Bacteriophages of Nitrogen-Cycling Bacteria and Engineered Biochar for Reducing Nitrogen Losses

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A thesis submitted to the National University of Sciences and Technology, Islamabad,
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Supervisor: Dr. Ghulam Haider

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Sadaf Khan

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amo A: Ammonia monooxygenase

AOA: Ammonia Oxidizing Archaea

AOB: Ammonia Oxidizing Bacteria

BC: Biochar

BNI: Biological Nitrification Inhibitor

CA: Conservation Agriculture

CO_2: Carbon dioxide

GHG: Green House Gases

HAO: Hydroxylamine Oxidoreductase

K: Potassium

N: Nitrogen

NH_2OH: Hydroxylamine

NH3: Ammonia

NH_4^+: Ammonium ions

NO_2^-: Nitrite

NO2: Nitrous Oxide

NO_3^-: Nitrate

NUE: Nitrogen Use Efficiency

NXR: Nitrite Oxidoreductase

OS: Organic Solution

P: Phosphorus

SNI: Synthetic Nitrification Inhibitor

ABSTRACT

The emission of Greenhouse gases (GHG) from agriculture is a huge problem. Nitrous oxide (N_2O) has a higher Global Warming Potential (GWP) than all other gases. The increased use of nitrogen fertilizers since the Green Revolution has intensified this issue, as lower Nitrogen Use Efficiency (NUE) results in nearly half of the applied nitrogen being lost through nitrate leaching, ammonia volatilization, and N_2O emissions. This study aims to mitigate N_2O emissions by controlling the rate of nitrification. The first strategy involved isolating the nitrifying bacteria (*Nitrosomonas europaea*) from active agricultural soil and its bacteriophage from sewage samples. The second strategy focused on the application of nitrogen-loaded biochar and sorghum extract-treated nitrogen-loaded biochar as biological nitrification inhibitors in a pot experiment with maize (*Zea mays*). Biochar (a carbon-rich compound) was loaded with nitrogen at three different levels (50kgha^{-1}, 100 kgha^{-1}, 150 kgha^{-1}). In another set, biochar was first treated with sorghum extract and then loaded with nitrogen at the same three levels. A maize Pot experiment was conducted to measure morphological parameters, study nutrient dynamics in soil and plant uptake, assess /photosynthetic parameters. The phylogenetic analysis of isolated bacteria confirmed their similarity to Pseudomonas spp., and a bacteriophage against this bacterium was isolated with positive results from the spot assay. The results of the biochar maize study revealed a 98.54% increase in shoot biomass with 100 kg/ha nitrogen-treated biochar and a 14.58% to 24.01% increase in plant height with 150 kg/ha nitrogen-treated biochar, compared to the control.while sorghum extract-doped nitrogen-treated biochar increased root biomass. The interaction of sorghum extract with nitrogen-loaded biochar significantly enhanced photosynthetic activity in maize, whereas nitrogen-treated biochar alone did not produce this effect. Soil extractable potassium was significantly increased by nitrogen-treated biochar while the organic solution has significantly reduced extractable potassium. Nitrogen-loaded biochar and organic solution have no significant effect on total phosphorus. However, their interaction has significantly reduced total phosphorus. Nitrogen-loaded biochar as well as organic solution (Sorghum extract) doped N-loaded biochar have significantly improved the Soil NO_3 and ammonical nitrogen.

In summary, nitrogen-loaded biochar, especially when treated with sorghum extract, shows promise in enhancing plant growth parameters, photosynthetic activity, and nutrient uptake, while contributing to the mitigation of N_2O emissions from agricultural sector.

Keywords: Greenhouse gas, Nitrogen Use Efficiency, *Nitrosomonas europaea*, Biochar

CHAPTER 1: INTRODUCTION

Agriculture and the environment are interdependent (Gallardo, 2024). For instance, agricultural activities like non-judicious use of agrochemicals can affect soil and aquatic environment (Tudi et al., 2021). It has become a fact that environmental conditions have a direct impact on agroecosystems where they can deplete soil biodiversity due to the release of toxic chemicals (McLaughlin & Mineau, 1995). The agricultural economic importance for a country diminishes after reaching a certain level of development (Dethier & Effenberger, 2011). Despite its crucial role in economic development, only 4% of the world's GDP was in the agricultural sector in 2019 (Gallardo, 2024).

The global population will reach 9 billion till 2035 (Grafton et al., 2015). 70% increase in agricultural production in developed countries and a 100% increase in developing countries are needed to feed such a huge population (Rodriguez & Sanders, 2015). To attain this significantly higher agricultural production, it is crucial to modify agricultural practices, reduce food wastage (FAO, 1998) and minimize nutrient losses. (Gruber & Galloway, 2008; Mahmud et al., 2021).

Nitrogen is a crucial element of many genetic, metabolic, and structural units in plant cells like proteins, enzymes, chlorophyll, and nucleotides. Among the six macro-nutrients (K, P, Mg, S, Ca, N), N is the most important for plants (ALnaass et al., 2021). It is present in amino acids and constitutes protein. It is also an essential constituent of chlorophyll, that maintains a balance in photosynthesis (Leghari et al., 2016) and imparts a green color to plants (Bloom, 2015). It aids in the foliage of crops and contributes to biomass yield

production (Anas et al., 2020). Nitrogen is crucial for plant metabolism (Marschner, 2011) and greater uptake of N by plants enhances photosynthetic carbon fixation (Andrews et al., 2013).

N is present abundantly present in the atmosphere in an inert form that the plants cannot uptake (Tyagi et al., 2022). NH4+, NO3- or NH3 uptake occurs by plants from soil (Adisa et al., 2019). To fulfill the plant's N requirements and to enhance crop productivity, nitrogen fertilizers have been used for more than five decades (Erisman et al., 2008).

Following the "Green Revolution", Nitrogen fertilizers have enhanced crop yield (Raghuram et al., 2022). The nitrogen fertilizer use intensity in South Asia is 121kg/ha and it is rapidly increasing. It is estimated to reach 268kg/ha by 2050 (Amjath-Babu et al., 2019). The fertilizer use efficiency ranges from 25-48% with the remainder lost to the environment (Bilal & Aziz, 2022). s

Since the 1980s, the emission of NH_3 and N_2O has almost doubled (Yang et al., 2022). Due to the extensive use of nitrogen fertilizers in this region (John & Babu, 2021). After the Green Revolution, although crop yield was enhanced but extensive use of fertilizers also resulted in loss of nitrogen to the environment and reduced nitrogen use efficiency (John & Babu, 2021). In Pakistan, Nitrogen Use Efficiency has sharply declined between 1960 to 2014 due to excess use of fertilizers (Shahzad et al., 2019).

The release of N into the environment results in various serious environmental issues (Galloway et al., 2008) like the increased anthropogenic emission of N_2O from agriculture (Shibata et al., 2015; Zaehle et al., 2011). N_2O has a global warming potential of about 300

times that of CO_2 (Shibata et al., 2015). The atmospheric nitrogen has increased by 20% from 270 (nL/L) in 1750 to 331 (nL/L) in 2018 (Tian et al., 2020). s

Nitrification is a biological process to convert NH_3 or NH_4^+ to NO_2^- or NO_3^-. Nitrifying bacteria i.e. *Nitrosomonas*, *Nitrospira*, and *Nitrobacter* and ammonia-oxidizing archaea such as *Nitrososphaera* carry out this process (Papadopoulou et al., 2020). Nitrifying bacteria in soil gain energy by converting ammonium to nitrate through the process of nitrification (Lancaster et al., 2018). The first step of nitrification involves the conversion of NH_3 or NH_4^+ to NO_2^- (Lancaster et al., 2018). This step is carried out by *Nitrosomonas* or *Nitrospira* due to the presence of Ammonia monooxygenase (AMO) enzymes (Lancaster et al., 2018). In the next step, the conversion of NO_2^- to NO_3^- and this step is carried out by *Nitrobacter* Nitrification indirectly contributes to GHG emissions as products of nitrification (NO_2^- and NO_3^-) are then subjected to denitrification, which is the responsible process for nitrous oxide emissions (Baggs, 2011; Hassan et al., 2022). During denitrification, anaerobic bacteria convert the NO_3^- to N_2, and N_2O is released (Signor & Cerri, 2013).

Maize (*Zea mays*) is one of the most important crops used globally for food, feed, and raw materials (Hou et al., 2023). The United States is the world's largest producer of corn (Ranum et al., 2014). A greater share of corn yield is annually used for ethanol production. Maize is the third most important crop for Pakistan after rice and wheat and is produced in bulk quantity in KPK and Punjab (Shakoor et al., 2017).

1.1 Strategies for Reducing Nitrous Oxide Emission

It is important to mitigate the emission of N2O because of its harmful effects on the environment and biodiversity. Some of the devised management strategies are:

1.1.1 Improved Method of N Application

The mode of application of N fertilizers can improve the NUE. There are many types of applications like Deep placement, granules, and foliar spraying of N fertilizer are some of the ways to apply N fertilizer that can improve its recovery (Yadav et al., 2017). The widespread practice of spreading nitrogen fertilizers causes significant N losses, such as NH3 volatilization, which lowers nitrogen recovery (McBratney et al., 2005). NUE can be improved by using urea super-granules, a modified form of N fertilizer, and deeply planting urea-based fertilizers (Yadav et al., 2017).

1.1.2 Crop residues

Crop residues are for plant growth and development because of their higher nutritional content (Mohanty & Mishra, 2014). Most legumes have higher N contents than cereals, their residues are useful sources of nitrogen (Dinnes et al., 2002). Within two months of application, legume residue can mineralize more quickly and provide plants with half of the available nitrogen (Ram et al., 2012).

1.1.3 Green Manuring

Several different kinds of legumes can be used as green manures because of their N fixation ability which makes them better green manure crops than non-leguminous crops (Vyn et al., 2000).

1.1.4 Integrated Nitrogen Management (INM)

Integrated Nutrient Management (INM) entails maximizing the utilization of native nitrogen sources along with chemical fertilizers, and their synergistic interactions to enhance nitrogen use efficiency (Olesen et al., 2004).

1.1.5 Improved Use Efficiency of Fertilizers

Nutrient use efficiency can be improved by minimizing its losses during synthesis and by enhancing its uptake by plants (Hirel et al., 2011). These fertilizers operate on two principles: they can either slow down the release rate of nutrients according to the plant's requirements or disrupt their biogeochemical processes to minimize losses (Yadav et al., 2017).

1.1.6 Slow-release fertilizer

The form of nitrogen fertilizers used has a big impact on managing different N losses, which impacts nitrogen recovery and availability (You et al., 2023). Nitrate fertilizers leach down more easily than other nitrogen fertilizers (Bibi et al., 2016). In contrast, Amide and ammonium fertilizers are more susceptible to volatilization than those containing nitrate (Jones et al., 2013). There are currently several slow-release fertilizers on the market that may lower different N losses and increase NUE (Giller et al., 2004). To enhance NUE and crop output, nitrification inhibitors can be added to the soil medium to prevent ammonium-N from being converted to nitrate-N and to guarantee a larger concentration of ammonical form of nitrogen (Shivay et al., 2001). A widely proven and commercially accessible

nitrification inhibitor that can be used in rice farming is called dicyandiamide (DCD) (Bharati et al., 2000).

1.1.7 Enhancement of NUE through Genetic Improvement

NUE may also be increased by improving crop cultivars through the introduction of different quality features that are essential for efficient N utilization (Oni et al., 2019). Different genotypes may result in varying grain yields while absorbing the same amount of N (Yadav et al., 2017).

1.1.8 Application of Biochar

Biochar is a high carbon-containing product produced through the pyrolysis of organic substances e.g. plant biomass (Oni et al., 2019). Biochar application in soil improves its physical, chemical, and biochemical properties influencing nitrous oxide production. (Case et al., 2012). Biochar has good adsorption capacity so ammonium and nitrate bind to it tightly making N unavailable for generation of nitrous oxide. (Hassan et al., 2022).

1.1.9 Biological Nitrification Inhibition

Many plants release root exudates into the rhizosphere that have inhibitory effects on the nitrification process (Devrim Coskun et al., 2017a). These exudates are composed of sorgoleone, , linoleic acid (LA), and brachialactone which cause a reduction in soil nitrification and prevent N leaching (G. V. Subbarao et al., 2013).

1.1.10. Bacteriophages as Nitrification Inhibitors

Bacteriophages are abundantly present in soil and play an important role in the dynamics of soil microbiota. It can regulate the bacterial community structure and functions and even the whole soil microbes (Wang et al., 2022). In soil different microbes compete for nutrients and bacteriophages play an important role in the release of nutrients from bacteria by lysis through the "Viral Shunt" mechanism (Wang et al., 2022).

Some prokaryotes, such as bacteria from the Nitrosomonas genus, oxidize NH3 to NO_2^- in the first step of nitrification. Bacteriophage application is one possible means of inhibiting nitrification; however, this approach has not been investigated. We present in this study the isolation of the first phage that infected a few species of Nitrosomonas. (Quirós et al., 2023)

Objectives

Based on the hypothesis our objectives were

1) Isolation and characterization of Nitrosomonas europaea from active agricultural soil
2) Isolation and identification of a virulent phage infecting species of nitrogen-cycling bacteria
3) To evaluate the effect of nitrogen-loaded biochar on the growth and nutrient uptake (NPK) of maize in a pot experiment
4) To assess the impact of sorghum extract, a biological nitrification inhibitor (BNI), combined with nitrogen-loaded biochar on soil nutrient dynamics and maize growth

CHAPTER 2: LITERATURE REVIEW

2.1 Role of Nitrogen in Plant Growth

N is widely distributed across the lithosphere, atmosphere, hydrosphere, and biosphere (Mengel & Kirkby, 1987; Singh & Gupta, 2018). The lithosphere holds 94% of Earth's nitrogen, with the atmosphere containing 6% and a small portion found in the hydrosphere and biosphere (Ward et al., 2005). In the biosphere, nitrogen ranks as the fourth most abundant element following oxygen, carbon, and hydrogen (Zayed et al., 2023). It is a crucial component of total biomass (Azadi & Ho, 2010; Singh & Gupta, 2018). The abundance of nitrogen in the biosphere highlights its significance to living organisms (Zayed et al., 2023).

In crop plants, N is crucial for many essential processes, including growth and yield (Anas et al., 2020). Nitrogen promotes the growth of new leaves from the stem's terminal meristem and the lateral buds of older leaves (Fathi, 2022). The growth of plants is affected if N is not available in sufficient amount (Fathi & Zeidali, 2021). Nitrogen is also a crucial element of amino acids and chlorophyll molecules, which is necessary for photosynthesis (Fathi & Zeidali, 2021). Because proteins and enzymes regulate nutrition and water intake, even the roots contain this vital element (Singh et al., 2018).

2.2 Sources of Nitrogen

In a terrestrial environment, soil acts as a reservoir of nutrients for plants, with less than 0.2% of the mineral nutrient supply dissolved in water, while the remaining 98% is bound in humus, organic matter, and insoluble substances (Lamers et al., 2012). The microbial

conversion of organic materials into inorganic N is primarily responsible for the soil's N content (Kox & Jetten, 2014). Root exudates, symbioses with microbes, root structure, and density all have an impact on a plant's availability of nitrogen (Jackson et al., 2008).

2.3 Uptake of Nitrogen

The absorption and assimilation of N are impacted by the availability of other nutrients, especially phosphorus. (Lambers et al., 1998). While plants may absorb both organic and inorganic nitrogen, most of the research has been focused on inorganic nitrogen sources. (NO_3^-) needs to be converted into NH_4^+ before plants can absorb and use it (Lambers et al., 1998). Terrestrial plants that thrive in environments with low pH and low redox potential tend to prefer the uptake of NH_4^+ (Kuzyakov & Xu, 2013). This preference results in the release of protons, which acidify the rhizosphere, subsequently impacting the microbes in the rhizosphere and decreasing nitrification rates (Kuzyakov & Xu, 2013). Conversely, plants that are adapted to higher pH soils generally favor the uptake of NO_3^-, as it leads to less soil acidification compared to NH_4^+ (Kox & Jetten, 2014; Lamers et al., 2012).

2.4 Nitrogen Use Efficiency (NUE)

NUE means the efficiency of using and retaining nitrogen by plants in soil (Sharma & Bali, 2017). This definition of NUE shows the tendency of plants to release N2O into the atmosphere without retaining nitrogen in their body (Daigger et al., 1976). NUE of leguminous crops is much higher than other crops because of nitrogen fixation and storage in its body (Hocking & Reynolds, 2012; Kumar et al., 2016).

NUE calculates the amount of nitrogen that plants absorb and the amount of nitrogen that is lost as a result of the plant's emissions of nitrous oxide (Choi et al., 2009). This determines how effectively nitrogen is absorbed and stored by plants (Choi et al., 2009). It is assumed that all nitrogen absorbed by the plant originates from the soil, and all nitrous oxide emissions are derived from soil nitrogen before the plant absorbs it, as plants take up N in the forms of NH_4^+ and NO_3^- (Choi et al., 2009). This approach aligns with the primary definition of nitrogen use efficiency (Sharma & Bali, 2017).

2.5 Nitrous Oxide Emission from Agriculture

Soil significantly contributes to the emission of N_2O, NO, and N_2 during denitrification (Ciais et al., 2013). Emissions of nitrogen oxides (NOx) from natural and agricultural soils are estimated to be 7.3 and 3.7 Tg N per year, respectively, which together constitute 23% of the global NOx emissions (Ciais et al., 2013). Nitrous oxide is nearly 300 times more harmful than CO_2 and about 12 times more potent than CH_4 over 100 years (Heil et al., 2016).

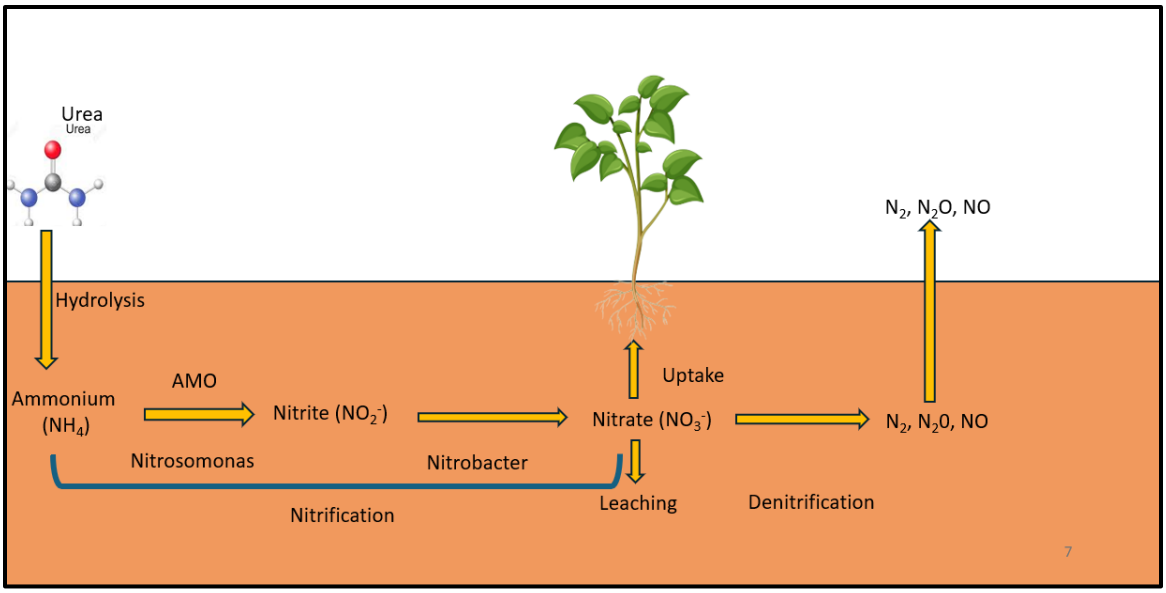

Figure 2.1: Mechanism of emission of nitrous oxide from agriculture

2.6 Nitrification

The process by which reduced nitrogen (N), usually present as NH_3 or NH_4^+, is converted into oxidized nitrogen, such as nitrite or nitrate, is known as nitrification. In this biochemical process converts NH_4^+ into negatively charged NO_2^- or NO_3^-. Due to their mobility in the soil, nitrification products are susceptible to leaching and denitrification losses.

Nitrification is a complicated process that combines chemical and biological processes. The interacting microorganisms' membrane location is where the biological process occurs (Amoo & Babalola, 2017). Primarily, NH3 is used as the major substrate (Fu et al., 2020). It is converted to hydroxylamine (NH_2OH) by the enzyme NH3 monooxygenase (amoA), and then hydroxylamine combines with water to make nitrite with the help of the enzyme hydroxylamine oxidoreductase (HAO) (Amoo & Babalola, 2017). Nitrite is converted to nitrate by nitrite oxidoreductase (NXR), which is present in NOB (Fu et al., 2020).

Eq. (1) illustrates the net result of NH_3 oxidation is nitrite.

$$1.5\ O_2 + NH_4^+ \text{----------} \rightarrow H_2O + 2H^+ + NO_2 \qquad (1)$$

The next step involves the transformation of NO_2^- to NO_3^- by NOB like Nitrobacter or Nitrospira and Nitrite oxidoreductase (NXR), a crucial enzyme, is involved in this step (Daims et al., 2015).

$$NO_2^- + 0.5\ O_2 \text{----------} \rightarrow NO_3 \qquad (2)$$

The net reaction of nitrification is

$$NH_4^+\ 2O_2 \text{----------} > NO_3^- + H_2O + 2H^+$$

2.7 Loss of Nitrogen

In soil, nitrogen is present in four different forms which are (1) soil organic matter (2) soil microbes, (3) ammonium ions (NH_4^+), and (4) mineral forms i.e. NH_4^+, nitrate (NO_3^-), and nitrite (NO_2^-) (Bishop & Manning, 2010). Nitrogen availability to plants and its release into the surrounding environment is influenced by the nitrogen gains, losses, and transformations that occur within the soil/plant system (Bateman & Baggs, 2005). Mineral-N is most vulnerable to losses in agriculture due to NH_3 volatilization, NO_3^- leaching, and denitrification (Cameron et al., 2013).

2.7.1 NH3 volatilization

In the process of ammonia volatilization, where gaseous NH_3 is lost from the soil surface (Bishop & Manning, 2010). Volatilized NH_3 typically returns to the earth via wet deposition or dry deposition, leading to acidification and eutrophication of natural

ecosystems (Bishop & Manning, 2010). Additionally, NH_3 emissions and their deposition on land and water indirectly contribute to N_2O emissions (Cameron et al., 2013). Agriculture accounts for approximately 50% of the global emission of ammonia (NH_3) into the atmosphere (Sommer et al., 2004).

2.7.2 Nitrate Leaching

Because of lower Nitrogen Use Efficiency (NUE), nitrate leaches into the soil and can also enter water bodies, leading to hazards for human health and environmental concerns (Andrews et al., 2007; Goulding et al., 2008). When nitrate gets into lakes or rivers, it can cause eutrophication, which can lead to fish population declines and algal blooms (Smith & Schindler, 2009). The extent of nitrate leaching from the soil is determined by two factors: the concentration of nitrate in the soil solution and the volume of drainage that occurs through the soil during a specific period (Cameron et al., 2013). The amount of N applied, the rates of nitrification and denitrification, etc., all affect how much nitrate is in the soil solution (Andrews et al., 2007).

2.7.3 Nitrification

The nitrification process results in a 50% nitrogen loss, decreasing the nitrogen accessible to plants (Beeckman et al., 2018). Despite these issues, the nitrification process remains crucial, primarily because it supplies nitrate, which is essential for plant growth (Ayiti & Babalola, 2022). While the soil contains various forms of nitrogen, nitrate is generally favored by most plants and soil organisms, enhancing ecosystem functionality when produced in appropriate amounts (Ayiti & Babalola, 2022).

2.7.4 Denitrification

Denitrification is the process by which soil bacteria transform nitrate into either dinitrogen or nitrous oxide (Vijay et al., 2022). It is more prevalent in areas with thicker soils, higher rainfall totals, and slower agricultural growth (Tufail et al., 2024). Denitrification can lose between 5% and 25% of the total amount of N applied, depending on the characteristics of the soil and the surrounding environment (Panday et al., 2022).

2.8 Reducing Strategies for Nitrous Oxide

Reduced soil nitrification rate and decreased likelihood of denitrification are two ways to mitigate $N_2O$and N_2 gas emissions (Saggar et al., 2009; Thomson et al., 2012).

2.8.1 Nitrogen Loaded Biochar

Biochar has high adsorption capacity and cation exchange capacity for NH_4^+, attributed to its dense pore structure and numerous oxygen-containing functional groups (Laghari et al., 2016; Lu et al., 2021). By adding 20g/kg of biochar to the soil, Laird et al. (2010) observed 11% reduction in total nitrogen leaching in a soil column modeling experiment. Coating chemical fertilizers with biochar is a promising method for creating controlled-release fertilizers (Soliman et al., 2022). This approach has been demonstrated to reduce nitrogen loss pathways and mitigate related environmental issues (Wang et al., 2015).

2.8.2 Chemical Nitrification Inhibitors

Different chemical inhibitors are used to limit the loss of N during the process of nitrification and denitrification (Devrim Coskun et al., 2017b). The effects of inhibitors are

observed by limiting the activity of genes and enzymes involved in the process (Liu et al., 2021).

Nitrapyrin (NP), 3,4-dimethylpyrazole phosphate (DMPP), and dicyandiamide (DCD) are prominent synthetic nitrification inhibitors (Lu et al., 2019). These inhibitors are typically applied in combination with organic waste or nitrogen fertilizers (Kong et al., 2018). Adding DMPP to organic waste slows down the rate of nitrification by inhibiting the initial enzymatic step of the process due to the presence of a copper-chelating element (Wu et al., 2018). Furthermore, the mechanisms of inhibition differ among various nitrification inhibitors (Rodrigues et al., 2018). For instance, using DCD with urea reduced the rate of NH_4^+ loss due to its inhibitory effect on AOB (Duncan et al., 2016). When applied alongside nitrogen fertilizers, DMPP significantly reduces soil nitrification, enhances *Zea mays* yield, and improves NUE, especially in soils with varying pH levels (Cui et al., 2022).

Another SNI is DMPP which is regarded as less toxic compared to DCD (Rodrigues et al., 2018). Yang et al. (2016) observed that in alkaline soil there was an increase in yield from the application of DMPP. However, there are several drawbacks to using chemical inhibitors like they are costly, they are pH and temperature-sensitive. Furthermore, they can also cause environmental pollution (Ayiti & Babalola, 2022).

2.8.3 Agrochemicals

Besides fertilizers and nitrification inhibitors, a range of other agrochemicals and substances applied on farms can affect the nitrification process (Ayiti & Babalola, 2022). These are often employed to enhance plant productivity (Ayiti & Babalola, 2022). Pesticides, including fungicides, insecticides, and rodenticides, are among these substances

(Youssef et al., 2019). For instance, the fungicide iprodione has an antagonistic impact on the amoA gene, decreasing its abundance (Zhang et al., 2018). Synthetic herbicides such as atrazine and glyphosate have been observed to significantly slow down nitrification process (Zhang et al., 2018)

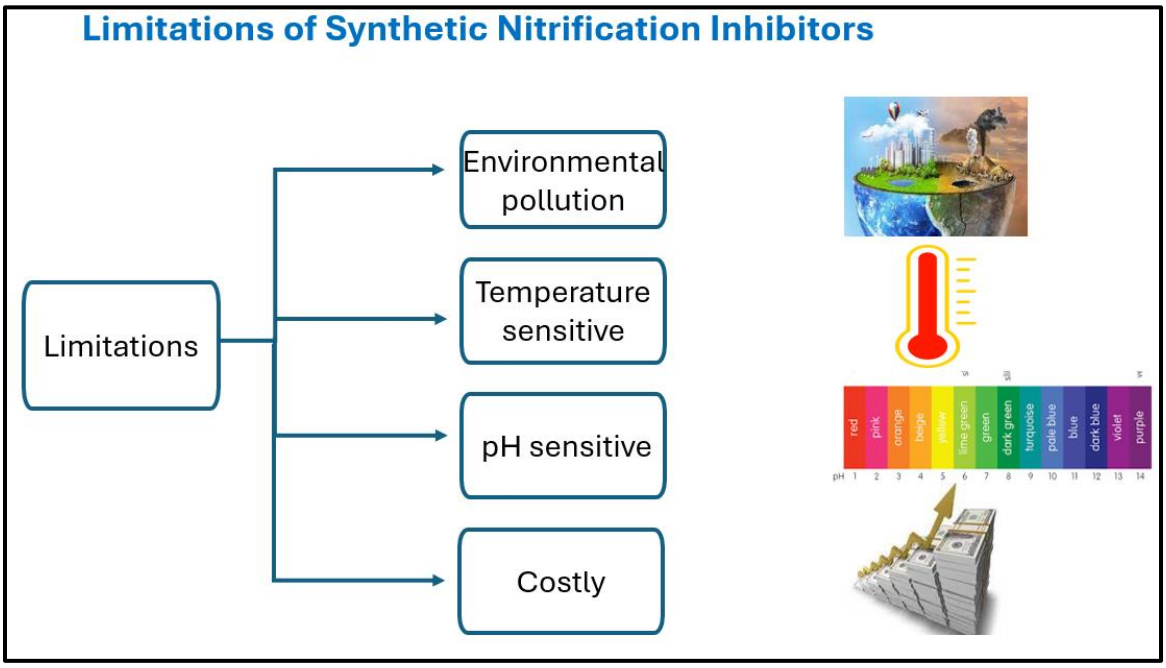

Figure 1.2: Limitations of Synthetic Nitrification Inhibitors

2.8.4 Biological Nitrification Inhibitors (BNIs)

Sorghum is effective as a soil nitrification inhibitor due to the presence of allelochemicals, especially phenolics which hinder the conversion of NH_4^+ to NO_3^- (Iqbal et al., 2022). These phenolic compounds slow down the nitrification process in soil by blocking enzymes in AOB and NOB (Subbarao et al., 2006). In 2008, researchers reported the isolation of the first nitrification inhibitor directly from root exudates of sorghum (*Sorghum bicolor*) (Zakir et al., 2008), a species noted for its high biological nitrification inhibition (BNI) capacity

compared to other crops (Subbarao et al., 2007). This compound, identified as methyl 3-(4-hydroxyphenyl) propionate (MHPP), is a phenylpropanoid with moderate BNI activity crops (Subbarao et al., 2007). Since this discovery, four additional BNI-active root exudates have been isolated and partially characterized. Two of these BNI-active root exudates are also isolated from sorghum. One is sorgoleone, a benzoquinone and the dominant BNI compound in the hydrophobic fraction of root exudates (G. Subbarao et al., 2013). The other is sakuranetin, a flavanone, which, like MHPP, was isolated from the hydrophilic fraction of root exudates. A third compound, brachialactone, a cyclic diterpene, is considered the most significant BNI in *B. humidicola* (Subbarao et al., 2009). The fourth and most recently discovered BNI compound is 1,9-decanediol, a fatty alcohol from rice (Sun et al., 2016). Despite the economic importance and high nitrogen use of the 'big three' crops—rice, wheat, and maize—relatively few studies have investigated biological nitrification inhibition in these species (D Coskun et al., 2017). The residual impact of biological nitrification inhibition (BNI) from B. humidicola pasture on subsequent Zea mays cultivation resulted in improved yields by reducing nitrification, which temporarily enhanced nitrogen uptake by the plants (Karwat et al., 2017).

Table 2.1: Biological Nitrification Inhibitors Found in S.bicolor

Plant	BNI	Type	References
S. bicolor	MHPP	phenylpropanoid	(Zakir et al., 2008)
	Sorgoleone	Benzoquinone	(G. Subbarao et al., 2013)
	Sakuranetin	Flavanone	(G. Subbarao et al., 2013)

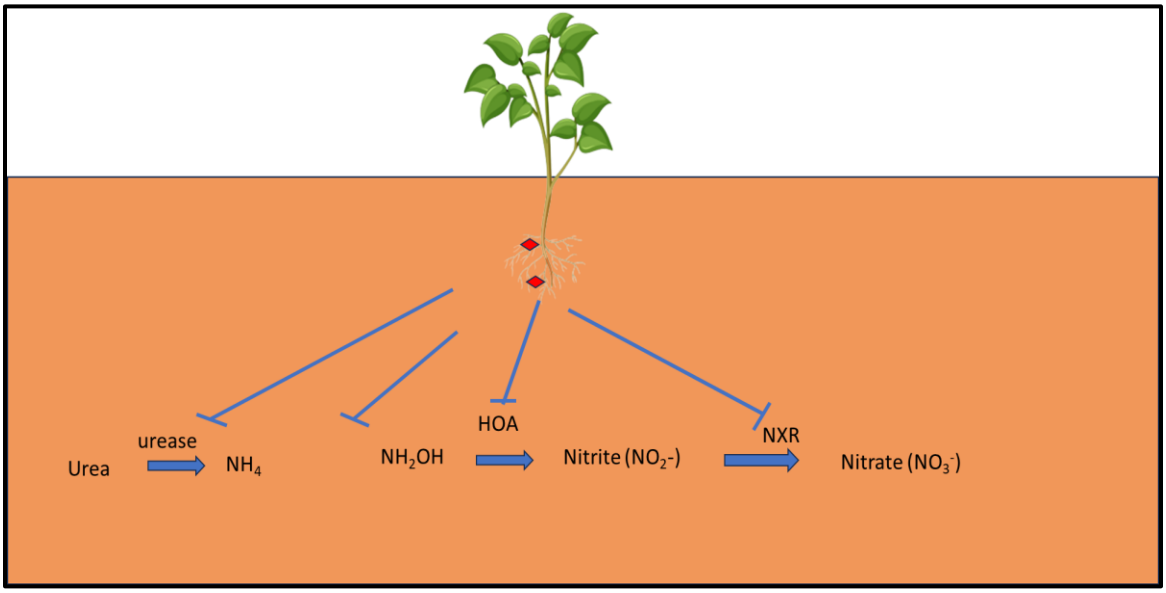

Figure 2.2: Mechanism of action of Biological Nitrification Inhibitors (BNI)

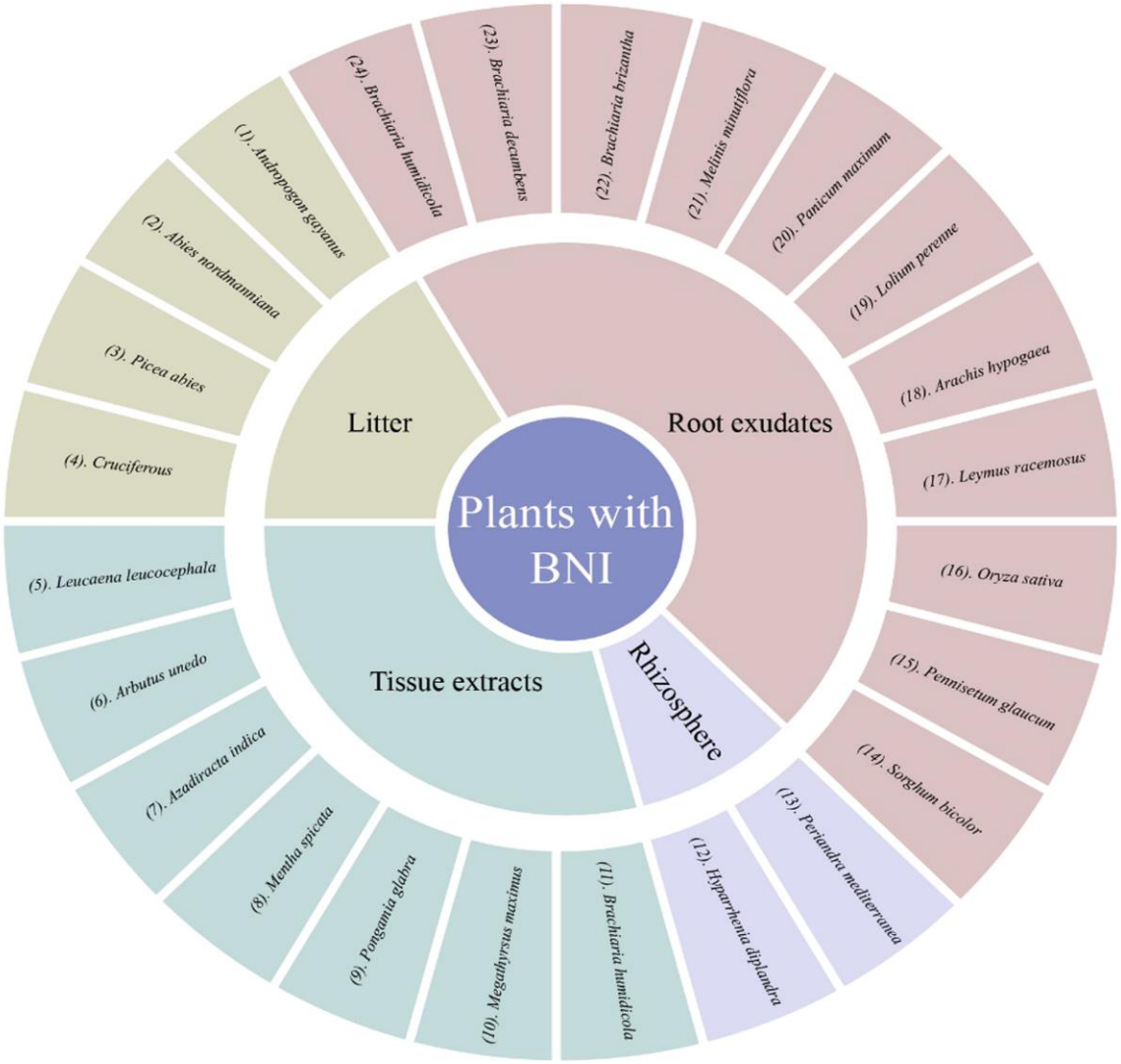

Figure 2.3: Plants which release BNIs to rhizosphere (Wang et al., 2021)

2.8.5 Bacteriophages as Nitrification Inhibitors

Soil viruses affect their hosts' ability to survive and/or adapt, just as land management practices and climate change affect the soil environment (Chu et al., 2022). Bacteriophages help their hosts by encoding auxiliary metabolic functions through horizontal gene transfer (Jin et al., 2019), viruses play an important role in shaping microbial communities by lysing bacterial hosts and releasing locked nutrients in their bodies (Betts et al., 2014). Viruses

that lyse their bacterial hosts release metabolic material and help microorganisms drive biogeochemical cycles; they also govern bacterial abundance and modify the composition of host communities (Jin et al., 2019). As the hosts are lysed, nutrients are released into the environment, where they are subsequently ingested by other microbes (Roux et al., 2016). This process, where bacteria are consumed by protists or other predators, bypassing the typical soil food web, is referred to as the 'viral shunt'. Consequently, the recycling of soil nutrients by viruses can affect soil ecology (Chu et al., 2022).

2.8.5.1 Role of phages in N cycling

Soil viruses are crucial for the biogeochemical cycles of essential nutrients, including nitrogen, phosphorus, and sulfur. Soil nitrogen flux is affected by rhizobiophages which infect rhizobia (Ali et al., 1998; Sharma et al., 2002). Enrichment of these phages in fields can reduce nodulation (Barnet, 1980). The dynamics of rhizobial populations in the soil, such as the success or failure of nodulation, and the overall efficacy of symbiosis and nitrogen fixation, are determined by the interactions between these rhizobiophages and their hosts (Williamson et al., 2017) .

2.8.5.2 Potential of Bacteriophages to Inhibit Nitrification

Because they lyse and infect bacterial cells, bacteriophages (phages) have been suggested as biocontrol agents (Vázquez et al., 2022; von lytischen Bakteriophagen, 2013) and can be used in a variety of fields with little to no negative effects on the microbial ecology of each biome (Greer, 2005). ΦNF-1 is the first virulent phage isolated that infects *Nitrosomonas* species, targeting at least three species within this genus. In contrast,

temperate phages have been previously described in the *Nitrosospira* genus and are known to infect ammonia-oxidizing archaea (AOA (Quirós et al., 2023).

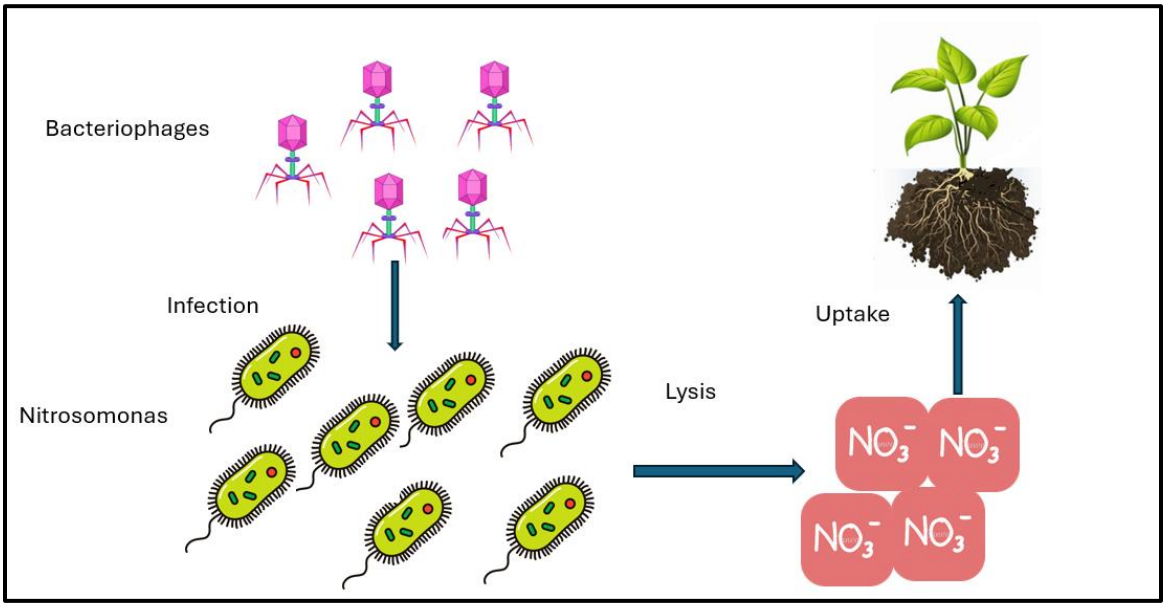

Figure 2.4: Bacteriophages as nitrification Inhibitors

CHAPTER 3: METHODOLOGY

3.1 Isolation of Bacteria from Active Agricultural Soil

Soil samples were serially diluted. Culturing of bacteria was done on nutrient agar media. Bacterial colonies were identified through 16S PCR and sequencing.

3.1.1 Soil sampling

Non-rhizosphere soil samples were collected from a soybean field at the National Agriculture Research Center (NARC) in Islamabad, Pakistan. For soil sampling, the entire area was traversed, and at random points, equal volumes of soil samples were collected using a sterilized auger. After collection, the samples were mixed in a large, sterilized plastic pail and then transferred to labeled zip-lock bags. These soil samples were stored at 4°C.

3.1.2 Serial Dilution

For the isolation of nitrifying bacteria (*Nitrosomonas europaea)*, 10 g of soil was mixed in 50 ml of sterilized Phosphate Buffer Solution (PBS). 0.1ml of these dilutions were spread on specific media for Nitrosomonas and incubated overnight at 37°C. (Kannan et al., 2018).

Table 3.1 Specific media for Nitrosomonas europaea

Sr.No	**Ingredients**	**Quantity (mg/L)**
1	NH_4Cl	535

2	KH_2PO_4	54
3	KCl	74
4	$MgSO_4.7H_2O$	49
5	$CaCl_2.2HO$	174
6	NaCl	584
7	Trace element solution	2ml
Trace element solution		
1	HCl	25
2	$MnSO4.4H_2O$	45
3	H_3BO_3	49
4	$ZnSO_4.7H_2O$	43
5	$(NH_4)6Mo7O_{24}. 4H_2O$	37
6	$FeSO_4.7H_2O$	973
7	$CuSO_4.5H_2O$	25

3.1.3 Pure Culture

After 24 hours of incubation at 37℃, colonies appear on specific media plates. The most prominent colonies were isolated and streaked on fresh media plates by using a bacterial loop in sterilized conditions. The next day, after streaking a loop full of bacteria is transferred to freshly prepared LB broth and placed on a shaking incubator at 37℃ overnight. The next day, OD was checked through a spectrophotometer at 600nm wavelength. Optical density was in the range of 0.5-0.8 showing exponential growth of bacteria.

3.1.4 Gram Staining

The protocol explained by Paray et al. (2023) was used for Gram staining. The microscope was used for microscopy. Optika Vision 2.0 software was used for visualizing the images.

3.1.5 DNA Extraction

Invitrogen by Thermo Fisher Scientific DNA extraction kit was used for DNA extraction from overnight culture of bacteria in LB broth following the manufacturer’s instructions.

3.1.6 16S PCR

PCR mixture was prepared with reagents and their quantities are mentioned in Table 3.1.Temperatures od all stages are mentioned in Fig 3.1 The sequence of the forward primer (16S F) was 5'-ACTCCTACGGGAGGCAGCA-3' and the sequence of 16S R is 5'-GGACTACHVGGGTWTCTAAT-3'.

Table 3.2: Reagents and Their Quantities Used in the PCR Reaction

Reagents	**Amount**
Master mix	10 µ
Nuclease free water	5 µl
DNA	2 µl
Forward primer	1.5 µl
Reverse primer	1.5 µl

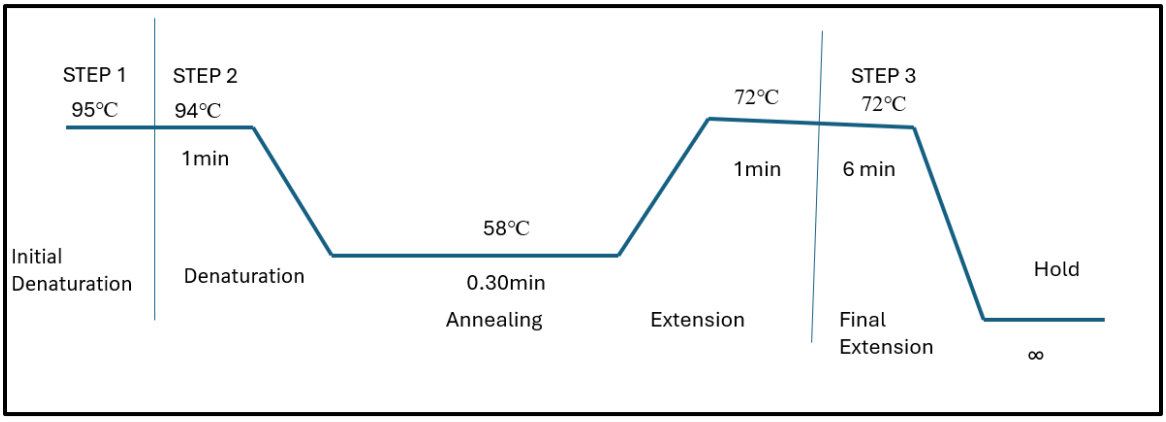

Figure 3.1: Cyclic conditions for Polymerase Chain Reaction

3.1.7 Gel Electrophoresis

Gel electrophoresis was conducted to check the amplification of 16S rRNA gene in PCR product. 1% agarose gel was prepared with 5µl ethidium bromide added. The gel tray was placed in a gel tank filled with 1X TAE buffer. 10µl of the sample was loaded into each

well. Additionally, 5µl of a 1 kb DNA ladder was loaded into the first well. The gel was run for 40 minutes at 80V, and the bands were visualized using a Gel Doc system.

3.1.8 Sequencing

The 16S PCR product was sent to Macrogen for sequencing.

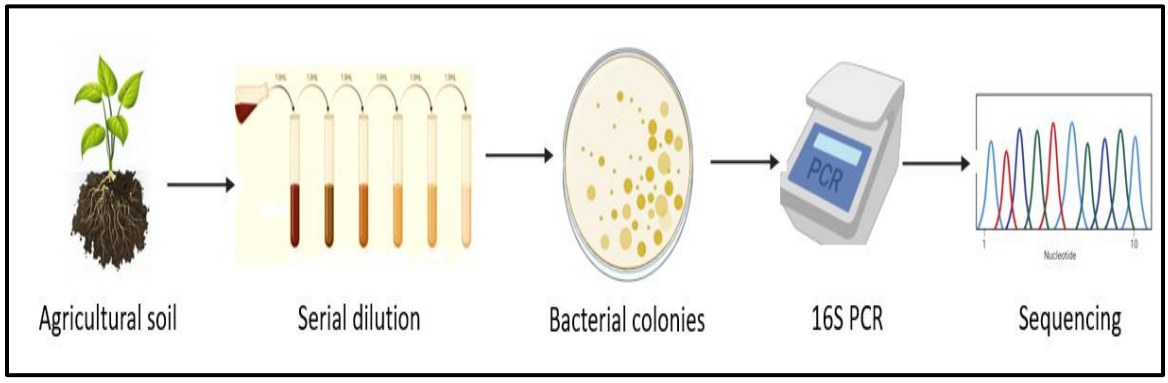

Figure 3.2: Isolation of bacteria from soil samples and its identification

3.2 Isolation of bacteriophages against *Nitrosomonas europaea*

3.2.1 Sewage sampling

For the isolation of bacteriophages, sewage samples were collected from Membrane Bioreacter (MBR) a wastewater treatment plant in NUST. In 50ml sterilized falcon tubes, samples were collected and stored at 4℃.

3.1.2 Isolation of bacteriophages

Bacteriophages were isolated using a protocol devised by Abatángelo et al. (2017). 20ml of sewage water was mixed with 20ml of phage buffer (composition is mentioned in Table 3.2), then incubated overnight at 225rpm. The next day the mixture was centrifuged for 20

mins at 4000rpm. The supernatant was filtered first through the 0.45µm syringe filter and then through the 2µm syringe filter. 400µl of 1% chloroform was added to the filtrate to remove the contamination. The filtrate was then incubated at 37℃ for 15 minutes and stored in the dark for further analysis.

Table 3.2: Tris- Magnesium Phage Buffer

Sr. No.	Ingredients	Quantity (ml/L)
1	5M NaCl	3
2	1M MgSO4	1
3	0.1 M CaCl2	1
4	1M Tris-Cl (PH=7.4)	4

3.2.3 Enrichment of bacteriophages

For the enrichment of bacteriophages, the protocol devised by Sjahriani et al. (2021) was used with slight modifications. Equal volumes (1:1) of phage filtrate and overnight cultured bacteria were mixed in 400 ml of LB broth and placed at 37℃ for 1 day. Next day, The mixture was centrifuged at 5000rpm for 5 minutes. The supernatant was then filtered through a 0.2µm syringe filter and phage lysate was obtained.

3.2.4 Agar Overlay Assay

200µl of bacteria was spread over the LB media plate and incubated at 30℃ for 40 mins. 50µl of phages were spotted on the surface of bacterial plates at 4 points and incubated overnight. After incubation, zones of inhibitions have appeared on plates (Khan Mirzaei & Nilsson, 2015).

3.2.5 Purification of bacteriophages

1 ml of bacterial culture was pipetted in 30 ml of LB broth. An isolated spot of agar from the agar overlay assay plate was added to the flask. The flask containing LB broth, bacteria, and agar spot was incubated overnight. Next day, 1% chloroform was added to that mixture and then centrifuged for 5 minutes at 5000rpm. The next step was the filtration of supernatant through a syringe filter of pore size 0.22µm. The purification was repeated 6 times to obtain purified phage lysate.

3.2.6 Plaque Assay

To perform this assay, 24-hour old bacterial culture was used and Phage lysates were serially diluted tenfold (from 10^-1 to 10^-10) using SM buffer. In a test tube, equal volumes of diluted phage lysate and the exponentially growing bacterial culture were mixed. The test tubes were incubated at 37°C for 15-20 minutes to allow the phages to adhere to their host bacteria. Then, 3-4 ml of semisolid agar was added to each test tube, and the mixture was immediately poured onto plates and incubated at 37°C for 24 hours. The next day, no plaques were observed instead bacterial colonies appeared indicating that the plaque assay was not successful.

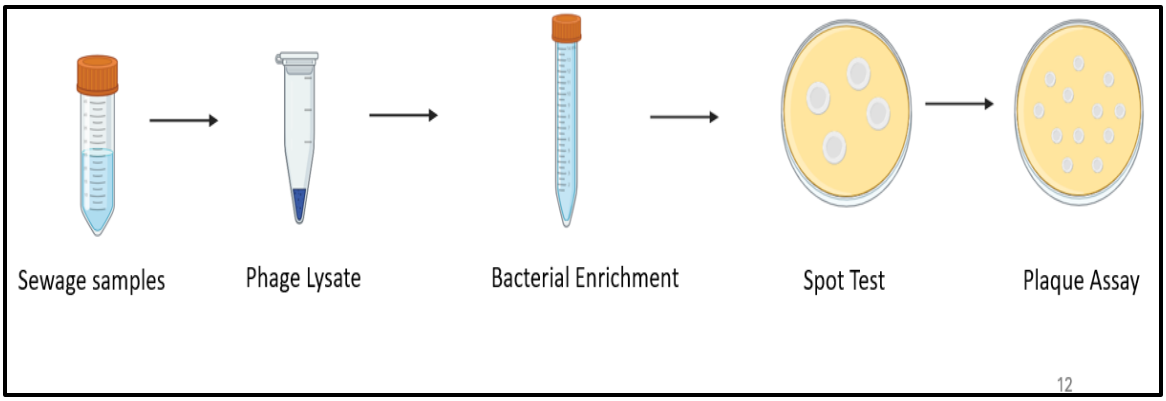

Figure 3.3: Isolation and purification of bacteriophages from sewage samples

3.3 Assessment of Nitrogen-Loaded and Sorghum Extract-Doped Biochar on Maize Growth and Soil Fertility

3.3.1 Soil Collection

Topsoil samples were collected from a peanut field at NARC. The dried soil was passed through a 2mm sieve.

Table 3.3: Soil Profiling Parameters and their Quantities

Parameter	Quantity
P^H	8.16
EC	0.169dS/m
NO3-N	1.38 mg/Kg
P	5.51 mg/Kg

K	68 mg/Kg
Organic Matter (OM)	0.59%

3.3.2 Water Holding Capacity

Water-holding capacity of the soil was measured according to the protocol explained by Estefan (2013), and it was 537.3ml/kg.

3.4. Biochar

Cotton stalk biochar was produced using a Kon Tiki flame curtain pyrolysis kiln under the temperature range of 650-750ºC. The resulting biochar underwent detailed physical and chemical property analysis at Eurofins Umwelt in Germany.

3.4.1 Preparation of Nitrogen-Loaded Biochar

Cotton stalk biochar prepared through slow pyrolysis is loaded with nitrogen. Urea solution was used as a source of nitrogen. Three different levels of nitrogen-loaded biochar were prepared (50Kgha^{-1}, 100kg ha^{-1} , 150kg ha^{-1}

3.4.2 Preparation of OS, N-50 Loaded Biochar (N-50)

40 grams of biochar was submerged in 400 milliliters of a 0.0164 M urea solution, maintaining a biochar to urea solution ratio of 1:10. The submerged biochar was dried at 40°C in drying oven.

3.4.3 Preparation of N-100 Nitrogen Loaded Biochar (N-100)

40g of biochar was submerged in 400 milliliters of a 0.0328 M urea solution, maintaining a biochar to urea solution ratio of 1:10. The submerged biochar was dried at 40°C in drying oven.

3.4.4 Preparation of N-150 Nitrogen Loaded Biochar (N-150)

40g of biochar was submerged in 400 milliliters of a 0.0494 M urea solution, maintaining a biochar to urea solution ratio of 1:10. The submerged biochar was dried at 40°C in drying oven.

3.5 Preparation of Organic Solution Doped-Nitrogen Loaded Biochar

Cotton stalk biochar prepared through slow pyrolysis is loaded with nitrogen. The sorghum plant extract was used as an organic solution. 1:3 (sorghum extract- distilled water) solution of sorghum plant extract solution was used. 120g of biochar was dipped in 242ml sorghum plant extract obtained from Multan and placed in an oven at 40℃ for 1 day. After drying, this biochar was divided into 3 equal parts and each part (40g) was loaded with three different levels of nitrogen. These levels were named OS, N-50, OS, N-100, and OS, N-150 based on the amount of nitrogen added per hectare i.e. 50 Kg/ha, 100 Kg/ha, and 150 Kg/ha.

3.5.1 Preparation of OS, N-50 Biochar (OS, N-50)

On the following day, the organic solution-doped biochar was removed from the oven. Subsequently, 40 grams of the organic solution-doped biochar were submerged in 400

milliliters of a 0.0164 M urea solution, maintaining a biochar-to-urea solution ratio of 1:10. The submerged biochar was dried in an oven at 40°C in 5 days.

3.5.2 Preparation of N-100 Nitrogen Loaded Biochar (OS, N-100)

40g of the organic solution-doped biochar were submerged in 400 milliliters of a 0.0328 M urea solution, maintaining a biochar to urea solution ratio of 1:10. The submerged biochar was dried in an oven at 40°C in 5 days.

3.5.3 Preparation of N-150 Nitrogen Loaded Biochar (OS, N-150)

40g of the organic solution-doped biochar were submerged in 400 milliliters of a 0.0494 M urea solution, maintaining a biochar to urea solution ratio of 1:10. The submerged biochar was dried in an oven at 40°C in 5 days.

3.5.4 Plant Material and Experimental Site

Maize was chosen for this study because of its economic importance in Pakistan. The maize (*Zea mays*) cultivar used was Hybrid 2222. Pot experiments were carried out between April 2024 to May 2024 at the experimental greenhouse of the Atta ur Rahman School of Applied Biosciences of the National University of Sciences and Technology (33.6425° N, 72.9930° E), Islamabad, Pakistan.

3.5.5 Design of Pot Experiment

Soil culture pot experiment was conducted to evaluate the ability of Nigrogen loaded biochar and organic solution doped Nitrogen loaded biochar on growth of maize plants and on soil nutrient dynamics.There was a total of 8 treatments and 4 replicates. Corn (*Zea*

mays) seeds are grown in compost sowing trays with an average temperature of 25 ± 2 °C. Seeds were germinated after 8 days. On the 10th day, the plants were transferred to pots containing 2 Kg of soil. To each pot, 239.6mg of KH_2PO_4 is added as a source of potassium and phosphorus. Nitrogen is applied in the form of nitrogen-loaded biochar. 5% w/w biochar is added to each pot. In one set of experiments biochar loaded with three different levels of N was applied while in another set Organic solution (sorghum extract) was added in combination with biochar loaded with three levels of N. In the first study, experimental treatments consist of i) N-0 (control) ii) N-50 biochar iii) N-100 biochar, and iv) N-150 biochar. In the second study, the experimental treatments consist of i) OS, N-0 (control) ii) OS, N-50, iii) OS, N-100, and iv) OS, N-150. There were a total of 8 treatments and four replicates of each treatment.

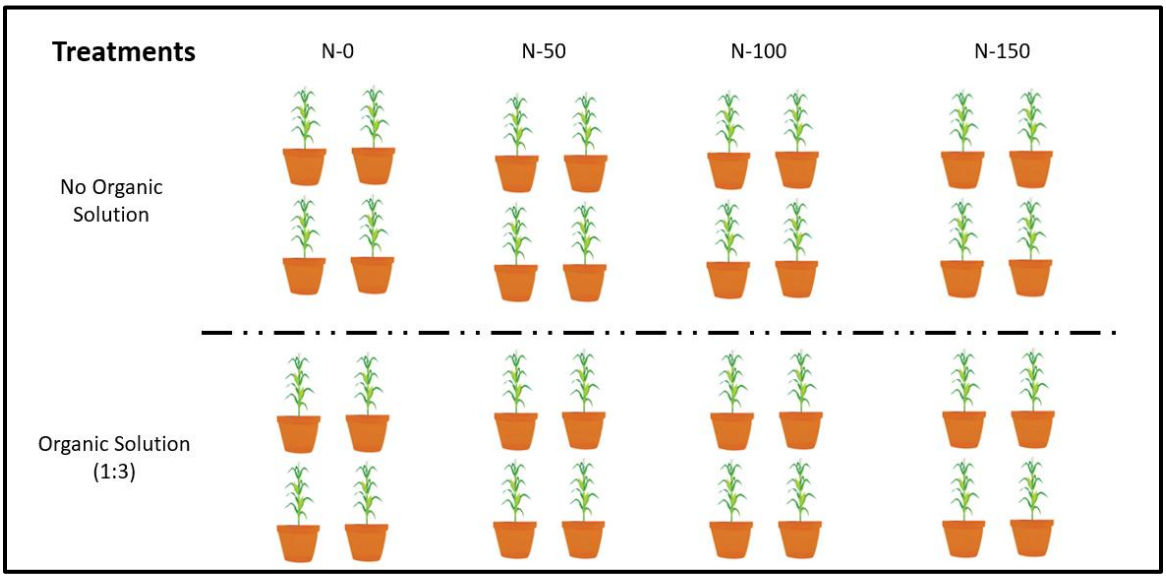

Figure 3.4: Experimental setup of pot treatments

3.6 Analysis of Plants and Soil Samples

On the 50th day just before harvesting the chlorophyll levels were measured by using a portable PhotosynQ, Multispeq v 2.0. After harvesting, the plants were carefully washed with deionized water. The fresh weights of the shoot and roots were measured with the help of digital balance. Plant heights were measured by a meter rod. Mean plant height was calculated by taking an average of four replications. The dry weight of the plant leaves and roots collected from each pot was measured after 48 h of drying at 70 °C in an oven with air circulation. Dry weights of roots and shoots were measured with the help of digital balance and used for data analysis thereafter.

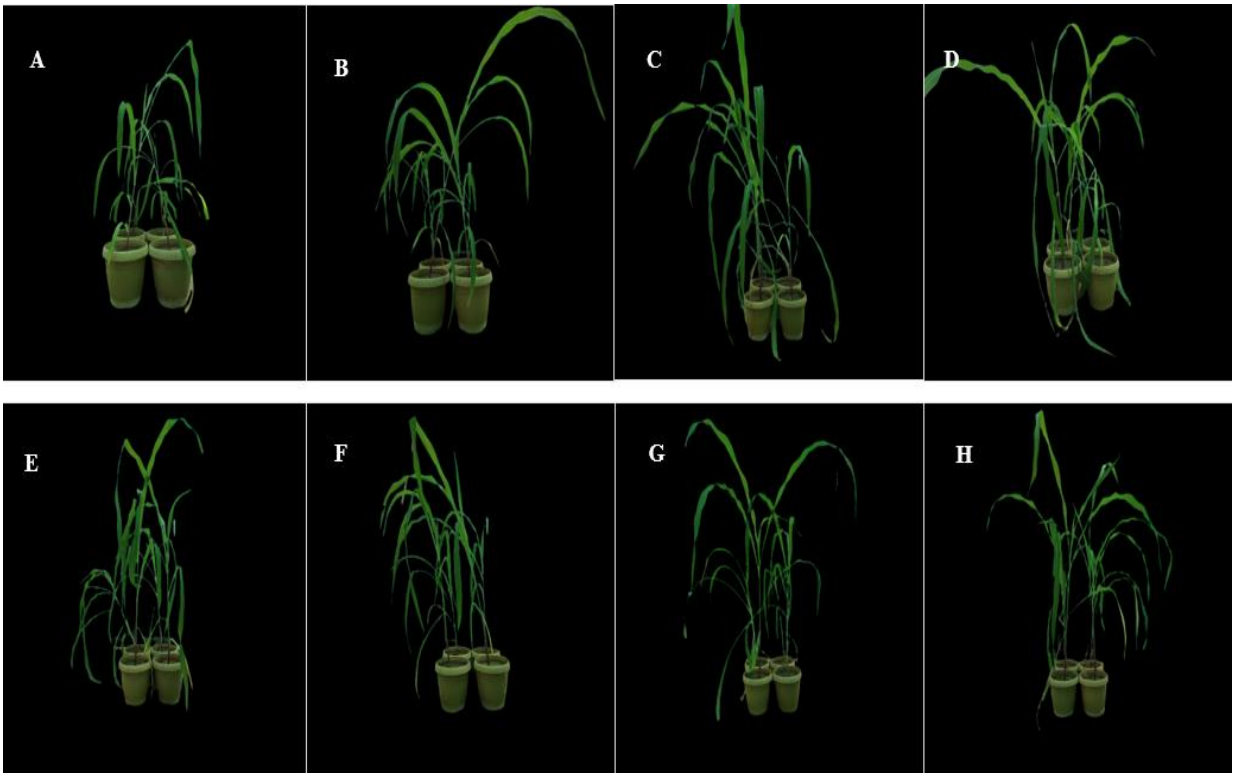

Figure 3.5: Maize Pot Experiment A) control: No OS, N-0, (B) No OS, N-50, (C) No OS, N-100 , (D) No OS, N-100, (E) OS, N-0, (F) OS, N-50 (G) OS, N-100, (H) OS, N-150

3.6.1 Nutrient Analysis in Soil and Plants

Nutrient analysis of both plant and soil samples was done after harvesting. Nitrogen was measured in plant and soil samples by the Kjeldahl method. Extractable was measured by the Olsen Blue method while in plant samples inorganic phosphate was determined by the Olsen Yellow method. For measurement of potassium in soil samples, extraction was done through ammonium acetate and readings were by flame photometer. Meanwhile, in plant samples extraction was done with acetic acid, and then readings were taken through a flame photometer.

3.6.2 Measurement of Phosphorus in soil

Olsen blue method (Olsen, 1954) was used to measure the amount of phosphorus that could be extracted from soil. L-ascorbic acid, antimony potassium tartrate, and ammonium heptamolybdate were combined to form the color development solution. To generate a calibration curve, standard solutions containing potassium dihydrogen phosphate were prepared. A sodium bicarbonate solution with a concentration of 0.5 M was used for soil extraction. Using a UV-VIS spectrophotometer, the absorbance was determined at 882 nm following the color development.

3.6.3 Measurement of Phosphorus in Plants

In plants for measurement of Total Phosphorus, the Olsen yellow method was used (Bowman, 1988). The color-developing reagent was made by adding 11.25g Ammonium heptamolybdate, 0.65g Ammonium metavanadate, and 125ml Nitric acid. 1ppm, 3ppm, 5ppm, and 7ppm standard solutions were made by dissolving KH_2PO_4 in distilled water.

Dry ashing of plant samples (1g each) was done in a muffle furnace at 550°C for 5 hours in crucibles. Then samples were taken out of the furnace, and 5 ml of 2N HCl was added to each sample. The samples were then kept submerged in HCl for 20-30 minutes. After that, 50ml dilutions were made by adding distilled water. Then dilutions were filtered with Whatman no. 1 filter papers. 1ml of extracts of each sample was then added to a solution of 8ml distilled water and 1ml of colour-developing reagent. Optical density readings of solutions were then noted, and graphs were made against the calibration curve.

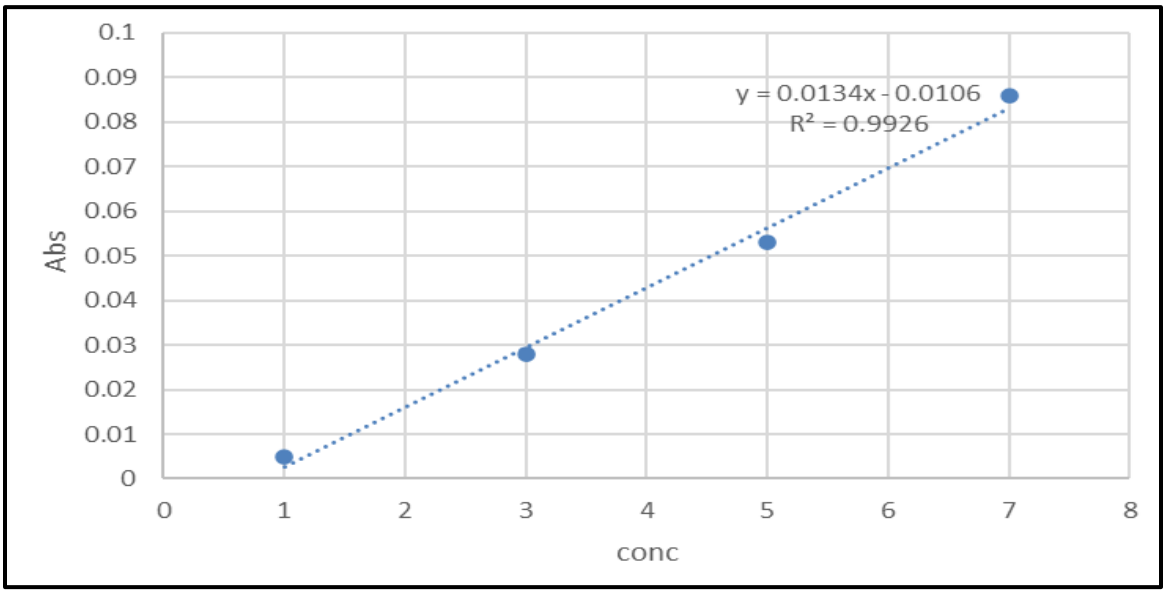

Figure 3.6: Calibration curve for measurement of phosphorus in plant samples

3.6.4 Measurement of potassium in soil

To extract potassium from air-dried soil, 10 grams of soil with a particle size of less than 2 millimeters was weighed into a 250-milliliter flask. 50 ml of 1 N ammonium acetate (NH4OAc) solution was added to the flask. The mixture was then agitated at a speed of 200 to 300 rpm for 30 minutes on a shaker. Following the shaking process, the suspension

was filtered, and the volume of the filtered extract was adjusted to 50 ml by adding 1 N NH4OAc solution if necessary. The extractable K was then determined through a pre-calibrated flame photometer.

3.6.5 Measurement of potassium in plants

Potassium (k^+) ions accumulation in plants was analyzed by extracting the dried leaf, stem samples (0.2g each) in 0.1M acetic acid solution by heating at 95 ℃ in a water bath for an hour. Suitable dilutions were made, and readings were measured using a flame photometer (PFP-7, Jenway) (Choudhary et al., 2016).

3.6.5 Kjeldahl Nitrogen of soil and plant samples

Total nitrogen of both soil and samples was done from Bahauddin Zakariya University, Multan, Pakistan through Kjeldahl. It involves three main steps: digestion, distillation, and titration.

In this protocol 0, 2, 4, 6, 8, and 10 ppm standards solution were used for making a calibration curve. For digestion, 0.25g of each dried plant sample was taken and added to a 50 ml digestion tube. Three to four pumice boiling granules were added followed by 5ml of concentrated sulfuric acid. The mixture was left to stand overnight. The tubes were heated at 100–150°C on a block digester. Periodically, the liquid was swirled to prevent foaming. If foaming got into the digesting tube's neck, 2 ml of 30% hydrogen peroxide was added. After cooling for a while and heating the tubes for 30 to 60 minutes on the block digester, 2 ml more hydrogen peroxide was added. After that, the block digester was heated to 280°C, and the tubes were kept at this temperature for ten minutes. The tubes were

heated for a further 10 minutes after cooling and receiving an additional 2 mL of hydrogen peroxide. After boiling the solution for ten minutes, this process was repeated until the solution remained clear. After cooling the tubes, the volume was brought to 50 ml by adding water. The clear filtrate was then collected by filtering the digest through filter paper.

To prepare the sample for analysis, 1 ml of the sulfuric acid-digested sample was placed in a test tube and diluted with 5 ml of distilled water. 0.1 ml of this diluted material was put into an Eppendorf tube. The tube was then filled with 0.5 ml of Reagent 2 (Take 1.25 g NaOH, 16.75 g Na_2HPO_4, and 2.5 ml NaOCl dissolved in 250 ml distilled water) and 0.5 mL of Reagent 1 (Take 2.5 ml Phenol in 12.65 mg Na Nitroprusside and dissolve it in 250 ml distilled water) right away, and the lid was quickly closed. After that, the tubes were submerged in a 37°C water bath for an hour. The sample color changed to blue after an hour. The wavelength of 625 nm was selected when the spectrophotometer was turned on. Plotting a graph and running standards were done. A volume of 0.1 ml was extracted from each standard, the same reagents were applied to the samples, and the samples were left in the water bath for an hour before readings were taken. Ultimately, each sample was tested individually, and the results were recorded.

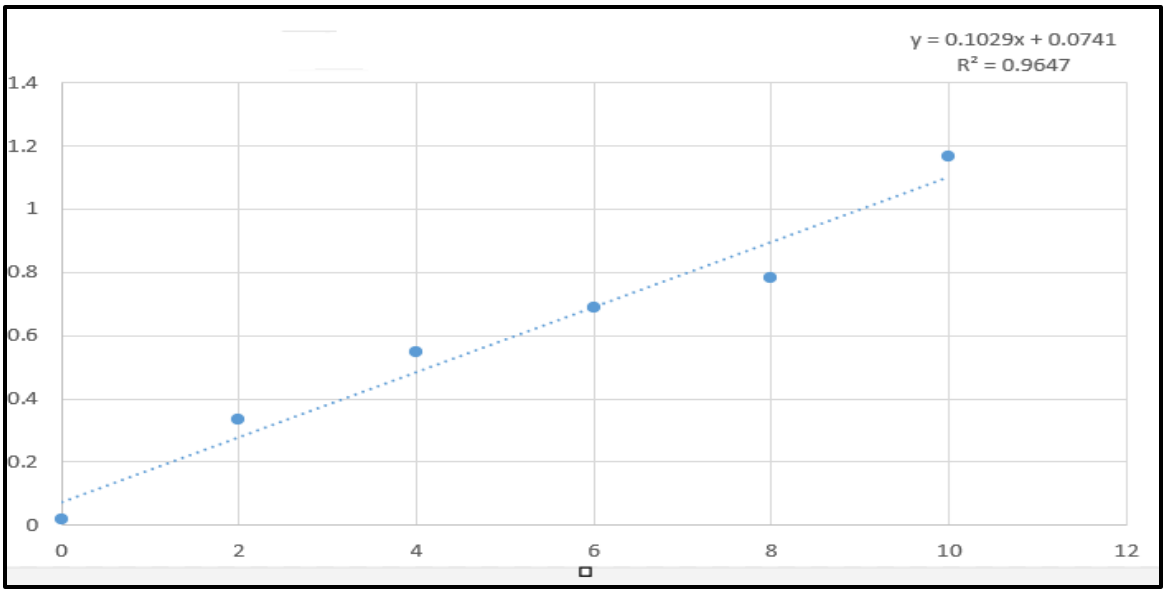

Figure 3.7: calibration curve for Kjeldahl Nitrogen

3.7 Statistical Analysis

This experimental design consisted of two factors (Nitrogen loading on biochar and Organic solution doping) so two-way ANOVA was applied. Means were deemed significantly different when P was less than 0.05. After performing two way ANOVA, the Fisher LSD post-hoc test was applied. Statistical analyses were conducted using Origin 2021 (version 9.8.0.200). Data visualization was carried out using GraphPad Prism (version 9.3.1471).

CHAPTER 4: RESULTS

4.1 Isolation of N-Cycling Bacteria

Serial dilutions were spread over media plates and incubated at 37℃ and bacterial colonies appeared over media after incubation which were then identified through 16S rRNA sequencing.

4.1.1 Bacterial Colonies

After incubation of agar plates in an incubator for 24h at 37℃, small densely packed bacterial colonies appeared on the plates. Colonies were creamy to off-white in color. These colonies were circular in shape, margins were smooth.

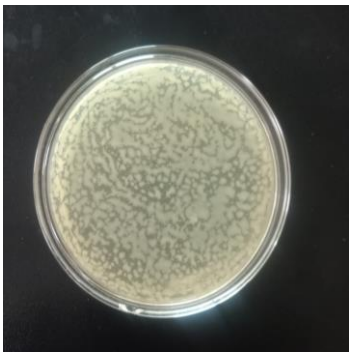

Figure 4.1 : Isolation of N-Cycling bacteria

4.1.2 Gram Staining

Gram staining was performed according to the standard protocol using a single bacterial colony. Observation under a microscope revealed that bacteria appeared pink in color characteristic of gram-negative bacteria.

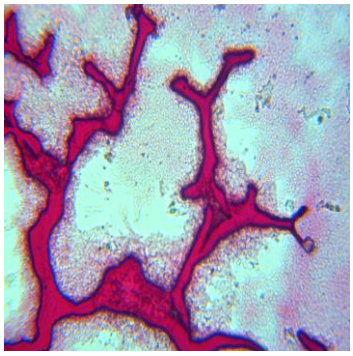

Figure 5 Figure 4.4: DNA extraction of bacteria, 1% gel. Lane 1: 1kb ladder; Lane 2 APEC DNA (positive control) and Lane 3: bacterial DNA (size above 460bp)

4.1.3 DNA Extraction

DNA was extracted according to the manufacturer's instructions. The purity of DNA was verified through nanodrop showing a concentration of 71ng/µl.

Figure 4.3: Bacterial DNA extraction

4.1.4 PCR and Gel Electrophoresis

The sequence of the forward primer (16S F) was 5'-ACTCCTACGGGAGGCAGCA-3' and the sequence of 16S R is 5'-GGACTACHVGGGTWTCTAAT-3'. The PCR product was run through 1% agarose gel and 1kb ladder was used. The band size was 469bp showing good amplification.

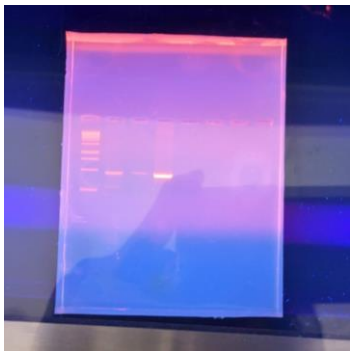

Figure 4.4: DNA extraction of bacteria, 1% gel. Lane 1: 1kb ladder; Lane 2 APEC DNA (positive control) and Lane 3: bacterial DNA (size above 460bp)

4.1.5 Sequencing

The sequences obtained through 16S rRNA sequencing data from Macrogen analyzed through NCBI, BLAST and a phylogenetic tress was constructed through MegaX 11 software using Neighbour Joining method revealing that the isolated bacteria was more predominantly related to strains of Pseudomonas instead of Nitrosomonas europaea as BLAST results indicated 97.64% similarity of isolated strain with *Pseudomonas mosselii* strain.

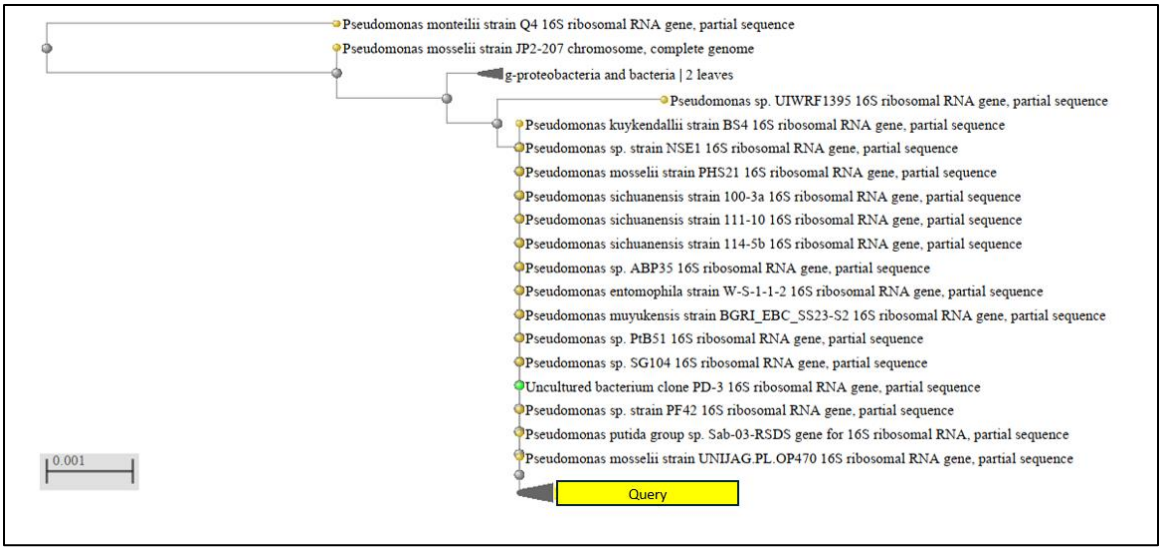

Figure 4.5: Phylogenetic tree constructed through Mega X 11 software using Neighbor Joining Method revealing that query sequence was more related to Pseudomonas strains instead of *Nitrosomonas europaea*

Score	Expect	Identities	Gaps	Strand
161 bits(87)	1e-34	200/254(79%)	9/254(3%)	Plus/Minus

```
Query  1     GTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGCAAGGAAGGGCAGTAAGGAAAT  60
             ||||||||||||||||||||||||||||||||||||||  ||||||||||||||||  |||
Sbjct  1097  GTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAAT  1038

Query  61    ACCAC-CACCCTCGACCATACCGAA-AGAATAAACACCGGATAACTCCCAGCCAGAAGCC  118
             |||   |    | |||  ||||||| ||||||| ||||||  ||||||   ||||| ||||
Sbjct  1037  ACCTTGCTGTTTTGACGTTACCGAACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCC  978

Query  119   GCGCTAAGACAGACAACGCAA-C---AATCGGAATTACCGCGCGTAAAGCGCACGAAGGC  174
             ||| ||| |||||    |||| |   |||||||||||| | ||||||||||| || |||
Sbjct  977   GCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGT  918

Query  175   CGATCAACAAGT-GCGACCTGAAAACCCCGGGCGCAACC-GGCAAACAGCAGCCAAAACT  232
              | ||   |||| | ||  ||||| |||||||| ||||| || |||| ||| ||||||||
Sbjct  917   GGTTCGTTAAGTTGGGATGTGAAAGCCCCGGGCTCAACCTGGGAAACTGCATCCAAAACT  858

Query  233   GACGA-CGAGAGTA  245
             | ||| | ||||||
Sbjct  857   GGCGAGCTAGAGTA  844
```

Figure 4.6: BLAST alignment of the 16S rRNA gene sequence from the soil isolate with Pseudomonas sp. strain RTY50. The alignment shows 79% identity over 254 base pairs with 3% gaps and an E-value of $1e^{-34}$

Sequences producing significant alignments | Download | Select columns | Show 10

select all 10 sequences selected | GenBank | Graphics | Distance tree of results | MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Uncultured bacterium partial 16S rRNA gene, clone SINW773_N11D0_16S_B	uncultured bacterium	795	795	94%	0.0	97.64%	1361	LN560201.1
Pseudomonas sp. strain BA7816 16S ribosomal RNA gene, partial sequence	Pseudomonas sp.	791	791	91%	0.0	98.45%	1301	KY849578.1
Pseudomonas monteilii strain J5 16S ribosomal RNA gene, partial sequence	Pseudomonas monteilii	791	791	91%	0.0	98.45%	871	KT027782.1
Pseudomonas sichuanensis strain 100_1 16S ribosomal RNA gene, partial sequence	Pseudomonas sichuanensis	791	791	91%	0.0	98.45%	1399	OP811679.1
Uncultured bacterium clone ncd2493a04c1 16S ribosomal RNA gene, partial sequence	uncultured bacterium	791	791	92%	0.0	98.24%	1358	JF215120.1
Pseudomonas sp. DR5 16S ribosomal RNA gene, partial sequence	Pseudomonas sp. DR5	791	791	92%	0.0	98.24%	591	FJ161952.1
Pseudomonas sp. RW5S2 partial 16S rRNA gene, strain RW5S2	Pseudomonas sp. RW5S2	791	791	92%	0.0	98.24%	1306	AM911660.1
Pseudomonas monteilii strain Q4 16S ribosomal RNA gene, partial sequence	Pseudomonas monteilii	787	787	91%	0.0	98.23%	899	KT027784.1
Uncultured Aeromonadaceae bacterium clone SIM046 16S ribosomal RNA gene, partial sequ...	uncultured Aeromonadaceae bacterium	787	787	93%	0.0	97.61%	1069	JF733211.1
Pseudomonas guariconensis strain LE3 16S ribosomal RNA gene, partial sequence	Pseudomonas guariconensis	785	785	91%	0.0	98.23%	1330	MT584853.1

Figure 4.7 Result of nucleotide BLAST

4.2 Isolation of Bacteriophages from Sewage Samples

4.2.1 Phage Lysate

Phage lysate was prepared by enriching phage filtrate with bacteria. After infection of bacterial cells for 24h at 37℃ in a shaking incubator, it was filtered through 0.22µm filters, and phage lysate was obtained.

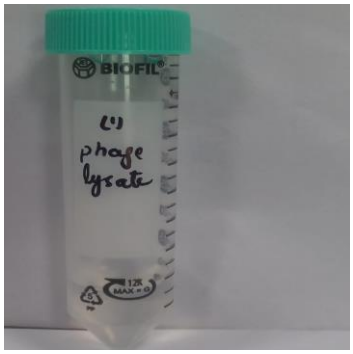

Figure 4.8: Phage lysate prepared by enrichment with bacterial cells

4.2.2 Agar Overlay Assay

Using 0.22µm filters, the phage that infects Nitrosomonas was isolated from samples of sewage water. The isolated phage's lytic activity was examined using a spot assay on a bacterial lawn.

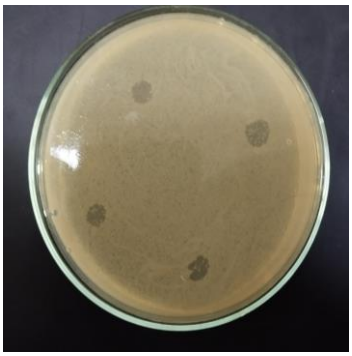

Figure 4.9: Spot assay showing clear zones of inhibitions over the bacterial lawn

4.2.3 Plaque Assay

The plaque assay was not successful as no proper plaques were formed.

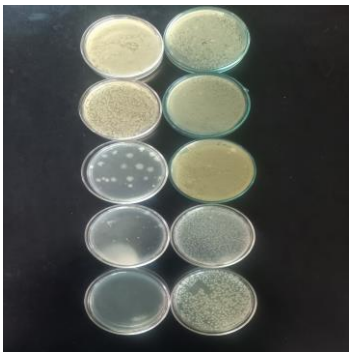

Figure 4.10: plaques assay, no proper plaques are formed

4.3. Effects of Nitrogen Loaded Biochar and Organic Solution Doped Nitrogen Loaded Biochar on Plant Morphological Characteristics of Mazie

Effects of Nitrogen-loaded biochar and organic solution-doped nitrogen-loaded biochar were investigated on plant morphological characteristics i.e. shoot and roots lengths, fresh and dry biomass. The nitrogen-loaded biochar has a significantly increase shoot fresh weight (P = 0.03795). Specifically, N-50 increased shoot fresh weight by 34.04%, N-100 by 98.59%, and N-150 by 27.99%. Additionally, the organic solution doping has also significantly (P = 0.03795) increased shoot fresh weight by 17.29%. However, the interaction between nitrogen-loaded biochar and organic solution doping did not have a significant effect on shoot fresh weight (Fig 4.11)

Nitrogen-loaded biochar significantly affected shoot dry weight (P = 0.01199). Specifically, N-50 increased shoot dry biomass by 18.18%, N-100 by 105.45%, and N-150 by 27.72% compared to the control (N-0). Organic solution doping also significantly increased shoot dry biomass by 52.72% (P = 0.00647). The combination of OS and N-50 increased shoot dry weight from 18.18% to 54.54%. However, the interaction of OS and N-100 decreased root dry weight from 105.45% to 94.54%, while the combination of OS and N-150 significantly increased shoot dry weight from 27.72% to 177.27% (Fig 4.12).

The application of Nitrogen-loaded biochar has no significant effect on root fresh weight. Organic solution doping has a significantly increased root fresh weight from 0% to 162.40% (P=0.00394). Their interaction has no significant (P=0.15252) effect on root fresh weight (Fig 4.13).

Nitrogen-loaded biochar has no significant effect on dry biomass of roots. Organic solution doping has a significant (P=0.05514) effect on root dry weight and increases the root dry

biomass from 0% to 308.98%. Their interaction has no significant (P=0.00336) effect on root dry weight (Fig 4.14).

Nitrogen loading has affected the shoot length of maize. N-50 has significantly increased shoot length from 3.28% to 12.76%. N-100 has significantly reduced shoot length from 33.73% to 25.53%, and N-150 has increased shoot length from 14.58% to 24.01% (Fig 4.15).

Nitrogen-loading biochar and organic solution have no significant effect on root length. N-50 interaction with organic solution has significantly increased root length from -15.68% to -7.80%. Interaction of N-100 with Organic solution has significantly reduced root length from 5.57% to -7.80%. N-150 interaction with organic solution has significantly increased from -10.78% to 21.18% (Fig 4.16).

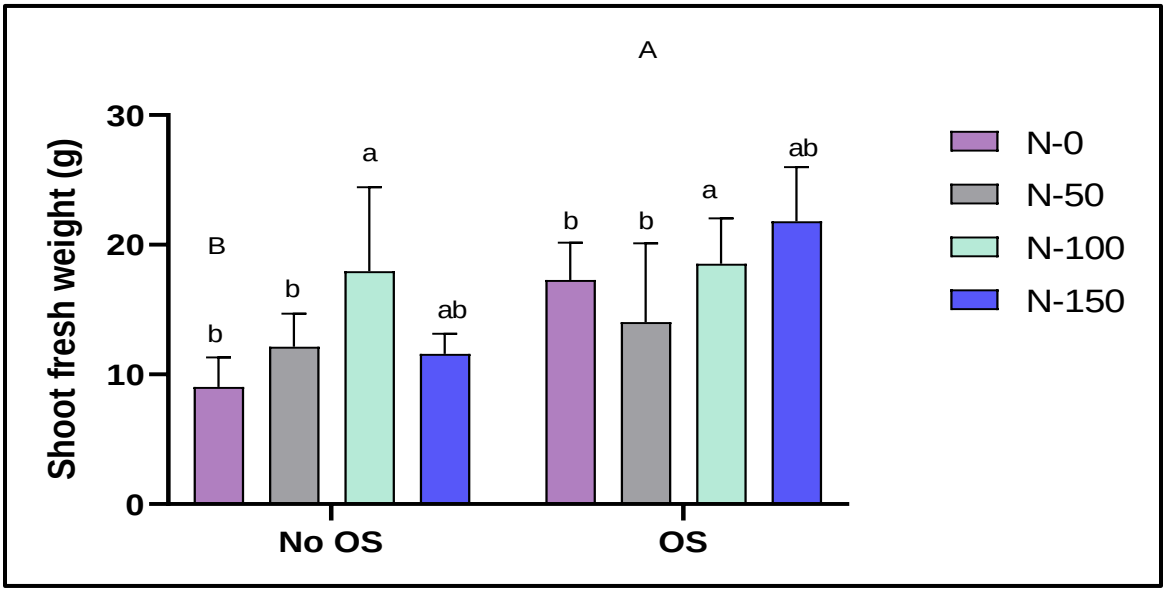

Figure 4.11: Effect of BC treated with N (0 kg ha-1,50kg ha-1, 100kg ha-1, 150kg ha-1) and Organic solution (0, 1:3) on Shoot Fresh Weight. Means are represented by bars. Mean bars sharing similar letters are not significantly different (P>0.05)

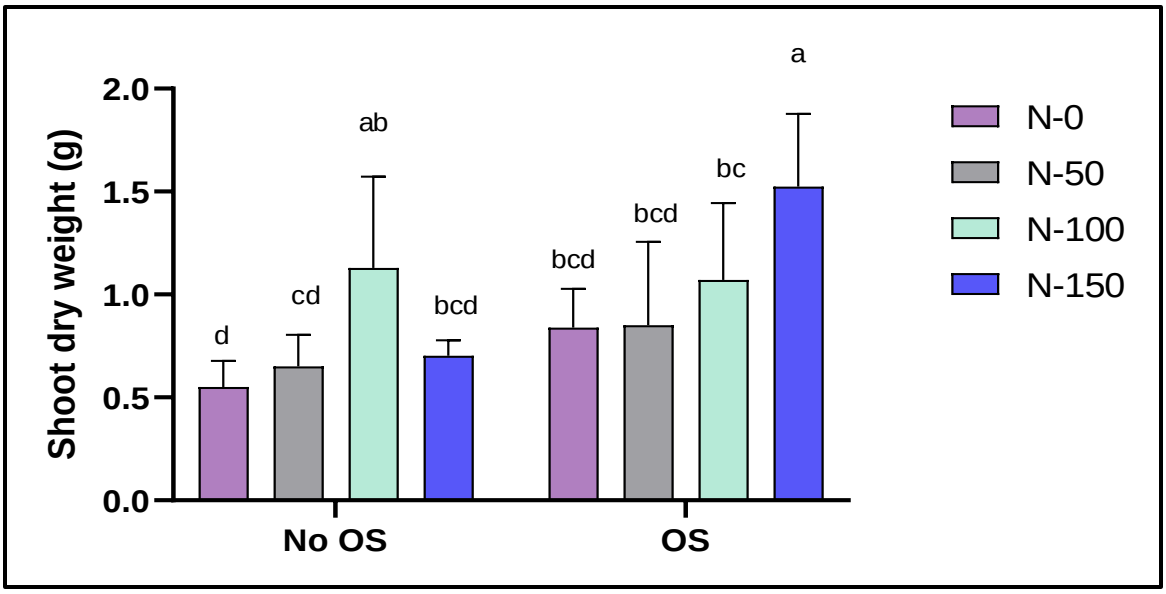

Figure 4.12: Effect of BC treated with N (0 kg ha-1,50kg ha-1, 100kg ha-1, 150kg ha-1) and Organic solution (0, 1:3) on Shoot Dry Weight. Means are represented by bars. Mean bars sharing similar letters are not significantly different (P> 0.05)

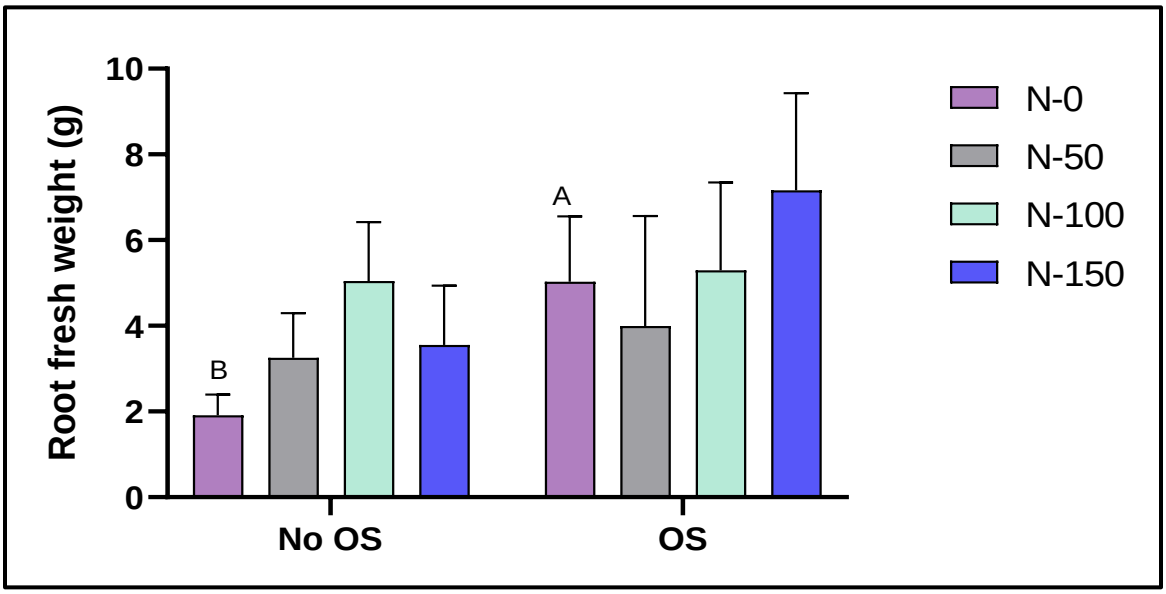

Figure 4.13: Effect of BC treated with N (0 kg ha-1,50kg ha-1, 100kg ha-1, 150kg ha-1) and Organic solution (0, 1:3) on Root Fresh Weight. Means are represented by bars. Mean bars sharing similar letters are not significantly different (P> 0.05)

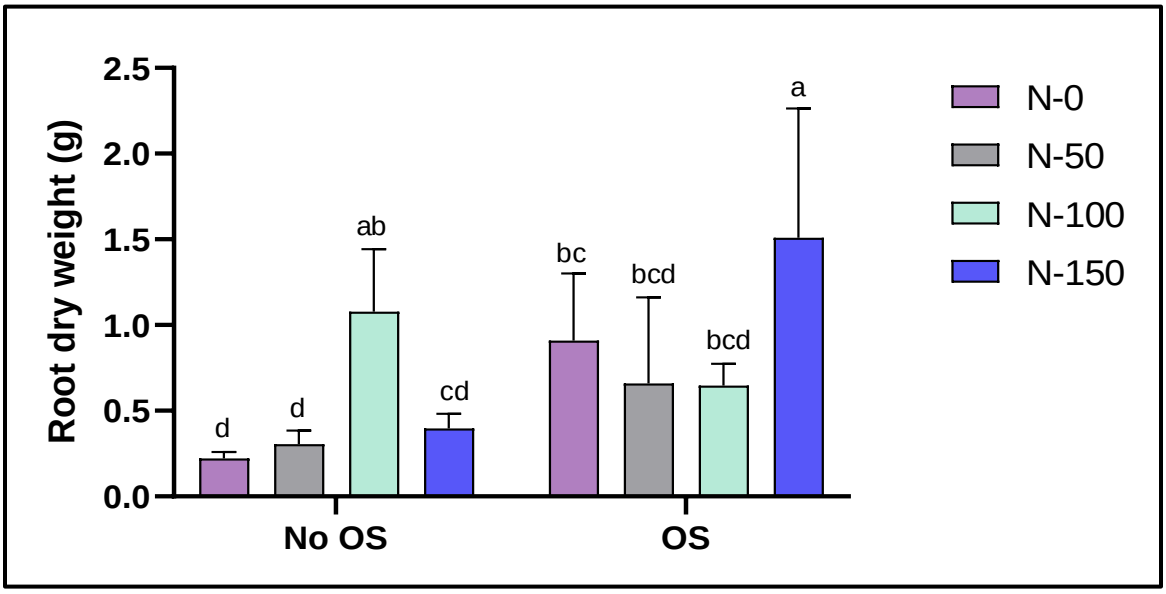

Figure 4.14: Effect of BC treated with N (0 kg ha-1,50kg ha-1, 100kg ha-1, 150kg ha-1) and Organic solution (0, 1:3) on Root dry weight. Means are represented by bars. Mean bars sharing similar letters are not significantly different (P> 0.05)

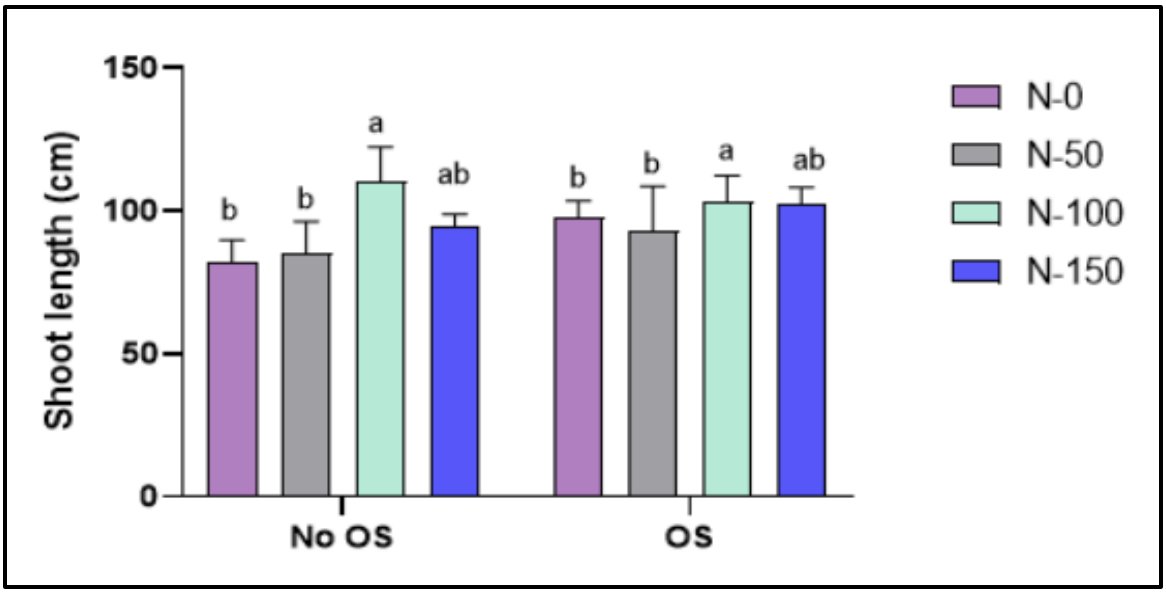

Figure 4.15: Effect of BC treated with N (0 kg ha^{-1},50kg ha^{-1}, 100kg ha^{-1}, 150kg ha^{-1}) and Organic solution (0, 1:3) on Shoot length. Means are represented by bars. Mean bars sharing similar letters are not significantly different (P>0.05)

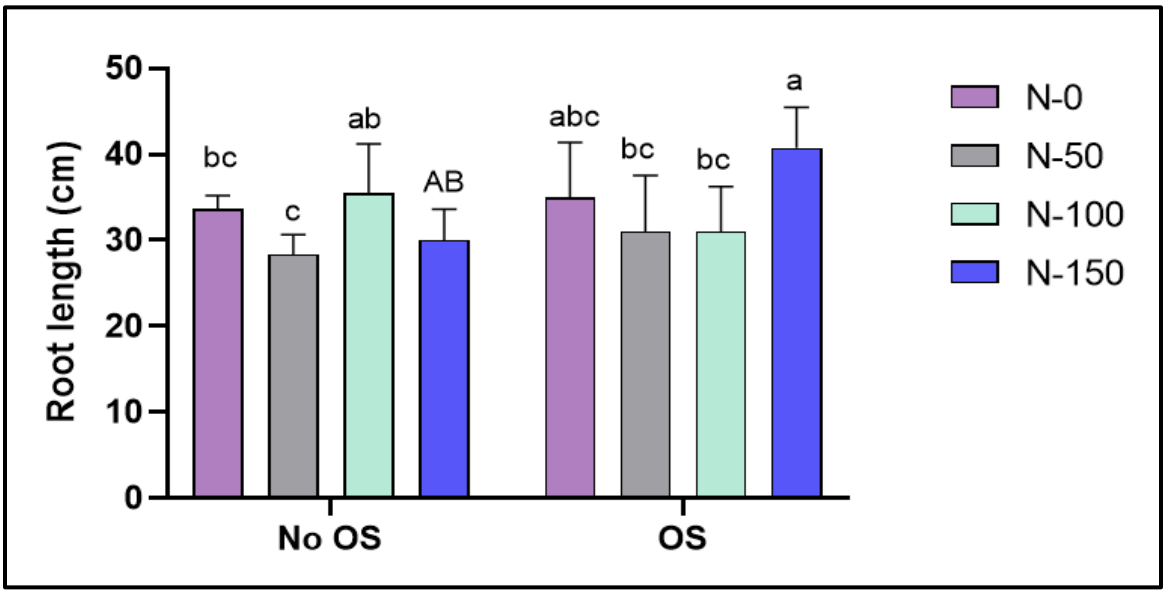

Figure 4.16: Effect of BC treated with N (0 kg ha-1,50kg ha-1, 100kg ha-1, 150kg ha-1) and Organic solution (0, 1:3) on Shoot length. Means are represented by bars. Mean bars sharing similar letters are not significantly different (P> 0.05)

4.4. Effects of Nitrogen Loaded Biochar and Organic Solution Doped Nitrogen Loaded Biochar on Plant Physiological Characteristics of Maize

Nitrogen-loaded biochar has no significant effect on Relative Chlorophyll content (SPAD index). Organic solution doping has no significant effect on the SPAD index. Their interaction has significantly (P=0.02029) increased the SPAD index. OS, N-50 has significantly increased the SPAD index from -6.39% to -3.47%. OS, N-100 has significantly increased the SPAD index from -14.86% to 32.27%. While the interaction of OS with N-150 has significantly reduced the SPAD index from -2.95% to -18.32% (Fig 4.17).

There was no significant effect of nitrogen-loaded biochar and OS-doped nitrogen-loaded biochar and their combination on the Quantum Yield of Non-Photochemical Quenching (Phi NPQ) (Fig 4.18), Non-photochemical Quenching (NPQt) (Fig 4.19), Phi NO (Fig 4.20), effective quantum yield of PSII (Phi 2) (Fig 4.21).

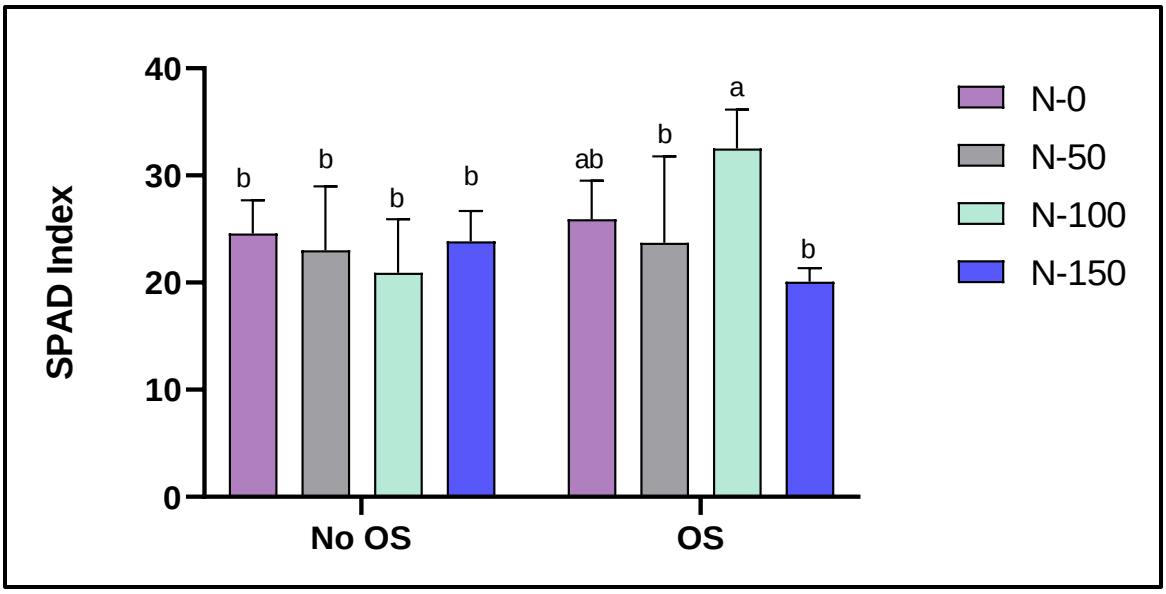

Figure 4.17: Effect of BC treated with N (0 kg ha-1,50kg ha-1, 100kg ha-1, 150kg ha-1) and Organic solution (0, 1:3) on SPAD Index. Means are represented by bars. Mean bars sharing similar letters are not significantly different (P>0.05)

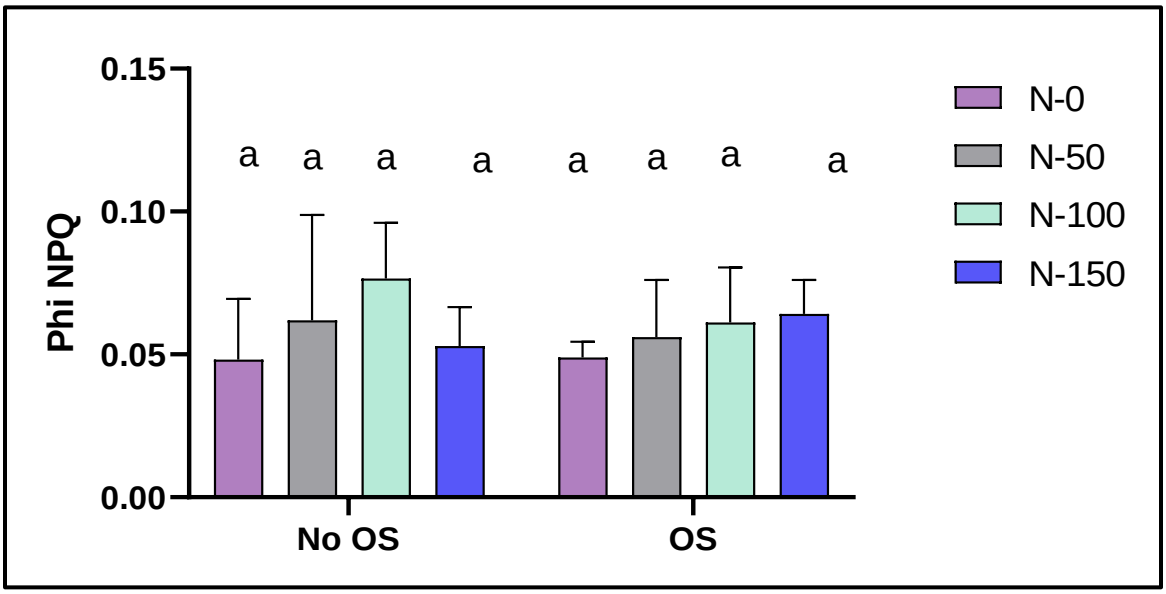

Figure 4.18: Effect of Biochar treated with Nitrogen (0 kg ha-1,50kg ha-1, 100kg ha-1, 150kg ha-1) and Organic solution (0, 1:3) on Phi NPQ. Means are represented by bars. Mean bars sharing similar letters are not significantly different (P>0.05)

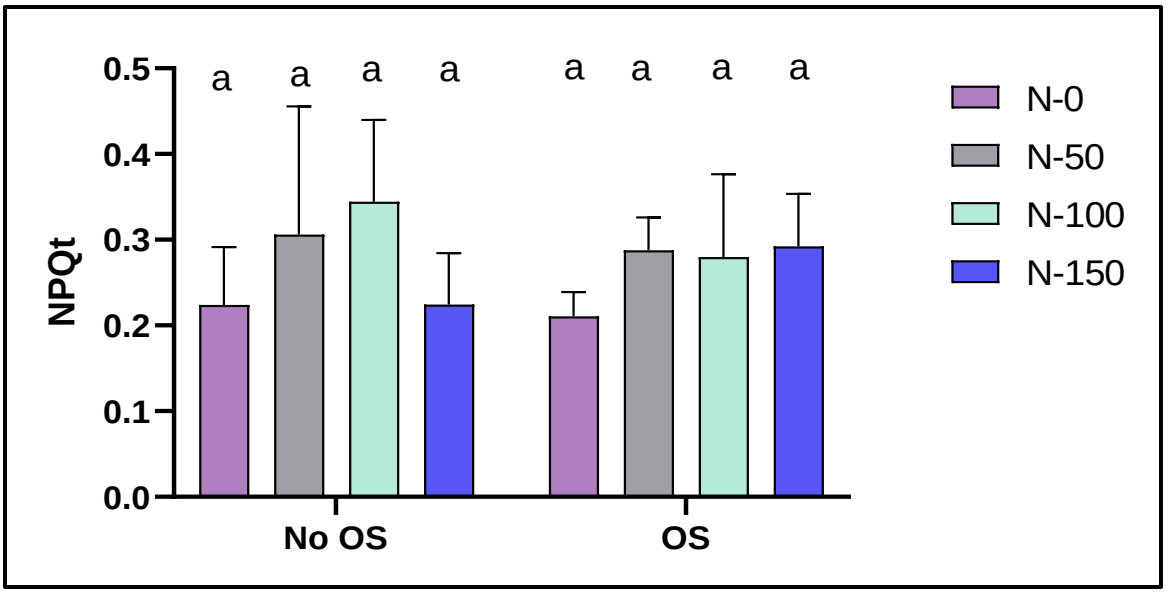

Figure 6 Figure 4.19: Effect of B treated with N (0 kg ha-1,50kg ha-1, 100kg ha-1, 150kg ha-1) and Organic solution (0, 1:3) on NPQt. Means are represented by bars. Mean bars sharing similar letters are not significantly different (P>0.05)

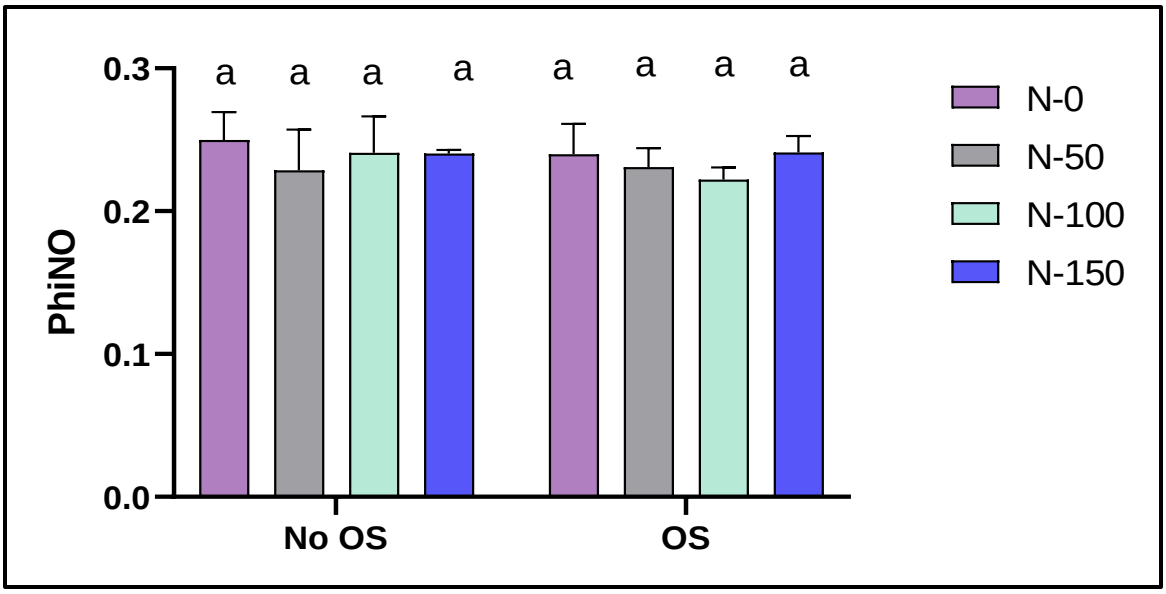

Figure 4.20: Effect of BC treated with N (0 kg ha-1,50kg ha-1, 100kg ha-1, 150kg ha-1) and Organic solution (0, 1:3) on Phi NO. Means are represented by bars. Mean bars sharing similar letters are not significantly different (P>0.05)

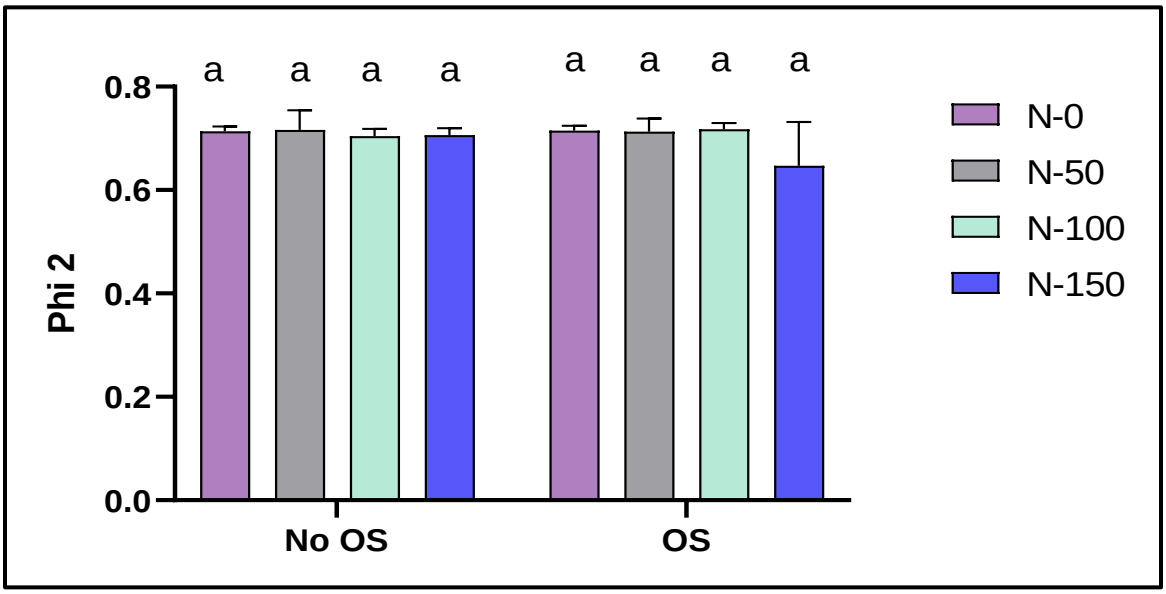

Figure 4.21: Effect of BC treated with N (0 kg ha-1,50kg ha-1, 100kg ha-1, 150kg ha-1) and Organic solution (0, 1:3) on PS II Quantum Yield. Means are represented by bars. Mean bars sharing similar letters are not significantly different (P>0.05)

4.5. Effects of Nitrogen Loaded Biochar and Organic Solution Doped Nitrogen Loaded Biochar on Soil and Plant Nutrient Dynamics

Soil extractable potassium was significantly influenced by nitrogen-treated biochar ($P < 0.0001$). The treatments showed the following increases: N-50 raised extractable potassium from 16.57% to 139.66%, N-100 increased it from 16.57% to 176.92%, and N-150 elevated it from 16.57% to 122.77%. Additionally, the organic solution has a significant impact ($P = 0.00163$), reducing extractable potassium from 0% to -6.93%. However, there was no significant effect of interaction between nitrogen-loaded biochar and organic solution doping and the organic solution on soil extractable potassium (Fig 22).

Percent potassium accumulation in plants is not impacted by any of the treatments and showed non-significant results in nitrogen-treated biochar, organic solution doped biochar, and interaction of nitrogen and organic solution (Fig 23).

Nitrogen loading on biochar has no significant effect on Soil Extractable Phosphorus. OS doping on biochar has significantly ($P= 0.00844$) reduced the soil available phosphorus from 0% to -31.28%. Also, the interaction between nitrogen and organic solution has significantly impacted the soil's available phosphorus ($P=0.01526$). The interaction of N-50 and organic solution has significantly increased the soil availability of phosphorus from -12.72% to 33.33%. N-100- 0S interaction has increased the soil available phosphorus from -20.61% to 41.67%. N-100- 0S interaction has increased the soil available phosphorus from -36.69% to 1.16% (Fig 24).

Nitrogen-loaded biochar and organic solution have no significant effect on inorganic phosphate. However, their interaction has a significant effect on total phosphorus (P= 0.01667). Interaction of organic solution doping with N-50 biochar has significantly reduced total phosphorus from 289.03% to 58%. Interaction of organic solution doping with N-100 biochar has significantly increased total phosphorus from 98.43% to 195.07%. Interaction of organic solution doping with N-150 biochar has significantly reduced total phosphorus from 266.99% to 160.41% (Fig 25).

Nitrogen loading, organic solution doping as well as their interaction have significantly increased the Soil NO_3. N-50 has significantly increased soil NO_3 from 0% to 289.03%. N-100 has significantly increased soil NO_3 from 0% to 98.43%. N-150 has significantly increased soil NO_3 from 0% to 266.99%. organic solution has significantly increased the Soil NO_3 from 0% to 231.39%. The interaction of organic solution and Nitrogen treatment has a significant effect on soil $N0_3$. Interaction of N-50 with organic solution has significantly decreased soil NO_3 from 289.03% to 58%. Interaction of N-100 with organic solution has significantly increased soil NO_3 from 98.43% to 195.07%. Interaction of N-150 with organic solution has significantly increased soil NO3 from 266.99% to 160.41% (Fig 26).

Nitrogen loading has a significantly ($P<0.0001$) increased Soil Total N (%). The organic solution has no significant effect on Soil Total N (%). N-50 has increased soil Total N (%) from 0% to 19.72%. N-100 has significantly increased soil Total Nitrogen from 0% to 33.13%. N-150 has significantly increased soil NO3 from 0% to 25.12%. The organic solution has increased the Soil Total N from 0% to 8.32%. OS, N-50 has significantly reduced Soil Total N from 19.72% to 16.12%. OS, N-100 has significantly increased Soil

Total N from 33.13% to 39.53%. OS, N-150 has significantly reduced Soil Total N from 25.12% to 21.72% (Fig 27).

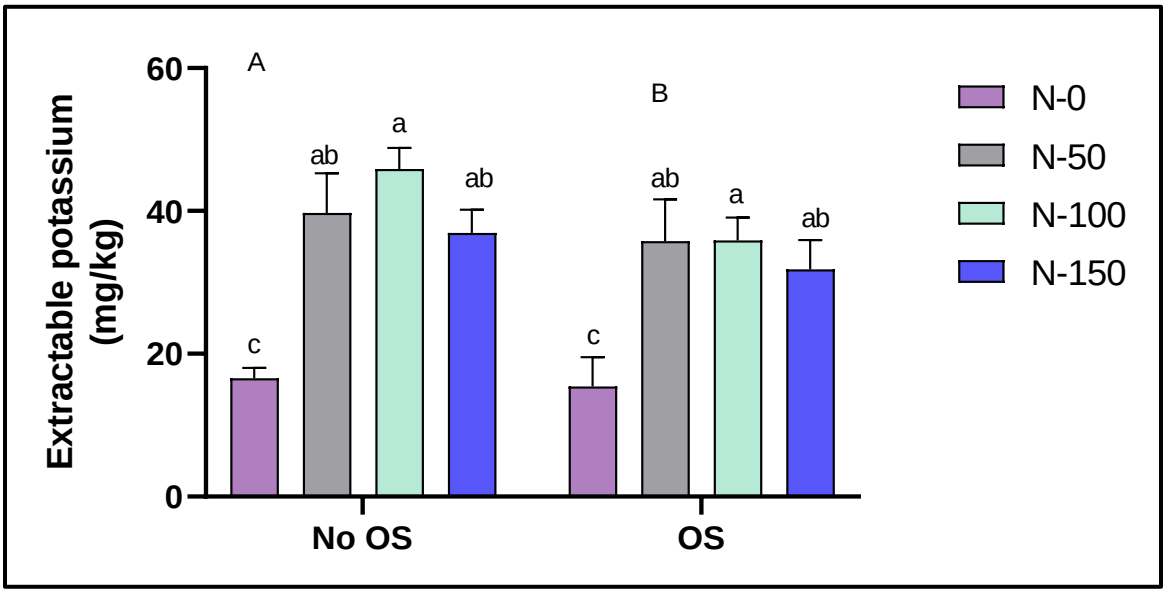

Figure 4.22: Effect of BC treated with N (0 kg ha-1,50kg ha-1, 100kg ha-1, 150kg ha-1) and Organic solution (0, 1:3) on Extractable Potassium in soil. Means are represented by bars. Mean bars sharing similar letters are not significantly different (P>0.05)

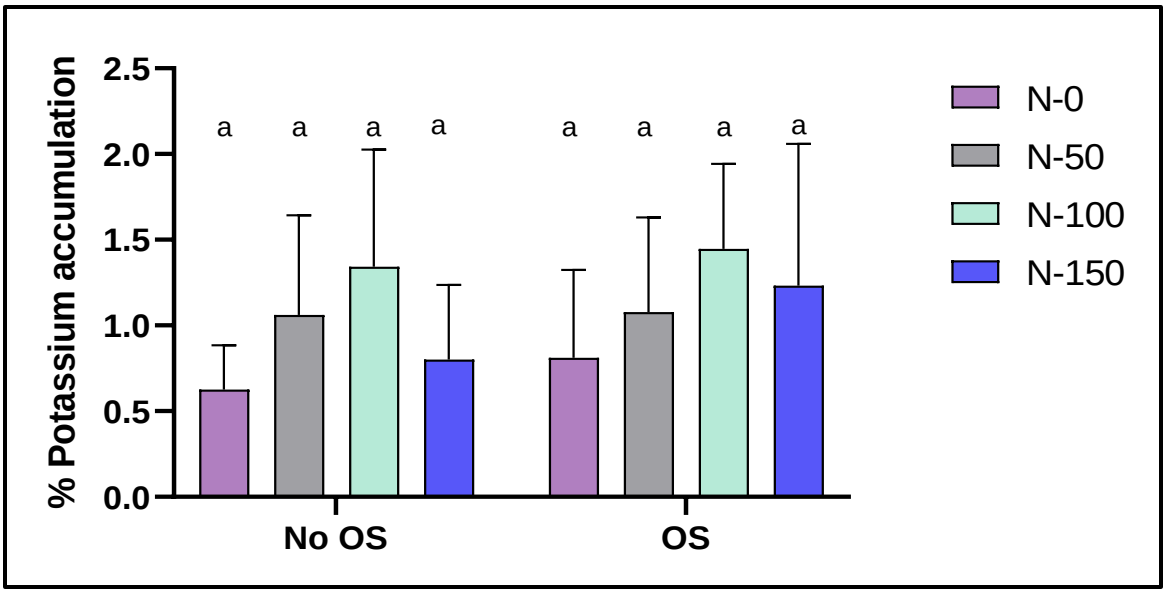

Figure 4.23: Effect of Bc treated with N (0 kg ha-1,50kg ha-1, 100kg ha-1, 150kg ha-1) and Organic solution (0, 1:3) on % K accumulation in plants. Means are represented by bars. Mean bars sharing similar letters are not significantly different (P>0.05)

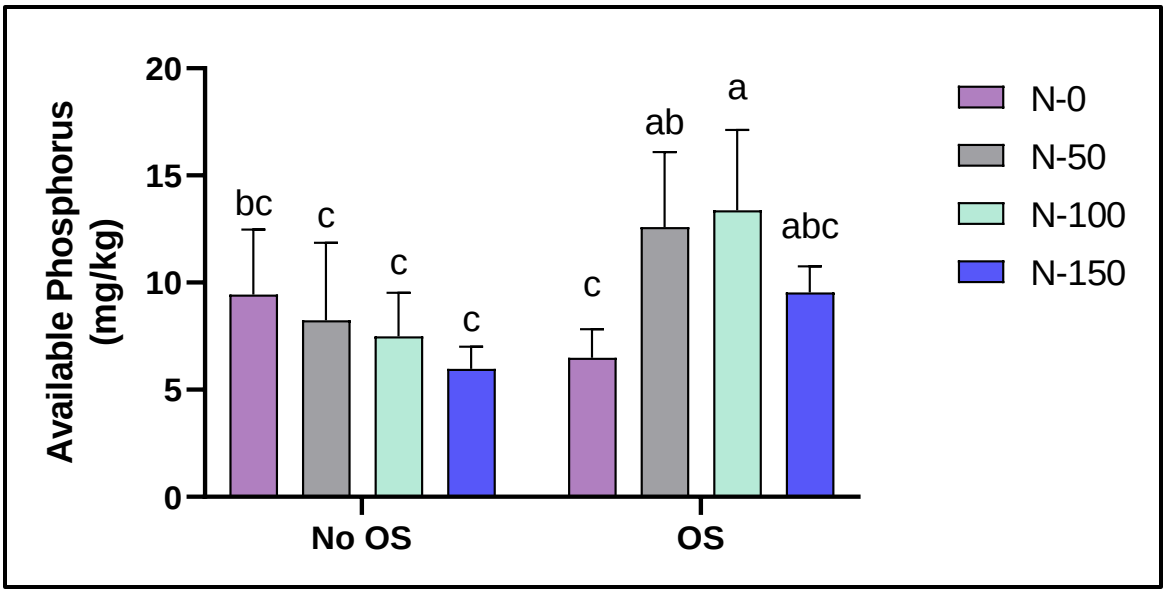

Figure 4.24: Effect of BC treated with N (0 kg ha-1,50kg ha-1, 100kg ha-1, 150kg ha-1) and Organic solution (0, 1:3) on Soil Available P. Means are represented by bars. Mean bars sharing similar letters are not significantly different (P>0.05)

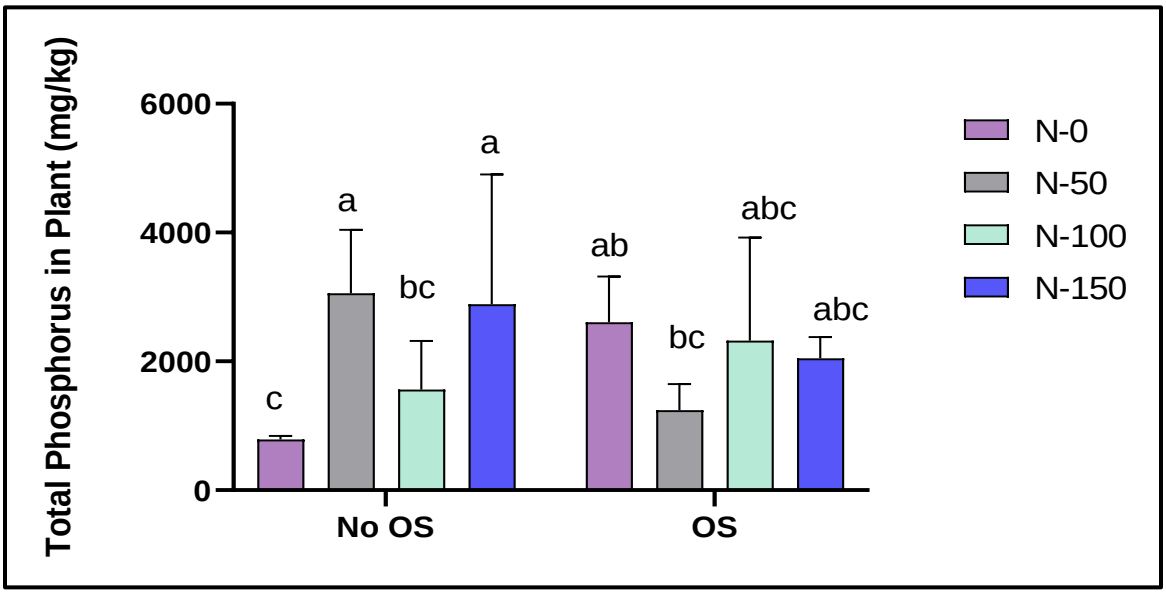

Figure 7 Figure 4.25: Effect of BC treated with N (0 kg ha-1,50kg ha-1, 100kg ha-1, 150kg ha-1) and Organic solution (0, 1:3) on Total P. Means of treatments are represented by vertical bars and error bars represent Standard Deviation among means. Mean bars sharing similar letters are not significantly different (P>0.05)

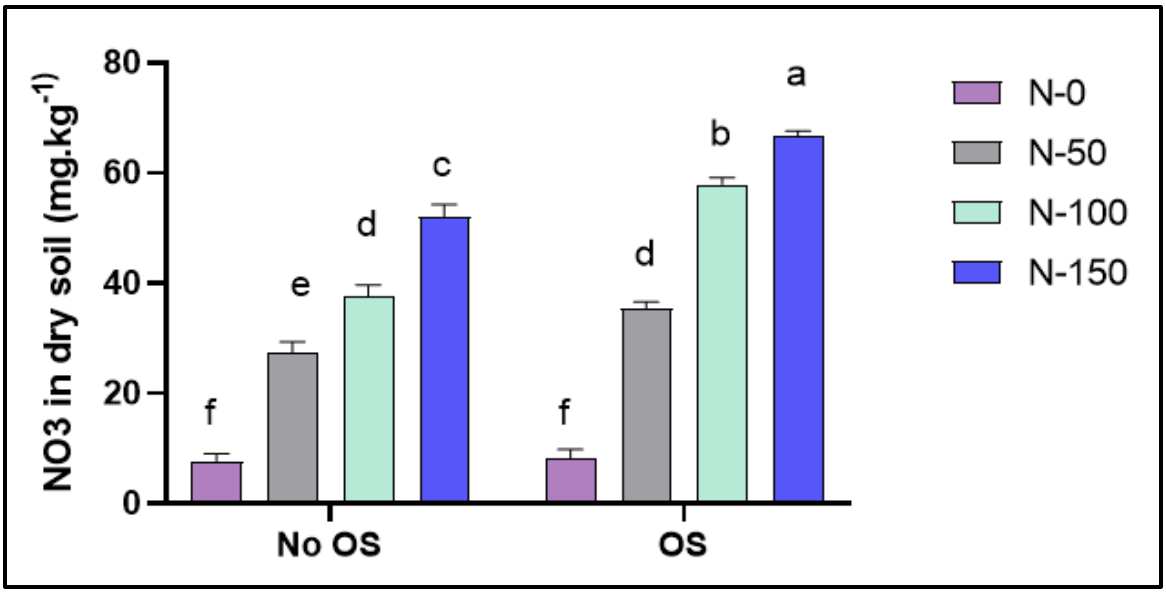

Figure 8 Figure 4.26: Effect of BC treated with N (0 kg ha-1,50kg ha-1, 100kg ha-1, 150kg ha-1) and organic solution (0, 1:3) on N3O in dry soil. Means are represented by bars. Mean bars sharing similar letters are not significantly different (P>0.05)

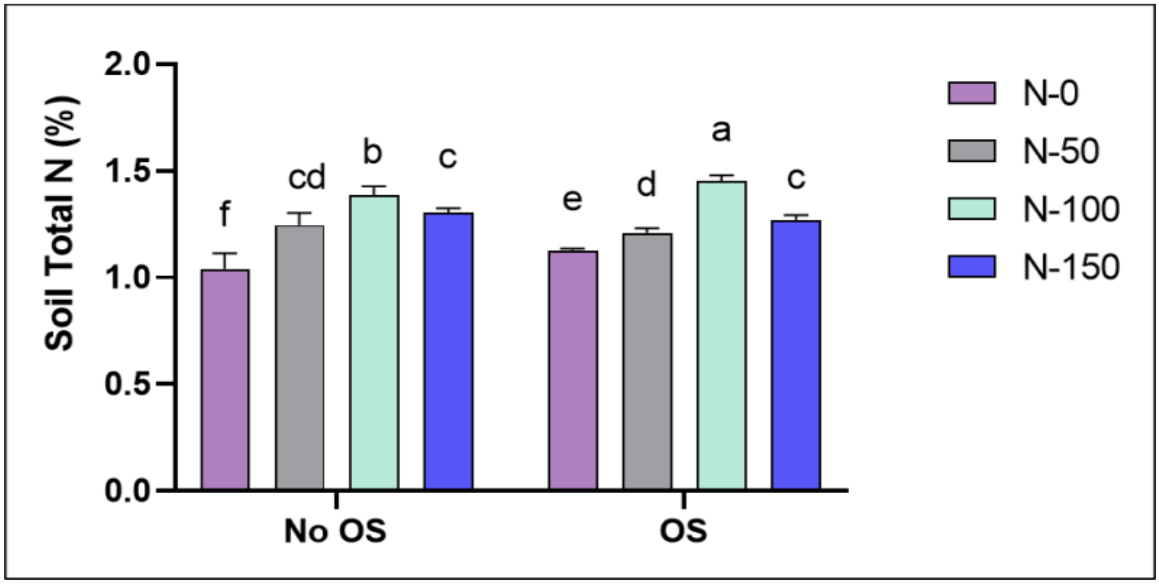

Figure 4.27: Effect of BC treated with N (0 kg ha-1,50kg ha-1, 100kg ha-1, 150kg ha-1) and organic solution (0, 1:3) on soil total N (%). Means are represented by bars. Mean bars sharing similar letters are not significantly different (P>0.05)

CHAPTER 5: DISCUSSION

Agriculture is the main contributor to N_2O emission due to the increased application of N fertilizers and lower NUE (Lawrence et al., 2021). N_2O emission is posing a serious threat to climate change mitigation and ozone layer protection (Tian et al., 2020). Immediate action to slow down N_2O emissions is crucial for safeguarding the planet's future (Tian et al., 2020).

Different Nitrification Inhibitors are generally used for this purpose and to improve NUE and reduce leaching and denitrification. These nitrification inhibitors are either in the form of Synthetic nitrification inhibitors (Beeckman et al., 2023) or biological nitrification inhibitors (root exudate) (Petroli et al., 2023). But these nitrification inhibitors are temperature and pH-sensitive and highly costly (Cui et al., 2022), so our study is focused on developing a new strategy for nitrification inhibition i.e. using bacteriophages for nitrification inhibition. In this study, we first isolated *Nitrosomonas europaea* from active agricultural soil, and phage against Nitrosomonas was isolated from sewage water. Furthermore, another strategy to limit the N_2O emission was also investigated i.e. use of N-loaded biochar and sorghum extract doped N-loaded biochar was used and their effects on maize (*Zea mays*) were investigated.

The bacterial strain was isolated from active agriculture soil but 16S rRNA sequencing results were not as expected instead of *Nitrosomonas europaea*, the isolated bacteria was more likely to be related to strains of *Pseudomonas*. This could be due to contamination during the isolation process, or a misidentification of the colonies picked for sequencing. Gram staining showed that the isolated bacteria was gram-negative, this could also be the

reason for misidentification because both Pseudomonas and Nitrosomonas are gram-negative.

A bacteriophage infecting *Pseudomonas sp*. was isolated from sewage samples. Phages against Pseudomonas sp. were also isolated by Akremi *et al*. (2022), from sewage samples in Tunisia. These results indicate that Pseudomonas sp. as well as their bacteriophages are abundantly present in wastewater.

The shoot fresh weight was significantly increased compared to the control by the application of nitrogen-loaded biochar. Similar results were observed in research conducted by Banik et al. (2023), where they used biochar-based fertilizer in a glasshouse experiment on maize. Additionally, doping biochar with sorghum extract led to an increase in shoot fresh weight, similar to findings by Iqbal et al. (2022). The shoot-dry biomass of maize was also significantly increased by the application of nitrogen-loaded biochar. This result is consistent with the research conducted by Zheng et al. (2013).

The root fresh weight was significantly increased by the application of sorghum-doped nitrogen-loaded biochar, this could be attributed to improved uptake of Nitrogen (Kuppe & Postma, 2024). Sorghum doping on nitrogen-loaded biochar has a significant effect on root dry weight. Similar findings were documented by Iqbal et al. (2022), where they applied sorghum in combination with nitrogen and improved the seedling dry weight of mashbeans.

Nitrogen loading on biochar has significantly improved the shoot length. Similar findings were documented by Cong et al. (2023), where biochar amendment has increased the height of maize plants. The reason could be the improved uptake of N. The root length is

significantly increased by the application of sorghum-doped nitrogen-loaded biochar similar to results reported by (Iqbal et al., 2022), where the application of sorghum mulch improved the root length of the mashbean.

The application of nitrogen-loaded biochar has no significant effect on the photosynthetic activity of maize. Similar results were reported by Hou et al. (2020) in their study on the combined effect of biochar and nitrogen fertilizers on *Carya illinoinensis*. In their study, biochar amendment showed a significant effect only after four months of treatment, but not before. Since our experimental duration was only 50 days, this explains why no significant effects of biochar were observed. The interaction of sorghum extract with nitrogen-loaded biochar has significantly improved the photosynthetic activity in maize. Similar results were reported by Liu et al. (2022), where the co-application of biochar and organic fertilizer improved the chlorophyll content, and photosynthetic activities were increased.

In our experiment, Nitrogen-loaded biochar (N-50, N-100, and N-150) application has significantly increased the soil extractable potassium and uptake of potassium by plants. This can be attributed to biochar's ability to increase soil CEC, enhancing the soil's capacity to hold and store K for plant uptake, and its inherent content of exchangeable K for plant use (Lentz & Ippolito, 2012).

Nitrogen-loaded biochar (N-50, N-100, and N-150) as well as organic solution (Sorghum extract) doped N-loaded biochar have significantly improved the Soil NO_3 and ammonical nitrogen. This may be due to the adsorption of NO_3 to biochar which retains nitrogen in soil and its slow release occurs according to plants' requirements (Abujabhah et al., 2018).

The increased uptake of N by plants may also be attributed to the reason that Sorghum extract acts as a Biological Nitrification Inhibitor so N is retained in soil (G. Subbarao et al., 2013).

Our findings indicate that the application of nitrogen-loaded biochar does not significantly impact soil-extractable phosphorus or plant uptake of phosphorus. This phenomenon can be attributed to the solubility dynamics of phosphorus in alkaline soils, which are primarily governed by its interactions with calcium ions (Ca^{2+}) (Vandecasteele et al., 2016). As biochar is rich in calcium and magnesium ions which interact with P resulting in calcium and magnesium phosphates (Vandecasteele et al., 2016). These compounds are less soluble, thereby reducing the availability of phosphorus for plant uptake (Chintala et al., 2014).

CHAPTER 6: CONCLUSION

We isolated bacteria from active agricultural soil but sequencing revealed that it was not Nitrosomonas instead the isolated bacteria was more related to Pseudomonas sp.. Bacteriophage was isolated from a sewage sample, and it produced lytic spots showing that it can infect bacteria but as the bacteria was Pseudomonas so isolated bacteriophage was also against Pseudomonas. Nitrogen loading on biochar at $50kgha^{-1}$, $100kgha^{-1}$, and $150kgha^{-1}$ has significantly improved the morphological parameters of maize. Similarly, the application of sorghum extracts doped nitrogen-loaded biochar ($50kgha^{-1}$, $100kgha^{-1}$, and $150kgha^{-1}$) has also enhanced morphological parameters like root biomass. The physiological parameters like chlorophyll content and photosynthetic activity were improved by the application of sorghum extract-doped nitrogen-treated biochar. Retention of nutrients by biochar was also improved by both nitrogen loading as well as by sorghum doping along with nitrogen loading on biochar. For instance, soil extractable potassium was significantly increased by nitrogen-treated biochar. Nitrogen-loaded biochar as well as organic solution (Sorghum extract) doped N-loaded biochar have significantly improved the NO3 and ammonical Nitrogen. Also, Soil Extractable Phosphorus was not affected by nitrogen loading on biochar but sorghum extract doping along with nitrogen loading on biochar has improved both the soil extractable Phosphorus and Total Phosphorus.

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by

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6.9.2024

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