EVALUATING THE IMPACT OF BIOFERTILIZER ON MAIZE (ZEA MAYS L) YIELD AND NITROGEN UPTAKE

By

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ABSTRACT

Synthetic fertilizers have played a significant role in sustaining the rapidly growing population; however, they have also led to significant environmental pollution. Nitrogen (N)-fixing biofertilizers have emerged as an effective alternative or partial substitute for synthetic N fertilizers without compromising crop productivity. A two-year field experiment was conducted to evaluate the effect of biofertilizer on maize yield, N uptake, and N use efficiency (NUE). We compared maize crop yield, N uptake, and NUE after the application of synthetic fertilizer (SF), a liquid blend of 28% N with a sulfur additive (26-0-0-2: N-P-K-S), and the co-application of Pivot PROVEN® 40 as a biofertilizer and SF (SF+Bio). In 2022, fields 1 and 2 for each treatment received the same amount of total N, 205 kg ha^{-1}. In 2023, fields 3 and 4 had total N rates of 229 kg ha^{-1} for SF and 268 kg ha^{-1} for SF+Bio. No significant differences were observed ($p > 0.05$) in maize yield and N uptake across all fields, with NUE being significant only in field 4. Maximum maize yields were 14.0, 13.8, 12.4, and 13.0 Mg ha^{-1} for fields 1, 2, 3, and 4, respectively. N uptake at R6 was 200, 195, 339, and 360 kg ha^{-1} for fields 1, 2, 3, and 4, respectively. The NUE values for fields 1, 2, and 3 were 0.99, 0.97, and 1.3, respectively. In field 4, the NUE for SF was 1.61, showing a 24% increase, while SF+Bio had an NUE of 1.3. Overall, substituting a portion of the SF with biofertilizer has positive implications. In the first year, it maintained yield, nitrogen uptake, and NUE, effectively promoting plant growth and development comparable to SF. In the second year, there was no benefit to increasing the N rate, suggesting that the substitution can consistently maintain yield and N uptake while maintaining NUE without the need for higher N inputs.

This thesis is dedicated to the Lord the most high, Ebenezer

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LIST OF ABBREVIATIONS

AOB	Ammonia-oxidizing bacteria
BNF	Biological nitrogen fixation
EFFs	Enhanced Fertilizer Efficiency
K	Potassium
Min	Mineral fertilizer
N	Nitrogen
N_2	Dinitrogen
N_2O	Nitrous oxide
NH_3	Ammonia
NH_4	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate
NOB	Nitrite-oxidizing bacteria
Nr	Reactive nitrogen
Nt	Total inorganic nitrogen
NUE	Nitrogen Use Efficiency
P	Phosphorus
SF	Synthetic fertilizer

1. INTRODUCTION

Since the 1950s, the global human population has increased and is expected to reach 9.8 billion by 2050 (United Nations, 2017). Meeting the food demands of this growing population will require a significant increase in global crop production, estimated to be between 60-110% from 2005 to 2050 (Pradhan et al., 2015).

Crops rely on essential nutrients such as nitrogen (N), phosphorus (P), potassium (K), and other minerals for their growth (Rai & Shukla, 2020). Nitrogen is fundamental to plant metabolism, serving as a crucial component of amino acids, which are the building blocks of proteins. Nitrogen is also crucial for nucleic acids, including DNA, and is necessary for the synthesis of chlorophyll, the molecule essential for photosynthesis (Leghari et al., 2016). Nitrogen plays a critical role in optimizing crop growth and development, which in turn enhances crop yield. It can be a limiting nutrient 2019), and nitrogen deficiency impacts yield more than any other nutrient (Ali et al., 2022; Lemaire and Gastal, 2019).

1.1. Nitrogen cycle in agriculture

Approximately 78% of the Earth's atmosphere is composed of N in the form of dinitrogen (N_2). However, this does not benefit plant productivity, as plants cannot directly use this N. Atmospheric N_2 has strong triple bonds that make it unavailable to plants. To be used by plants, N_2 must undergo a transformation process to convert it into a soluble, non-toxic chemical form that can be readily absorbed and assimilated by the plant (Vassilev et al., 2022).

Understanding the integral role of N in crop productivity with a particular focus on the interactions between soil and plants necessitates an understanding of the N cycle (Zhang et al., 2021). Key processes within this cycle include N fixation, mineralization, nitrification, immobilization, volatilization, and denitrification (Aryal et al., 2022). Each process can influence N levels in the soil and availability, impacting soil health and plant growth within agricultural ecosystems.

Dinitrogen is converted into NH_3 in the process of N fixation. There are three main ways to fix nitrogen: biologically, industrially, and through natural processes. Biological N fixation (BNF) occurs using the enzyme nitrogenase, and it involves either free-living or symbiotic microorganisms. Free-living microorganisms, such as *Azotobacte*r, actinomycetes, and cyanobacteria., live independently in the soil, obtain energy from organic compounds, and do not form associations with plant hosts. In contrast, symbiotic bacteria like *Rhizobium* form mutually beneficial relationships with host plants, such as legumes. These bacteria colonize the roots of the host plant and form nodules where the conversion takes place (Santillano-Cázares et al., 2022). The Haber–Bosch process, is the main industrial fixation converting N_2 to NH_3 and then N fertilizer, by a reaction with hydrogen (H_2) using an iron metal catalyst (Cocking, 2000). Additionally, N can be fixed through natural processes such as lightning, which provides the energy to break the bonds of N_2, leading to the formation of N oxides (NO and NO_2). These oxides are then deposited into the soil through precipitation, ultimately forming nitrates (NO_3-) (Barth et al., 2023).

Mineralization, where organic N compounds from decomposing plant and animal residues are transformed into plant -available form is a process mediated by soil microorganisms. Soil bacteria or fungi decompose nitrogen -rich organic compounds such as proteins to amino acid compounds which are further broken down to NH_4^+ (Miransari, 2012). Nitrification is a two-step aerobic process in which ammonia-oxidizing bacteria (AOB) first converts NH_4^+ to nitrite (NO_2^-), and then nitrite-oxidizing bacteria (NOB) further converts NO_2^- to NO_3^- (Miransari, 2012). These processes are essential for converting organic N compounds into inorganic forms readily assimilated by plants (Pate, 1973). Immobilization converts inorganic N compound back into organic forms within microbial biomass; it is carried out by soil microorganisms that absorb the inorganic N to synthesize essential compounds for their growth and reproduction (Paul & Juma, 1981), affecting the availability of N for plant uptake (Cocking, 2000). Denitrification 'completes' the cycle by reducing NO_3^- to gaseous N forms (N_2 and N_2O) by denitrifying bacteria, thereby returning N_2 to the atmosphere. Nitrate is reduced to NO_2^- by nitrate reductase, NO_2^- is then converted to nitric oxide (NO) by nitrite reductase. This is followed by the conversion of NO into nitrous oxide (N_2O) by nitric oxide reductase, and finally, nitrous oxide reductase reduces N_2O to N_2.

1.2. Nitrogen in crops

There are four primary sources of N inputs for crops: synthetic fertilizers, manure, BNF, and natural atmospheric deposition (Zhang et al., 2015). In the early 20th century , agriculture was revolutionized by he development of the Haber-Bosch process by enabling the large-scale production of synthetic fertilizers, providing a readily available source of N for plants that has enabled crop yields to increase (Prasad, 2013). Globally, synthetic fertilizers are now the predominant source of N applied to croplands (Hirel et al., 2011). However, this advancement has led to adverse impacts from N on the environment through nitrogen leaching, runoff, volatilization, and denitrification (Cameron et al., 2013).

Maize relies on N at every stage of its development throughout the growing season (Aziiba et al., 2019). The steady global increase in maize yield has been strongly associated with the widespread application of N supplied from synthetic fertilizers (Robertson & Vitousek, 2009). Stewart et al. (2005) conducted a review and analyzed data from 362 seasons of crop production and reported that the application of N fertilizer results in a 41% increase in maize yield.

A reduction in grain yield occurs if maize is deficient in N, especially during the rapid vegetative growth stages, while the accumulation of biomass during reproductive stages is closely linked to the N content in above-ground plant tissue (Ciampitti & Vyn, 2013). By the time maize reaches the flowering stage (R1), it has accumulated about 65% of its total N requirement for the season (Burns et al., 2022) with the remaining amount being taken up during the grain-filling period (R1 to R6) (Ciampitti et al., 2013).

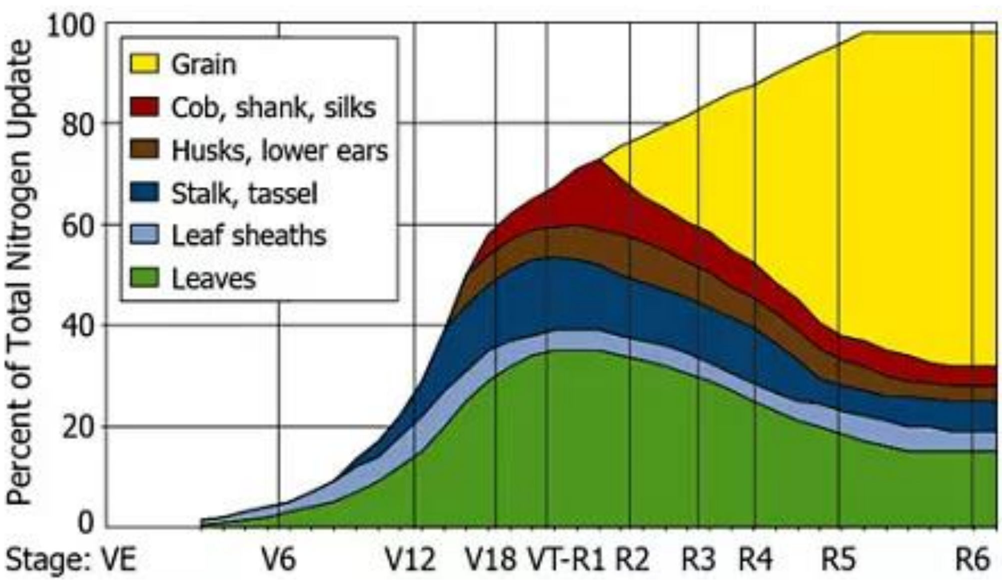

Figure 1. Total maize nitrogen uptake and growth stages (Butzen, 2019).

1.3. Nitrogen-use-efficiency

Nitrogen-use-efficiency (NUE) is an effective metric to gauge N management (Congreves et al., 2021). Nitrogen losses from applied N fertilizer are associated with volatilization, denitrification as well as surface runoff, erosion, and leaching. Nitrogen-use-efficiency is defined as the ratio of the total amount of N in a harvested plant to the total amount of N applied to the soil (Congreves et al., 2021). The overall NUE of a cropping system can be enhanced by improving the uptake efficiency of N from applied inputs and minimizing N losses (Cassman et al., 2002).

More than 60% of synthetic fertilizer N applied for crop production is lost to the environment (Kant et al., 2011). These losses are influenced by various factors such as soil type, climate, and agricultural practices (Byrnes, 1990). Of the options to reduce these losses, management of N is readily accessible and under direct farmer control. More precise management of the rate, timing, source, and placement of the N fertilizer can help improve nitrogen-use-efficiency (NUE), reducing N losses and ensuring a greater fraction of the N applied is delivered to the crop. The correct response to each of these N management strategies is still challenging and differs geographically between and within fields (van Es et al., 2020). With regards to source, enhanced-efficiency fertilizers (EEFs) can help synchronize the supply of N fertilizer with crop needs to increase NUE (Sela & Van Es, 2018). These include nitrification inhibitors, urease inhibitors and controlled-release coatings. More recently there has been great interest and commercial activity surrounding biofertilizers to reduce inputs of synthetic N fertilizer.

1.4. Biofertilizers

Biofertilizers are comprised of living microorganisms (bacteria, fungi, or algae) that are applied to seeds, plant surfaces, or soil and can colonize the rhizosphere or plant interior, forming a relationship with the plant and promoting plant growth by increasing the supply or accessibility of nutrients to the plant.

Biofertilizers can be classified into different types based on their function, and include N-fixing, phosphate, potassium, zinc, and sulfur. By far the most dominant are N-fixing organisms (diazotrophs), due to the essential requirement of many crops for large amounts of N (O'Callaghan et al., 2022). N-fixing organisms fix atmospheric dinitrogen (N_2) using the enzyme nitrogenase to produce ammonia (NH_3). Microorganisms may establish symbiotic or associative relationships with plant roots or exist freely in the soil.

Symbiotic N-fixing bacteria form nodules on their plant host roots and directly supply the fixed N_2(Zhao et al., 2024). Associative N-fixing (ANF) bacteria associate with the roots but do not form nodules, instead making fixed N available in the rhizosphere (Dommelen & Vanderleyden, 2007; Franche et al., 2009). Several ANF bacteria have been found associated with cereal crops (Guo et al., 2023). ANF could theoretically provide substantial amounts of N to a cereal crop under suitable conditions; however, because to the high energy needed for this process, the high amounts of synthetic N in agricultural soil selects against and represses BNF (Weese et al., 2015)and the bacteria effectively assimilate the fixed N into their microbial biomass (Batista & Dixon, 2019). Therefore, diazotrophs that can express nitrogenase genes under N fertilized field conditions are rarely mentioned in the literature (Bloch et al., 2020). N fixed by microbes may not be readily available to crop plants, as it is primarily assimilated into microbial biomass and not easily taken up by the crop. Despite this, there are numerous commercial products currently sold that contain non-symbiotic N-fixing bacteria (Basu et al., 2021). Common products available in the U.S. Midwest include Utrisha (Corteva Agrisciences, Indianapolis, IN), MicroAZ-ST (TerraMax, Bloomington, MN), Envita (Azotic, Guelph, Ontario, Canada), and Proven®40 (Pivot Bio, Berkeley, CA). Nitrogen-fixing biofertilizer use holds promise, however inconsistent performance (Schütz et al., 2018) has hindered widespread adoption among farmers. Further research is therefore required.

In our study we used Proven®40, a bacterial N replacement inoculant (asymbiotic N-fixing product) for maize, that contains the bacteria *Kosakonia sacchari* and *Klebsiella variicola*. Modified strains of these bacteria were obtained from non-transgenic gene editing to remove their N-sensing and restrict their N retention abilities, such that the N they fix is excreted into the maize root environment (Pivot Bio, 2024; Wen et al., 2021).

Pivot Bio Proven®40 consists of nitrogen-producing microbes applied during planting. Once the seed and microbes are together in the ground, the roots immediately have access to the Pivot Bio Proven®40 microbes, and polysaccharide molecules adhere microbes to the root structure, forming a mutually beneficial relationship with the roots. Microbes capture atmospheric N_2, convert it into a form that the plant can use, and feed it directly to maize roots daily throughout the growing season.

1.5. Hypotheses and Objectives

The study evaluates the impact of biofertilizer (Bio) when co-applied with synthetic fertilizer (SF) and synthetic fertilizer alone on maize crop yield, maize N uptake, and agronomic NUE over two consecutive years, 2022 and 2023. In 2022, the SF+Bio treatment received a reduced amount of SF, with the assumption that the biofertilizer would fix the additional N (i.e., the amount SF was reduced by) needed to match the 'total N' applied in the SF treatment. Conversely, in 2023, both SF and SF+Bio received the same amount of SF; with the SF+Bio treatment potentially having more N available due to the biofertilizer contributing additional fixed N. The hypothesis is that in 2022 the SF+Bio treatment will result in similar maize N uptake and crop yield to SF, and that the NUE will increase in the SF+Bio treatment due to the lower SF rate. In 2023, it is expected that maize yield and N uptake will increase in the SF+Bio treatment compared to SF, with a similar NUE. Therefore, the objectives of this study were to compare the co-application of synthetic fertilizer and biofertilizer (SF+Bio) with synthetic N fertilizer (SF) to evaluate i) N availability in the soil, ii) maize yield and N uptake, and iii) agronomic NUE.

2. METHODS

2.1. Study Sites and Management

Experiments were conducted in four commercial maize fields in Portland, MI during the 2022 and 2023 crop growing seasons. In 2022, experiments were conducted in Field 1 (42°53’24.2” N 85°01’03.1” W) and Field 2 (42°52’57.2” N 85°01’06.7” W). In 2023, they were conducted in Field 3 (42°49’38.3” N 84°56’46.1” W) and Field 4 (42°52’07.7” N 85°03’39.3” W). The soil in all fields is classified as clay loam. The climate in the study area is continental, with an average daily temperature of 7.8°C and an annual average precipitation of 895 mm. All fields are rainfed, with conventional tillage 10 cm deep.

2.2. *Experimental Design*

Two treatments were evaluated in each field. Treatment 1: synthetic N fertilizer (SF), a liquid blend of 28 %, N with a sulfur additive (26-0-0-2: N-P-K-S) was applied, and treatment 2: the same SF applied along with the Proven®40 biofertilizer (SF+Bio). For SF+Bio, in all fields, the biofertilizer was co-applied in its entirety at planting along with a portion of the SF at crop growth stages V6 and V8, SF was applied as a side-dressing (Table 1). In 2022, the SF+Bio treatment received a reduced amount of SF compared to the SF only treatment (166 kg N ha^{-1} vs 205 kg N ha^{-1}) based on the assumption that the inoculant would fix the additional 39 kg ha^{-1} of N. In 2023, the SF+Bio treatment received the same amount of fertilizer as the SF treatment (229 kg N ha^{-1}), with the assumed contribution of an extra 39 kg ha^{-1} of fixed N from the Bio. The SF was applied in a strip area, while the remaining field area received the co-application of SF and biofertilizer (Fig. 3). Points were randomly selected using ArcGIS after the fertilizer was applied in each of the two treatment areas and were sampled for soil and maize plant biomass at the same location throughout the growing seasons.

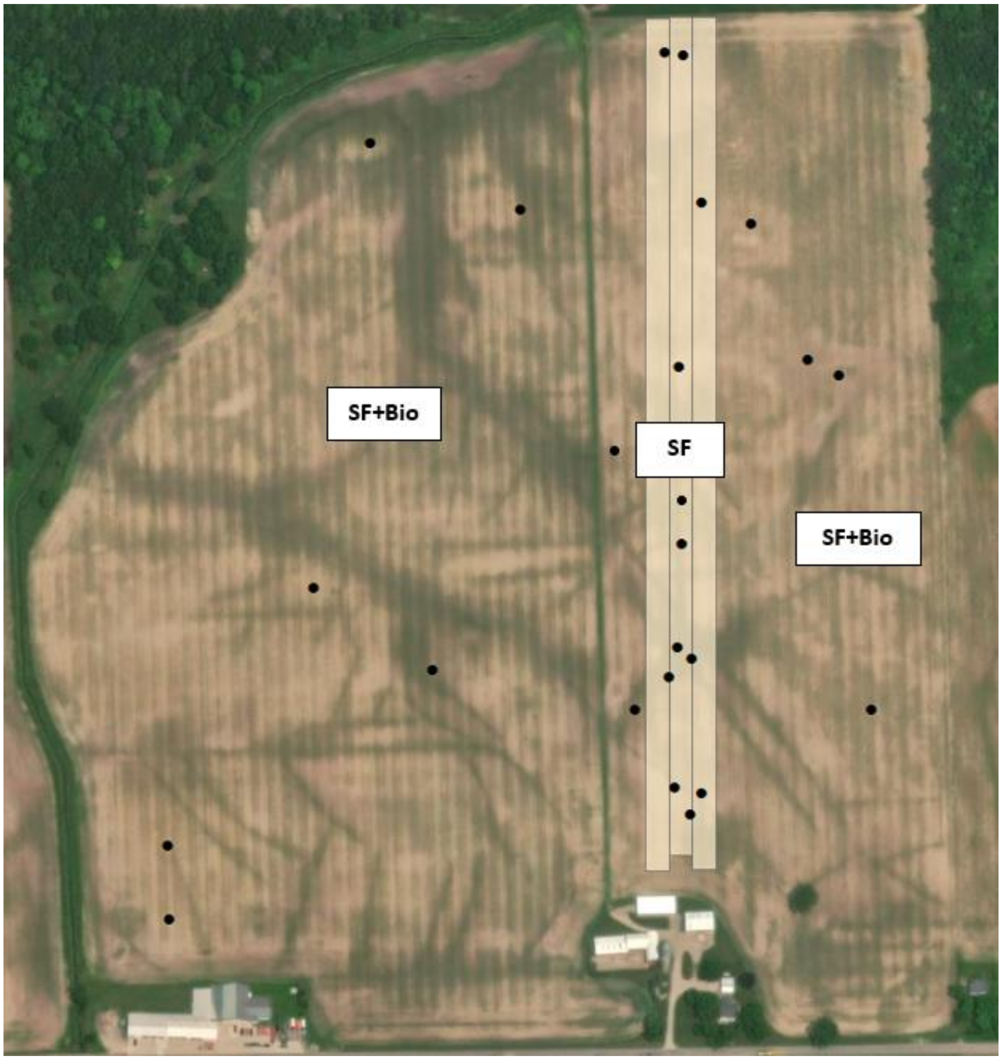

Figure 2. Experiment conducted in Field 1 in 2022. The synthetic N fertilizer (SF) was applied in the highlighted strips, while the remaining field area received the co-application of SF and PROVEN® 40 biofertilizer (SF+Bio). The black dots depict randomly selected sites that were sampled consistently for soil and biomass throughout the season.

Table 1. Nitrogen rates (kg ha^{-1}), sources, and time applied in Fields 1, 2, 3 and 4 in 2022 and 2023.

Year	Field	Treatment	Biofertilizer	Synthetic N applied at:			Total
			planting	Planting	V6	V8	N*
2022	1 and 2	SF	0	34	123	48	206
		SF+Bio	40	34	123	9	206
2023	3 and 4	SF	0	78	93	58	229
		SF+Bio	40	78	93	58	269

* Includes contribution from synthetic N fertilizer (SF) and biofertilizer (Bio; assumed to be 40 kg N ha^{-1}).

2.3. Soil sampling

Soil samples were collected from each points in each treatment in each field and composited at each of four depths (0-7 cm, 7-15 cm, 15-26 cm, and 26-40 cm) on four occasions; at crop growth stages V5, V14, R3, and R6 for Fields 1 and 2, and at stages V5, R1, R3, and R6 for Field 3 and 4. The composite soil samples were sieved (5 mm) and extracted with 100 ml of 2M KCL that was added to 10 g of the soil in a graduated cylinder. The extract was shaken for 60 minutes and filtered through a Whatman No 42filter paper, then were sent to a commercial analytical laboratory (Woods End Laboratories, Mount Vernon, ME) to determine inorganic N (N-NO_3 and N-NH_4).

2.4. Plant sampling and Satellite Imagery

Maize biomass samples were collected at growth stages V2, V3, V5, V9, V14, R1, R3, and R6. Maize (6-10 plants) was destructively sampled for above ground biomass over a 1 m length of row closest to each of the pre-selected points in each treatment within each field to determine cumulative plant biomass. A subsample was collected from the collective sample at each point, and oven dried (65°C) and weighed. Dried samples were ground using a Wiley Mill grinder (Arthur H. Thomas Co), and a subsample of 100 g was sent to Woods End Laboratories to determine total N content in the grain (%Ng) and crop biomass (stem, leaves, cob, and husk) (%Nb).

Satellite images were downloaded from Planet in red edge, NIR, and R, G, and B bands for each field and date when biomass samples were collected. Images were imported in ArcGIS to extract the normalized difference vegetation index (NDVI, Eq. 1) of each point in each field.

$$NDVI = \frac{(IR-R)}{(IR+R)} \text{ .... Eq. 1}$$

2.5. Nutrient use-efficiency

Our study used the partial N Balance (PNB), a fertilizer-based index, to calculate NUE (Congreves et al., 2021). PNB is defined as the ratio of N in the harvested crop to the N applied as fertilizer (Eq. 2). One limitation of this index is that it does not account for background soil nitrogen. This approach is suitable for our study as it provides insights into the efficiency of N utilization by focusing solely on N inputs and outputs since we were assessing the NUE for a single growing season. It will help us to understand the efficiency of fertilization treatment.

$$\text{NUE} = \frac{Total\ N\ uptake}{Fertilizer\ N} \text{ .... Eq. 2}$$

2.6. Statistical analysis

All data was evaluated with a mixed effect model approach in PROC GLIMMIX procedures of SAS 9.4 (SAS Institute, Cary, NC, USA). The analysis was performed with analysis of variance and Tukey's honest significant difference to directly compare fertilization treatments. Field number, crop growth stage, treatment, and all interactions were used as fixed effects. The sample locations were nested within each treatment and field and included as a random effect in the model. A spatial autocorrelation (sp (exp)) test was carried out using the sample location coordinates to account for spatial dependency in the data and the spatial autocorrelation was used to have a spatial covariance structure over time. To examine the interaction between field and treatment, the effect of the treatment was evaluated separately for each field. Mean separation between groups was analyzed using Tukey's method.

3. RESULTS

3.1. Soil Nitrogen dynamics

Total inorganic nitrogen (Nt) was significantly affected by the fertilization treatment in Field 1 and Field 2. In Field 1, the SF +Bio treatment showed higher Nt compared to SF at V5 and the SF treatment showed higher Nt compared to SF+Bio at R3. (Figure 3a, Appendix Table 6). In Field 2, the SF+Bio treatment showed higher Nt compared to SF at R3 (Figure 3b). Soil Nt ranged from 25.8 to 51.4 kg ha^{-1}, from 26.7 to 62. kg ha^{-1}, from 1.78 to 36.5 kg ha^{-1}, and from 2.2 to 26.1 kg ha^{-1} in Field 1, 2, 3, and 4, respectively.

Fertilization treatments did not significantly affect Nt across most growing stages, except for Field 1 and Field 2 (Figure 3a and b). In Field 1, SF+Bio showed 57% more Nt at V5 compared to SF, but it had 20% lower levels of Nt at R3 (Figure 3a). In Field 2, SF+Bio had 38% more Nt than SF (Figure 3b).

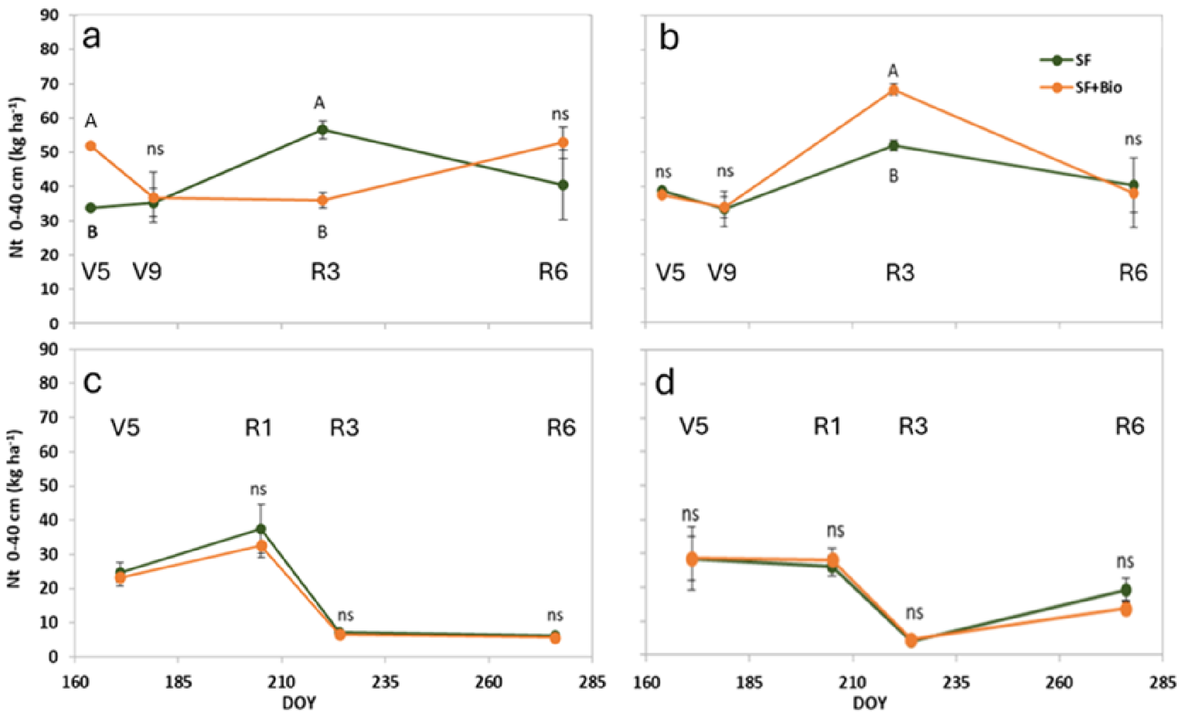

Figure 3. Total inorganic N (Nt, kg ha^{-1}) by fertilization treatment for a) Field 1, b) Field 2, c) Field 3, and d) Field 4. Fertilization treatments were the co-application of synthetic N fertilizer and Biofertilizer (SF+Bio) and synthetic fertilizer only (SF). Soil Nt was determined from samples collected at growth stages V5, V9, R3 and R6 for Field 1 and 2 and at V5, R1, R3, and R6 for Field 3 and 4. Soil samples were collected at four depths: 0-7, 7-15, 15-26, and 26-40 cm. ns denote not significant differences (p>0.05) and capital letters indicate significant differences (p<0.05) between treatments. Errors bars indicate +/- 1 standard error.

Soil N from NH_4 was not significantly affected by fertilization treatment across all fields within each year. Overall, there was a varying pattern of N-NH_4 over the growing season, and most stages showed a similar trend between the two treatments for all fields (Figure 4). In 2022, the amount of N-NH_4 ranged from 5.1 to 8.8 kg ha^{-1}, whereas in 2023 it ranged from 0.9 to 13.5 kg ha^{-1}. The amount of N-NH_4 ranged from 5.1 to 8.4 kg ha^{-1}, from 5.6 to 8.8 kg ha^{-1}, from 0.9 to 5.1 kg ha^{-1}, and from 1.3 to 13.5 kg ha^{-1}, in Field 1, 2, 3, and 4, respectively. No significant differences were observed between the fertilization treatments at each stage (Figure 4), except for Field 4 at R6 (Figure 4d). In Field 1 and 2, N-NH_4 levels were similar across maize growing stages, in contrast, Fields 3 and 4 showed more variability across different stages.

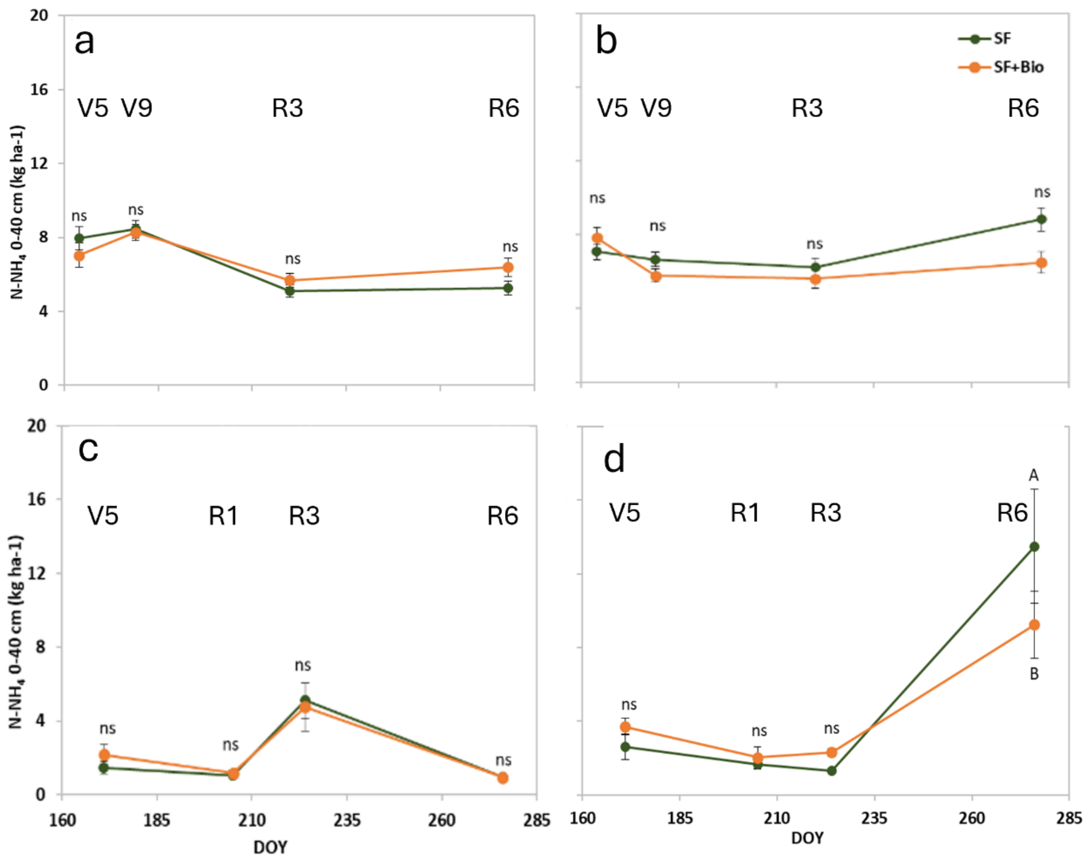

Figure 4. Soil nitrogen from ammonium (N-NH4) (kg ha^{-1}) by fertilization treatment for a) Field 1, b) Field 2, c) Field 3, and d) Field 4. Fertilization treatments were the co-application of synthetic N fertilizer and Biofertilizer (SF+Bio) and synthetic fertilizer only (SF). N-NH4 was determined from soil samples collected at growth stages V5, V9, R3 and R6 for Fields 1 and 2 and at V5, R1, R3 and R6 for Fields 3 and 4. Soil samples were collected at four depths: 0-7, 7-15, 15-26, and 26-40 cm. ns denote not significant differences (p>0.05), and capital letters indicate significant differences between treatments (p<0.05). Errors bars indicate +/- 1 standard error.

Further analysis examined the effects treatment by depth and growing stage. Soil N-NH_4 showed significant effect of treatment in Field 1 at R3 in the 15-26 cm depth (Appendix Figure 17c), Field 2 at R6 in the 26-40 cm depth (APPENDIX Figure 18d), Field 3 at V5 in the 26-40 cm depth (Appendix Figure 19a), and in Field 4 at R3 (APPENDIX Figure 20c).

Across all fields, the fertilization treatment did not significantly affect N-NO3 levels, except in Field 1 at V5 and R3 and in Field 2 at R3. In Field 1, at V5 N-NO_3 was higher in SF+Bio treatment compared to SF whereas at R3 SF treatment N-NO_3 was higher compared to SF+Bio. In Field 2 SF+Bio showed higher N-NO_3 levels compared with SF. Soil N-NO_3 levels varied across Fields 1, 2, 3, and 4 from 25.8 to 51.4 kg ha^{-1}, 26.7 to 62.6 kg ha^{-1}, 1.78 to 36.5 kg ha^{-1}, and 2.2 to 26.1 kg ha^{-1}, respectively (Figure 6).

Analyzing N-NO3 levels stage, there were no significant differences between the fertilization treatments, except for Field 1 at V5 and R3 (Figure 6a), and Field 2 at R3 (Figure 6b). In Field 1, SF+Bio had 19.2 kg ha^{-1} more N-NO_3 than SF at V5, while at R3, SF had 21.0 kg ha^{-1} more N-NO_3 than SF+Bio (Figure 6a). In field 2, N-NO_3 levels of the SF+Bio treatment was 16.9 kg ha^{-1} higher than SF. In Field 1 and 2, N-NO_3 levels peaked at R3 stage and decreased by R6. In Field 3, N-NO_3 levels were highest at R1, with lower levels observed at R3 and R6. In Field 4, N-NO_3 levels were similar and higher at V5 and R1 and decreased in the subsequent stages.

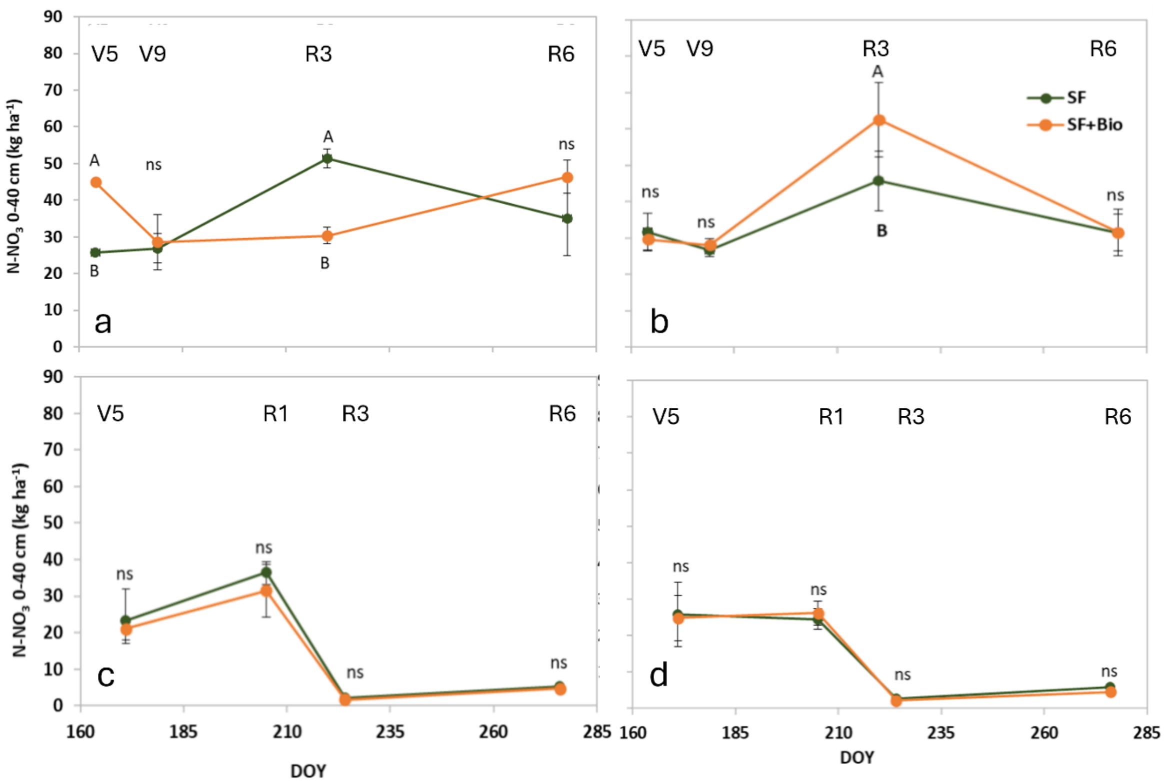

Figure 5. Soil Nitrogen from Nitrate (N-NO_3) (kg ha^{-1}) by fertilization treatment for a) Field 1, b) Field 2, c) Field 3, and d) Field 4. Fertilization treatments were the co-application of synthetic N fertilizer and Biofertilizer (SF+Bio) and synthetic fertilizer only (SF). N-NO_3 was determined from soil samples collected at growth stages V5, V9, R3 and R6 for Field 1 and 2 and at V5, R1, R3, and R6 for Field 3 and 4. Soil samples were collected at four depths: 0-7 cm, 7-15 cm, 15-26 cm and 26-40 cm. ns denote not significant differences (p>0.05) and capital letters indicate significant differences between treatments (p<0.05). Errors bars indicate +/- 1 standard error.

An analysis to assess the effect treatment on N-NO3 was performed by depth and growing stage (Figure 20-23). Fertilization treatment had a significant effect only in Field 1 at and in Field 4 (APPENDIX Figure 20 and 23). In Field 1, N-NO3 levels were 2.5 and 2 times higher in SF+Bio at V5 and R6 growing stages in the 26-40 cm depth (APPENDIX Figure 20a and d). In Field 4, N-NO3 levels were 20% lower in SF+Bio at R3 in the 15-26 and 26-40 cm depths (APPENDIX Figure 23c), and 22% lower at R6 in the 15-26 and 26-40 cm depths (APPENDIX Figure 23d).

3.2. *Biomass accumulation, nitrogen uptake, and yield*

Across all fields, throughout the growing season, there were similar increases in biomass accumulation that were not significantly influenced by fertilization treatment (Figure 6), except in Field 1 (2022) and Field 4 (2023) at R6 (Figures 6a and d), where SF had more (Field 4) and less (Field 1) biomass (2.1 Mg ha^{-1}) in both cases.

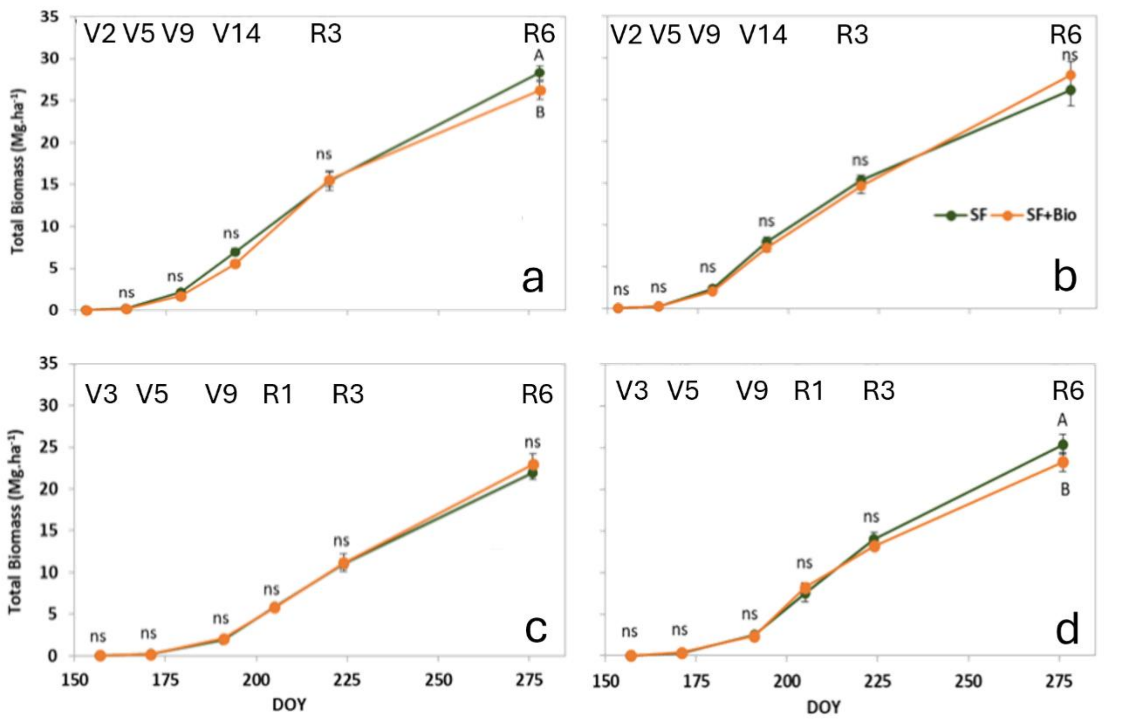

Figure 6. Maize plant biomass (Mg ha^{-1}) accumulation by fertilization treatment for a) Field 1, b) Field 2, c) Field 3, and d) Field 4. Fertilization treatments were the co-application of synthetic N fertilizer and biofertilizer (SF+Bio) and synthetic fertilizer only (SF). Maize biomass was determined from destructive samples collected at growth stages V2, V5, V9, V14, R3 and R6 in Field 1 and 2, and at V3, V5, V9, R1, R3, and R6 in Field 3 and 4. ns denote not significant differences (p>0.05), and capital letters indicate significant differences between treatments (p<0.05). Error bars indicate +/- 1 standard errors.

Across all fields and stages, maize N uptake (Nup) trends throughout the growing seasons did not significantly differ between the fertilization treatments (Figure 7). In 2022, Nup grain ranged from 118.7 to 135.3 kg ha^{-1} and from 208.9 to 244.4 kg ha^{-1} in 2023, with mean Nup grain for Fields 1, 2, 3, and 4 of 127.7 kg ha^{-1}, 127.0 kg ha^{-1}, 211.4 kg ha^{-1}, and 233.5 kg ha^{-1}, respectively. Nup biomass ranged from 71.4 to 78.9 kg ha^{-1} in 2022 and from 125.3 to 129.0 kg ha^{-1} in 2023, with mean Nup biomass for Fields 1, 2, 3, and 4 of 73.9 kg ha^{-1}, 71.8 kg ha^{-1}, 127.9 kg ha^{-1}, and 126.8 kg ha^{-1}, respectively. Total Nup (Nup grain + Nup biomass) ranged from 190.9 to 207.8 kg ha^{-1} in 2022 and from 337.8 to 369.7 kg ha^{-1} in 2023. Total Nup for Fields 1, 2, 3, and 4 were 200.3 kg ha^{-1}, 195.3 kg ha^{-1}, 339.3 kg ha^{-1}, and 360.2 kg ha^{-1}, respectively.

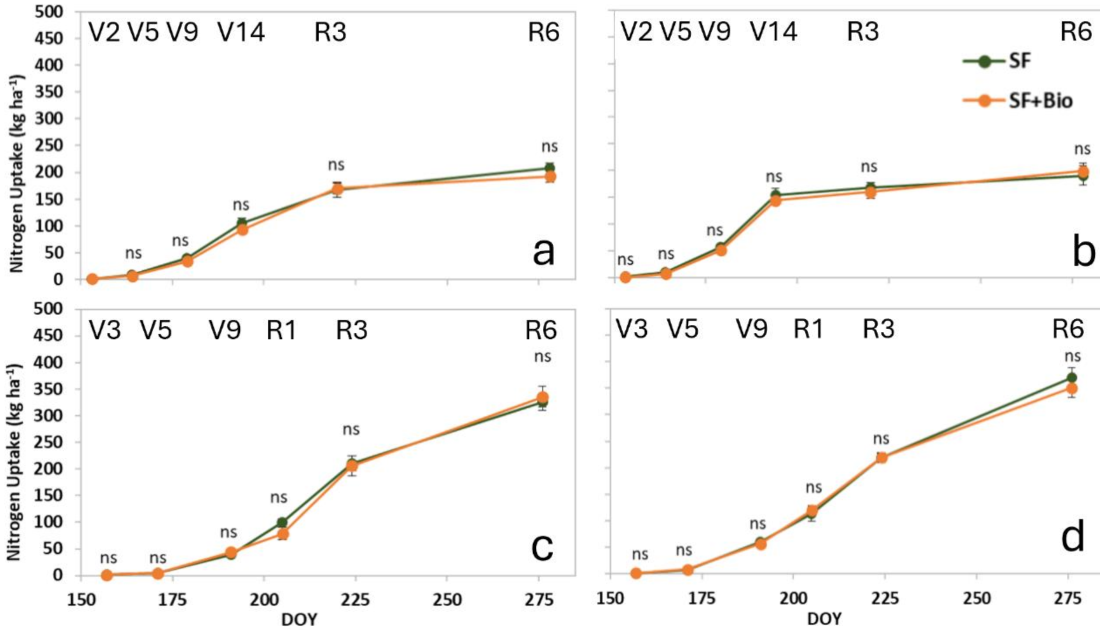

Figure 7. Maize total nitrogen uptake (kg ha^{-1}) by fertilization treatment for a) Field 1, b) Field 2, c) Field 3, and d) Field 4. Fertilization treatments were the co-application of synthetic N fertilizer and biofertilizer (SF+Bio) and synthetic fertilizer only (SF). Nitrogen uptake was determined from destructive samples collected at growth stages V2, V5, V9, V14, R3 in Field 1 and 2, and at R6, and at V3, V5, V9, R1, R3, and R6 in Field 3 and 4. ns denote not significant differences (p>0.05), and capital letters indicate significant differences between treatments (p<0.05). Error bars indicate +/- 1 standard errors.

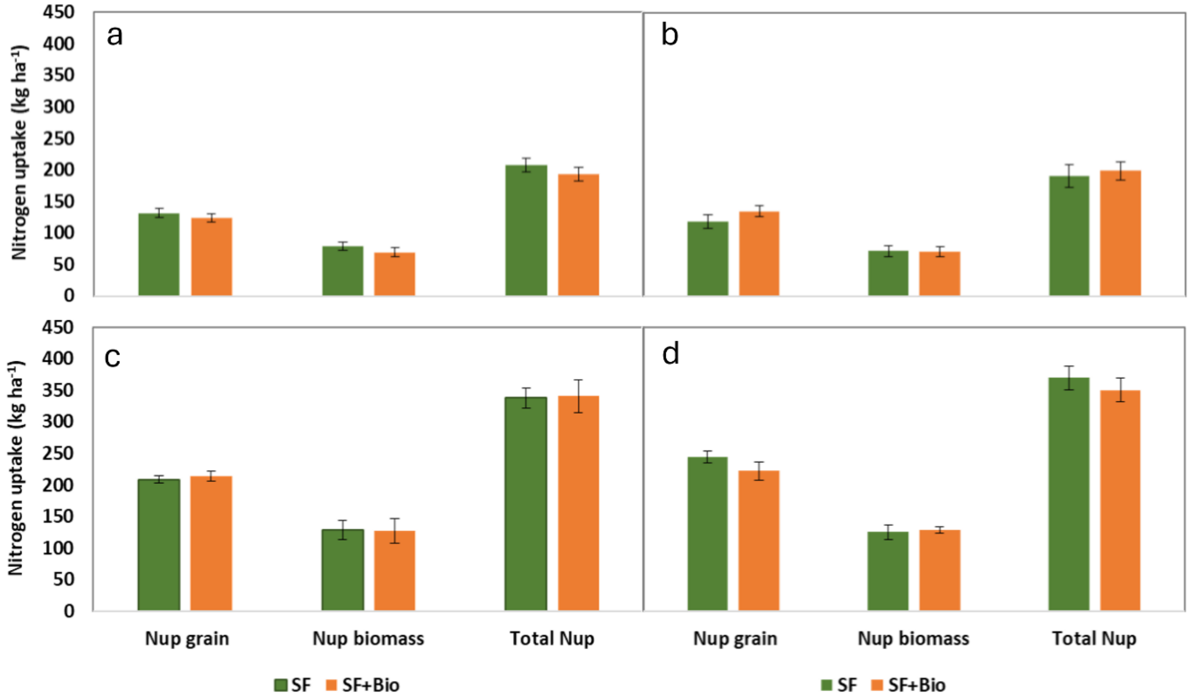

Figure 8. Maize nitrogen uptake (kg ha^{-1}) in the grains (Nup grain), biomass (Nup biomass), and total (total Nup) measured at R6 by fertilization treatment for a) Field 1, b) Field 2, c) Field 3, and d) Field 4. Fertilization treatments were the co-application of synthetic N fertilizer and biofertilizer (SF+Bio) and synthetic fertilizer only (SF). Error bars indicate +/- 1 standard errors.

Maize grain yield was not significantly affected by the fertilization treatment (Figure 9). In 2022, maize yield for both treatments ranged from 13.0 to 14.0 Mg ha^{-1}. In 2023, it ranged from 12.2 to 13.7 Mg ha^{-1}. The mean maize yield for Fields 1, 2, 3, and 4 were 14.1 Mg ha^{-1}, 14.0 Mg ha^{-1}, 12.4 Mg ha^{-1}, and 13.0 Mg ha^{-1}, respectively.

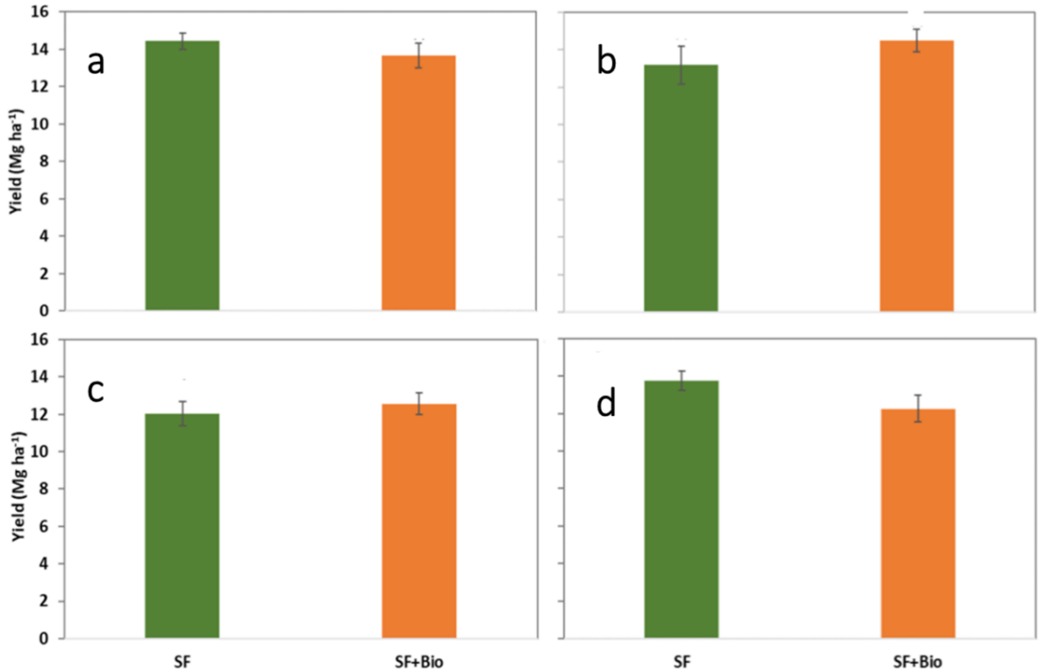

Figure 9. Maize grain yield (Mg ha^{-1}) by fertilization treatment for a) Field 1, b) Field 2, c) Field 3, and d) Field 4. Fertilization treatments were the co-application of synthetic N fertilizer and biofertilizer (SF+Bio) and synthetic fertilizer only (SF). Error bars indicate +/- 1 standard errors.

The NUE for Fields 1 and 2 in 2022 were not significantly different (1.02 and 0.95 for SF, respectively, and 0.96 and 1.00 in Fields 1 and 2 for SF+Bio) (Figure 10a and b). In 2023, NUE for Fields 3 and 4 were 1.37 and 1.61 for SF and 1.30 for SF+Bio (both fields; Figure 10c and d), with a significant difference in Field 4. In Field 4, crops with SF treatment had a high NUE than crops with SF+Bio treatment. The NUE for SF was 1.61, showing a 24% increase, while SF+Bio had an NUE of 1.3.

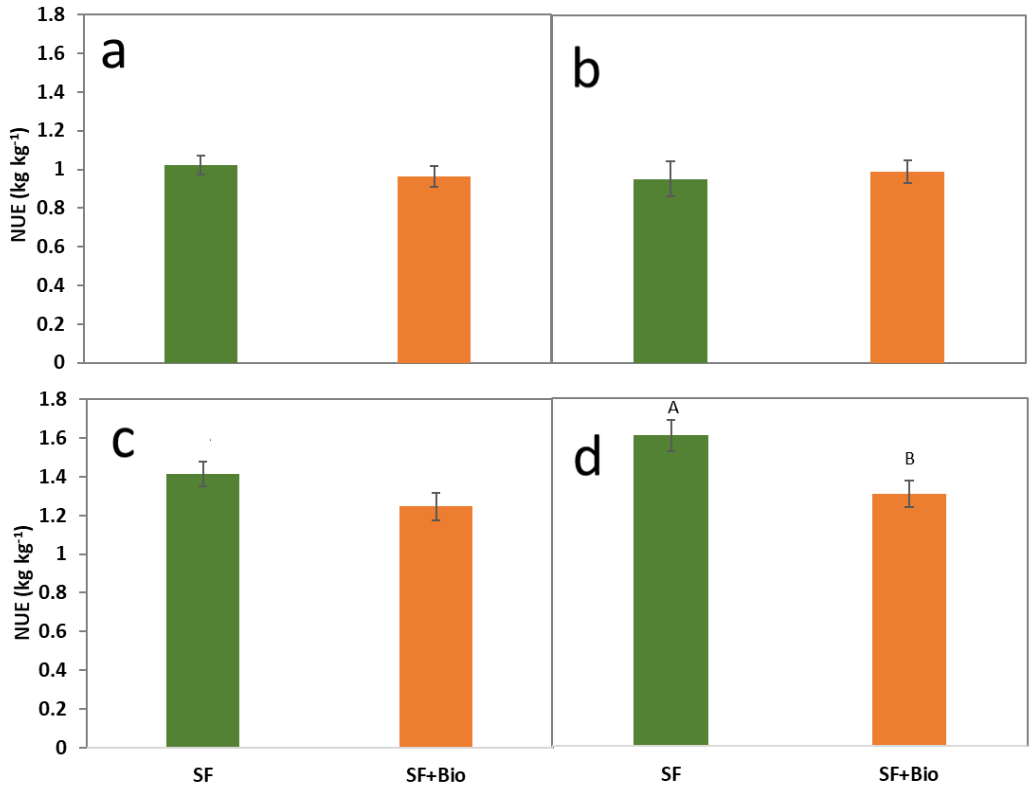

Figure 10. Nitrogen Use Efficiency (NUE) (kg kg^{-1}) by fertilization treatment a) Field 1, b) Field 2, c) Field 3, and d) Field 4. Fertilization treatments were the co-application of synthetic N fertilizer and Biofertilizer (SF+Bio) and synthetic fertilizer only (SF). Errors bars indicate +/- 1 standard errors. (NUE =Total N uptake at R6/ N applied).

3.3. Maize biomass accumulation vs NDVI

The total biomass and NDVI relationship were explained with an exponential curve and did not show significant differences between fertilization treatments (Figure 11); however, the NDVI was significant across all fields. The results show that as the NDVI increases, total biomass increases exponentially, demonstrating a positive correlation between NDVI and biomass. The mean NDVI values were 0.27, 0.49, 0.58, 0.84 and 0.86, in Field 1; 0.27, 0.53, 0.67, 0.89, and 0.85 in Field 2 for V2, V5, V9.V14 and R3, respectively. The mean NDVI values were 0.32, 0.39, 0.65, 0.77, and 0.79 in Field 3, and 0.28, 0.39, 0.79, 0.83, and 0.87 in Field 4 for V3, V5, V9, R1, R3 and R6, respectively.

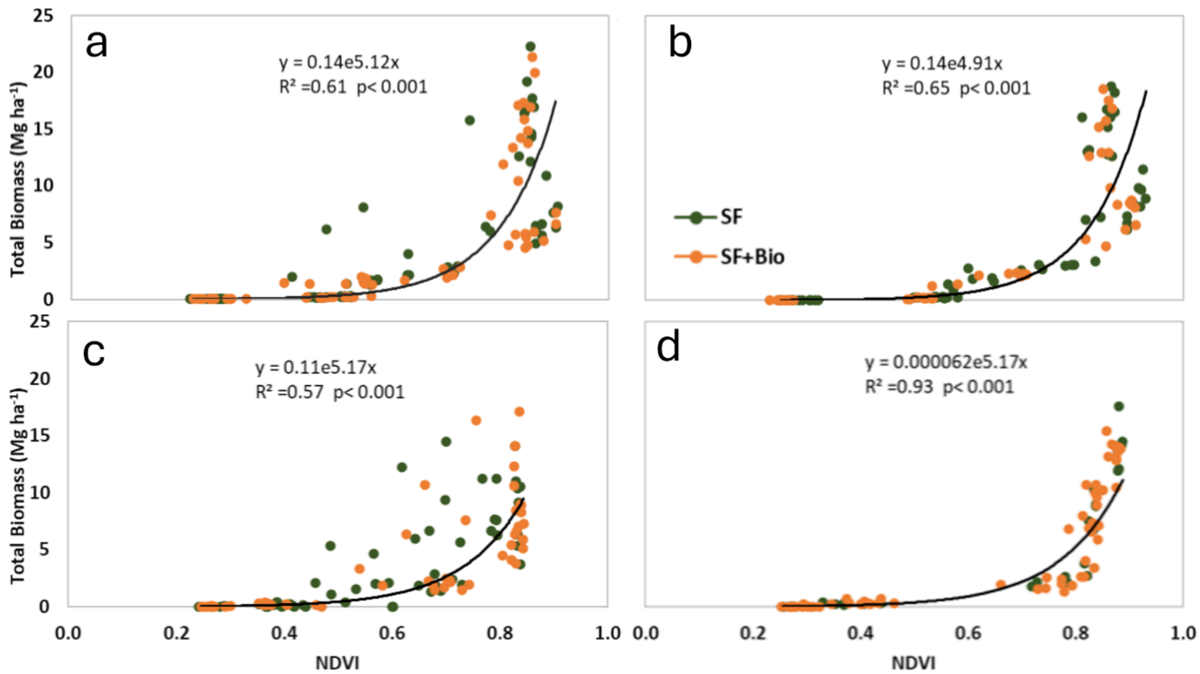

Figure 11. Relationship between NDVI determined at different maize growing stages (V2, V5, V9, V14, R1, and R3) and aboveground total biomass (Mg ha^{-1}) in a) Field 1, b) Field 2, c) Field 3, and d) Field 4 by fertilization treatment. Fertilization treatments were the co-application of synthetic N fertilizer and Biofertilizer (SF+Bio) and synthetic fertilizer only (SF). The fitted line is for the average between the two treatments.

4. DISCUSSION

Nitrogen is essential for crop growth and yield, with its deficiency impacting yields more than any other nutrient. Under current management practices, a significant portion of synthetic N fertilizer applied for crop production is not available to the plants. Nitrogen fixing biofertilizers offer a promising alternative to improve nitrogen use efficiency and reduce environmental impact, while maintaining or enhancing yield. This study evaluated the effects of co-applying synthetic fertilizer (SF) and a nitrogen fixing biofertilizer (Bio) versus using only synthetic fertilizer on soil N dynamics, crop biomass, yield, and N uptake.

The effect of the N fixing biofertilizer on total soil inorganic N (Nt) showed inconsistent results in the first year (Fields 1 and 2 in 2022) and no significant differences in the second year (Fields 3 and 4 in 2023). This inconsistency or lack of differences might be related to a slower NO_3- production rate induced by the biofertilizer, N losses not investigated (e.g., NO_3- leaching or N_2O emissions), or sampling that did not effectively capture soil N dynamics near the maize roots. Several studies have demonstrated that biofertilizers provide available N at a slower rate when compared to urea (Chu et al., 2007). Although soil samples in our study were taken near the plant's roots, this may not have effectively captured the N released by the root-associated microbes. Additionally, the experiments were conducted in commercial fields, and it was not feasible to include a control treatment with zero N application. These limitations hindered our ability to effectively and accurately differentiate between the N made available by the biofertilizer and the N from mineralization of inherent soil N or N fertilizer addition. The result observed in Field 1 (i.e. Nt in the SF+Bio at R3 was lower than SF) aligned with findings reported by (Sun et al., 2020a), who evaluated the use of biofertilizer containing *Bacillus subtilis* in a wheat-maize rotation. Their study found that the biofertilizer treatment reduced N losses (runoff and leaching) by decreasing the accumulation of N-NO_3. Similarly, Hall et al., (2023), showed that NO_3- leaching was reduced at high N rates with Proven40 (i.e., it varied little with increasing N rate) when compared to no biofertilizer addition.

The accumulation of maize biomass did not show significant treatment differences during the growing seasons, although final biomass was higher in the SF treatment in two instances. While comparative studies are limited, our results differ from Heiniger et al. (2022) who reported that the use of Proven40 increased maize biomass accumulation by 4.5% and 40.0% at two farm sites in North Carolina. In Arkansas, whole maize plant biomass increases of 20.9% and 17.5% at V4-10 and V11-VT stages, respectively, when Proven40 was used compared to untreated controls (Pivot Bio, 2023).

There was no difference in maize yield between fertilizer treatments in either year in any field. This is in partial agreement with our hypothesis that predicted no yield differences in 2022, but higher yields in the SF+Bio treatment in 2023. This agrees broadly with Franzen et al. (2023), who summarized N fixing biofertilizer product trials in maize over the US Midwest and found that 51 of the 53 paired maize N rate field experiments showed no yield change with biofertilizer addition; of the 13 experiments with Proven40 in Illinois, none showed a yield increase. It has however been noted that not all these trials were conducted with appropriate product management (Pivot Bio, 2024). A meta-analysis of 171 peer reviewed publications(Schütz et al., 2018), reported average cereal crop yield increases of 14.0 ± 1.0% across all biofertilizers, and in all crops, a 14.5% (± 1.5%) yield increase with N fixing biofertilizers.

Research in Mexico, conducted by Santillano-Cázares et al. (2022) evaluated several biofertilizer treatments, both alone and with synthetic fertilizer. They concluded that biofertilizers are effective only in certain locations and that, even then, only some biofertilizers show a response. This multi-site, multi-year study suggests that the absence of response in certain environments may due to factors such as precipitation, organic matter content, and residual soil fertility. In their meta-analysis, (Schmidt & Gaudin, (2018) noted that climate regions influence biofertilizer efficacy, with greater effectiveness in dry compared to wet climates. Our experiments conducted in a Continental climate at sites in SW Michigan had rainfall of 265 mm in 2022 and 293 mm in 2023 during the maize growing season, being 41 and 35% lower than the historical precipitation for the same period. These soils, some of the most productive in Michigan, are tile drained, which may have resulted in loss of N below the measurement zone and help explain differing results from other studies where tile drainage was absent.

Crop N uptake through the growing season in this study was unaffected by the biofertilizer treatment in all fields. Again, this is in partial agreement with the hypotheses that predicted a similar N uptake in 2022 and an increased N uptake in SF+Bio in 2023. In contrast, (Sun, Gu, et al. (2020b) reported a 20% increase in N uptake in the biofertilizer treatment when compared to synthetic fertilizer, similar to Heiniger et al. (2022) who observed a 24% and 37% increase in maize N uptake. There were no significant NUE differences in 3 of the 4 fields. Field 4 showed a 24% increase in the SF treatment when compared to SF+Bio. This contrasts with (Schütz et al., 2018) who determined a NUE increase of 5.8 ± 0.6 kg yield per kg N fertilizer through biofertilization, when compared with changes in NUE in our fields that ranged from less than 1% to 20% in favor of SF.

Remote sensing data during crop growth periods has been successfully shown to estimate crop biomass (Mihai & Florin, 2016; Zillmann et al., 2006) and detect differences in biomass accumulation related to N fertilization (Verhulst et al., 2011) . Our results indicated no significant differences in NDVI between the fertilization treatments, suggesting that biomass accumulation at several crop growing stages was similar for both SF and SF+Bio treatments.

Nitrogen fixing biofertilizers vary from synthetic and organic N fertilizers in that they do not provide nutrients to crops directly. Instead, they are living cultures of bacteria and fungi that can increase N availability to plant roots (Chen 2006).Their effectiveness can be influenced by various factors, including climate and soil conditions. While our results generally aligned with our predictions for 2022, they did not for 2023. These inconsistent findings, alongside limited studies on nitrogen dynamics in the literature, highlight the need for further research across diverse soil types and management practices. The interaction between soil nitrogen, nitrogen-fixing biofertilizers, and their effect on yield is complex and cannot be easily predicted by a single metric (Schmidt & Gaudin, 2018b). The efficiency of biofertilizers remains highly variable due to factors such as the different functions and traits of biofertilizers and varying soil conditions (Santillano-Cázares et al., 2022).

The inconsistency of biofertilizers is partly because microorganisms are highly dynamic (Lau et al., 2012), making it challenging to fully understand their effectiveness, which relies on microorganisms successfully colonizing the plant rhizosphere(Hassan et al., 2019). While significant results have been observed in controlled environments, further studies are needed to comprehend the relationships between different types of microorganisms, climatic conditions, and field soil conditions. Mixed results suggest that a one-size-fits-all approach to biofertilizers is not optimal. Generally, their efficiency depends on the type of microorganism in the biofertilizer, the type of crop, the type of soil, and the specific climate and site conditions.

5. CONCLUSIONS

This study aimed to assess the impact of co-applying biofertilizer with synthetic fertilizer (SF+Bio) compared to using synthetic fertilizer (SF) alone on maize crop yield, N uptake, and nitrogen use efficiency. Results from the two-year experiment indicated that in the first year, partial substitution of SF with biofertilizer sustained yield, N uptake, and NUE. In the second year, increasing the N rate did not provide additional benefits, as maize yield and N uptake were comparable between treatments, and NUE was not affected.

Overall, the study demonstrated that substituting a portion of SF with biofertilizer can maintain yield and N uptake while preserving NUE without the need for higher N inputs. This suggests that nitrogen fixing biofertilizers have the potential to effectively replace a fraction of synthetic N fertilizers, promoting plant growth and development comparable to synthetic fertilizer use alone.

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APPENDIX

Table 2. Significance of soil N-NO_3(kgha^{-1}), soil N-NH4 (kg ha^{-1}) and Soil total Inorganic N (kg ha^{-1}), biomass accumulation (Mg ha^{-1}) the total nitrogen uptake (kg ha^{-1}) for 2022 and 2023.

Effect	Soil Nitrogen						Crop Nitrogen			
	N-NO3		N-NH4		Nt		Biomass		Total N Uptake	
	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
Field	ns	ns	ns	*	ns	ns	ns	*	*	ns
Trt	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Stage	ns	ns	ns	ns	ns	ns	*	*	*	ns
Field*Trt	*	*	*	ns	*	*	ns	ns	ns	ns
Field*Stage	ns	ns	*	ns	ns	*	ns	*	*	ns
Trt*Stage	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Field*Trt*Stage	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 3. Significance at R6: ANOVA of biomass, yield and of nitrogen uptake in the grains (N_{upt}g) (Mg ha^{-1}), biomass (N_{upt}b) kg ha^{-1}), total nitrogen uptake (Total N_{upt}) (kg ha^{-1}) nitrogen use efficiency (NUE) (kg kg^{-1}) measured at R6 (Ritchie et al., 1986) for 2022 and 2023. differences were denoted as follows: ns for not significant.

	Biomass		Yield		N_{upt}g		N_{upt}b		Total N_{upt}		NUE	
	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
Field	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
Trt	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Field*Trt	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns: denotes non-significant differences (p>0.05), *denotes significant differences (p<0.05).

Table 4. Analysis of variance of N-NO_3(kg ha^{-1}), N-NH_4 (kg ha^{-1}), Nt (kg ha^{-1}), biomass (kg ha^{-1}), yield (Mg ha^{-1}), N Uptake (kg ha^{-1}) and NUE (kg $kg^{-1)}$2022 and 2023 growing seasons.

Year	Source of variation	N-NH_4	N-NO_3	Nt	Biomass	N Uptake	Yield	NUE_
2022	Field	0.6145	0.3985	0.3674	0.365	0.0502	0.9065	0.7701
	Trt	0.2236	0.9215	0.9991	0.7224	0.9703	0.9229	0.8934
	Field*Trt	0.4853	0.502	0.4584	0.2175	0.3359	0.2104	0.5229
	Stage	0.0151	0.0086	0.0459	<.0001	<.0001	N/A	N/A
	Field*Stage	<.0001	0.1045	0.0721	0.8386	0.001	N/A	N/A
	Trt*Stage	0.2335	0.1519	0.1767	0.4932	0.8486	N/A	N/A
	Field*Trt*Stage	0.4778	0.2586	0.3037	0.4244	0.5066	N/A	N/A
2023	Field	0.0001	0.4044	0.4444	0.0022	0.0329	0.4246	0.1023
	Trt	0.7869	0.4295	0.3972	0.7707	0.1138	0.1897	0.0031
	Field*Trt	0.6333	0.528	0.7107	0.4414	0.6987	0.4563	0.3650
	Stage	<.0001	<.0001	<.0001	<.0001	<.0001	N/A	N/A
	Field*Stage	<.0001	0.1538	0.008	0.0049	0.1072	N/A	N/A
	Trt*Stage	0.064	0.9955	0.9586	0.9135	0.0275	N/A	N/A
	Field*Trt*Stage	0.0801	0.901	0.7693	0.2907	0.9936	N/A	N/A

Table 5. Analysis of variance of $N\text{-}NO_3$(kg ha^{-1}), $N\text{-}NH_4$ (kg ha^{-1}), Nt (kg ha^{-1}) , biomass (kg ha^{-1}), yield (Mg ha^{-1}), N Uptake (kg ha^{-1}), and NUE(kg kg^{-1})at four field experiment.

Field	Source	N-NH4	N-NO3	Nt	Biomass	N Uptake	Yield	NUE
Field 1	Trt	0.6359	0.5693	0.5989	0.33	0.3763	0.3574	0.4711
	Stage	<.0001	0.1917	0.1389	<.0001	<.0001	N/A	N/A
	Trt*Stage	0.1716	0.2363	0.2797	0.9637	0.9641	N/A	N/A
Field 2	Trt	0.2477	0.6974	0.6028	0.3529	0.5244	0.3219	0.7752
	Stage	0.02	0.0041	0.0256	<.0001	<.0001	N/A	N/A
	Trt*Stage	0.5831	0.1908	0.2107	0.2375	0.3896	N/A	N/A
Field 3	Trt	0.7548	0.3005	0.3264	0.6801	0.1158	0.6075	0.0943
	Stage	<.0001	<.0001	<.0001	<.0001	<.0001	N/A	N/A
	Trt*Stage	0.8591	0.894	0.9163	0.9343	0.1627	N/A	N/A
Field 4	Trt	0.7282	0.9355	0.7651	0.5313	0.4522	0.1873	0.0168
	Stage	<.0001	<.0001	<.0001	<.0001	<.0001	N/A	N/A
	Trt*Stage	0.0512	0.9887	0.8462	0.2989	0.3228	N/A	N/A

Table 6. Analysis of variance of N-NO_3(kg ha^{-1}), N-NH_4 (kg ha^{-1}), and Nt (kg ha^{-1}) at four field experiments.

Field	Stage	Trt	Depth	Trt*Depth
Field 1	V5	0.1443	0.0003	0.5226
	V9	0.4419	0.028	0.623
	R3	0.2653	<.0001	0.2780
	R6	0.7608	0.0145	0.9237
Field 2	V5	0.9949	0.0134	0.9253
	V9	0.1293	0.0003	0.3188
	R3	0.9123	0.0294	0.7833
	R6	0.241	<.0001	0.002
Field 3	V5	0.1823	0.067	0.3605
	R1	0.5678	0.8078	0.508
	R3	0.8251	0.0016	0.6539
	R6	0.8386	<.0001	0.9684
Field 4	V5	0.2145	0.0003	0.4931
	R1	0.6742	0.5084	0.7585
	R3	0.0034	0.0188	0.8478
	R6	0.2309	0.0002	0.5458

Table 7. Mean of N-NH_4 (kg ha^{-1}), N-NO_3(kg ha^{-1}), and Nt(kg ha^{-1}) at four field for every sampled stage.

		N-NH_4 (kg ha^{-1})		N-NO_3 (kg ha^{-1})		Nt (kg ha^{-1})	
Field	**Stage**	**SF**	**SF+Bio**	**SF**	**SF+Bio**	**SF**	**SF+Bio**
Field 1	V5	7.97	7.04	25.78	44.95	33.75b	51.99a
	V9	8.44	8.27	26.92b	28.56a	35.36	36.83
	R3	5.11	5.68	51.44a	30.34b	56.55a	36.02b
	R6	5.26	6.39	35.1	46.39	40.36	52.78
Field 2	V5	7.08	7.85	31.74	29.71	38.83	37.56
	V9	6.67	5.79	26.68	28.09	33.34	33.87
	R3	6.25	5.62	45.74b	62.63a	51.98b	68.25a
	R6	8.83	6.5	31.5	31.55	40.33	38.06
Field 3	V5	1.46	2.17	23.21	21.08	24.67	23.25
	R1	1.02	1.17	36.48	31.49	37.5	32.67
	R3	5.1	4.75	2.11	1.78	7.21	6.53
	R6	0.94	0.93	5.2	4.72	6.14	5.65
Field 4	V5	2.61	3.71	25.76	24.84	28.36	28.53
	R1	1.68	2.03	24.54	26.14	26.22	28.18
	R3	1.33	2.32	2.75	2.21	4.08	4.54
	R6	13.5a	9.24b	5.78	4.55	19.28	13.79

Table 8. Mean of Total Biomass (Mg ha^{-1}), N Uptake (kg ha^{-1}) at four field for every stage Mean of total biomass (Kg ha^{-1}), and total N uptake (Kg ha^{-1}) at four field for every sampled stage.

Field	Stage	Total Biomass (Mg h^{-1})		N Uptake (Kg h^{-1})	
		SF	SF+Bio	SF	SF+Bio
Field 1	V2	0.04	0.03	1.68	1.3
	V5	0.24	0.18	9.58	7.06
	V9	2.19	1.71	40.66	33.78
	V14	6.97	5.56	104.62	93.01
	R3	15.42	15.61	167.97	170.17
	R6	28.33a	26.23b	207.76	192.95
Field 2	V2	0.03	0.03	1.5	1.18
	V5	0.26	0.20	9.5	7.32
	V9	2.37	2.07	56.5	51.1
	V14	7.92	7.26	153.47	144.46
	R3	15.39	14.69	168.74	159.86
	R6	26.20	27.99	190.93	199.63
Field 3	V3	0.04	0.04	1.19	1.31
	V5	0.28	0.23	5.15	4.2
	V9	1.90	2.10	39.11	44.19
	R1	5.89	5.79	99.15	78.57
	R3	11.04	11.15	210.72	205.85
	R6	21.97	22.94	325.36	335.6
Field 4	V3	0.03	0.03	1.25	1.18
	V5	0.30	0.37	6.9	8.45
	V9	2.52	2.34	59.48	56.51
	R1	7.53	8.22	113.23	119.83
	R3	14.01	13.17	218.62	219.6
	R6	25.36a	23.27b	369.67	350.71

Table 9. Mean of maize yield (Mg ha-1), nitrogen uptake in grain (Nup grain) (kg ha-1), nitrogen uptake in biomass (Nup biomass) (kg ha-1), total nitrogen uptake (Total Nup)(kg ha-1) and Nitrogen Use Efficiency (NUE) (kg kg-1N).

Field	Yield		Nup grain		Nup biomass		Total Nup		NUE	
	SF	SF+Bio	SF	SF+Bio	SF	SF+Bio	SF	SF+Bio	SF	SF+Bio
Field 1	14.44a	13.65a	131.35a	124.01a	78.92a	68.94a	207.76a	192.95a	1.02a	0.96a
Field 2	13.18a	14.48a	118.67a	135.33a	72.26a	71.4a	190.93a	199.63a	0.95a	1.0a
Field 3	12.65a	12.23a	208.91a	213.92a	128.97a	126.83a	337.88a	340.75a	1.47a	1.27a
Field 4	13.77a	12.27a	244.36a	222.44a	125.31a	128.27a	369.71a	350.71a	1.61a	1.3b

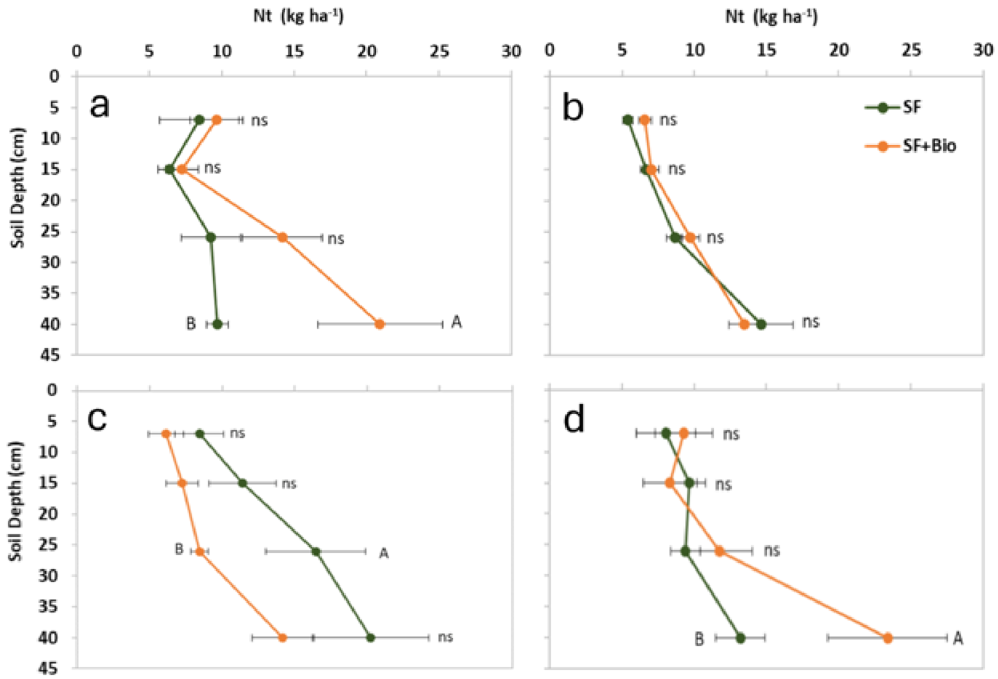

Figure 12. Total inorganic nitrogen (Nt) (kg ha^{-1}) by fertilization treatment for Field 1 at V5 (a), V9 (b), R3 (c), and R6 (d). Fertilization treatments were the co-application of synthetic N fertilizer and Biofertilizer (SF+Bio) and synthetic fertilizer only (SF). Soil total N was determined from soil samples were at four depths: 0-7 cm, 7-15 cm, 15-26 cm and 26-40 cm. ns denote not significant differences (p>0.05), and capital letters indicate significant differences between treatments (p<0.05). Errors bars indicate +/- 1 standard error.

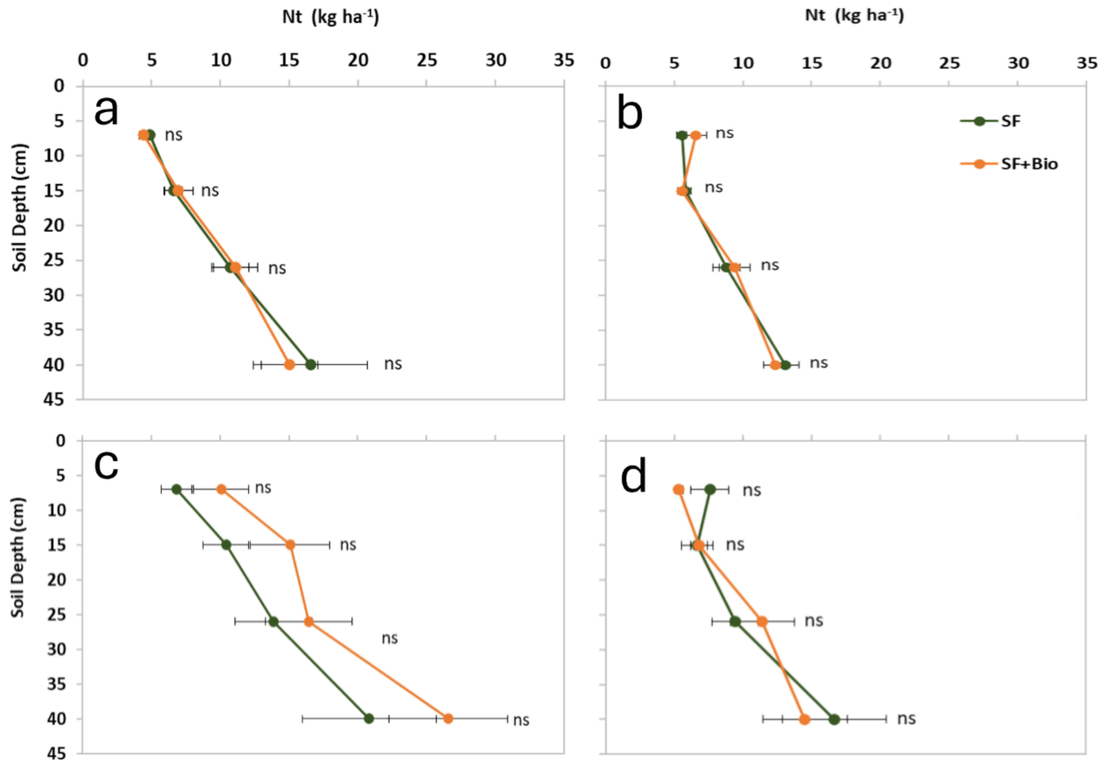

Figure 13. Total inorganic nitrogen (Nt) (kg ha^{-1}) by fertilization treatment for Field 2 at V5 (a), V9 (b), R3 (c), and R6 (d). Fertilization treatments were the co-application of synthetic N fertilizer and Biofertilizer (SF+Bio) and synthetic fertilizer only (SF). Soil total N was determined from soil samples were at four depths: 0-7 cm, 7-15 cm, 15-26 cm and 26-40 cm. ns denote not significant differences (p>0.05), and capital letters indicate significant differences between treatments (p<0.05). Errors bars indicate +/- 1 standard error.

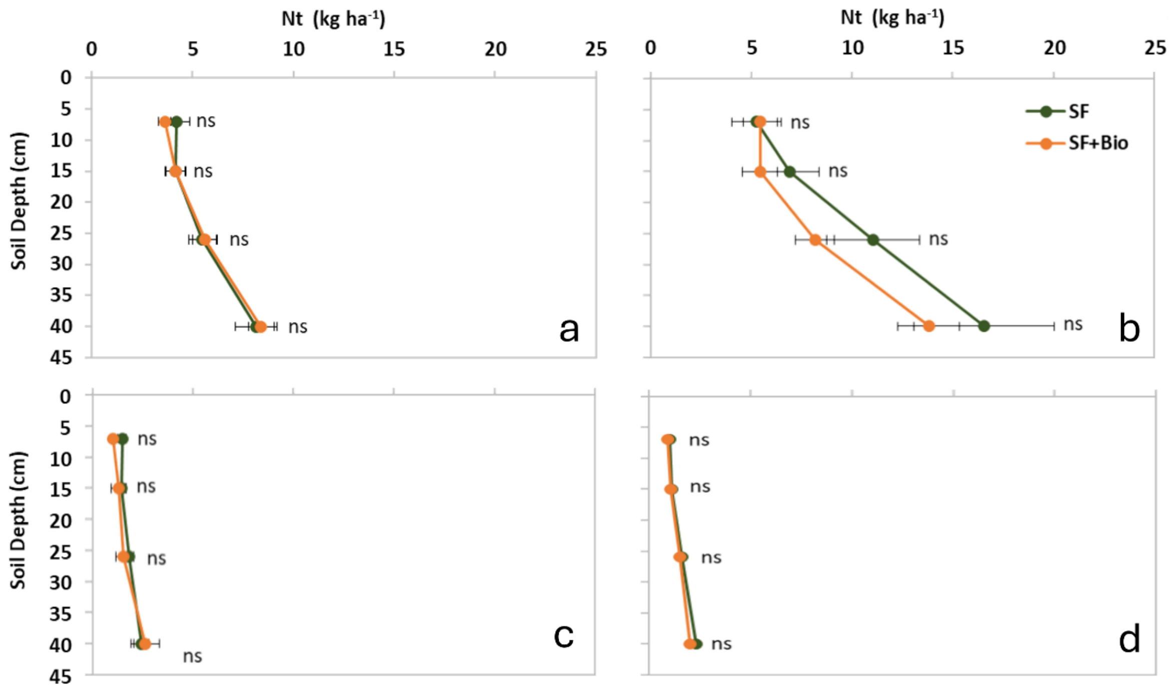

Figure 14. Total inorganic nitrogen (Nt) (kg ha^{-1}) by fertilization treatment for Field 3 at V5 (a), R1 (b), R3 (c), and R6 (d) maize growing states. Fertilization treatment was the co-application of synthetic N fertilizer and Biofertilizer (SF+Bio) and synthetic fertilizer only (SF). N-NH4 was determined from soil samples were at four depths: 0-7 cm, 7-15 cm, 15-26 cm and 26-40 cm. ns denote not significant differences (*p*>0.05), and capital letters indicate significant differences between treatments (*p*<0.05). Errors bars indicate +/- 1 standard error.

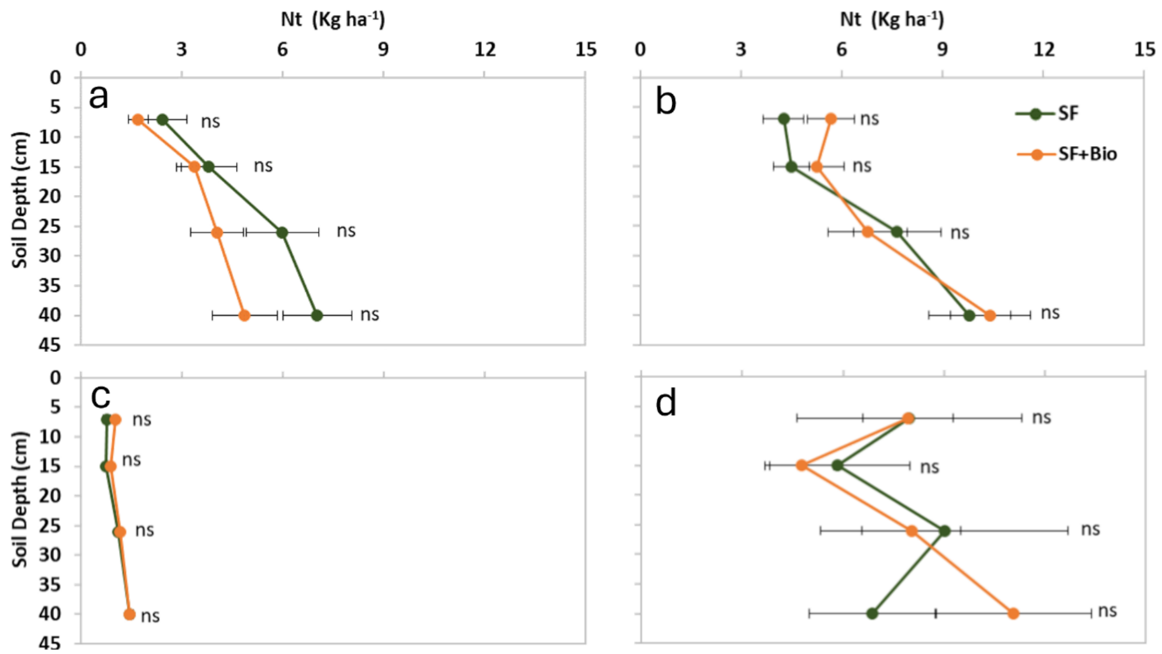

Figure 15. Total inorganic nitrogen (Nt) (kg ha^{-1}) by fertilization treatment for Field 4 at at V5 (a), R1 (b), R3 (c), and R6 (d) maize growing states. Fertilization treatment was the co-application of synthetic N fertilizer and Biofertilizer (SF+Bio) and synthetic fertilizer only (SF). N-NH4 was determined from soil samples were at four depths: 0-7 cm, 7-15 cm, 15-26 cm and 26-40 cm. ns denote not significant differences (p>0.05), and capital letters indicate significant differences between treatments (p<0.05). Errors bars indicate +/- 1 standard error.

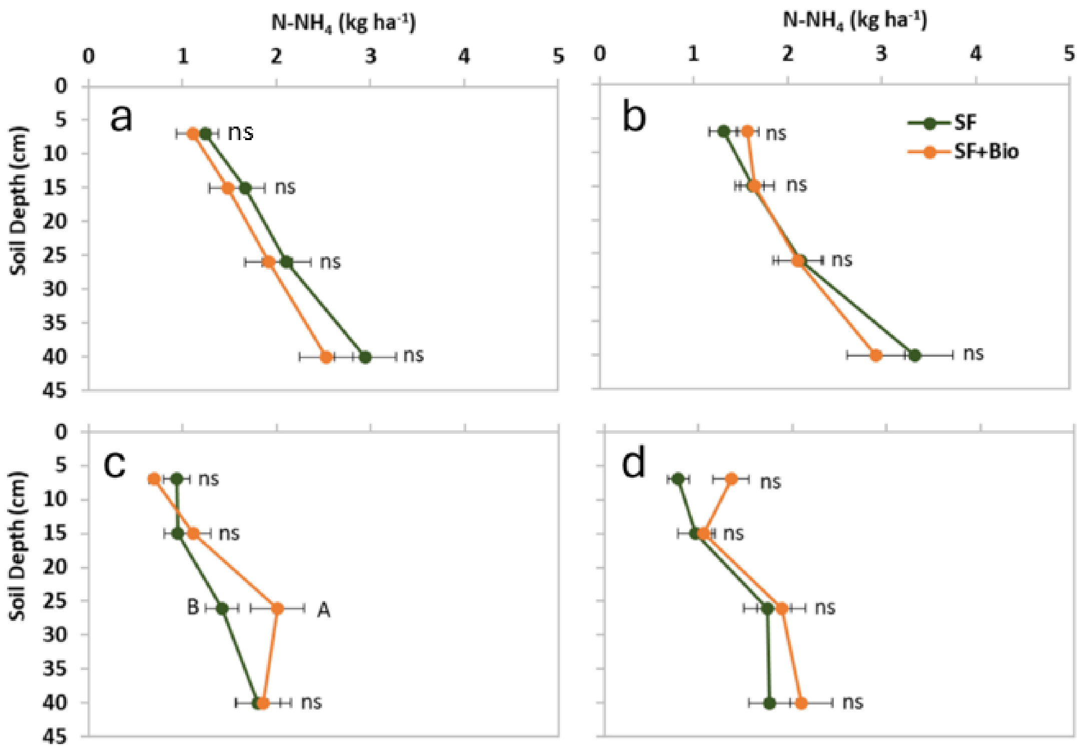

Figure 16. Soil Nitrogen from ammonium (N-NH_4) (kg ha^{-1}) by fertilization treatment for Field 1 at V5 (a), V9 (b), R3 (c), and R6 (d) growing stages. Fertilization treatments were the co-application of synthetic N fertilizer and Biofertilizer (SF+Bio) and synthetic fertilizer only (SF). Soil N-NH_4 was determined from soil samples collected at four depths: 0-7, 7-15, 15-26, and 26-40 cm. ns denote not significant differences (p>0.05), and capital letters indicate significant differences between treatments (p<0.05). Errors bars indicate +/- 1 standard error.

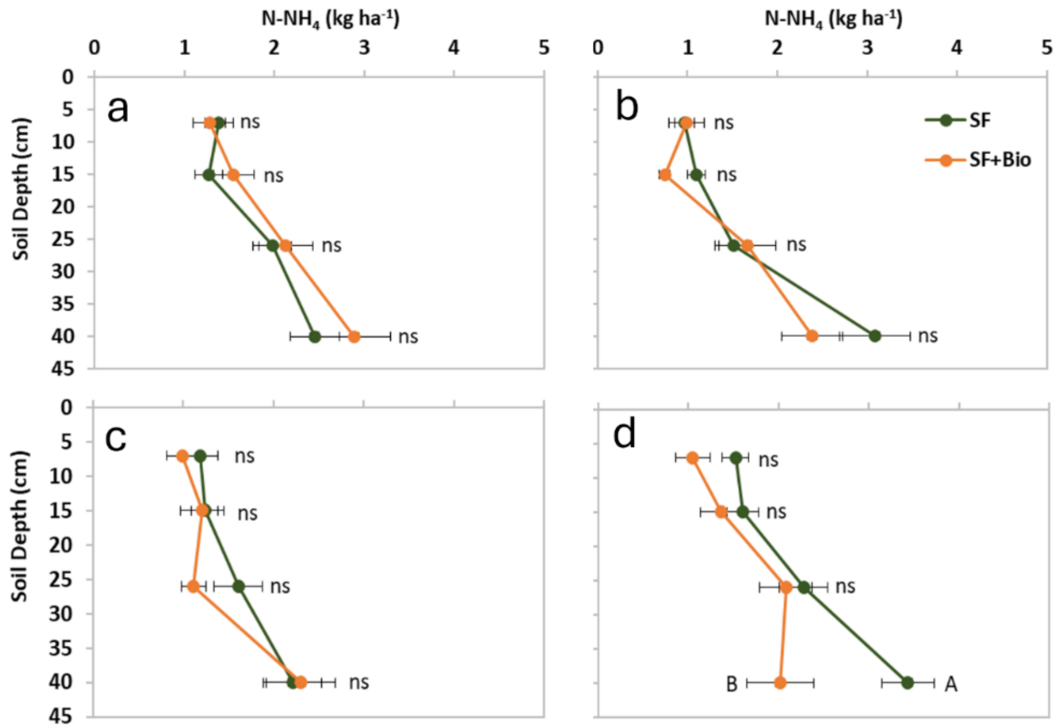

Figure 17. Soil Nitrogen from ammonium (N-NH_4) (kg ha^{-1}) by fertilization treatment for Field 2 at V5 (a), V9 (b), R3 (c), and R6 (d) growing stages. Fertilization treatments were the co-application of synthetic N fertilizer and Biofertilizer (SF+Bio) and synthetic fertilizer only (SF). Soil N-NH_4 was determined from soil samples at four depths: 0-7, 7-15, 15-26, and 26-40 cm. ns denote not significant differences (p>0.05), and capital letters indicate significant differences between treatments (p<0.05). Errors bars indicate +/- 1 standard error.

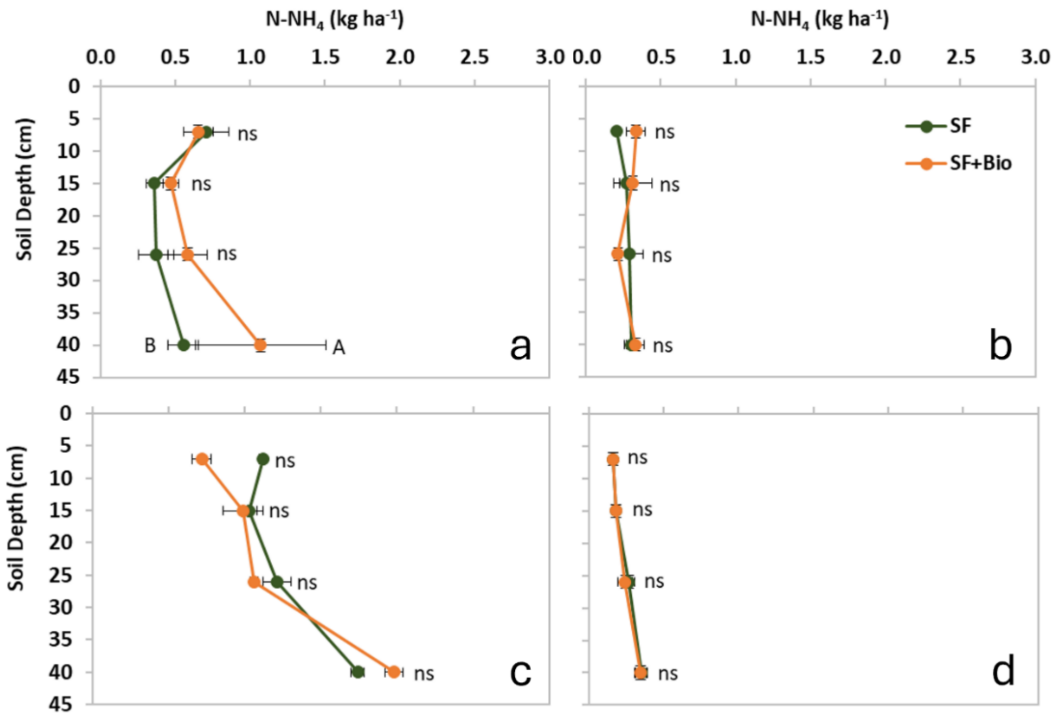

Figure 18. Soil Nitrogen from ammonium (N-NH_4) (kg ha^{-1}) by fertilization treatment for Field 3 at V5 (a), R1 (b), R3 (c), and R6 (d) growing stages. Fertilization treatments were the co-application of synthetic N fertilizer and Biofertilizer (SF+Bio) and synthetic fertilizer only (SF). Soil N-NH_4 was determined from soil samples at four depths: 0-7, 7-15, 15-26, and 26-40 cm. ns denote not significant differences (p>0.05), and capital letters indicate significant differences between treatments (p<0.05). Errors bars indicate +/- 1 standard error.

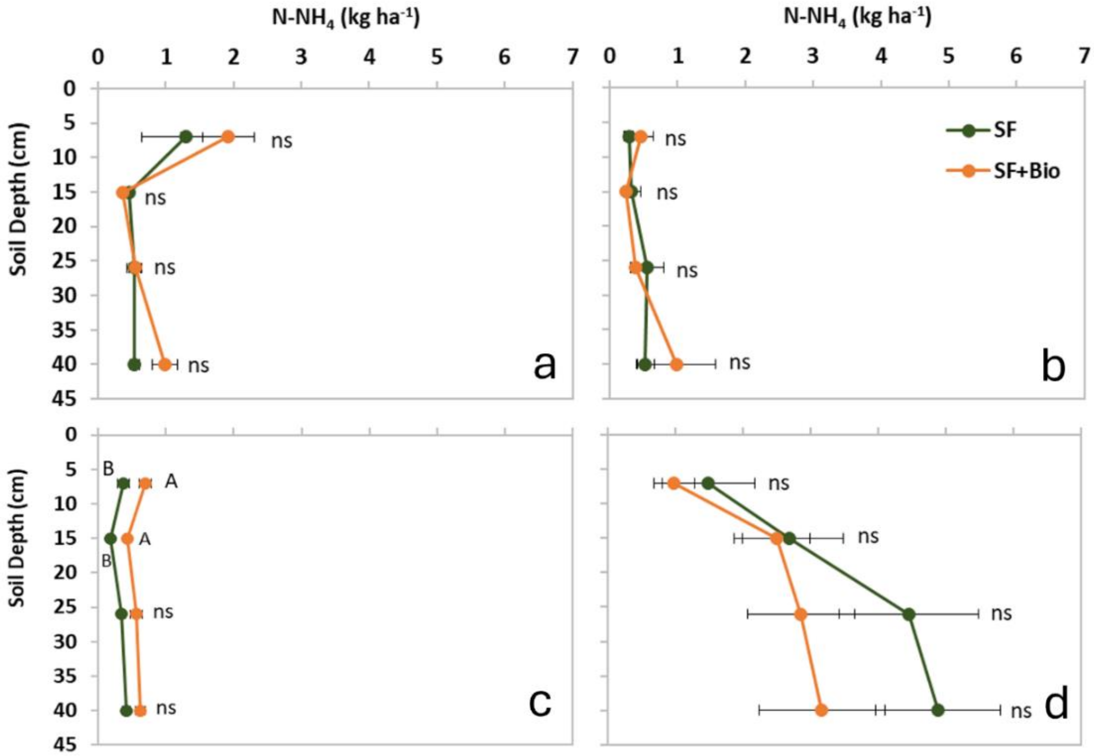

Figure 19. Soil Nitrogen from ammonium (N-NH_4) (kg ha^{-1}) by fertilization treatment for Field 4 at V5 (a), R1 (b), R3 (c), and R6 (d) growing stages. Fertilization treatments were the co-application of synthetic N fertilizer and Biofertilizer (SF+Bio) and synthetic fertilizer only (SF). Soil N-NH_4 was determined from soil samples at four depths: 0-7, 7-15, 15-26, and 26-40 cm. ns denote not significant differences (p>0.05), and capital letters indicate significant differences between treatments (p<0.05). Errors bars indicate +/- 1 standard error.

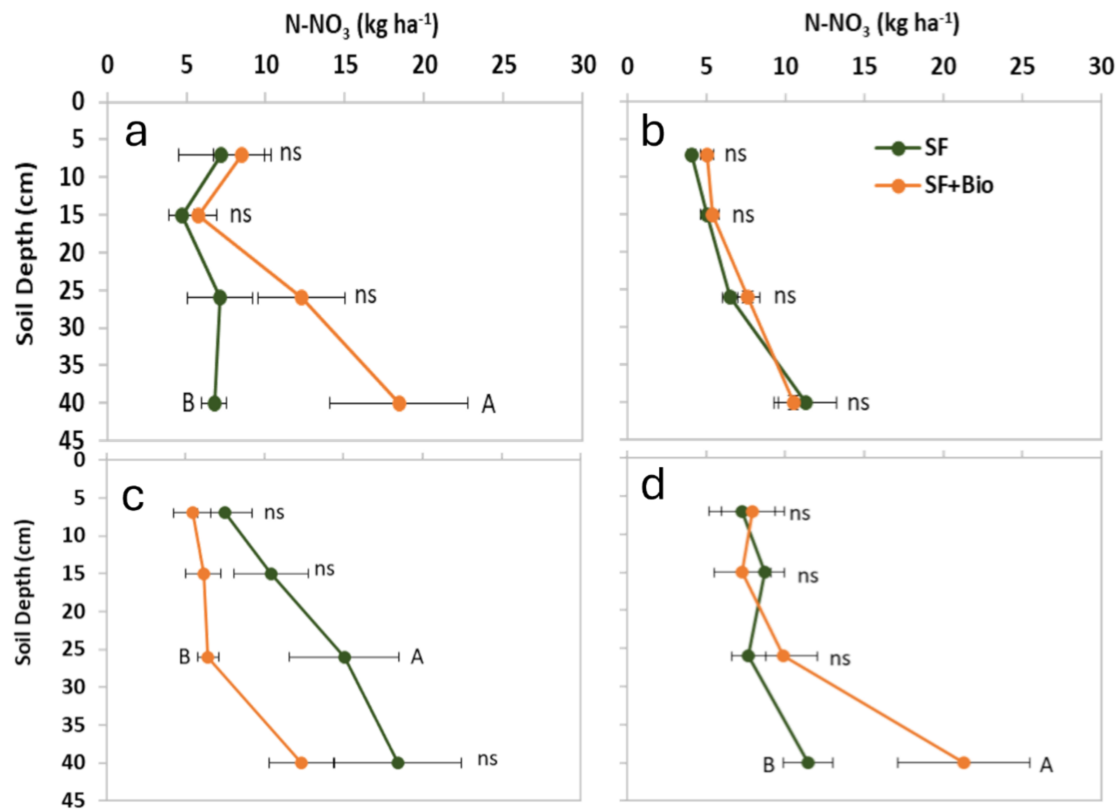

Figure 20. Soil N from nitrates ($N\text{-}NO_3$) ($kg\ ha^{-1}$) by fertilization treatment for Field 1 at V5 (a), V9 (b), R3 (c), and R6 (d) growing stages. Fertilization treatments were the co-application of synthetic N fertilizer and Biofertilizer (SF+Bio) and synthetic fertilizer only (SF). Soil $N\text{-}NO_3$ was determined from soil samples at four depths: 0-7, 7-15, 15-26, and 26-40 cm. ns denote not significant differences ($p>0.05$), and capital letters indicate significant differences between treatments ($p<0.05$). Errors bars indicate +/- 1 standard error.

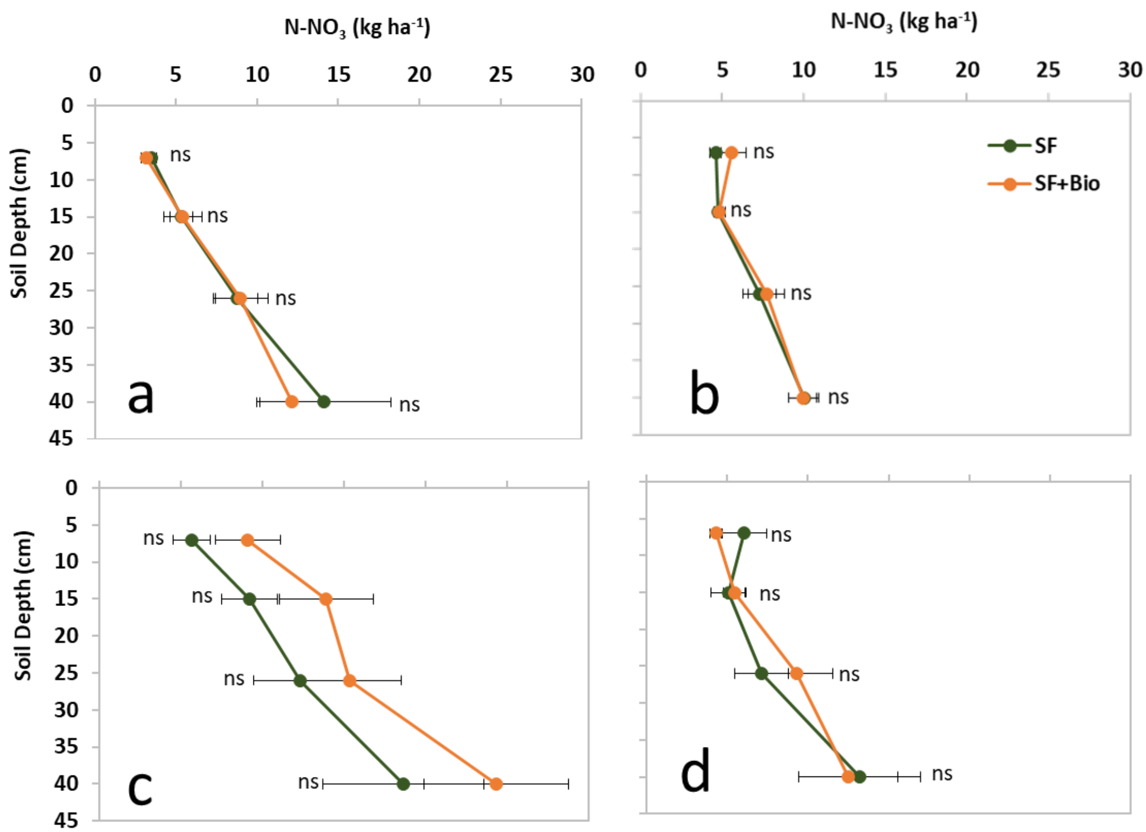

Figure 21. Soil Nitrogen from nitrate ($N\text{-}NO_3$) (kg ha^{-1}) by fertilization treatment for Field 2 at V5 (a), V9 (b), R3 (c), and R6 (d) growing stages. Fertilization treatments were the co-application of synthetic N fertilizer and Biofertilizer (SF+Bio) and synthetic fertilizer only (SF). Soil $N\text{-}NO_3$ was determined from soil samples at four depths: 0-7, 7-15, 15-26, and 26-40 cm. ns denote not significant differences (p>0.05), and capital letters indicate significant differences between treatments (p<0.05). Errors bars indicate +/- 1 standard error.

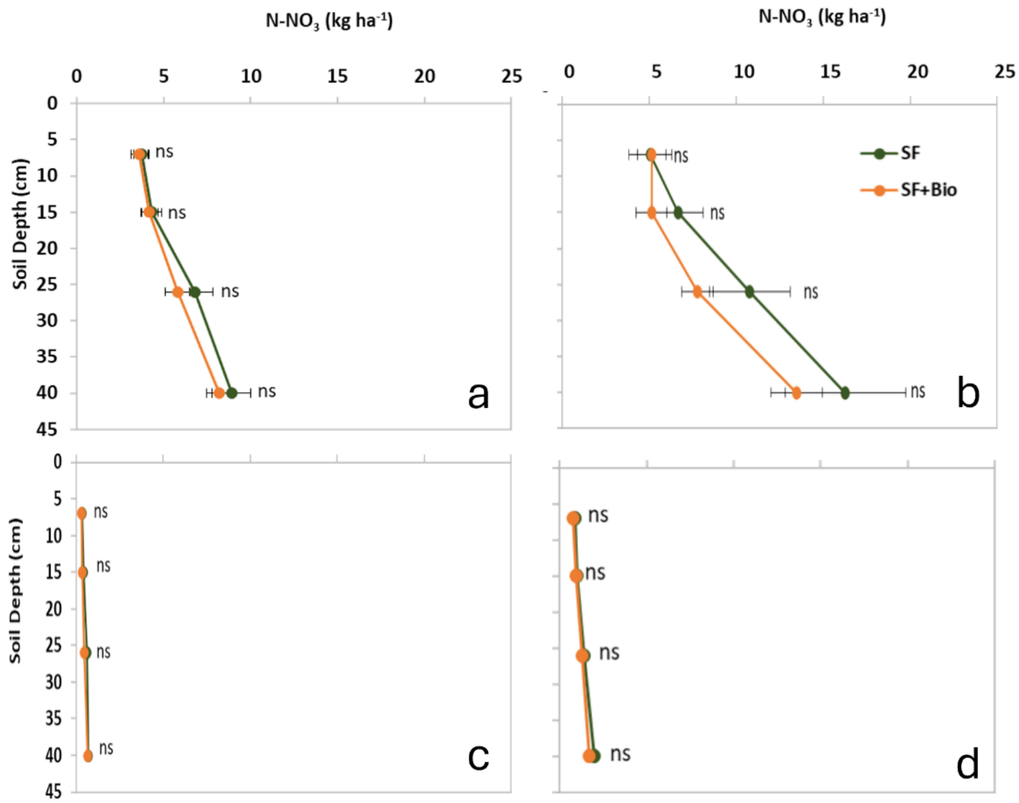

Figure 22. Soil Nitrogen from nitrate (N-NO_3) (kg ha^{-1}) by fertilization treatment for Field 3 at V5 (a), R1 (b), R3 (c), and R6 (d) growing stages. Fertilization treatments were the co-application of synthetic N fertilizer and Biofertilizer (SF+Bio) and synthetic fertilizer only (SF). Soil N-NO_3 was determined from soil samples at four depths: 0-7, 7-15, 15-26, and 26-40 cm. ns denote not significant differences (p>0.05), and capital letters indicate significant differences between treatments (p<0.05). Errors bars indicate +/- 1 standard error.

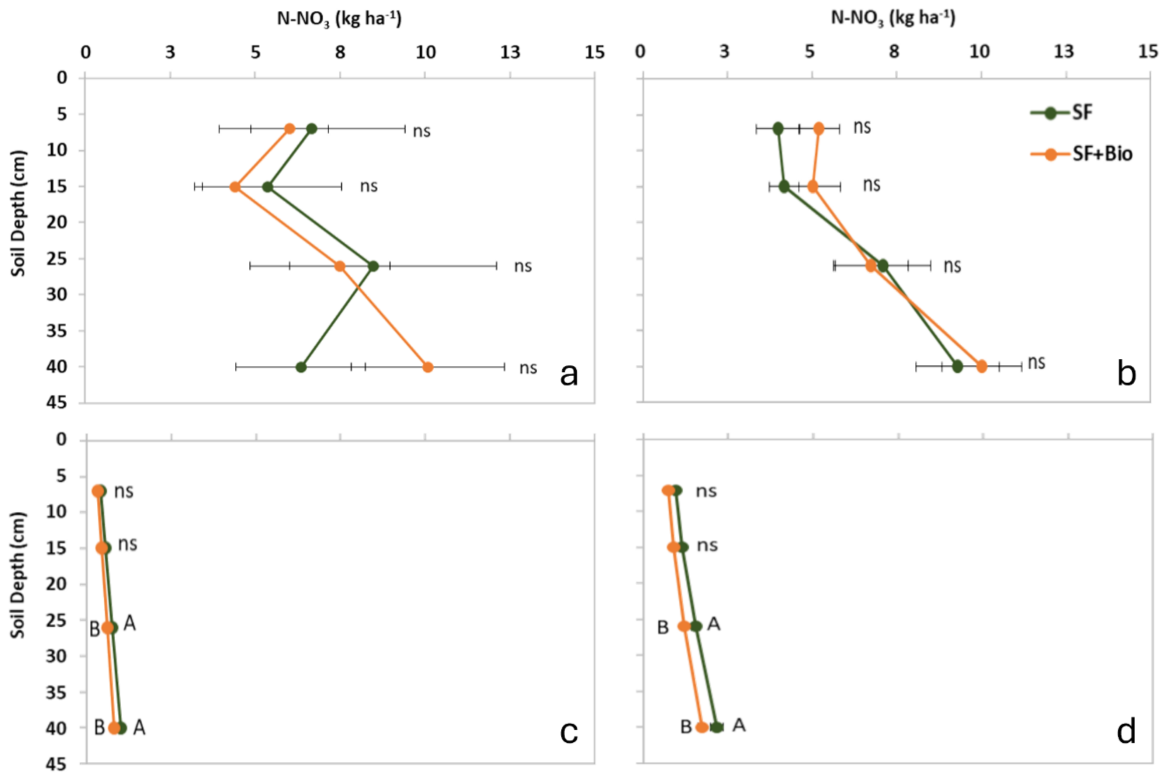

Figure 23. Soil Nitrogen from nitrate (N-No3) (kg ha-1) by fertilization treatment for Field 4 at V5 (a), R1 (b), R3 (c), and R6 (d) growing stages. Fertilization treatments were the co-application of synthetic N fertilizer and Biofertilizer (SF+Bio) and synthetic fertilizer only (SF). Soil N-NO_3 was determined from soil samples at four depths: 0-7, 7-15, 15-26, and 26-40 cm. ns denote not significant differences (p>0.05), and capital letters indicate significant differences between treatments (p<0.05). Errors bars indicate +/- 1 standard error.