly, PCo1 (31.6 %) and PCo2 (12.4 %) differentiate the fungal communities in terms of Jaccard-distances (Fig. 4.7b). No significant differences were found between the control and the treatment groups ($p > 0.05$; Table 4.4). For the taxonomic identification, the fungal communities were classified up to the phylum and class level. In the present study, the results from the phylum level classification revealed that the potentially beneficial fungal taxa were assigned to 15 phyla (Fig. 4.8a). Of the total relative abundance, the highest number of sequences were affiliated to phyla *Ascomycota* (37-44 %), and *Streptophyta* (21-33 %), followed by *Basidiomycota* (11-19 %), *Chlorophyta* (8-12 %), *Bacillariophyta* (3-6 %), and *Phaeophyta* (0.3-0.8 %) (Fig. 9a). The dominant fungal classes include Oribiliomycetes (6.5-16.6 %), *Saccharomycetes* (2.6-13.2 %), *Agaricomycetes* (6.6-12 %) *Lilliopsida* (1.7-9.2 %), *Sordariomycetes* (4.7-8.7 %), *Lecanoromycetes* (1.8-4.9 %), *Dothideomycetes* (2.3-4.6 %), *Prasinophyceae* (0.9-4.8 %), *Eurotiomycetes* (1.3-3.8 %), *Cryptophyta* (0.9-3.5 %), *Dinophyceae* (0.3-0.8 %) (Fig. 8b). Among the treatments, a higher relative abundance of *Saccharomycetes* (13 %), *Eurotiomycetes* (3.8 %), *Cryptophyta* (3.5 %), *Bryopsida* (2 %), *Oligohymenophorea* (2 %), *Leotiomycetes* (1 %), *Zygnemophyceae* (0.6 %), *Jungermanniopsida* (0.6 %), *Tremellomycetes* (0.6 %), *Cosmopogonophyceae* (0.1 %), *Ulvophyceae* (0.1 %) were observed in Al(100 %) (Fig. 20b).While as *Sordariomycetes* (8 %), *Lilliopsida* (9 %), *Basidiomycota* (1 %), *Trebouxiophyceae* (0.7 %), *Ustilaginomycetes* (0.7 %), *Bangiophyceae* (0.6 %) were higher in Al(50 %)+CF(50 %) (Fig. 4.8b).

Among the fungal community, Ascomycota was the most abundant in all the treatments and was reported as a dominant phylum of chili rhizosphere microbiome (Chen et al., 2021). It was

reported that Ascomycota has an important role in the degradation of organic matter in rhizospheric soil, soil stability, plant biomass decomposition, and endophytic interactions with plants (Challacombe et al., 2019). Moreover, Basidiomycota members are significant lignocellulosic agricultural waste degraders, recycling C and other nutrients in the soil ecosystem (Ramamoorthy and Theradimani, 2022). The fungal diversity was high in Al(100 %) in comparison to the Cnt. This shows that microalgal bio-inoculants improved rhizosphere fungal response to microalgal bio-inoculants. Similar, to bacteria, by using the Spearman's rank-order correlation analysis, it was found 81 potential fungal classes were significantly associated with soil fertility ($p < 0.05$) (Fig. 4.9b). All these fungal groups are highly enriched in soils treated with microalgal bio-inoculants, suggesting their significant role in enhancing soil fertility and, therefore chili plant growth and performance (Fig. 4.9b). In this study, the higher bacterial diversity in Al(50 %)+CF(50 %) and higher fungal diversity in Al(100 %), may be related to higher bacterial and fungal colony counts in these two treatments, respectively. It has been reported that plant growth and yield may result from shifts in population densities of core microbiomes (Hu et al., 2021, Sun et al., 2022).

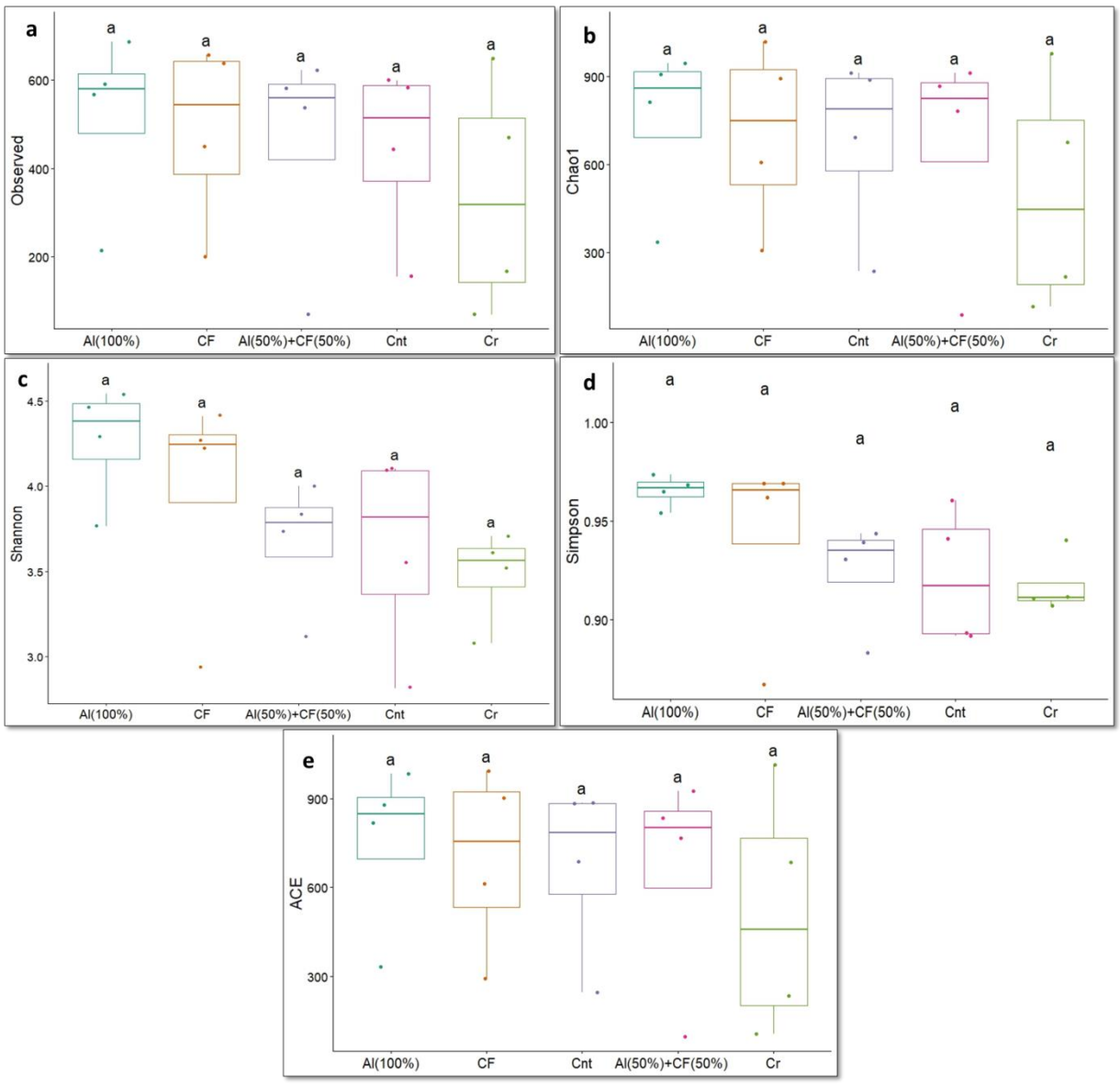

Figure 4.6 The boxplot of a) Observed index, b) Chao1 index, c) Shannon index, d) Simpson diversity index and e) ACE index of fungal community between different treatments. Plots denoted by a different letter are significantly different at $p < 0.05$. Cnt, Control; Cr, Carrier; CF, Chemical fertilizer; Al(50 %)+CF(50 %), Combination of 50 % microalgal consortia and 50 % Chemical fertilizer; Al(100 %), 100 % Microalgal consortia.

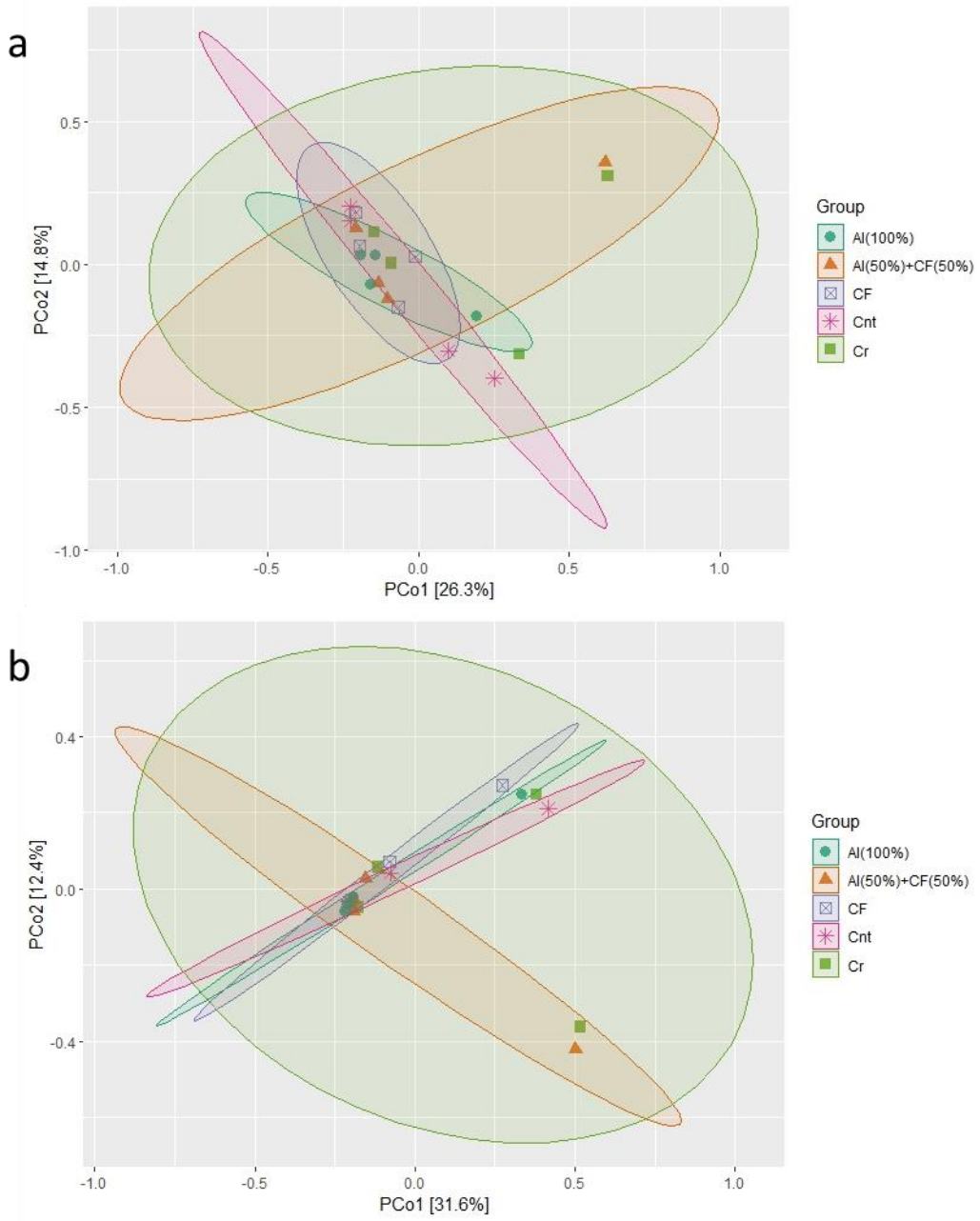

Figure 4.7 Principal coordinate analysis (PCoA) of five treatments based on the (a) composition of fungal community using Bray-curtis distance (b) composition of bacterial community using Jaccard distance. Cnt, Control; Cr, Carrier; CF, Chemical fertilizer; Al(50 %)+CF(50 %), Combination of 50 % microalgal consortia and 50 % Chemical fertilizer; Al(100 %), 100 % microalgal consortia

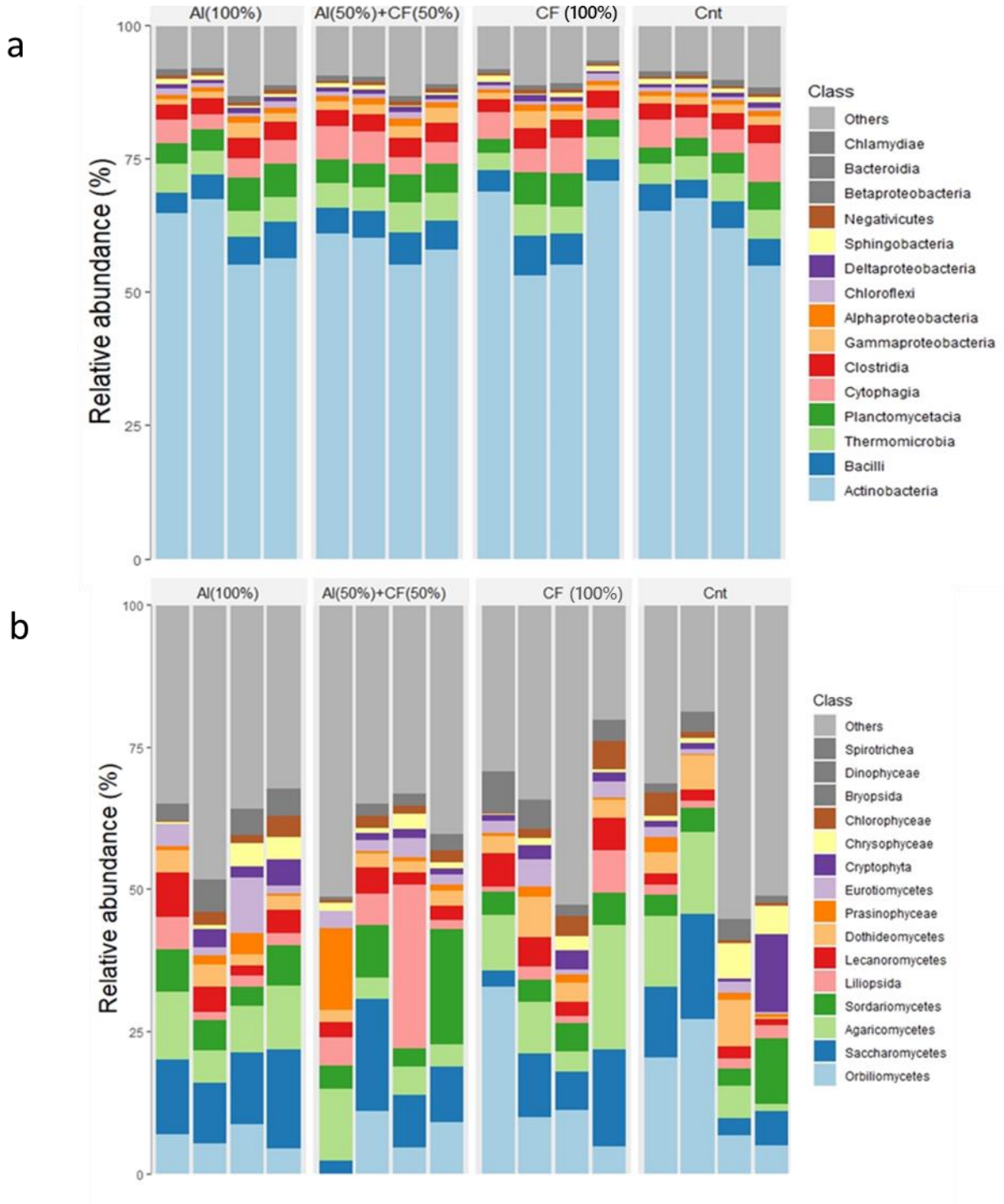

Figure 4.8 Relative abundance of dominant (a) phylum (b) class in the rhizosphere fungal community under control and treatments. Cnt, Control; Cr, Carrier; CF, Chemical fertilizer; Al(50 %)+CF(50 %), Combination of 50 % microalgal consortia and 50 % Chemical fertilizer; Al(100 %), 100 % Microalgal consortia.

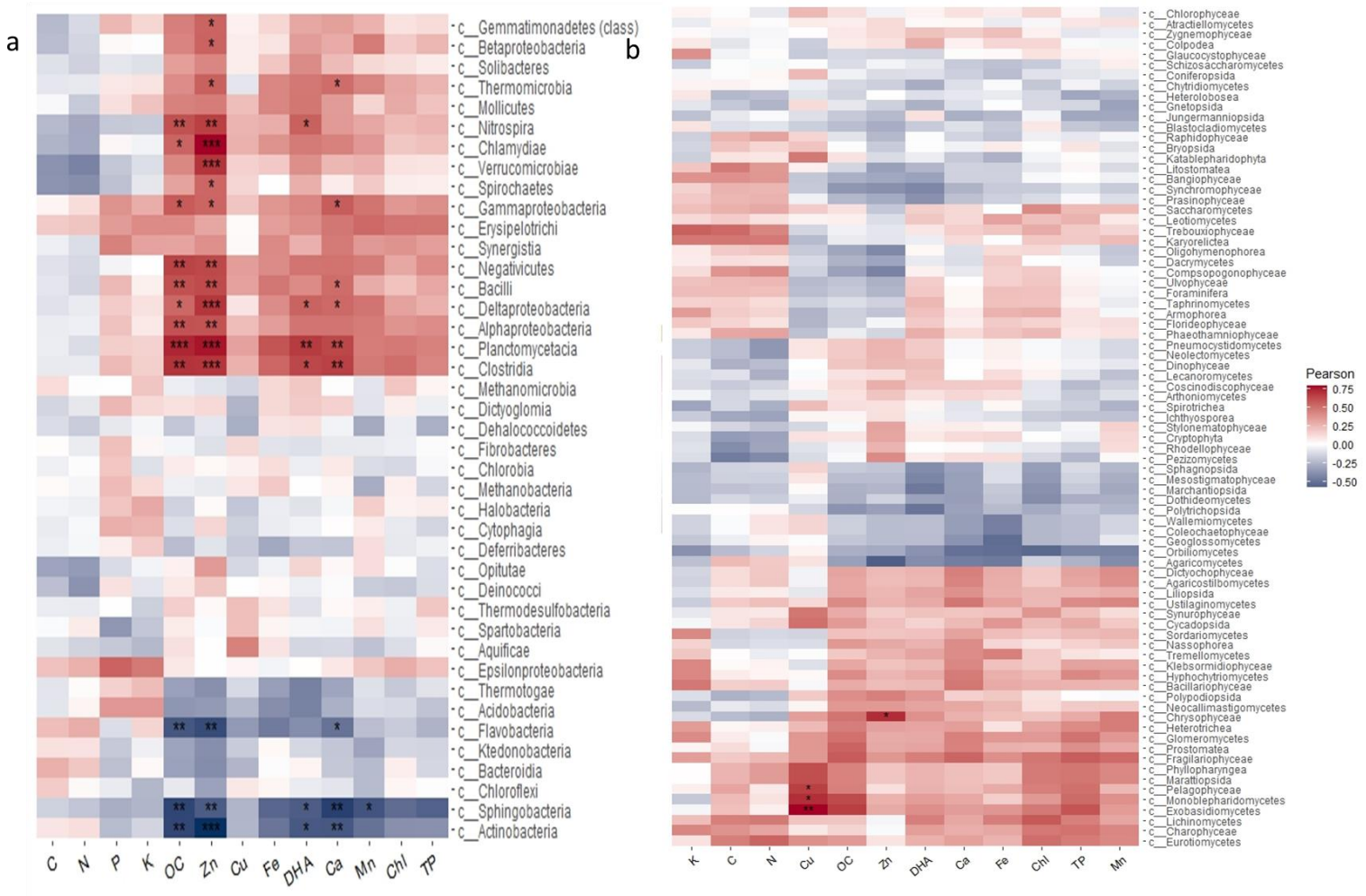

Figure 4.9 Heatmap analysis of spearman's correlation coefficients between relative abundances of (a) bacterial community and environmental factors in the soil samples at class level (b) fungal community and environmental factors in the soil samples at class level. p value: ***p < 0.001; **p < 0.01; *p < 0.05. K, Potassium; C, Carbon; N, Nitrogen; DHA, Dehydrogenase activity; OC, Organic carbon; TP, Total polysaccharides; Chl, Soil chlorophyll; Ca, Calcium; Cu, Copper; Fe, Iron; Mn, Manganese; Zn, Zinc.

Table 4.4 Permutational multivariate analysis of variance (PERMANOVA) table showing the significant effect between the treatments in comparison with bacterial community and fungal community**.**

	PERMANOVA			
	Bacterial community		**Fungal community**	
Groups	**R^2**	**p-value**	**R^2**	**p-value**
Cnt vs Cr	0.364	0.054*	0.12	0.185
Cnt vs CF	0.227	0.207	0.14	0.160
Cnt vs Al (50 %) + CF(50 %)	0.230	0.303	0.140	0.122
Cnt vs Al(100 %)	0.262	0.133	0.162	0.102
Cr vs CF	0.059	0.841	0.170	0.015*
Cr vs Al (50 %) +CF (50 %)	0.161	0.263	0.143	0.118
Cr vs Al(100 %)	0.124	0.468	0.200	0.008*
CF vs Al(50 %)+CF(50 %)	0.105	0.614	0.165	0.005*
CF vs Al(100 %)	0.065	0.837	0.018	0.044*

Al(50 %)+CF(50 %) vs Al(100 %)	0.068	0.917	0.166	0.022*

Cnt, Control; Cr, Carrier; CF, Chemical fertilizer; Al(50 %)+CF(50 %), Combination of 50 % microalgal consortia and 50 % Chemical fertilizer; Al(100 %), 100 % microalgal consortia.

***p < 0.001, ** p < 0.01, * p < 0.05.

LefSe analysis was performed to identify the taxa that showed significant differences among treatments, and the analysis revealed distinct taxa between the microalgal treatments and the control treatments. For bacteria, Al(50 %)+CF(50 %), has the highest number of twelve differential taxa, while Al(100 %) has three, CF has two, Cnt has two, and Cr has four (Appendix 7). At the genus level, *Microtetraspora, Salinibacterium, Prauserella, Thermobaculum, Ochrobactrum, Parascardovia, Aminobacterium, Synechococcus*, and *Dolichosermum* were identified as the most differential taxa in Al(50 %)+CF(50 %). The genomic features in Al(100 %) identified the most differential taxa as *Nostoc, Peptoniphilus, Prosthecobacter* (Appendix 7a). Similarly, *Fischerella* in CF, *Rhodopirellula*, and *Geodermatophilus* in Cr and *Hymenobacter* in Cnt were identified as the main taxonomic contributors (Appendix 7a). In the case of fungi, Al(100 %) showed five differential taxa such as *Paxillaceae, Paxillus*, *Tmesipteris*, *Psilotales*, *Psilotaceae* (Appendix 7b). Al(50 %)+CF(50 %) showed *Chaetothyriales,* Cnt showed *Omphalotaceae* and *Omphalotus,* Cr showed *Diplococcium* and *Helminthosphaeriaceae* as differential taxa (Appendix 7b). However, for CF, none of the significant taxonomic contributors was found. According to LEfSe analysis, the application of microalgal bio-inoculants favored the existence of certain microbial taxa associated with plant growth, development and yield performance, such as *Nostoc, Peptoniphilus, Prosthecobacter, Microtetraspora, Salinibacterium, Thermobaculum, Ochrobactrum, Paxillus* etc. (Szuba et al., 2016, Burygin et al., 2019, Katsenios et al., 2021, Zhang et al., 2021, Yurgel et al., 2022, Orozco-Mosqueda et al., 2023). Overall, our findings suggest that microalgal bio-inoculants have the potential to stimulate specific microorganisms that contribute to plant growth and yield performance. The possible reasons for such findings

may be attributed to changes in soil properties, particularly C/N (Wu et al., 2021, Sun et al., 2022), enhanced enzyme activity (Mia et al., 2019), niche development by bio-inoculants (Tilman, 2004), and enhanced metabolic activity upon the application of microalgal bio-inoculants (Shade et al., 2013, Singh et al., 2015).

4.3.7 Correlation between microbial community composition and soil properties

Spearman's correlation analysis was performed to establish the relationship between the microbial communities and soil parameters, and the results suggest that soil chemistry played an important role in shaping the microbiome structure and composition. The relationships between the bacterial community composition and soil properties were significantly different between different treatments (Appendix 9). Spearman's correlation analysis between different bacterial classes and soil parameters showed a significant positive correlation between *Planctomycetacia* and DHA ($\rho = 0.742$, $p = 0.006$), *Clostridia* and DHA ($\rho = 0.704$, $p = 0.01$), *Deltaproteobacteria* and DHA ($\rho = 0.602$, $p = 0.03$), and *Nitrospira* and DHA ($\rho = 0.611$, $p = 0.03$). While, *Bacilli* ($\rho = 0.666$, $p = 0.005$), *Planctomycetacia* ($\rho = 0.802$, $p = 0.001$), *Clostridia* ($\rho = 0.772$, $p = 0.001$), *Gammaproteobacteria* ($\rho = 0.634$, $p = 0.01$), *Alphaproteobacteria* ($\rho = 0.661$, $p = 0.005$), *Deltaproteobacteria* ($\rho = 0.591$, $p = 0.02$), *Negativicutes* ($\rho = 0.724$, $p = 0.002$), *Chlamydiae* ($\rho = 0.590$, $p = 0.02$), and *Nitrospira* ($\rho = 0.672$, $p = 0.005$) showed positive correlation with OC (Appendix 9 and 10). The detailed correlation of other different bacterial and fungal classes is given in the supplementary table (Appendix 9 and 10). It is important to mention that the structural and functional composition of soil microbial communities is significantly associated with changes in soil chemistry (Lauber et al., 2008, Campbell et al., 2010). Therefore, it is interesting to highlight that in the present study, different soil parameters such as DHA, OC, TP and Chl were positively associated with changes in the soil microbial community, as previously observed by Yang et al. (2019) and Zhang et al. (2019). Moreover, the presence of various differentially abundant taxa in different treatment groups provides links towards the response of these microbial taxa to different microalgal treatments (Wang et al., 2023). Moreover, in the present study, the microalgal bio-inoculation stimulated and enriched potentially beneficial

microbial taxa in Al(50 %)+CF(50 %) and Al(100 %) treatments that were significantly positively associated with the improvement in chili plant growth and yield (Appendix 4 and 5).

4.4 Conclusion

Microalgal bio-inoculation produced changes in the bacterial and fungal community, correlated with increased soil enzyme activity, nutrient concentration, plant growth and yield performance. The microalgal amended soils were characterized by a higher relative abundance of different microbial taxa such as Firmicutes, Bacteroidetes, Chloroflexi, Planctomycetes, Proteobacteria, Basidiomycota, Chlorophyta, and Bacillariophyta in the rhizosphere. All these microbes are nutrient-solubilizing and major contributors to soil fertility. This work has evidenced the potential of microalgal bio-inoculants in boosting the native soil microbiome, which in turn contributes to plant growth, crop yield and sustainable agriculture. These findings also served to emphasize the critical roles that soil microbial activity and nutrient availability play in enhancing plant development and yield. Future work should focus on unravelling the mechanistic interactions of bio-inoculants with plant, microbes and their surrounding environment that will pave the way for more eco-friendly agriculture and crop production.

CHAPTER FIVE

Evaluating the impact of microalgal bio-inoculant on chili rhizosphere using shotgun metagenomics

5.1 Introduction

The soil microbiome is complex, multivariate, and multidimensional, thus necessitating extensive research to understand the interaction among different microorganisms to predict their functions in the plant rhizosphere (Chen et al., 2022). Cyanobacteria and microalgae are highly diverse group of microorganisms that can interact with the microbial community in the rhizosphere to improve soil quality and plant growth (Ramakrishnan et al., 2023). The increased abundance of soil microbial communities upon microalgal bio-inoculations has been reported through the elevated soil enzyme activities (Rana et al., 2015). The microalgal amendments in soil are reported to enhance enzyme activities compared to non-amended soil samples (Manjunath et al., 2016). Thus, if the practice is carried out over time, it may also have long-term positive impact on soil quality (Osorio-Reyes et al., 2023).

Numerous genes that can positively affect soil fertility and plant growth have been identified by researchers, including *nif* (N-fixation), *nod* (nitric oxide dioxygenase), *gcd* (P cycling), *amo*A and *amo*B (ammonium oxidizing), and many more (Igiehon and Babalola, 2018, Zhong et al., 2020). A few strains of cyanobacteria, both marine (*Crocosphaera*) and terrestrial (*Cyanothece* and *Synechococcus*), are known to possess *nif* gene clusters (Ramakrishnan et al., 2023). Additionally, the genomes of the *Actinobacteria* and *Proteobacteria* lineages, as well as those of *Firmicutes, Chloroflexi, Chlorobi*, and *Bacteroidetes*, feature *nif* gene clusters (Ramakrishnan et al., 2023). The only diazotrophic lineage that fixes N is cyanobacteria, which also produces molecular oxygen as a metabolic by-product (Ramakrishnan et al., 2023). The most popular method, 16S and ITS gene amplicon sequencing provides insight into species richness, diversity, similarities and differences among the microbial communities in different treatments (Lv et al., 2020). As a result, there isn't yet a generally acknowledged method for taxonomic, functional, high-resolution, and economical microbiome investigation (Hillmann et al., 2018). Moreover,

Shotgun metagenomics facilitates the characterization and identification of functional genes in microalgae, the interaction between microalgae and microbes, a thorough analysis of the variations in various functional genes in the C, N, and P-cycling process of soil, and the role that these organisms play in C, N and P metabolism to promote sustainable agriculture. However, to the best of our knowledge no research has been published on the response of rhizosphere microorganisms to microalgal biomass as bio-inoculants, using shotgun metagenomics. In order to bridge this technological gap, we assessed the impact of microalgal bio-inoculants on shotgun metagenomic sequencing and offer comprehensive functional profile predictions as an alternative to 16S and ITS sequencing for microbiome research.

5.2 Materials and methods

5.2.1 Experimental design

The experimental design for this experiment has been explained in detail in section 4.2.2. Briefly, the pot-experiment was conducted in a greenhouse at Botanic gardens, Durban, South Africa (29^0.85′ S and 31^0 14 ′ E). The experiment was carried in five replicates with five treatments including (1) control (Cnt) with no treatment; carrier (Cr) control containing vermiculite: compost (1:1); (3) chemical fertilizer (CF) control; (4) 50 % microalgal consortium biomass + 50 % chemical fertilizer (Al(50 %)+CF(50 %)); (5) 100 % microalgal consortium biomass (Al(100 %)). The microalgal consortium were composed of two native N-fixing cyanobacteria *viz*. *Nostoc* sp. and *Calothrix* sp., and a non-N-fixing green microalga *Desmodesmus armatus.* The application of the recommended dose of chemical fertilizer was 2.3.2 (22) NPK kg ha^{-1}.The microalgal formulations for Al(100 %) were prepared by mixing wet consortial biomass with vermiculite and compost (1:1) carrier in polyethylene bags. The developed formulations were thoroughly mixed with 2 kg of soil in the respective pots. Prior to inoculation, the chili seeds were soaked in distilled water overnight. Equal numbers of five seeds were sown in each treatment, and the seeds were germinated for 14 days in a climate-controlled culture bed maintained at 22 ± 2 °C. The enzymes activity and microbial community response were recorded at the beginning and mid of the experiment (at 50 days after planting).

5.2.2 Soil sampling, DNA extraction and shotgun sequencing

The soil sample collection and total genomic DNA extraction were done as mentioned in previous chapter (section 4.2.5). The shotgun sequencing was done using Illumina NextSeq500 platform in a commercial lab (Inqaba Biotech, South Africa). Briefly, the genomic DNA samples were fragmented following the kit (NEBNext® Ultra™ II FS DNA Library Prep Kit for Illumina). Resulting DNA fragments were size selected (>200 bp), using AMPure XP beads. The fragments were end repaired and Illumina specific adapter sequences were ligated to each fragment. Each sample was individually indexed, and a second size selection step was performed. Samples were then quantified using a fluorometric method (NEBNext Library Quant Kit for Illumina), diluted to a standard concentration (4nM) and then sequenced on Illumina's NextSeq500 platform using a NextSeq mid out kit (300 cycle), following a standard protocol as described by the manufacturer. 2Gb of data (2x150bp paired-end reads) were produced for each sample.

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5.2.3 Bioinformatic Data processing

a processing

The Illumina generated raw reads were demultiplexed and quality filtered using by Fast QC v0.11.9 (Andrews, 2010). The quality filtered reads were then imported to SqueezeMeta v1.6.3, a fully automated metagenomic pipeline that covers all steps of the analysis (Tamames and Puente-Sanchez, 2018). The pipeline involved the use of MetaSpades for assembly, MetaProdigal for ORF prediction, Diamond for taxonomy assignment against the NCBI RefSeq database, and Diamond/Hidden markov model (HMM) for functional prediction against the clusters of orthologs genes, COG database (Buchfink et al., 2021). Using these methods, three different databases were annotated such as Kyoto encyclopedia of genes and genomes (KEGG)

annotations provide in-depth information about microbial communities' metabolic capability, COG annotations provide functional categories related to metabolism, cellular processes and signalling, information storage and processing and PFAM annotations of protein families (Kanehisa et al., 2016). Statistical analysis was performed by using R software (R Core, 2020) and all the tests were deemed significant at 95 % significance level ($P \leq 0.05$).

5.3 Results and Discussion

5.3.1 Taxonomic abundance

The major microbial community abundance at genus level involved in the C, N, and P metabolism is depicted in Fig. 5.1 (a-c). Microbial community groups involved in the three metabolism and their abundance with respect to different treatments, such as Initial soil (CNT_I), Midcrop soil (Cnt), Vermiculite and compost (Cr), Chemical fertilizer (CF), 100 % microalgal treatment (Al(100 %)) and 50 % microalgal treatment with 50 % chemical fertilizer (Al(50 %)+CF(50 %)) were compared. These includes unclassified microbial community such as *Micromonosporaceae* (3 % in Al(50 %)+CF(50 %)), *Betaproteobacteria* (64 % in Al(100 %) and 60 % in Al(50 %)+CF(50 %)), *Acidobacteria* (31 % in Al(100 %) and 28 % in Al(50 %)+CF(50 %)), *Myxococcales* (106 % in Al(50 %)+CF(50 %)), *Actinomycetia* (1 % in Al(50 %)+CF(50 %)), *Gammaproteobacteria* (61 % in Al(50 %)+CF(50 %) and 56 % in Al(100 %)AL(100 %)), *Alphaproteobacteria* (62 % in Al(100 %) and 40 % in Al(50 %)+CF(50 %)), *Actinobacteria* (0.6 % in Al(100 %)), *Proteobacteria* (82 % in Al(50 %)+CF(50 %)), *Hyphomicrobiales* (66 % in Al(100 %) and 37 % in Al(50 %)+CF(50 %)) and other bacteria (74 % in Al(50 %)+CF(50 %)). Among the different treatments except CF, Al(100 %) and Al(50 %)+CF(50 %) showed higher abundance of *Micromonosporaceae, Betaproteobacetria, Acidobacteria, Myxococcales, Actionomycetia, Gammaproteobacteria, Alphaproteobacteria, Actinobacteria, Proteobacteria, Hyphomicrobiales* when compared to CNT_I. A higher abundance of microbes was also shown in CF, as compared to CNT_I. Thus C, N, and P metabolism, a similar group of unclassified microbes such as *bacteria, proteobacteria, hyphomicrobiales, alphaproteobacteria, gammaproteobacteria, betaproteobacteria,*

actinomycetia, and *acidobacteria* were highly abundant in Al(50 %)+CF(50 %) and Al(100 %) in comparison to CNT_I (Fig. 5.1a-c).

The soil microbial biomass serves as a source and sink of nutrients as well as act as a catalyst for soil organic matter transformation and C, N, and P cycling (Kallenbach and Grandy, 2011). A previous study by Mishra et al. (2023) reported that *alpha, beta, gammaproteobacteria*, and *actinobacteria* actively fix atmospheric C in the form of CO_2 in the soil. Furthermore, in C and N metabolism, the microbial communities such as *actinobacteria*, and *proteobacteria* are highly abundant (Miralles et al., 2018). Our findings are also in line with the microbial community in P cycling with abundant phyla of *actinobacteria, acidobacteria, proteobacteria* (Unno and Shinano, 2013). Overall, microalgal bio-inoculants enhance the microbial community profiles of the rhizosphere, thereby improving the plant growth and soil fertility (Nain et al., 2009, Bidyarani et al., 2016). Thus, the bio-inoculants shifted gene diversity in this study might reveal alterations in the plant rhizosphere and show plant growth promoting rhizobacteria with C, N and P sources.

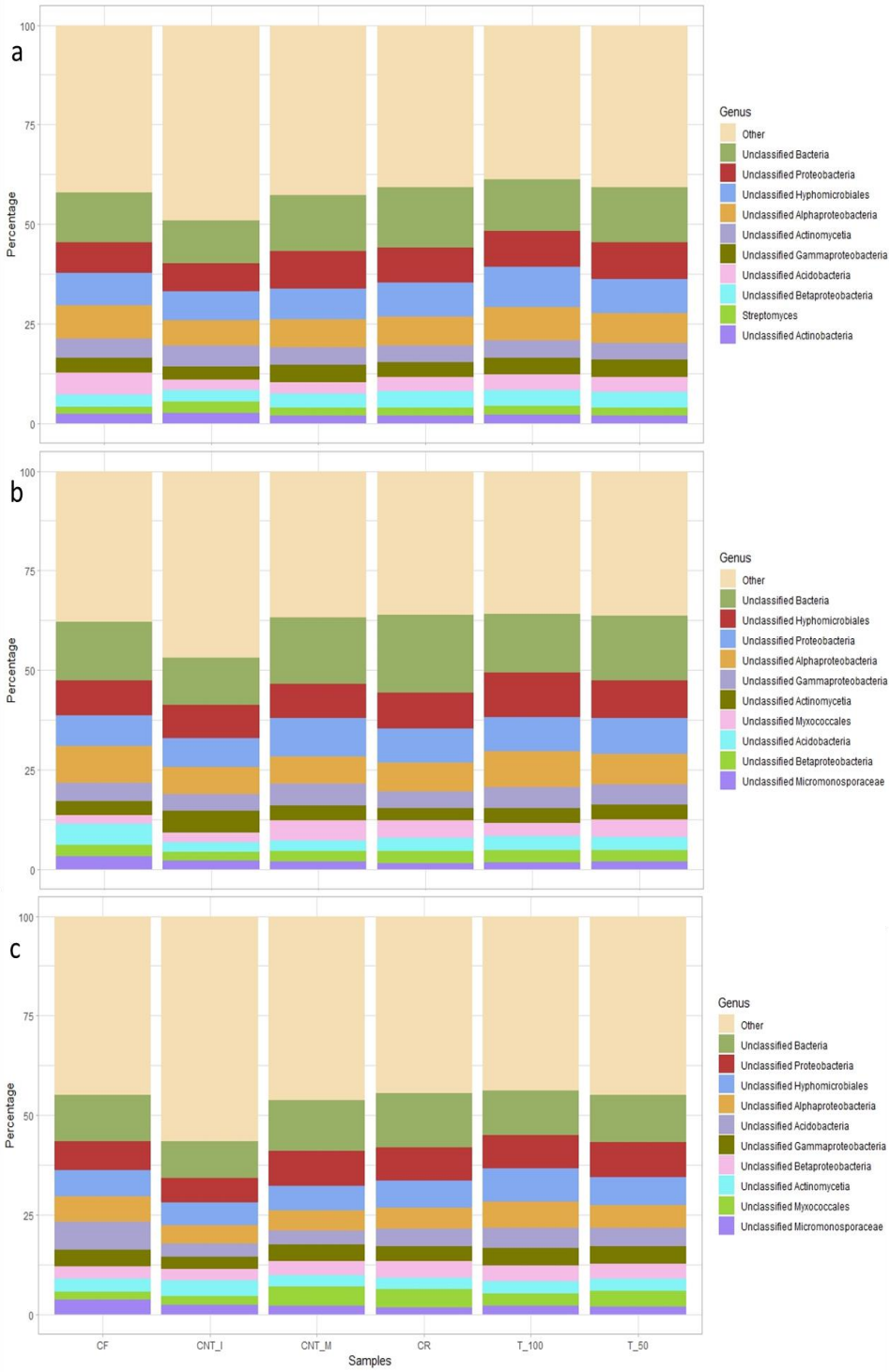


Figure 5.1 Microbial community abundance at genus level in different treatments that is responsible for a) C metabolism, b) N metabolism and c) P metabolism

5.3.2 Changes related to nitrogen cycling

The resulting taxonomic profiles were subjected to SqueezeMeta analysis for functional predictions in order to evaluate the metabolic capability of the bacterial communities and to pinpoint the functional aspects that were differentially abundant in the communities after microalgal amendment (Tamang et al., 2021). The predicted genes were further categorized using KEGG orthologs (KOs), which were collected from the KEGG database and further classified (Puente-Sanchez et al., 2020). The KEGG database showed a total number of 8562 KOs, 30977 COGs and 6810 PFAMs. When compared to the initial soil sample (CNT_I) both the microalgal treatments 100 % microalgal treatment (Al(100 %)) and 50 % microalgal treatment (Al(50 %)+CF(50 %)) showed higher abundance in enzyme activities. For example, glutamine synthetase [EC:6.3.1.2] (16 %), glutamate synthase (NADPH) large and small chain [EC:1.4.1.13] (21 %), nitrate reductase/nitrite oxidoreductase, alpha subunit [EC:1.7.5.1 1.7.99.-] (23 %), nitrate/nitrite transport system ATP-binding protein [EC:3.6.3.-] (79 %) highly abundant in Al(100 %) and nitrate reductase large subunit [EC:1.7.1.15] (45 %), glutamate dehydrogenase [EC:1.4.1.2], assimilatory nitrate reductase catalytic subunit [EC:1.7.99.-] (35 %), two component response system; *NtrC* family; N regulation response regulator *NtrX* (49 %) higly abundant in Al(50 %)+CF(50 %) when compared to CNT_I (Fig.5.2). All these enzymes play a significant role in N metabolism. For example, the important N source, ammonium is assimilated by two pathways. One is Glutamine synthetase, which plays a key role in N assimilation in all organisms, because it is the only enzyme that catalyzes the incorporation of ammonium into glutamate to synthesize L-glutamine (Geisseler et al., 2009). The other one is Glutamate dehydrogenase that serves as a link between C and N metabolism, in its role of assimilating ammonia into glutamine or deaminating glutamate into 2-oxoglutarate and ammonia (Magadlela et al., 2019). The nitrate reductase is also a crucial metabolic enzymes that helps to convert inorganic N (Pinto et al., 2014). Based on the results, it is evident that the microalgal treatments influenced soil microbial community and enhanced glutamine synthetase, glutamate synthase, nitrate reductase, nitrate/nitrite transport system, nitrate reductase large subunit, glutamate dehydrogenase, assimilatory nitrate reductase catalytic subunit that play a crucial role in N metabolism.

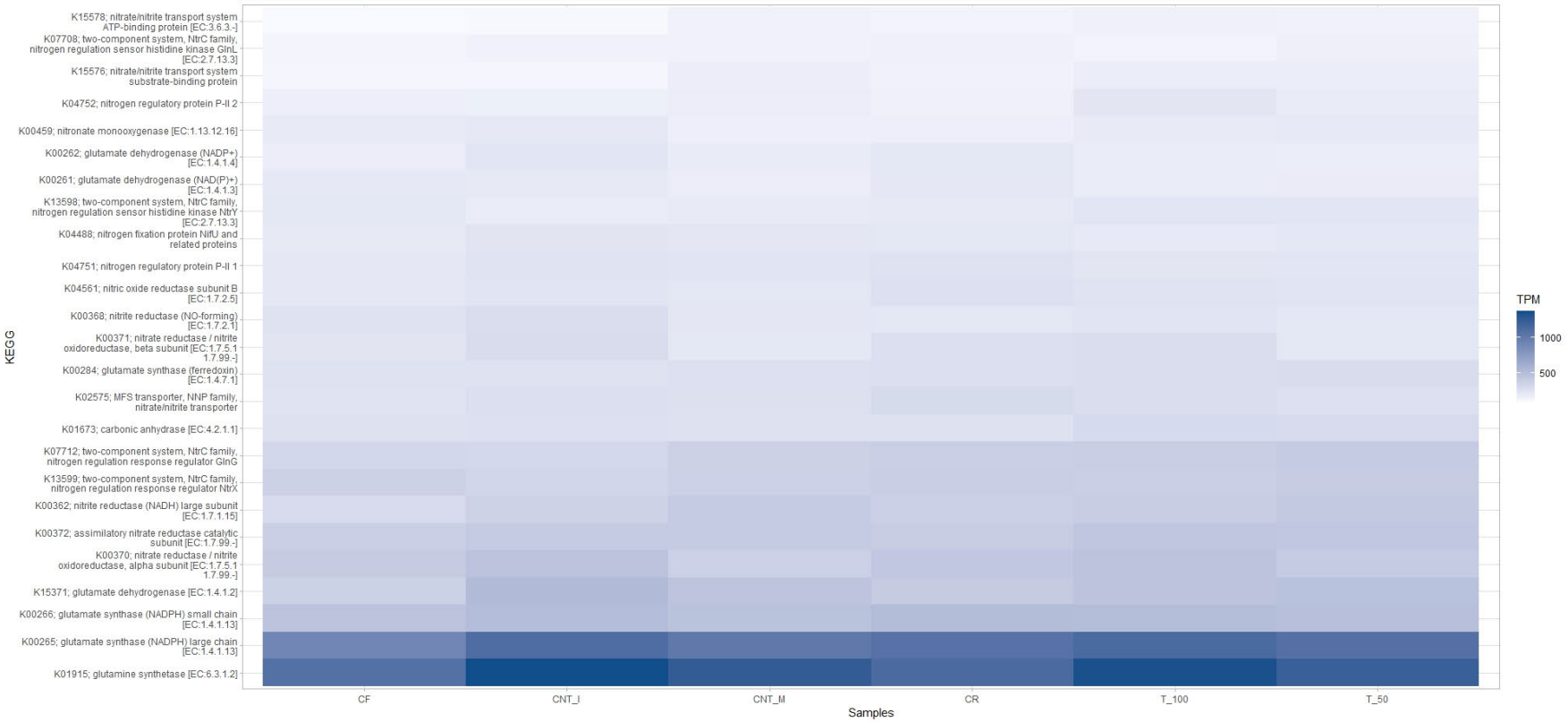


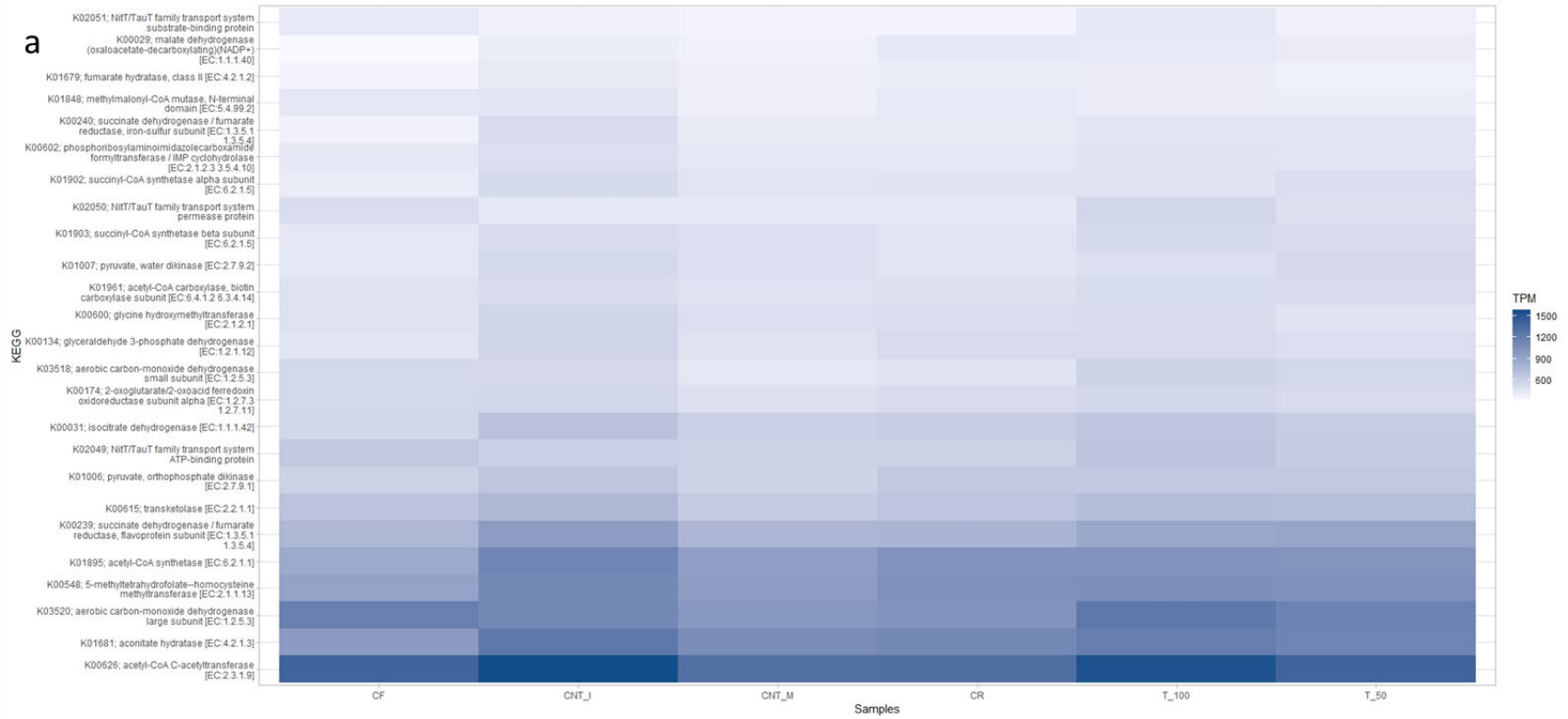
Figure 5.2 The enzyme abundance in different treatments that is responsible for N metabolism. TPM- Transcripts per million. CF, Chemical fertilizer; CNT_I, Initial soil control; CNT_M, Midcrop soil control; CR, Carrier; T_100, 100 % microalgal treatment; T_50, 50 % microalgal treatment.

5.3.3 Changes related to carbon cycling and phosphorus cycling

Similar to N cycling, the abundance of the enzymatic activities in C cycling was analyzed in the different treatments. When compared to the CNT_I the microalgal treatments (Al(100 %) and Al(50 %)+CF(50 %)) were showing higher abundance. For example, Acetyl-CoA C-acetyltransferase [EC:2.3.1.9] (13 % in Al(100 %) and 5 % in Al(50 %)+CF(50 %)), aconitate hydratase [EC:4.2.1.3] (15 % in Al(50 %)+CF(50 %) and 14 % in Al(100 %)), aerobic carbon monoxide dehydrogenase, large subunit [EC:1.2.5.3] (25 % in Al(100 %) and 19 % in Al(50 %)+CF(50 %)), 5-methyltetrahydrofolate-homocysteine methyltransferase [EC:2.1.1.13] (16 % in Al(100 %) and 15 % in Al(50 %)+CF(50 %)), acetyl Co-A synthetase [EC:6.2.1.1] (7 % in Al(50 %)+CF(50 %) and 6 % in Al(100 %)), succinate dehydrogenase/fumarate reductase, flavoprotein subunit [EC:1.3.5.1 1.3.5.4] (14 % in Al(50 %)+CF(50 %) and 11 % in Al(100 %)), transketolase [EC:2.2.1.1] (14 % in Al(50 %)+CF(50 %) and 12 % in Al(100 %)), pyruvate, orthophosphate dikinase [EC:2.7.9.1] (15 % in Al(50 %)+CF(50 %) and 13 % in Al(100 %)), NiT/TauT family transport system, ATP-binding protein [EC:1.2.5.3] (31 % in Al(100 %) and 20 % in Al(50 %)+CF(50 %)), isocitrate dehydrogenase [EC:1.1.1.42] (17 % in Al(100 %) and 10 % in Al(50 %)+CF(50 %)) were showing higher abundance than the CNT_I (Fig. 5.3). Moreover, all these enzymes play a key role in C metabolism (Fig. 5.3). For example, acetyl-CoA is the key intermediate in the metabolic processes needed to break down non-fermentable C sources (Martin, 2020). Likewise, succinate dehydrogenase is the enzyme present in most of the bacterial cell and the only enzyme present in both citric acid cycle and electron transport chain (Moosavi et al., 2019).The shotgun data showed changes in the abundance of enzyme activity that involved in the metabolism of C compounds, indicating that C dynamics have improved due to microalgal amendment (both 50 % and 100 %) in comparison with the CNT_I. Thus, the previous findings showed that the microalgae-cyanobacteria consortium significantly increased the organic C pool in soil, enhanced the growth of microbial flora, microbial activity and plant growth (Yilmaz and Sönmez, 2017).

The shotgun data also revealed a higher abundance of enzymes involved in P cycle with respect to treatment, such as Al(50 %)+CF(50 %) and Al(100 %) in comparison with CNT_I. The main

changes comprised to acetolactate synthase I/II/III large subunit [EC:2.2.1.6] (28 % in Al(50 %)+CF(50 %) and 25 % in Al(100 %)), ribonucleoside-diphosphate reductase alpha chain [EC:1.17.4.1] (13 % in Al(50 %)+CF(50 %) and 11 % in Al(100 %)), carbamoyl-phosphate synthase large subunit [EC:6.3.5.5] (14 % in Al(100 %) and 9 % in Al(50 %)+CF(50 %)), K(+)-stimulated pyrophosphate- energized sodium pump (22 % in Al(100 %) and 19 % in Al(50 %)+CF(50 %)), glucosamine--fructose-6-phosphate aminotransferase [EC:2.6.1.16] (11 % in Al(50 %)+CF(50 %) and 8 % in Al(100 %)), transketolase [EC:2.2.1.1] (14 % in AL(50 %)+CF(50 %) and 12 % Al(100 %)), pyruvate orthophosphate dikinase [EC:2.7.9.1] (15 % in Al(50 %)+CF(50 %) and 13 % in Al(100 %)), quinoprotein glucose dehydrogenase [EC:1.1.5.2] (62 % in Al(50 %)+CF(50 %) and 44 % in Al(100 %)), UDP-glucose 4-epimerase [EC:5.1.3.2] (29 % in Al(50 %)+CF(50 %) and 21 % in Al(100 %)), glyceraldehyde 3-phosphate dehydrogenase [EC:1.2.1.12] (5 % in Al(50 %)+CF(50 %) and 8 % in Al(100 %)), pyruvate water dikinase [EC:2.7.9.2] (26 % in Al(50 %)+CF(50 %) and 16 % in Al(100 %)), GTP pyrophosphate kinase [EC:2.7.6.5] (6 % in Al(50 %)+CF(50 %) and 5 % in Al(100 %)). Thus, microalgal amendment showed influence on the various enzymes in P metabolism as well. For example, acetolactate synthase is the catalytic enzyme involved in various amino acid synthesis (Dezfulian et al., 2017). Carbamoyl phosphate is a compound combining ammonia, carbonate, and phosphate in a single molecule. As part of the fermentation process, several microbes use carbamoyl phosphate to transfer their phosphate group from adenosine diphosphate (ADP) to produce adenosine triphosphate (ATP) (Shi et al., 2018). All the other enzymes also play a pivotal role in different pathways, such as pentose phosphate pathway, ribonucleotide synthesis, NADPH production (Riyapa et al., 2019). Moreover, cyanobacteria are able to immobilize tricalcium phosphate insoluble source of inorganic phosphate (Vaishampayan et al., 2001). Overall, the microalgal enzymes that liberate inorganic P from organic P compounds could have an impact on the soil P-cycling process as well as the availability of P for plants.


a
K02051; NitT/TauT family transport system substrate-binding protein
K00029; malate dehydrogenase (oxaloacetate-decarboxylating)(NADP+) [EC:1.1.1.40]
K01679; fumarate hydratase, class II [EC:4.2.1.2]
K01848; methylmalonyl-CoA mutase, N-terminal domain [EC:5.4.99.2]
K00240; succinate dehydrogenase / fumarate reductase, iron-sulfur subunit [EC:1.3.5.1 1.3.5.4]
K00602; phosphoribosylaminoimidazolecarboxamide formyltransferase / IMP cyclohydrolase [EC:2.1.2.3 3.5.4.10]
K01902; succinyl-CoA synthetase alpha subunit [EC:6.2.1.5]
K02050; NitT/TauT family transport system permease protein
K01903; succinyl-CoA synthetase beta subunit [EC:6.2.1.5]
K01007; pyruvate, water dikinase [EC:2.7.9.2]
K01961; acetyl-CoA carboxylase, biotin carboxylase subunit [EC:6.4.1.2 6.3.4.14]
K00600; glycine hydroxymethyltransferase [EC:2.1.2.1]
K00134; glyceraldehyde 3-phosphate dehydrogenase [EC:1.2.1.12]
K03518; aerobic carbon-monoxide dehydrogenase small subunit [EC:1.2.5.3]
K00174; 2-oxoglutarate/2-oxoacid ferredoxin oxidoreductase subunit alpha [EC:1.2.7.3 1.2.7.11]
K00031; isocitrate dehydrogenase [EC:1.1.1.42]
K02049; NitT/TauT family transport system ATP-binding protein
K01006; pyruvate, orthophosphate dikinase [EC:2.7.9.1]
K00615; transketolase [EC:2.2.1.1]
K00239; succinate dehydrogenase / fumarate reductase, flavoprotein subunit [EC:1.3.5.1 1.3.5.4]
K01895; acetyl-CoA synthetase [EC:6.2.1.1]
K00548; 5-methyltetrahydrofolate--homocysteine methyltransferase [EC:2.1.1.13]
K03520; aerobic carbon-monoxide dehydrogenase large subunit [EC:1.2.5.3]
K01681; aconitate hydratase [EC:4.2.1.3]
K00626; acetyl-CoA C-acetyltransferase [EC:2.3.1.9]
KEGG
CF
CNT_I
CNT_M
CR
T_100
T_50
Samples
TPM
1500
1200
900
600

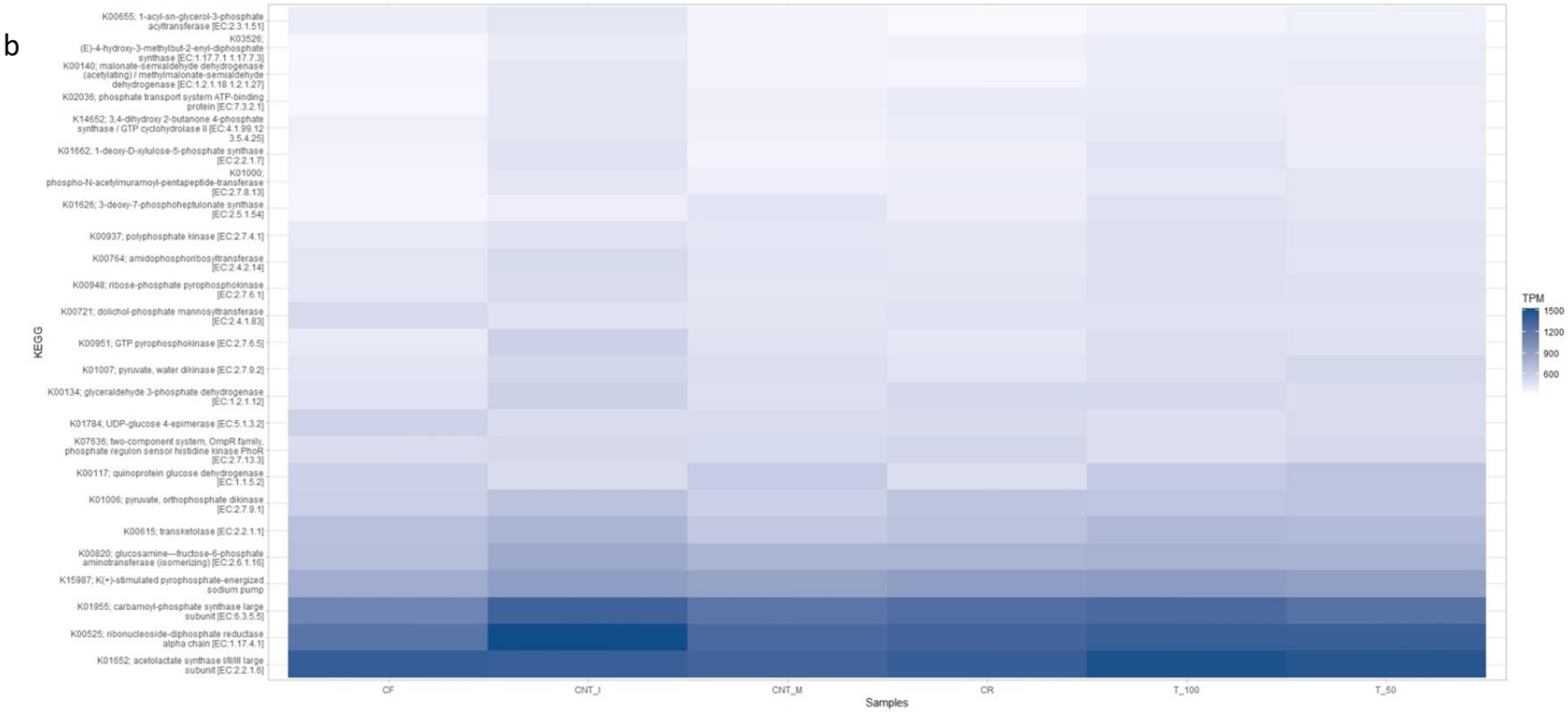


Figure 5.3 The enzyme abundance in different treatments that is responsible for a) C and b) P metabolism. TPM- Transcripts per million. CF, Chemical fertilizer; CNT_I, Initial soil control; CNT_M, Midcrop soil control; CR, Carrier; T_100, 100 % microalgal treatment; T_50, 50 % microalgal treatment.

5.3.4 Predictive functional pathway for nitrogen, and phosphorus cycling

The main metabolic events in N cycling together with enzymes involved are summarized in Fig. 5.4. Despite the high levels of N in the atmosphere, most species, including plants, cannot use it (Renuka et al., 2018). As a result, N must first be converted to ammonia (NH_3) before it can be absorbed into biological molecules (Alvarez et al., 2021). The atmospheric N was reduced to NH_3 through biological N-fixation by microorganisms (Berman-Frank et al., 2003). Cyanobacteria are well known for their N-fixing ability using a specialized cell called heterocyst (Alvarez et al., 2021). Cyanobacteria or cyanobacterial consortia significantly enhance the soil N and maintain a balance in the soil (Renuka et al., 2016). Thus, the main reactions and the enzymes involved in the N metabolism, together with the most abundant treatments, are described in this section and summarized in Fig. 5.4.

N-fixation takes place by converting N to NH_3 in a single step reaction catalysed by enzyme nitrogenase [EC: 1.18.6.1] forming *NifDK* heterotetrameric complex constitutes the dinitrogenase component of molybdenum-type (MoFe) nitrogenases. Al(50 %)+CF(50 %) showed higher abundance in this protein complex. The nitrification process takes place by the oxidation of NH_3 to hydroxylamine by ammonia monooxygenase [EC: 1.14.99.9] and further to nitrite by hydroxylamine dehydrogenase [EC: 1.7.2.6]. All these reactions have higher abundance in Al(100 %) and CF. The nitrite is then converted to nitrate by assimilatory nitrate reductase [EC: 1.7.99.-]. This final step showed enzymatic abundance in Al(50 %)+CF(50 %) and CF. During denitrification of N the gene *Nrt*, transports nitrate from plasma to cytosol. The nitrate is then converted into nitrite by the enzymes nitrate reductase/nitrite oxidoreductase [EC: 1.7.5.1]. *NirK* decomposed it into nitric oxide, which was then reduced to nitrous oxide by nitric oxide reductase [EC: 1.7.2.5], then *NosZ* finally turned nitrous oxide into N. Enzyme activity and gene abundance were high in the Al(100 %) and CF in all of these reactions.

The NH_3 is further catalyzed by glutamine synthetase [EC: 6.3.1.2] to form L-Glutamine and further converted to L-Glutamate by glutamate synthase [EC: 1.4.1.13]. In both reactions, enzyme abundance was higher in Al(100 %) and CF. Furthermore, glutamate is the most abundant amino acid in soil, which is also found in root exudates and as the pool of dissolved organic N (Forde and Lea, 2007, Magadlela et al., 2019). The ammonia also is converted into L-

Glutamate in a single step reaction with glutamate dehydrogenase [EC: 1.4.1.2] (Fig. 5.4). This enzyme showed higher abundance in Al(50 %)+CF(50 %) and CF. Overall, the N metabolism showed the influence of microalgal treatments in all the major reactions and enhanced the abundance of enzyme activity and the genes involved in the N metabolism.

The P is the second most important limiting nutrient in agriculture after N. Soil P is found in two forms, organic and inorganic, but it is unavailable to plants and makes it available with the help of P solubilizing microorganisms (Gyaneshwar et al., 2002). Cyanobacteria and microalgae have the ability to solubilize and release the inorganic P from organic P compounds in the soil (Mandal et al., 1999, Yandigeri et al., 2011). The most recognized source of organic P in soil is in the form of inositol phosphates (Reusser et al., 2020). Although myo-inositol hexakisphosphate is the most common form in nature, it also exists as inositols in different states of phosphorylation (attached to between one and six phosphate groups) (Turner et al., 2002). Thus, the inositol phosphate metabolism and the influence of different treatments were summarized in Fig. 5.5. 1-Phosphatidyl 1D-*myo*-inositol-5P is phosphorylated to 1-Phosphatidyl-1D-*myo*-inositol-4,5P_2 by the enzyme 1-phosphatidylinositol-5-phosphate 4-kinase [EC: 2.7.1.149] and it is further transferred to 1D-*myo*-Inositol-1,4,5P_3 using phosphoinositide phospholipase C [EC: 3.1.4.11].

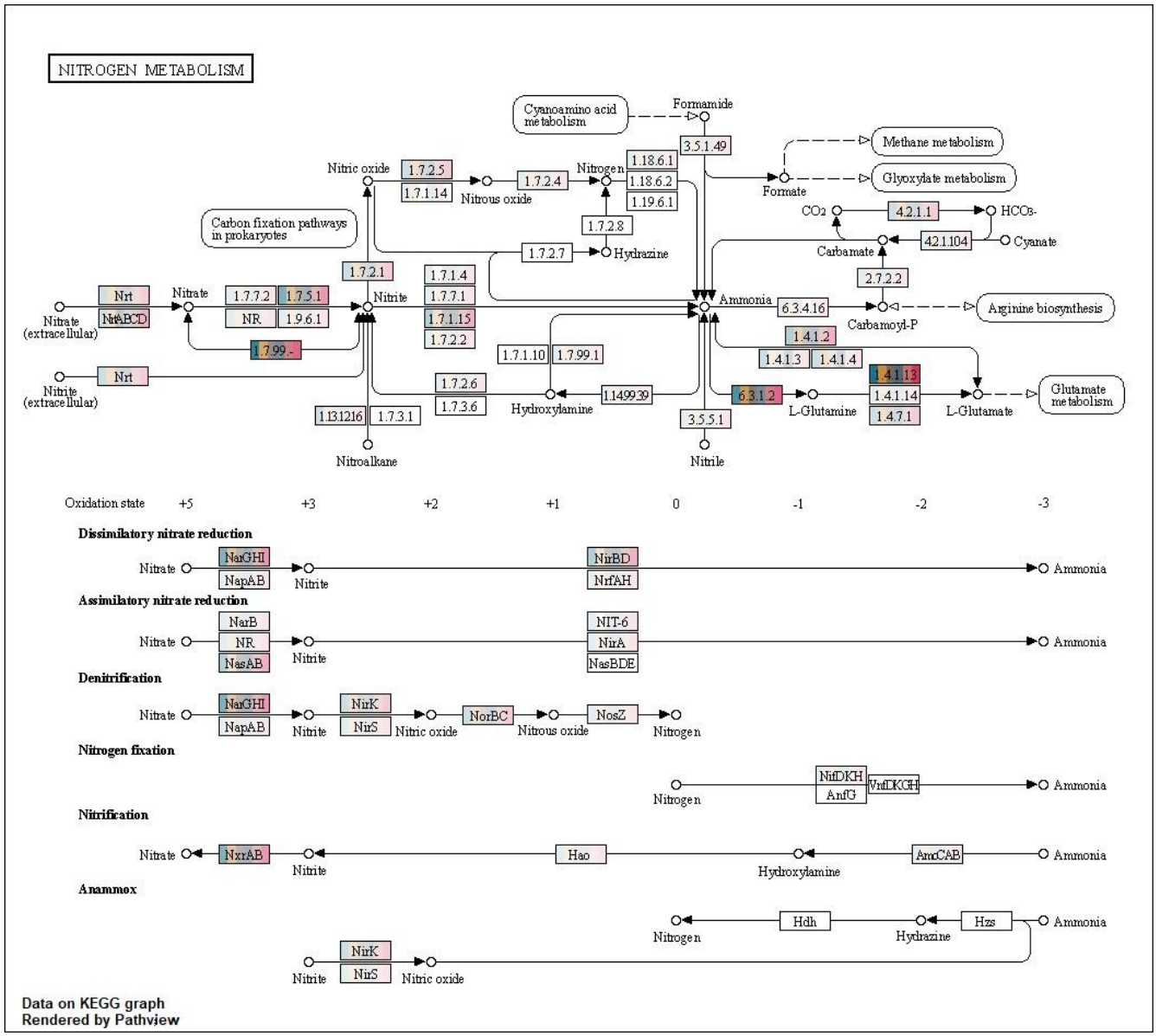


Figure 5.4 Enzymes involved in nitrogen metabolism detected in soil metagenome

It again phosphorylates to 1D-*myo*-Inositol-1,3,4,5,6P_5 using inositol-polyphosphate multikinase [EC: 2.7.1.151], which is then transformed to inositol hexakisphosphate (phytate) by inositol-pentakisphosphate 2-kinase [EC: 2.7.1.158], and phytate degrades into two components. The enzyme 4-phytase is used by inositol-1,2,3,4,5P_5 [EC: 3.1.3.26], and 3-phytase is used by inositol-1,2,4,5,6P_5 [EC: 3.1.3.8]. These are the main hydrolytic enzymes that catalyze the

breakdown of phytases to orthophosphate and are found in microorganisms, plants and animals (Rivera-Solís et al., 2014). Al(50 %)+CF(50 %) and Al(100 %) treatments are more prevalent in these enzyme reactions. Few other reactions also influenced by Al(50 %)+CF(50 %) treatments such as D-Glucose-6P into 1D-myo-inositol-3P using inositol-3-phosphate synthase [EC: 5.5.1.4], and it is further transformed to *myo*-Inositol by inositol phosphate phosphatase [EC: 3.1.3.25]. This compound transformed to its various isomeric forms, such as *scyllo*-Inositol, D-*chiro*-Insoitol and 1-Keto-D-*chiro*-inositol using *scyllo*-inositol 2-dehydrogenase (NAD^+) [EC: 1.1.1.370], D-*chiro*-inositol 1-dehydrogenase [EC: 1.1.1.369] and 2-keto-*myo*-inositol isomerase [EC: 5.3.99.11]. These isomers are converted to *scyllo*-Inosose, further transformations take place, and finally, two products are formed; one is malonic semialdehyde, which converts to acetyl Co A and enters the TCA cycle, where it is oxidized for energy production. The other product is dihydroxyacetone phosphate, which transforms into glyceraldehyde-3P and enters glycolysis. These final reactions showed higher enzyme abundance in both Al(50 %)+CF(50 %) and Al(100 %) treatments. Thus, the microalgal treatments showed a higher influence on the conversion of organic P and the release of phytate, the sole storage form of P in cereals and legumes (Zhu and Wakisaka, 2020). Therefore, by P-solubilization and P-mineralization, this finding would aid in estimating the potential contributions of microalgal bio-inoculant to soil P-cycling.

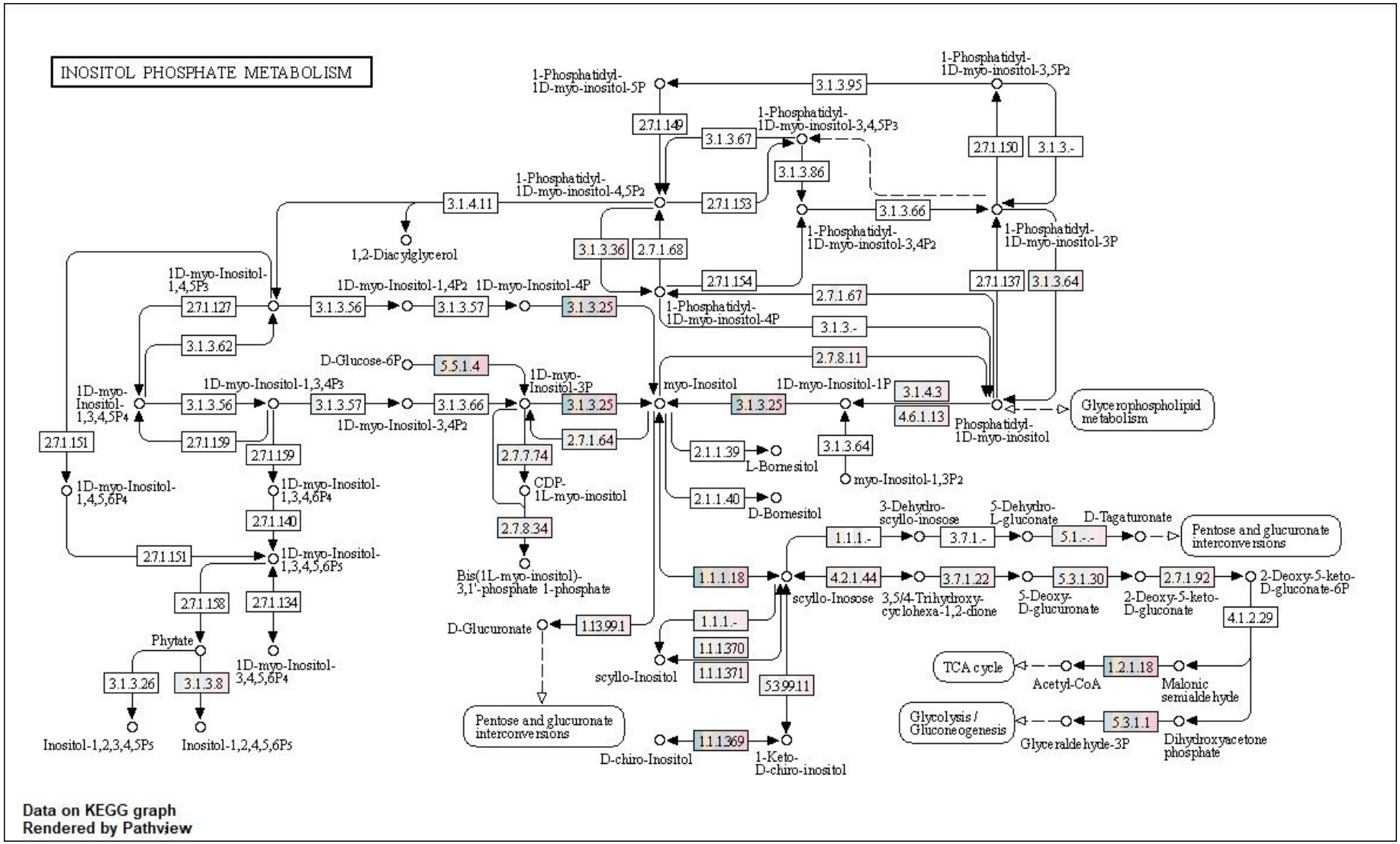


Figure 5.5 Enzymes involved in phosphorus metabolism detected in soil metagenome

5.4 Conclusion

The present study revealed the influence of a cyanobacteria-microalgae consortium in soil on the abundance of various functional genes related to C, N, and P metabolism in the chili plant rhizosphere and their influence on the predicted functional pathways. The bio-inoculant improved the gene diversity, which may have revealed changes in the rhizosphere of the plants and demonstrated rhizobacteria with supplies of C, N, and P that promote plant growth. The microalgal treatments (both Al(50 %)+CF(50 %) and Al(100 %)) enhanced specific unclassified groups of the microbial community that are mainly involved in the C, N, and P metabolism. The enzyme abundance for C, N, and P metabolism was also high in Al(100 %) as well as Al(50 %)+CF(50 %) compared to the CNT_I. Modifications in microbial taxa and functional genes indicated that microalgal bio-inoculant was a major driver of microbial metabolic change. In the predicted Kegg pathway of N and P metabolism, the main catalytic reactions were highly influenced by microalgal bio-inoculants. Thus, this work has evidenced the potential of cyanobacteria-microalgae consortia involved in the C, N, and P metabolism and directly measures changes in functional gene abundance among soil microbiomes, indicating the potential shifts of soil microbiome that pave the way to the development of sustainable agriculture.

ating the potential shifts of soil microbiome that pave the way to the development of sustainable agriculture.

CHAPTER SIX

Conclusions and Recommendations

6.1 Conclusions

- In this study, 13 indigenous cyanobacteria and green microalgae were isolated and screened for their potential as bio-inoculants. The results of this study showed that microalgal growth, biomass production, exopolymers, phytohormones, and nutrient production varied among species. The IAA production in the biomass extracts from cyanobacteria was significantly higher than in green microalgae. The best performing microalgal strains (*Desmodesmus armatus, Chlorella* sp., *Nostoc* sp., and *Calothrix* sp.) were used to develop the consortia.
- Among the eight consortia developed, the consortium of *Desmodesmus armatus, Nostoc* sp., and *Calothrix* sp (T7) demonstrated the highest growth and biomass production under N deficient conditions. Compared to individual strains, the consortium biomass was found to contain a higher amount of IAA, C, N, P, K, Ca, Cu, Fe, and Mn. The developed consortia were used as biostimulant and bio-inoculant.
- A significant increase in seedling length and leaf number was observed when seeds were treated with biomass extracts of microalgae consortium compared with controls. Additionally, the pot culture study showed that microalgal bio-inoculants exhibited a positive effect on soil fertility, chili plant growth, and the native soil microbiome. Compared to controls, the Al(100 %) and Al(50 %)+CF(50 %) treatments showed an increase in soil DHA, OC, Chl, TP, C, N, P, K and Mn content. These findings supported that the microalgal bio-inoculant stimulated the seedling growth and increased soil nutrient content.
- A significant increase in shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, root length and the number of leaves was observed in Al (50 %)+CF(50 %) treatment compared to Cnt. The shoot length increased in both microalgal treatments Al(100 %) and Al(50 %)+CF(50 %) compared to the Cnt. Photosynthetic efficiency (Fv/Fm) and rETR of chili plant leaves showed 14 % and 2 % increase in Al(50

%)+CF(50 %) compared to Cnt, respectively. The chili fruit parameters such as the length of chili fruit, fresh weight of chili, dry weight of chili, and number of fruits per plant were higher in Al(50 %)+CF(50 %) and Al(100 %) than the Cnt. The findings from the study suggested that microalgal bio-inoculation improved the chili plant growth.

- Microalgal bio-inoculation produced changes in the soil native (bacterial and fungal) community structure, which correlated with increase in soil enzyme activity, nutrient concentration, plant growth and yield performance. The microalgal amended soils were characterized by a higher relative abundance and diversity of potentially beneficial microbes. Favourable response of potentially beneficial microbes indicated the significance and importance of microalgal applications to soil and plants. Thus, the results obtained from 16SrRNA and ITS amplicon sequencing suggested that microalgal bio-inoculation improved the diversity and composition of native soil microbiome.
- Shotgun metagenomics study revealed the influence of microalgal treatment to enhance the taxonomic abundance of the microbial community responsible for C, N, and P metabolism. It also showed a higher abundance in the enzymatic activities of the C, N and P metabolism in soil rhizosphere microbial communities. Changes in functional genes and microbial taxa suggested that microalgal bio-inoculation was a key factor in the microbial metabolic shift. The predicted metabolic pathway of N and P showed the influence of microalgal bio-inoculant by enhancing the enzyme activity and genes responsible for N and P metabolism.
- e of microalgal bio-inoculant by enhancing the enzyme activity and genes responsible for N and P metabolism.

6.2 Recommendations

- To develop efficient microalgal consortia for various agricultural crops, it is necessary to isolate and screen indigenous microalgal strains with enhanced N-fixation, EPS production, phytohormone production, and stress tolerance. Creating a database

containing pertinent information about the strains would facilitate their use in agricultural applications.

- The development of consortia in N-deficient environments is becoming increasingly popular. This study demonstrated the importance of consortia (combinations of cyanobacteria and green microalgae) in increasing biomass yield and phytohormone production. In spite of this, additional laboratory studies and large-scale experiments are required before this can be recommended for commercial use.
- An in-depth understanding of soil-plant dynamics and the influence of microalgae on microbes-soil-plant interactions requires the application of advanced platforms such as OMICS tools. The use of OMICS tools offers researchers insights into the genetic composition, functional capabilities, and metabolic profiles of microorganisms, as well as the mechanisms by which microalgae affect their interactions.
- A greater understanding of genomics, transcriptomics, proteomics, and metabolomics may enable researchers to create unique modified strains (super strains) with desirable characteristics for soil and agricultural applications.
- Microalgal potential for improving soil fertility and plant growth is well established using pot studies. Adopting this technology for sustainable agriculture can be highly advantageous for large-scale field applications; however, additional research and development in the form of long-term field trials is recommended.

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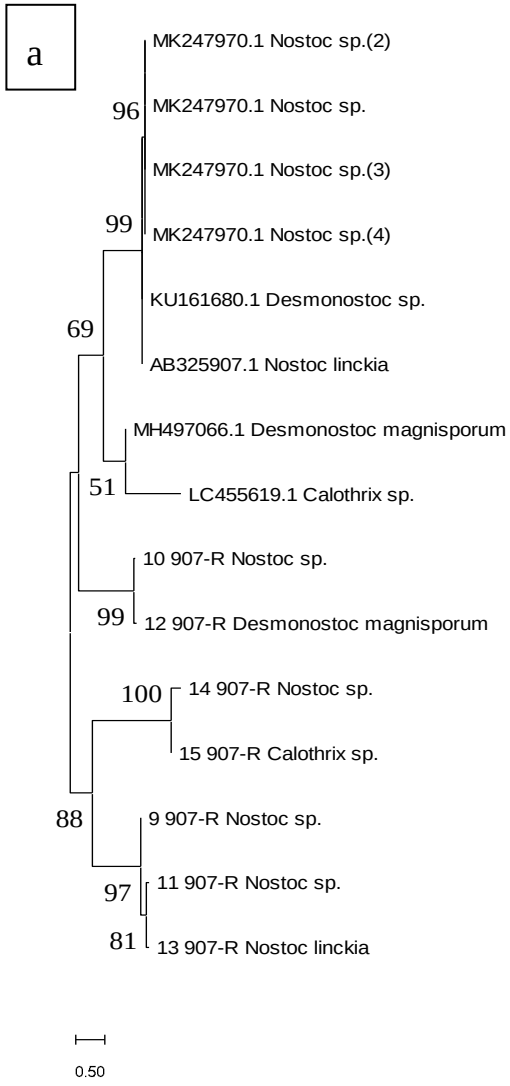
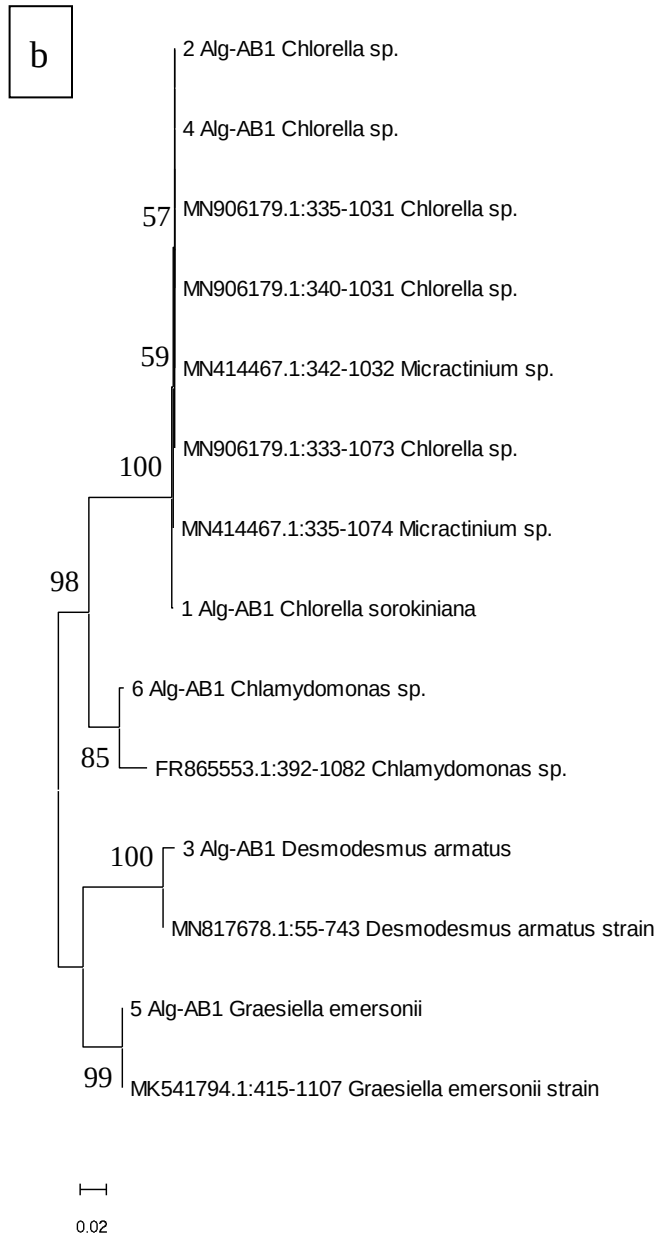
APPENDICES

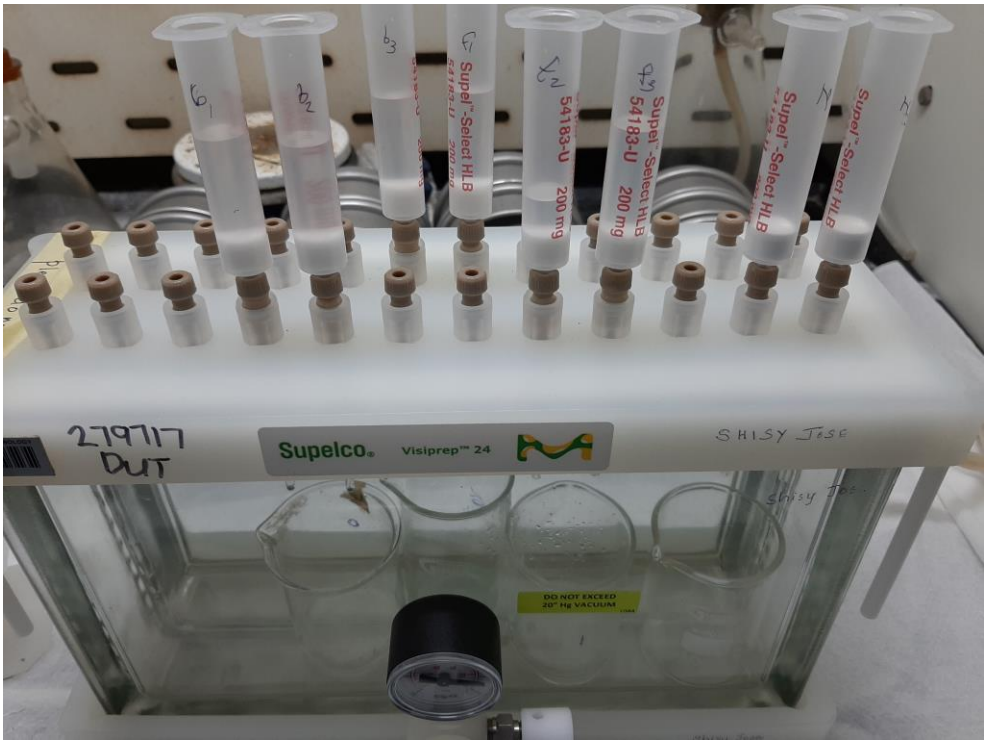
Appendix 1 Phylogenetic tree depicts results of Neighbour-joining analysis (Mega 11) (Chapter 3)





A: Cyanobacteria phylogenetic tree, B: Microalgae phylogenetic tree

Appendix 2 Solid phase extraction protocol (Chapter 3)

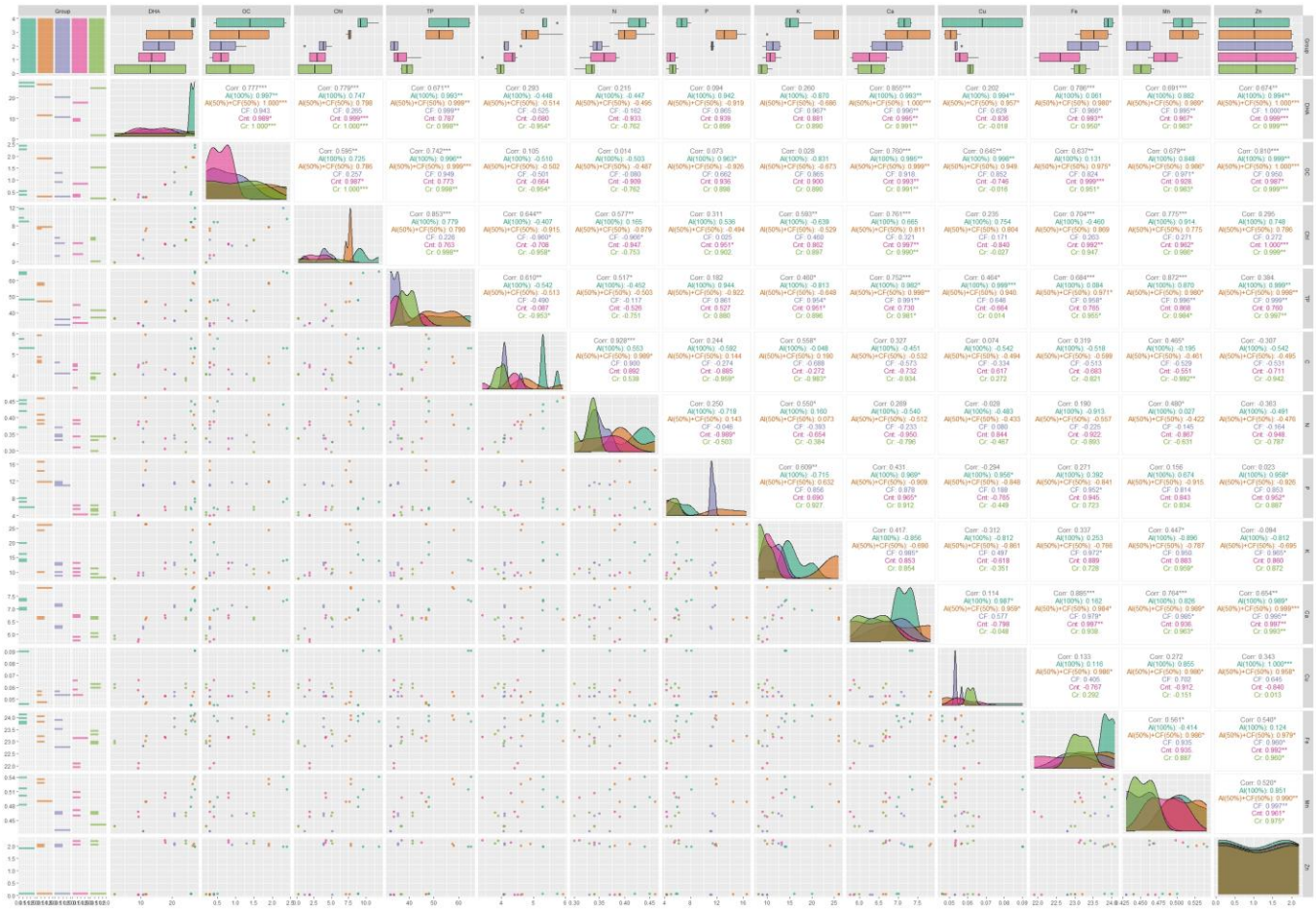


Supel-Select SPE 200mg/6mL cartridges, capable of retaining different range from reverse phase to a combination of reverse phase and ion exchange were used (Merck KGaA, Darmstadt, Germany) to purify and enrich plant growth regulators in microalgae. The experiment was carried out with standard phytohormones mixtures of Indole acetic acid, zeatin and microalgal sample to see the response of the cartridge. The pre-treated dried microalgal extract re-dissolved in 20 % methanol. Initially the cartridge is conditioned by 4 mL of methanol and equilibrated using 20 % methanol adjusted to pH 3 by adding formic acid 0.1 %. Then the 4 mL of microalgal sample is loaded in the cartridge. The hormones retained the column were washed with 2 mL water and eluted with 1 mL methanol. The concentrated phytohormones extracts were determined using UHPLC LCMS/MS system.

Appendix 3 Initial characteristics of soil used in the experiment (Chapter 4)

Soil parameters	Initial charcteristics
Carbon	4.6 %
Nitrogen	0.38 %
Potassium	9 mg kg^{-1}
Dehydrogenase activity	8.9 µg g^{-1}
Organic carbon	0.29 %
Soil chlorophyll	1.8 mg g^{-1}
Total polysaccharides	33.5 µg g^{-1}
Calcium	6.1 mg kg^{-1}
Copper	0.06 mg kg^{-1}
Iron	22.3 mg kg^{-1}
Manganese	0.46 mg kg^{-1}
Zinc	0.06 mg kg^{-1}
Bacterial colony count	215 x 10^{-4} CFU mL^{-1}
Fungal colony count	144 x 10^{-3} CFU mL^{-1}

Appendix 4 Correlation of soil parameters in different treatments (Chapter 4)



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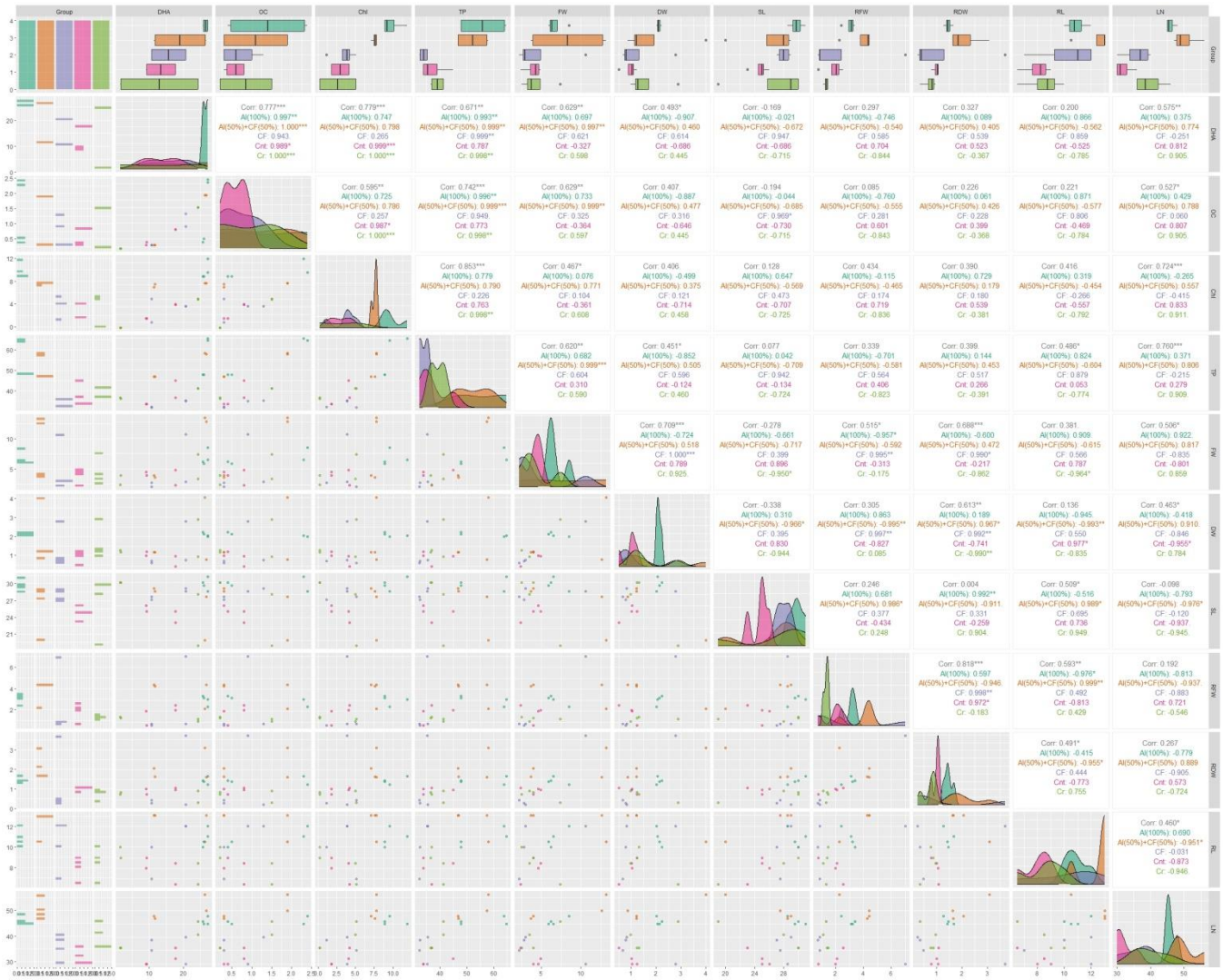
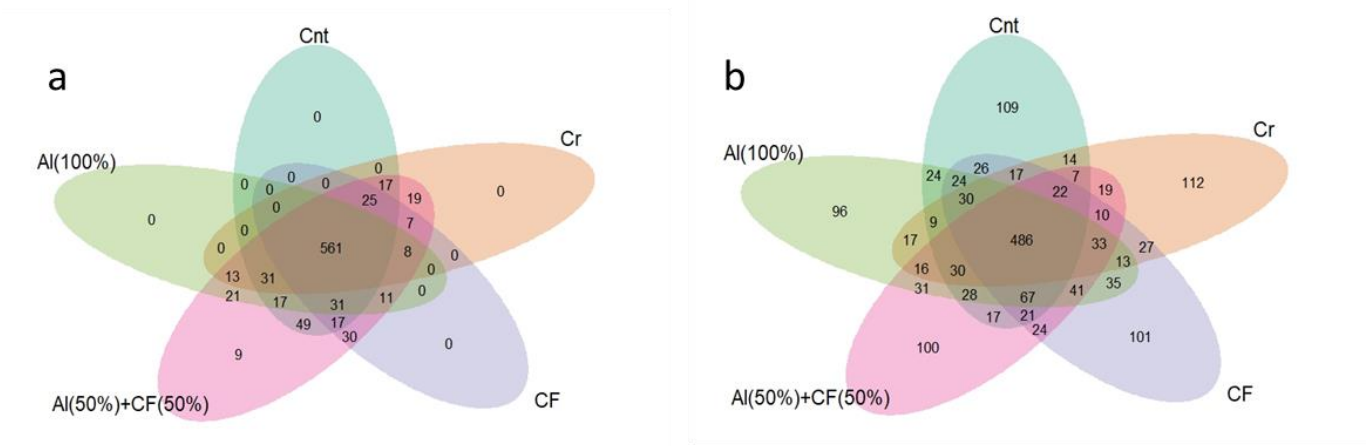
Corr, Correlation; Cnt, Control; Cr, Carrier; CF, Chemical fertilizer; Al(50 %)+CF(50 %), Combination of 50 % microalgal consortia and 50 % chemical fertilizer; Al(100 %), 100 % microalgal consortia. DHA, Dehydrogenase activity; OC, Organic carbon; Chl, Soil chlorophyll; TP, Total polysaccharides; C, Carbon; N, Nitrogen; P, Phosphorus; K, Potassium; Ca, Calcium; Cu, Copper; Fe, Iron; Mn, Mangaese; Zn, Zinc. ***p < 0.001, ** p < 0.01, * p < 0.05.

Appendix 5Correlation of soil parameters with plant parameters (Chapter 4)



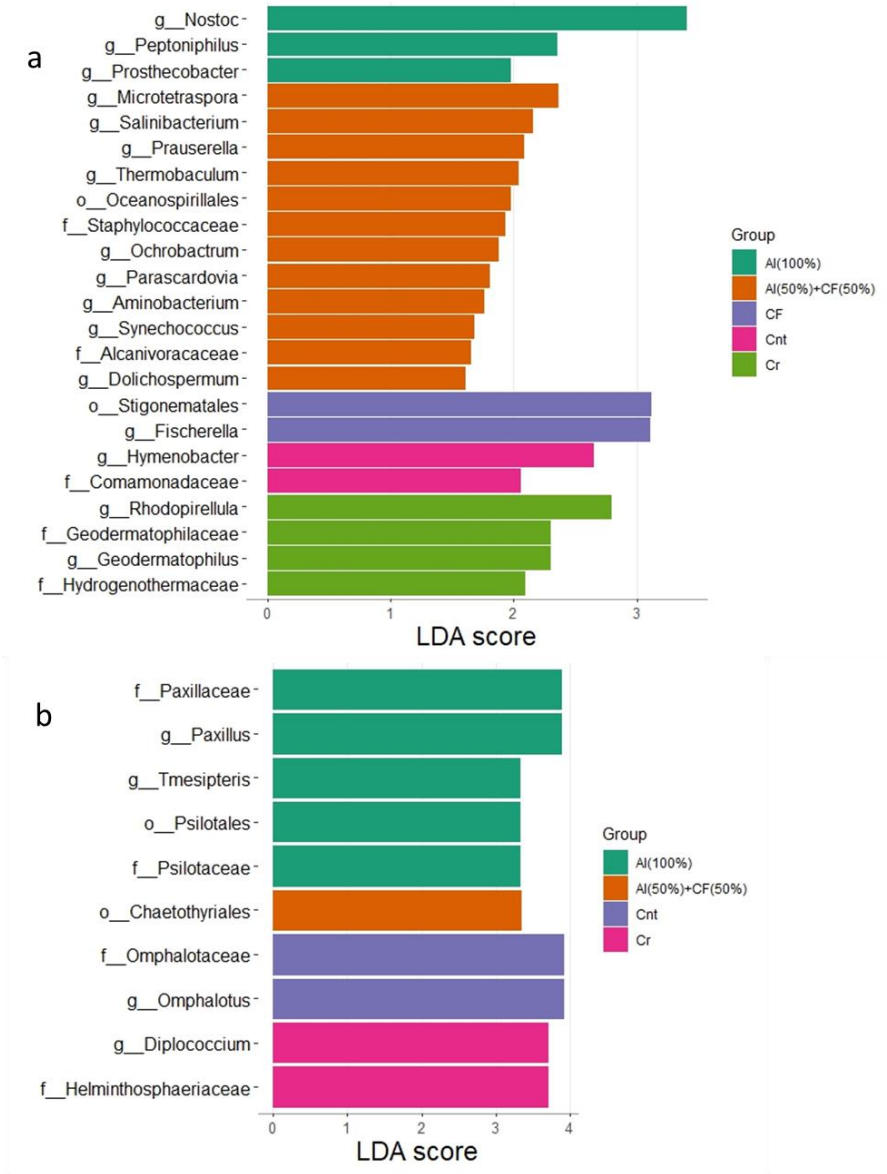
Corr, Correlation; Cnt, Control; Cr, Carrier; CF, Chemical fertilizer; Al(50 %)+CF(50 %), Combination of 50 % microalgal consortia and 50 % chemical fertilizer; Al(100 %), 100 % microalgal consortia, DHA, Dehydrogenase activity; OC, Organic carbon; Chl, Soil chlorophyll; TP, Total polysaccharides; FW, Plant fresh weight; DW, Plant dry weight; SL, Shoot length; RFW, Root fresh weight; RDW, Root dry weigth; RL, Root length; LN, Leaf number.***p < 0.001, ** p < 0.01, * p < 0.05.

Appendix 6 Venn diagram of bacterial and fungal community (Chapter 4)



Venn diagram showing the OTUs distribution between the treatments of pot experiment (A) Bacterial community (B) Fungal community. Cnt, Control; Cr, Carrier; CF, Chemical fertilizer; Al(50 %)+CF(50 %), Combination of 50 % microalgal consortia and 50 % Chemical fertilizer; Al(100 %), 100 % Microalgal consortia

Appendix 7 LefSe analysis of bacterial and fungal community (Chapter 4)



LEfSe (Linear discriminant analysis effect size) analysis depicting (**a**) bacterial and (**b**) fungal microbial community changes between different treatment groups:

Cnt, Control; Cr, Carrier; CF, Chemical fertilizer; Al(50 %)+CF(50 %), Combination of 50 % microalgal consortia and 50 % Chemical fertilizer; Al(100 %), 100 % microalgal consortia.

Appendix 8 Macronutrients and micronutrients in the chili plant (Chapter 4)

Treatment	Nitrogen (mg/kg)	Phosphorus (mg/kg)	Potassium (mg/kg)	Calcium (mg/kg)	Copper (mg/kg)	Iron (mg/kg)	Magnesium (mg/kg)	Manganese (mg/kg)	Zinc (mg/kg)
Cnt	4.72±0.1	20±3	94±4	12.6 ± 0	0.16 ± 0.1	3.12 ± 0	5.8 ± 0.1	0.2 ± 0.1	6.5 ± 0.1
Cr	4.50±0.06	32±1	172±1	13 ± 0.1	0.22 ± 0.1	4 ± 0.1	7.9 ± 0.3	0.22 ± 0.01	14 ± 0.3
CF	5.17±0.05	32±1	162±9	15 ± 0.1	0.17 ± 0.1	7.4 ± 0.9	7.3 ± 0.1	0.28 ± 0.02	18.9 ± 0.5
Al(50 %)+ CF(50 %)	5.27±0.13	44±2	224±2	16 ± 0.4	0.23 ± 0.1	7.1 ± 0.2	7.4 ± 0	0.28 ± 0.01	18.6 ± 0.1
Al(100 %)	4.52±0.08	46±4	174±11	13 ± 0.2	0.18 ± 0	6.1 ± 0.1	7.2 ± 0.1	0.27 ± 0.01	17.6 ± 0.3

Appendix 9 Correlation of soil parameters and bacterial community (Chapter 4)

Type	Kingdom	Phylum	Class	Env	Correlation	Pvalue	AdjPvalue	Significance
V1	Bacteria	Actinobacteria	Actinobacteria	DHA	-0.68	1.1E-03	0.01	*
V2	Bacteria	Firmicutes	Bacilli	DHA	0.45	4.6E-02	0.12	
V3	Bacteria	Chloroflexi	Thermomicrobia	DHA	0.52	1.8E-02	0.07	
V4	Bacteria	Planctomycetes	Planctomycetacia	DHA	0.75	1.6E-04	0.01	**
V5	Bacteria	Bacteroidetes	Cytophagia	DHA	-0.05	8.3E-01	0.92	
V6	Bacteria	Firmicutes	Clostridia	DHA	0.70	5.5E-04	0.01	*
V7	Bacteria	Proteobacteria	Gammaproteobacteria	DHA	0.47	3.9E-02	0.11	
V8	Bacteria	Proteobacteria	Alphaproteobacteria	DHA	0.54	1.4E-02	0.07	

V9	Bacteria	Chloroflexi	Chloroflexi	DHA	-0.07	7.7E-01	0.91	
V10	Bacteria	Proteobacteria	Deltaproteobacteria	DHA	0.60	5.0E-03	0.03	*
V11	Bacteria	Bacteroidetes	Sphingobacteria	DHA	-0.66	1.6E-03	0.02	*
V12	Bacteria	Firmicutes	Negativicutes	DHA	0.54	1.4E-02	0.07	
V13	Bacteria	Proteobacteria	Betaproteobacteria	DHA	0.38	9.9E-02	0.20	
V14	Bacteria	Bacteroidetes	Bacteroidia	DHA	0.04	8.7E-01	0.93	
V15	Bacteria	Chlamydiae	Chlamydiae	DHA	0.52	2.0E-02	0.08	
V16	Bacteria	Gemmatimonadetes	Gemmatimonadetes	DHA	0.34	1.5E-01	0.29	
V17	Bacteria	Verrucomicrobia	Verrucomicrobiae	DHA	0.44	5.5E-02	0.12	
V18	Bacteria	Bacteroidetes	Flavobacteria	DHA	-0.45	4.9E-02	0.12	
V19	Bacteria	Nitrospirae	Nitrospira	DHA	0.61	3.9E-03	0.03	*
V20	Bacteria	Spirochaetes	Spirochaetes	DHA	0.31	1.8E-01	0.34	
V21	Bacteria	Acidobacteria	Solibacteres	DHA	0.45	4.8E-02	0.12	
V22	Bacteria	Chloroflexi	Ktedonobacteria	DHA	-0.15	5.3E-01	0.75	
V23	Bacteria	Verrucomicrobia	Opitutae	DHA	0.16	5.0E-01	0.75	
V24	Bacteria	Aquificae	Aquificae	DHA	-0.15	5.2E-01	0.75	
V25	Bacteria	Proteobacteria	Epsilonproteobacteria	DHA	0.01	9.8E-01	0.99	
V26	Bacteria	Deinococcus-Thermus	Deinococci	DHA	-0.06	8.0E-01	0.91	
V27	Bacteria	Verrucomicrobia	Spartobacteria	DHA	-0.04	8.8E-01	0.93	
V28	Bacteria	Chloroflexi	Dehalococcoidetes	DHA	0.12	6.2E-01	0.81	
V29	Bacteria	Synergistetes	Synergistia	DHA	0.30	1.9E-01	0.34	

V30	k__Archaea	Euryarchaeota	Methanobacteria	DHA	0.00	9.9E-01	0.99	
V31	Bacteria	Tenericutes	Mollicutes	DHA	0.53	1.6E-02	0.07	
V32	k__Archaea	Euryarchaeota	Methanomicrobia	DHA	0.21	3.7E-01	0.61	
V33	Bacteria	Dictyoglomi	Dictyoglomia	DHA	0.20	3.9E-01	0.62	
V34	Bacteria	Chlorobi	Chlorobia	DHA	-0.06	7.9E-01	0.91	
V35	Bacteria	Firmicutes	Erysipelotrichi	DHA	0.44	5.5E-02	0.12	
V36	Bacteria	Acidobacteria	Acidobacteria	DHA	-0.50	2.7E-02	0.08	
V37	Bacteria	Thermotogae	Thermotogae	DHA	-0.50	2.5E-02	0.08	
V38	Bacteria	Thermodesulfobacteria	Thermodesulfobacteria	DHA	-0.11	6.5E-01	0.81	
V39	Bacteria	Fibrobacteres	Fibrobacteres	DHA	-0.12	6.3E-01	0.81	
V40	Bacteria	Deferribacteres	Deferribacteres	DHA	-0.21	3.7E-01	0.61	
V41	k__Archaea	Euryarchaeota	Halobacteria	DHA	-0.11	6.6E-01	0.81	
V42	Bacteria	Actinobacteria	Actinobacteria	OC	-0.76	1.1E-04	0.00	**
V43	Bacteria	Firmicutes	Bacilli	OC	0.67	1.3E-03	0.01	**
V44	Bacteria	Chloroflexi	Thermomicrobia	OC	0.44	5.0E-02	0.11	
V45	Bacteria	Planctomycetes	Planctomycetacia	OC	0.81	1.5E-05	0.00	***
V46	Bacteria	Bacteroidetes	Cytophagia	OC	-0.12	6.1E-01	0.81	
V47	Bacteria	Firmicutes	Clostridia	OC	0.77	7.3E-05	0.00	**
V48	Bacteria	Proteobacteria	Gammaproteobacteria	OC	0.63	2.9E-03	0.01	*
V49	Bacteria	Proteobacteria	Alphaproteobacteria	OC	0.67	1.3E-03	0.01	**
V50	Bacteria	Chloroflexi	Chloroflexi	OC	-0.08	7.3E-01	0.84	

V51	Bacteria	Proteobacteria	Deltaproteobacteria	OC	0.59	6.7E-03	0.02	*
V52	Bacteria	Bacteroidetes	Sphingobacteria	OC	-0.76	9.1E-05	0.00	**
V53	Bacteria	Firmicutes	Negativicutes	OC	0.72	3.5E-04	0.00	**
V54	Bacteria	Proteobacteria	Betaproteobacteria	OC	0.48	3.2E-02	0.09	
V55	Bacteria	Bacteroidetes	Bacteroidia	OC	-0.36	1.2E-01	0.21	
V56	Bacteria	Chlamydiae	Chlamydiae	OC	0.60	5.4E-03	0.02	*
V57	Bacteria	Gemmatimonadetes	Gemmatimonadetes	OC	0.50	2.6E-02	0.08	
V58	Bacteria	Verrucomicrobia	Verrucomicrobiae	OC	0.46	4.0E-02	0.10	
V59	Bacteria	Bacteroidetes	Flavobacteria	OC	-0.68	8.8E-04	0.01	**
V60	Bacteria	Nitrospirae	Nitrospira	OC	0.67	1.2E-03	0.01	**
V61	Bacteria	Spirochaetes	Spirochaetes	OC	0.36	1.2E-01	0.21	
V62	Bacteria	Acidobacteria	Solibacteres	OC	0.37	1.1E-01	0.21	
V63	Bacteria	Chloroflexi	Ktedonobacteria	OC	-0.35	1.3E-01	0.21	
V64	Bacteria	Verrucomicrobia	Opitutae	OC	0.05	8.2E-01	0.84	
V65	Bacteria	Aquificae	Aquificae	OC	0.12	6.2E-01	0.81	
V66	Bacteria	Proteobacteria	Epsilonproteobacteria	OC	0.09	7.2E-01	0.84	
V67	Bacteria	Deinococcus-Thermus	Deinococci	OC	0.09	7.2E-01	0.84	
V68	Bacteria	Verrucomicrobia	Spartobacteria	OC	0.07	7.6E-01	0.84	
V69	Bacteria	Chloroflexi	Dehalococcoidetes	OC	-0.22	3.6E-01	0.54	
V70	Bacteria	Synergistetes	Synergistia	OC	0.35	1.3E-01	0.21	
V71	k__Archaea	Euryarchaeota	Methanobacteria	OC	-0.06	8.1E-01	0.84	

V72	Bacteria	Tenericutes	Mollicutes	OC	0.49	3.0E-02	0.09
V73	k__Archaea	Euryarchaeota	Methanomicrobia	OC	-0.06	7.9E-01	0.84
V74	Bacteria	Dictyoglomi	Dictyoglomia	OC	0.12	6.3E-01	0.81
V75	Bacteria	Chlorobi	Chlorobia	OC	0.07	7.7E-01	0.84
V76	Bacteria	Firmicutes	Erysipelotrichi	OC	0.45	4.7E-02	0.11
V77	Bacteria	Acidobacteria	Acidobacteria	OC	-0.41	7.2E-02	0.16
V78	Bacteria	Thermotogae	Thermotogae	OC	-0.36	1.1E-01	0.21
V79	Bacteria	Thermodesulfobacteria	Thermodesulfobacteria	OC	0.19	4.3E-01	0.63
V80	Bacteria	Fibrobacteres	Fibrobacteres	OC	-0.04	8.7E-01	0.87
V81	Bacteria	Deferribacteres	Deferribacteres	OC	-0.25	2.8E-01	0.44
V82	k__Archaea	Euryarchaeota	Halobacteria	OC	-0.17	4.8E-01	0.68
V83	Bacteria	Actinobacteria	Actinobacteria	Chl	-0.44	5.5E-02	0.39
V84	Bacteria	Firmicutes	Bacilli	Chl	0.23	3.2E-01	0.80
V85	Bacteria	Chloroflexi	Thermomicrobia	Chl	0.34	1.5E-01	0.54
V86	Bacteria	Planctomycetes	Planctomycetacia	Chl	0.52	1.8E-02	0.19
V87	Bacteria	Bacteroidetes	Cytophagia	Chl	-0.08	7.4E-01	0.87
V88	Bacteria	Firmicutes	Clostridia	Chl	0.56	9.5E-03	0.19
V89	Bacteria	Proteobacteria	Gammaproteobacteria	Chl	0.40	8.0E-02	0.41
V90	Bacteria	Proteobacteria	Alphaproteobacteria	Chl	0.42	6.7E-02	0.39
V91	Bacteria	Chloroflexi	Chloroflexi	Chl	0.04	8.8E-01	0.90
V92	Bacteria	Proteobacteria	Deltaproteobacteria	Chl	0.36	1.2E-01	0.54

V93	Bacteria	Bacteroidetes	Sphingobacteria	Chl	-0.59	6.3E-03	0.19
V94	Bacteria	Firmicutes	Negativicutes	Chl	0.34	1.4E-01	0.54
V95	Bacteria	Proteobacteria	Betaproteobacteria	Chl	0.20	4.1E-01	0.80
V96	Bacteria	Bacteroidetes	Bacteroidia	Chl	0.07	7.9E-01	0.87
V97	Bacteria	Chlamydiae	Chlamydiae	Chl	0.22	3.6E-01	0.80
V98	Bacteria	Gemmatimonadetes	Gemmatimonadetes	Chl	0.11	6.4E-01	0.87
V99	Bacteria	Verrucomicrobia	Verrucomicrobiae	Chl	0.11	6.4E-01	0.87
V100	Bacteria	Bacteroidetes	Flavobacteria	Chl	-0.20	4.1E-01	0.80
V101	Bacteria	Nitrospirae	Nitrospira	Chl	0.26	2.6E-01	0.77
V102	Bacteria	Spirochaetes	Spirochaetes	Chl	0.07	7.7E-01	0.87
V103	Bacteria	Acidobacteria	Solibacteres	Chl	0.12	6.2E-01	0.87
V104	Bacteria	Chloroflexi	Ktedonobacteria	Chl	-0.15	5.2E-01	0.87
V105	Bacteria	Verrucomicrobia	Opitutae	Chl	-0.01	9.6E-01	0.96
V106	Bacteria	Aquificae	Aquificae	Chl	-0.12	6.0E-01	0.87
V107	Bacteria	Proteobacteria	Epsilonproteobacteria	Chl	0.31	1.8E-01	0.56
V108	Bacteria	Deinococcus-Thermus	Deinococci	Chl	-0.21	3.7E-01	0.80
V109	Bacteria	Verrucomicrobia	Spartobacteria	Chl	-0.06	7.9E-01	0.87
V110	Bacteria	Chloroflexi	Dehalococcoidetes	Chl	-0.06	7.9E-01	0.87
V111	Bacteria	Synergistetes	Synergistia	Chl	0.42	6.5E-02	0.39
V112	k__Archaea	Euryarchaeota	Methanobacteria	Chl	0.06	8.0E-01	0.87
V113	Bacteria	Tenericutes	Mollicutes	Chl	0.33	1.6E-01	0.54

V114	k__Archaea	Euryarchaeota	Methanomicrobia	Chl	0.22	3.6E-01	0.80
V115	Bacteria	Dictyoglomi	Dictyoglomia	Chl	0.07	7.8E-01	0.87
V116	Bacteria	Chlorobi	Chlorobia	Chl	-0.15	5.4E-01	0.87
V117	Bacteria	Firmicutes	Erysipelotrichi	Chl	0.54	1.4E-02	0.19
V118	Bacteria	Acidobacteria	Acidobacteria	Chl	-0.14	5.5E-01	0.87
V119	Bacteria	Thermotogae	Thermotogae	Chl	-0.21	3.9E-01	0.80
V120	Bacteria	Thermodesulfobacteria	Thermodesulfobacteria	Chl	-0.06	8.1E-01	0.87
V121	Bacteria	Fibrobacteres	Fibrobacteres	Chl	-0.06	8.0E-01	0.87
V122	Bacteria	Deferribacteres	Deferribacteres	Chl	-0.08	7.3E-01	0.87
V123	k__Archaea	Euryarchaeota	Halobacteria	Chl	0.05	8.3E-01	0.87
V124	Bacteria	Actinobacteria	Actinobacteria	TP	-0.43	5.8E-02	0.30
V125	Bacteria	Firmicutes	Bacilli	TP	0.34	1.5E-01	0.59
V126	Bacteria	Chloroflexi	Thermomicrobia	TP	0.29	2.2E-01	0.59
V127	Bacteria	Planctomycetes	Planctomycetacia	TP	0.49	3.0E-02	0.30
V128	Bacteria	Bacteroidetes	Cytophagia	TP	-0.16	5.0E-01	0.85
V129	Bacteria	Firmicutes	Clostridia	TP	0.47	3.5E-02	0.30
V130	Bacteria	Proteobacteria	Gammaproteobacteria	TP	0.43	5.6E-02	0.30
V131	Bacteria	Proteobacteria	Alphaproteobacteria	TP	0.44	5.0E-02	0.30
V132	Bacteria	Chloroflexi	Chloroflexi	TP	-0.01	9.8E-01	0.98
V133	Bacteria	Proteobacteria	Deltaproteobacteria	TP	0.30	2.0E-01	0.59
V134	Bacteria	Bacteroidetes	Sphingobacteria	TP	-0.63	2.6E-03	0.11

V135	Bacteria	Firmicutes	Negativicutes	TP	0.45	4.7E-02	0.30
V136	Bacteria	Proteobacteria	Betaproteobacteria	TP	0.28	2.3E-01	0.59
V137	Bacteria	Bacteroidetes	Bacteroidia	TP	-0.15	5.2E-01	0.85
V138	Bacteria	Chlamydiae	Chlamydiae	TP	0.24	3.1E-01	0.67
V139	Bacteria	Gemmatimonadetes	Gemmatimonadetes	TP	0.13	5.7E-01	0.87
V140	Bacteria	Verrucomicrobia	Verrucomicrobiae	TP	0.10	6.6E-01	0.90
V141	Bacteria	Bacteroidetes	Flavobacteria	TP	-0.29	2.1E-01	0.59
V142	Bacteria	Nitrospirae	Nitrospira	TP	0.29	2.2E-01	0.59

Appendix 10 Correlation of soil parameters and fungal community (Chapter 4)

Type	Taxa	Env	Correlation	Pvalue	AdjPvalue
V1	k__Eukaryota|Ascomycota|Orbiliomycetes	DHA	-0.38	0.10	0.61
V2	k__Eukaryota|Ascomycota|Saccharomycetes	DHA	0.16	0.49	0.69
V3	k__Eukaryota|Basidiomycota|Agaricomycetes	DHA	-0.41	0.08	0.61
V4	k__Eukaryota|Ascomycota|Sordariomycetes	DHA	0.22	0.36	0.62
V5	k__Eukaryota|Streptophyta|Liliopsida	DHA	0.32	0.17	0.61
V6	k__Eukaryota|Ascomycota|Lecanoromycetes	DHA	0.21	0.37	0.62
V7	k__Eukaryota|Ascomycota|Dothideomycetes	DHA	-0.33	0.16	0.61
V8	k__Eukaryota|Chlorophyta|Prasinophyceae	DHA	-0.39	0.09	0.61
V9	k__Eukaryota|Ascomycota|Eurotiomycetes	DHA	0.27	0.25	0.61
V10	k__Eukaryota||Cryptophyta	DHA	0.08	0.74	0.90
V11	k__Eukaryota||Chrysophyceae	DHA	0.39	0.09	0.61
V12	k__Eukaryota|Chlorophyta|Chlorophyceae	DHA	0.04	0.86	0.94

V13	k__Eukaryota|Streptophyta|Bryopsida	DHA	-0.01	0.98	0.99
V14	k__Eukaryota||Dinophyceae	DHA	0.23	0.33	0.62
V15	k__Eukaryota||Spirotrichea	DHA	0.04	0.88	0.94
V16	k__Eukaryota|Ascomycota|Pezizomycetes	DHA	0.06	0.79	0.92
V17	k__Eukaryota|Bacillariophyta|Coscinodiscophyceae	DHA	0.19	0.43	0.64
V18	k__Eukaryota||Oligohymenophorea	DHA	0.03	0.91	0.96
V19	k__Eukaryota|Streptophyta|Coniferopsida	DHA	-0.11	0.66	0.85
V20	k__Eukaryota|Ascomycota|Leotiomycetes	DHA	0.20	0.41	0.64
V21	k__Eukaryota||Stylonematophyceae	DHA	0.05	0.83	0.93
V22	k__Eukaryota|Streptophyta|Zygnemophyceae	DHA	0.21	0.36	0.62
V23	k__Eukaryota|Streptophyta|Jungermanniopsida	DHA	0.10	0.66	0.85
V24	k__Eukaryota|Chlorophyta|Trebouxiophyceae	DHA	0.09	0.71	0.89
V25	k__Eukaryota|Basidiomycota|Tremellomycetes	DHA	0.32	0.18	0.61
V26	k__Eukaryota|Chytridiomycota|Chytridiomycetes	DHA	-0.29	0.22	0.61
V27	k__Eukaryota|Streptophyta|Polytrichopsida	DHA	-0.50	0.03	0.61
V28	k__Eukaryota||Compsopogonophyceae	DHA	0.05	0.83	0.93
V29	k__Eukaryota|Chlorophyta|Ulvophyceae	DHA	0.17	0.47	0.66
V30	k__Eukaryota|Ascomycota|Arthoniomycetes	DHA	0.10	0.68	0.87
V31	k__Eukaryota|Basidiomycota|Ustilaginomycetes	DHA	0.30	0.20	0.61
V32	k__Eukaryota|Glomeromycota|Glomeromycetes	DHA	0.30	0.20	0.61
V33	k__Eukaryota||Bangiophyceae	DHA	-0.33	0.16	0.61
V34	k__Eukaryota||Synchromophyceae	DHA	-0.43	0.06	0.61
V35	k__Eukaryota|Bacillariophyta|Bacillariophyceae	DHA	0.24	0.30	0.62
V36	k__Eukaryota|Basidiomycota|Exobasidiomycetes	DHA	0.39	0.09	0.61
V37	k__Eukaryota|Streptophyta|Sphagnopsida	DHA	-0.41	0.07	0.61
V38	k__Eukaryota|Ascomycota|Taphrinomycetes	DHA	0.25	0.29	0.62

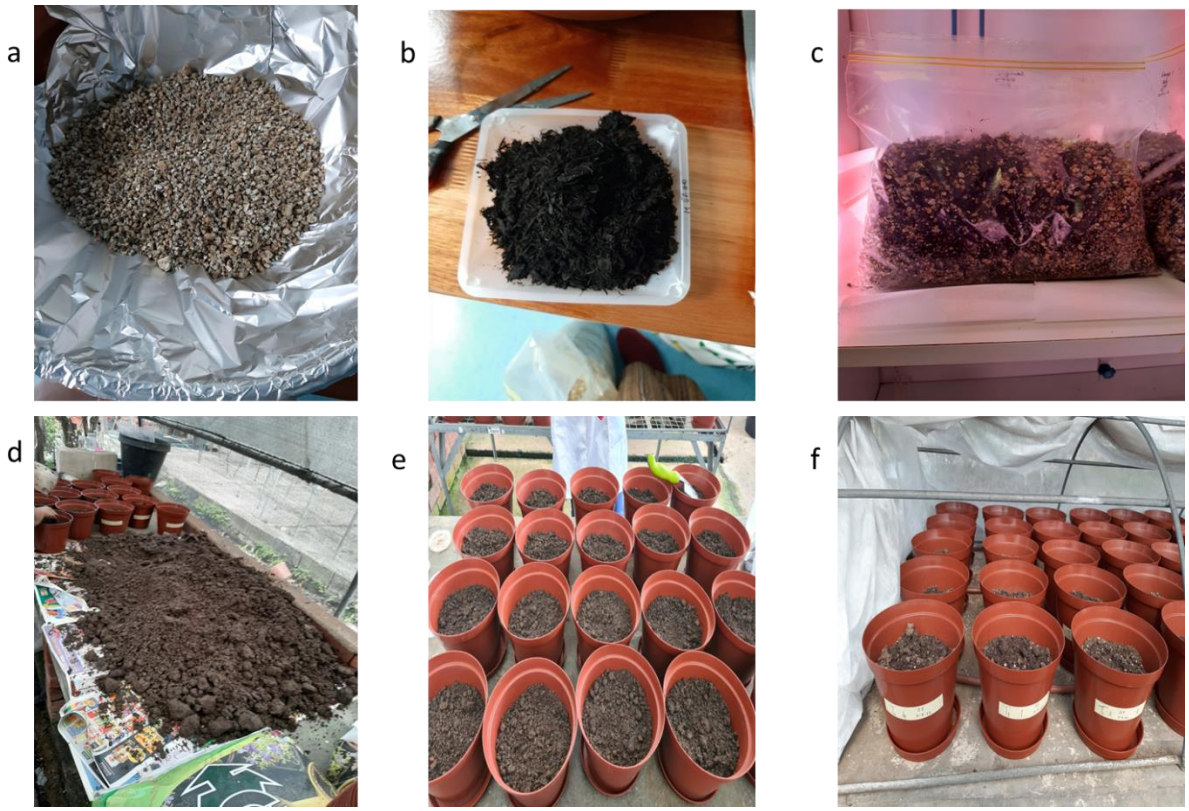
V39	k__Eukaryota|Bacillariophyta|Fragilariophyceae	DHA	0.45	0.05	0.61
V40	k__Eukaryota||Litostomatea	DHA	-0.25	0.28	0.62
V41	k__Eukaryota|Ascomycota|Lichinomycetes	DHA	0.45	0.05	0.61
V42	k__Eukaryota||Heterolobosea	DHA	-0.11	0.66	0.85
V43	k__Eukaryota|Basidiomycota|Dacrymycetes	DHA	-0.07	0.78	0.92
V44	k__Eukaryota||Synurophyceae	DHA	0.21	0.37	0.62
V45	k__Eukaryota|Streptophyta|Marchantiopsida	DHA	-0.48	0.03	0.61
V46	k__Eukaryota||Foraminifera	DHA	0.19	0.43	0.64
V47	k__Eukaryota||Dictyochophyceae	DHA	0.26	0.26	0.61
V48	k__Eukaryota|Streptophyta|Polypodiopsida	DHA	0.36	0.11	0.61
V49	k__Eukaryota||Karyorelictea	DHA	-0.06	0.80	0.92
V50	k__Eukaryota||Phaeothamniophyceae	DHA	0.28	0.23	0.61
V51	k__Eukaryota|Blastocladiomycota|Blastocladiomycetes	DHA	-0.18	0.45	0.66
V52	k__Eukaryota|Streptophyta|Gnetopsida	DHA	-0.27	0.25	0.61
V53	k__Eukaryota|Chytridiomycota|Monoblepharidomycetes	DHA	0.34	0.14	0.61
V54	k__Eukaryota||Heterotrichea	DHA	0.31	0.18	0.61
V55	k__Eukaryota||Katablepharidophyta	DHA	-0.06	0.80	0.92
V56	k__Eukaryota|Streptophyta|Cycadopsida	DHA	0.15	0.52	0.71
V57	k__Eukaryota||Colpodea	DHA	0.29	0.22	0.61
V58	k__Eukaryota||Ichthyosporea	DHA	-0.04	0.88	0.94
V59	k__Eukaryota||Raphidophyceae	DHA	-0.01	0.96	0.99
V60	k__Eukaryota||Pelagophyceae	DHA	0.27	0.25	0.61
V61	k__Eukaryota|Streptophyta|Charophyceae	DHA	0.34	0.15	0.61
V62	k__Eukaryota|Basidiomycota|Atractiellomycetes	DHA	0.02	0.93	0.97
V63	k__Eukaryota|Ascomycota|Schizosaccharomycetes	DHA	-0.08	0.74	0.90
V64	k__Eukaryota||Glaucocystophyceae	DHA	-0.15	0.54	0.73

V65	k__Eukaryota||Nassophorea	DHA	0.27	0.26	0.61
V66	k__Eukaryota||Prostomatea	DHA	0.32	0.17	0.61
V67	k__Eukaryota||Hyphochytriomycetes	DHA	0.29	0.21	0.61
V68	k__Eukaryota||Florideophyceae	DHA	0.19	0.43	0.64
V69	k__Eukaryota||Armophorea	DHA	0.22	0.36	0.62
V70	k__Eukaryota|Neocallimastigomycota|Neocallimastigomycetes	DHA	0.32	0.17	0.61
V71	k__Eukaryota||Rhodellophyceae	DHA	0.00	0.99	0.99
V72	k__Eukaryota|Basidiomycota|Wallemiomycetes	DHA	-0.23	0.34	0.62
V73	k__Eukaryota|Basidiomycota|Agaricostilbomycetes	DHA	0.25	0.28	0.62
V74	k__Eukaryota|Streptophyta|Klebsormidiophyceae	DHA	0.24	0.31	0.62
V75	k__Eukaryota|Streptophyta|Marattiopsida	DHA	0.26	0.26	0.61
V76	k__Eukaryota||Phyllopharyngea	DHA	0.26	0.26	0.61
V77	k__Eukaryota|Streptophyta|Mesostigmatophyceae	DHA	-0.44	0.05	0.61
V78	k__Eukaryota|Streptophyta|Coleochaetophyceae	DHA	-0.23	0.34	0.62
V79	k__Eukaryota|Ascomycota|Neolectomycetes	DHA	0.18	0.44	0.64
V80	k__Eukaryota|Ascomycota|Pneumocystidomycetes	DHA	0.18	0.44	0.64
V81	k__Eukaryota|Ascomycota|Geoglossomycetes	DHA	-0.23	0.33	0.62
V82	k__Eukaryota|Ascomycota|Orbiliomycetes	OC	-0.28	0.23	0.63
V83	k__Eukaryota|Ascomycota|Saccharomycetes	OC	0.10	0.66	0.85
V84	k__Eukaryota|Basidiomycota|Agaricomycetes	OC	-0.33	0.15	0.60
V85	k__Eukaryota|Ascomycota|Sordariomycetes	OC	0.22	0.36	0.76
V86	k__Eukaryota|Streptophyta|Liliopsida	OC	0.30	0.19	0.60
V87	k__Eukaryota|Ascomycota|Lecanoromycetes	OC	0.10	0.69	0.85
V88	k__Eukaryota|Ascomycota|Dothideomycetes	OC	-0.28	0.24	0.63
V89	k__Eukaryota|Chlorophyta|Prasinophyceae	OC	-0.25	0.29	0.70
V90	k__Eukaryota|Ascomycota|Eurotiomycetes	OC	0.35	0.13	0.55

V91	k__Eukaryota||Cryptophyta	OC	0.09	0.71	0.85
V92	k__Eukaryota||Chrysophyceae	OC	0.49	0.03	0.38
V93	k__Eukaryota|Chlorophyta|Chlorophyceae	OC	0.11	0.64	0.85
V94	k__Eukaryota|Streptophyta|Bryopsida	OC	-0.01	0.98	0.98
V95	k__Eukaryota||Dinophyceae	OC	0.20	0.39	0.76
V96	k__Eukaryota||Spirotrichea	OC	0.11	0.64	0.85
V97	k__Eukaryota|Ascomycota|Pezizomycetes	OC	-0.02	0.93	0.94
V98	k__Eukaryota|Bacillariophyta|Coscinodiscophyceae	OC	0.13	0.58	0.83
V99	k__Eukaryota||Oligohymenophorea	OC	-0.31	0.18	0.60
V100	k__Eukaryota|Streptophyta|Coniferopsida	OC	-0.05	0.84	0.90
V101	k__Eukaryota|Ascomycota|Leotiomycetes	OC	0.06	0.81	0.89
V102	k__Eukaryota||Stylonematophyceae	OC	-0.02	0.93	0.94
V103	k__Eukaryota|Streptophyta|Zygnemophyceae	OC	0.08	0.73	0.87
V104	k__Eukaryota|Streptophyta|Jungermanniopsida	OC	-0.18	0.44	0.76
V105	k__Eukaryota|Chlorophyta|Trebouxiophyceae	OC	-0.05	0.84	0.90
V106	k__Eukaryota|Basidiomycota|Tremellomycetes	OC	0.19	0.42	0.76
V107	k__Eukaryota|Chytridiomycota|Chytridiomycetes	OC	-0.13	0.58	0.83
V108	k__Eukaryota|Streptophyta|Polytrichopsida	OC	-0.38	0.10	0.55
V109	k__Eukaryota||Compsopogonophyceae	OC	-0.33	0.16	0.60
V110	k__Eukaryota|Chlorophyta|Ulvophyceae	OC	-0.21	0.38	0.76
V111	k__Eukaryota|Ascomycota|Arthoniomycetes	OC	0.10	0.67	0.85
V112	k__Eukaryota|Basidiomycota|Ustilaginomycetes	OC	0.41	0.07	0.51
V113	k__Eukaryota|Glomeromycota|Glomeromycetes	OC	0.50	0.03	0.38
V114	k__Eukaryota||Bangiophyceae	OC	-0.28	0.24	0.63
V115	k__Eukaryota||Synchromophyceae	OC	-0.37	0.11	0.55
V116	k__Eukaryota|Bacillariophyta|Bacillariophyceae	OC	0.29	0.21	0.60

V117	k__Eukaryota|Basidiomycota|Exobasidiomycetes	OC	0.67	0.00	0.11
V118	k__Eukaryota|Streptophyta|Sphagnopsida	OC	-0.18	0.44	0.76
V119	k__Eukaryota|Ascomycota|Taphrinomycetes	OC	-0.11	0.64	0.85
V120	k__Eukaryota|Bacillariophyta|Fragilariophyceae	OC	0.57	0.01	0.22
V121	k__Eukaryota||Litostomatea	OC	-0.14	0.55	0.83
V122	k__Eukaryota|Ascomycota|Lichinomycetes	OC	0.20	0.40	0.76
V123	k__Eukaryota||Heterolobosea	OC	-0.15	0.53	0.82
V124	k__Eukaryota|Basidiomycota|Dacrymycetes	OC	-0.27	0.26	0.63
V125	k__Eukaryota||Synurophyceae	OC	0.29	0.21	0.60
V126	k__Eukaryota|Streptophyta|Marchantiopsida	OC	-0.27	0.25	0.63
V127	k__Eukaryota||Foraminifera	OC	-0.19	0.42	0.76
V128	k__Eukaryota||Dictyochophyceae	OC	0.32	0.17	0.60
V129	k__Eukaryota|Streptophyta|Polypodiopsida	OC	0.40	0.08	0.51
V130	k__Eukaryota||Karyorelictea	OC	-0.07	0.78	0.89
V131	k__Eukaryota||Phaeothamniophyceae	OC	-0.06	0.80	0.89
V132	k__Eukaryota|Blastocladiomycota|Blastocladiomycetes	OC	-0.23	0.34	0.76
V133	k__Eukaryota|Streptophyta|Gnetopsida	OC	-0.08	0.74	0.87
V134	k__Eukaryota|Chytridiomycota|Monoblepharidomycetes	OC	0.63	0.00	0.13
V135	k__Eukaryota||Heterotrichea	OC	0.45	0.05	0.45
V136	k__Eukaryota||Katablepharidophyta	OC	0.10	0.69	0.85
V137	k__Eukaryota|Streptophyta|Cycadopsida	OC	0.35	0.13	0.55
V138	k__Eukaryota||Colpodea	OC	0.07	0.77	0.89
V139	k__Eukaryota||Ichthyosporea	OC	0.03	0.89	0.92
V140	k__Eukaryota||Raphidophyceae	OC	-0.09	0.70	0.85
V141	k__Eukaryota||Pelagophyceae	OC	0.45	0.05	0.45
V142	k__Eukaryota|Streptophyta|Charophyceae	OC	0.15	0.53	0.82

Appendix 11 Preparation of formulations and soil for pot experiment (Chapter 4)



Preparation of formulations using (a) Vermiculite and (b) Compost (c) Inoculation of microalgae into carrier (d) Soil preparation for potting (e) Potted soil (f) Seed germination on climate-controlled culture bed

Appendix 12 Midcrop analysis of chili plant (Chapter 4)

a) Chili plants at green house b) The different treatments of chili plant at midcrop stage. T0, Control; T1, Carrier; T2, Chemical fertilizer; T3, 50 % microalgal treatment and 50 % Chemical fertilizer; T4, 100 % microalgal treatment.

Appendix 13 Chili harvesting (Chapter 4)

a) Chili plants at harvest b) Different treatments of chilli plants c) Chili harvesting d) Chili plants of different treatments taken for analysis d) Chili fruit of different treatments. T0, Control; T1, Carrier; T2, Chemical fertilizer; T3, 50 % microalgal treatment and 50 % Chemical fertilizer; T4, 100 % microalgal treatment.

Appendix 14 Publication front page

Pedosphere
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Review Article

Microalgal bioinoculants for sustainable agriculture and their interaction with biotic and abiotic components of the soil

Shisy JOSE [1], Nirmal RENUKA [1 2], Sachitra Kumar RATHA [1 3], Sheena KUMARI [1], Faizal BUX [1]

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ABSTRACT

Modern agricultural practices have posed a detrimental impact on the environment due to their intensive use to meet the food demands of an ever-increasing population. In this context, microalgal bioinoculants, specifically cyanobacteria and green microalgae, have emerged as sustainable options for agricultural practices to improve soil organic carbon, nutrient availability, microbial quality, and plant productivity. Among the benefits of microalgal bioinoculants include releasing agronomically important metabolites (exopolymers and phytohormones) as well as solubilizing soil nutrients. Furthermore, they function as biocontrol agents against soil-borne pathogens and facilitate the

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Bioprospecting of microalgae from agricultural fields and developing consortia for sustainable agriculture

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ABSTRACT

Bioinoculation of soil with microalgae, such as cyanobacteria and green microalgae has been identified as an innovative approach to promote sustainable agriculture. In this study, indigenous nitrogen-fixing cyanobacteria and non-nitrogen-fixing green microalgae were isolated from agricultural fields and analyzed for metabolites of agricultural significance for the development of suitable microalgal consortia under nitrogen-deficient conditions. The amount of indole acetic acid (IAA) in biomass extracts from cyanobacteria *Calothrix* sp. (2.54 ng g^{-1}) and *Nostoc* sp. (1.52 ng g^{-1}) was significantly higher than in extracts from green microalgae (*Chlorella* sp. (0.32 ng g^{-1}) and *Desmodesmus armatus* (0.20 ng g^{-1}). A completely randomized design was used to develop and evaluate eight microalgal consortia on a nitrogen-deficient medium with the selected microalgal strains. The microalgal consortium with superior biomass productivity was evaluated for the production of exopolymers, phytohormones, macronutrients, and micronutrients. A significant improvement in the production of IAA was observed in the consortium compared to the individual isolates, with the highest production of 21.06 ng g^{-1} occurring in the late log phase. The selected microalgal consortium was further analyzed for biostimulant properties using seed germination assay in *Capsicum annuum*. A significant increase in seedling length and leaf number was observed in seeds treated with biomass extracts of microalgae consortium compared to the control. The present study paved the basis for developing cyanobacteria and green microalgae-based consortia for agricultural applications.