Evaluating the impact of nitrogen-fixing microbial inoculants on nitrous oxide emissions and nitrogen dynamics in corn cropping systems

by

Irosha Wanithunga

PgDip., Sri Lanka Institute of Marketing, 2011
A.A.S., Cloud County Community College, 2017
M.S., Kansas State University, 2020

AN ABSTRACT OF A DISSERTATION

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Abstract

Nitrogen (N) is a critical nutrient for plant growth and essential for synthesizing amino acids, proteins, and nucleic acids, which are fundamental for crop yield and quality. The introduction of N fertilizers in the early 20th century significantly boosted agricultural productivity, meeting the demands of a growing global population. However, the widespread use of N fertilizers has led to environmental concerns, particularly the emission of nitrous oxide (N_2O), a potent greenhouse gas with a global warming potential approximately 300 times greater than carbon dioxide (CO_2). Our comprehensive research study integrates three related investigations, each exploring different aspects of N management in corn cropping systems, including the efficacy of N-fixing microbial inoculants in mitigating N_2O emissions, enhancing fertilizer N recovery, and modeling N dynamics using the Denitrification-Decomposition (DNDC) model.

The first study assessed the effectiveness of N-fixing microbial inoculants in reducing N_2O emissions and improving N recovery in corn production. Conducted from 2021 to 2023 at the Agronomy North Farm at Kansas State University, the experiment employed a randomized complete block design with N application rates of 0, 56, 112, and 168 kg N ha^{-1}, both with and without bioinoculants. N_2O fluxes were measured bi-weekly using the static chamber technique. N_2O emissions increased significantly with higher N fertilizer rates, with the highest cumulative emissions reaching 1.13 kg N_2O-N ha^{-1} in 2022. Most emissions occurred within the first 40 days after planting, particularly in 2022, when precipitation events were more frequent. In 2023, N_2O emissions were lower and more evenly distributed throughout the growing season. Bioinoculant-treated plots consistently exhibited lower N_2O emissions than untreated plots, although these reductions were not statistically significant. Emission factors (EF%) remained below the IPCC

default emission factor value of 1% across all treatments in 2022 and 2023. N_2O emission-yield curves had a significant linear trend with yield without bioinoculant application. However, there was no apparent trend with bioinoculant application. These findings highlight the significant role of N fertilizer rates in driving N_2O emissions. The effect of bioinoculant on N_2O flux was not clear and non-significant.

The second study focused on N recovery in above-ground biomass and soil N dynamics using a stable isotope ^{15}N technique, which explored the effects of N-fixing microbial inoculant across different growth stages and N application rates. Conducted over the same period and location as the previous study, this study used micro-plots to accommodate ^{15}N fertilizer treatments. In 2022, fertilizer N recovery ranged from 6.7% to 11.62% at R6 corn growth stage, with the bioinoculant having minimal impact. However, in 2023, N recovery in biomass increased significantly, ranging from 17% to 30.9%, with the greatest recovery observed at the 168 kg N ha^{-1} rate with bioinoculant application. The lower N recovery in 2022 might be due to significant early-season precipitation, which can potentially increase N losses by way of leaching and denitrification. N_2O emissions were higher in 2022, and more fertilizer N was recovered at the 15-30 cm soil layer. Well-distributed precipitation in 2023 facilitated better N recovery in above-ground biomass. Nitrogen immobilization was profoundly greater at 15-30 cm compared to 0-5cm and 5-15cm, especially at lower N rates. Fertilizer N recovery as organic N ranged from 8.7% to 28.3% at the harvest stage in 2023. Overall fertilizer N recoveries for both above-ground biomass and below-ground soil pools were lower than most other ^{15}N research studies. The effect of N-fixing bioinoculant in N recovery was not clear. Long-term field trials need to be implemented to investigate the impact of N-fixing microbial inoculant on N recovery in corn biomass and soil profiles.

The third study employed the Denitrification-Decomposition (DNDC) model to simulate cumulative N_2O emissions under no-till, continuous corn management without bioinoculants. The objective was to evaluate the model's accuracy in predicting N_2O emissions and to assess its sensitivity to varying environmental conditions, particularly precipitation. The model was calibrated using 2022 data, incorporating daily precipitation and temperature inputs and fine-tuning soil parameters such as bulk density, pH, and soil organic carbon obtained from the Web Soil Survey database. The calibration process demonstrated strong accuracy, with a Mean Absolute Error (MAE) of 0.016, Root Mean Squared Error (RMSE) of 0.023, Mean Bias Error (MBE) of 0.016, and a Coefficient of Determination (R^2) of 0.997. Validation using 2023 data showed a slight decline in accuracy, with MAE of 0.056, RMSE of 0.067, MBE of -0.051, and R^2 of 0.934, indicating a minor underprediction bias. The model effectively captured the influence of precipitation on N_2O emissions, accurately reflecting emission peaks during periods of high rainfall. These findings validate the DNDC model's reliability in predicting N_2O emissions under specified agricultural practices and highlight the need for continuous evaluation and recalibration to maintain predictive accuracy across different growing seasons.

In conclusion, these studies provide comprehensive insights into N management in corn cropping systems, emphasizing the critical role of N application rates, the potential benefits and limitations of bioinoculants, and the utility of modeling tools like the DNDC model in predicting greenhouse gas emissions. The findings underscore the importance of integrating N-fixing bioinoculants with precise N management strategies to enhance N use efficiency, improve crop yields, and mitigate environmental impacts. However, the stage-specific and N rate-dependent effectiveness of bioinoculants and the need for continuous model recalibration highlight the complexity of N dynamics in agricultural systems and the necessity for ongoing targeted research to optimize these practices for sustainable agriculture.

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Major Professor
Dr. Charles W. Rice

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In conclusion, these studies provide comprehensive insights into N management in corn cropping systems, emphasizing the critical role of N application rates, the potential benefits and limitations of bioinoculants, and the utility of modeling tools like the DNDC model in predicting greenhouse gas emissions. The findings underscore the importance of integrating N-fixing bioinoculants with precise N management strategies to enhance N use efficiency, improve crop yields, and mitigate environmental impacts. However, the stage-specific and N rate-dependent effectiveness of bioinoculants and the need for continuous model recalibration highlight the complexity of N dynamics in agricultural systems and the necessity for ongoing targeted research to optimize these practices for sustainable agriculture.

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Dedication

This dissertation is dedicated to all the soil scientists, researchers, and educators who are tirelessly working to advance our understanding of climate change and its impacts. Their relentless efforts to research, educate, and raise awareness inspire and motivate me. I am deeply grateful for their commitment to addressing one of the most pressing challenges of our time.

Chapter 1 - Introduction

Nitrogen (N) is a biologically essential element vital for the biotic synthesis of the foundation of life components such as protein, RNA, DNA, etc. The element N is present in the atmosphere (~78%) as di-nitrogen gas with a triple covalent bond, which requires higher breaking energy to make it available as reactive N (N_r) (Kitadai and Maruyama, 2017). All living organisms directly utilize the reduced form of N as ammonium or as an enzymatically reduced version of N during the N assimilation process (Zerkle and Mikhail, 2017). Nitrogen-fixing microorganisms are responsible for fixing atmospheric N to Nr through natural biological processes while additional N is fixed anthropogenically in the modern day by the Haber-Bosch process. However, before the evolution of N-fixing microorganisms, N_r transformed abiotically in the earth's primordial atmosphere through photochemical reactions. The processes of the N cycle are tightly interconnected with both biotic and abiotic interactions within the soil ecosystem (Stein and Klotz, 2016; Doane, 2017). The N cycle has been studied extensively as it can be considered the cornerstone of productivity, which is coupled with the lithosphere, hydrosphere, and atmosphere; yet there are many challenges to be solved in the N cycle itself with the changing climate (Gruber and Galloway, 2008; Zerkle and Mikhail, 2017; Nations Environment Programme, 2019).

Nitrogen is critical for crop growth and food production. It is crucial to manage the global agricultural lands for food production to meet the food demand with the increasing global population. Ensuring economic and agronomic profitability while minimizing environmental impact is crucial for sustainable crop production in a carbon-based market. Farmers can generate carbon credits by adopting practices that reduce greenhouse gas emissions and enhance carbon sequestration, benefiting from carbon incentives or selling these credits (Ekins and Zenghelis, 2021; Department of Agriculture, 2023; Phelan et al., 2024).

Many studies have shown that 50% or more of N fertilizer applied in cropping systems is lost (Tilman et al., 2002; Mălinaș et al., 2022; Griesheim et al., 2023). Schlesinger, (2009) reported 150 Tg N yr^{-1} was applied to the land surface anthropogenically, and a portion of that accumulated in the terrestrial biosphere for several decades. Further, the study revealed that 10% of N applied globally was available in food, and another portion was lost through runoff to watersheds, streams, and oceans, leached to groundwater, or denitrified as gases to the atmosphere.

Global agriculture occupies ~37% of the earth's surface and accounts for considerable greenhouse gas (GHG) emissions, including N_2O (Smith et al., 2008). The US EPA estimated that 10.6% of total GHG emissions in the US were attributed to the agriculture sector (US EPA, 2023). The excessive use of fertilizer in agriculture systems has degradedsoil systems, specifically in agricultural lands (Kopittke et al., 2019). Energy use, land use, land use change, lifestyles (transportation and diet preferences), and patterns of consumption have led to an increase in global GHG concentrations, which cause climate change (Calvin et al., 2023).Continuing global population growth with an increase in per capita food production and the demand for meat consumption can increase N_2O emissions in the future (Reay et al., 2012. Xia et al., 2023). Further, extreme weather conditions in crop lands could emit 80 times more emissions than the baseline scenario based on a deep-learning study by Elberling et al. (2023). Improving N use efficiency in agricultural production is a crucial strategy to mitigate N_2O emissions (Reay et al., 2012).

Soil microbial communities play a vital role in ecosystem functions and services, which are integrated with the plant-soil nutrient cycles and resistance to soil pathogenic effects (Jacoby et al., 2017; Arif et al., 2020). To address the growing demand for food while reducing the environmental footprint, a deeper understanding of the N cycle (Figure 1.1) and the development of innovative N management strategies are essential.

1.1 Nitrogen fixation

There are four main types of N sources for the entire biosphere, natural atmospheric deposition, organic fertilizer inputs such as manure, synthetic fertilizer produced through the Haber-Bosch process, and biological N fixation. (BNF) (Clark et al., 2013). Total overall N fixation globally was estimated to be 413 Tg N Yr^{-1} (Flower et al., 2013).The Haber-Bosh process generates 120 Tg N Yr^{-1}. In the agricultural systems, BNF is responsible for 60 Tg N Yr^{-1}. In addition to that BNF generates 58 Tg N Yr^{-1} and 140 Tg N Yr^{-1} to terrestrial and marine ecosystems[1] (Flower et al., 2013).

Nr compounds in the atmosphere are returned to earth during atmospheric deposition through rain or snow. In-cloud scavenging and below-cloud scavenging are two types of wet depositions. Further, gaseous and N_r components are deposited back to the earth's surface without being involved in precipitation events. Overall natural atmospheric N deposition is lower than BNF human-induced N fixation methods. Lighting-induced N_r deposition is also considered an atmospheric deposition, accounting for approximately about 5 Tg N yr^{-1}. Further, the dry and wet deposition of N_r accounted for 70 Tg N Yr^{-1} to the earth's surface and 30 Tg N Yr^{-1} to the marine ecosystem (Flower et al., 2013; Bebber, 2021).

Adding excessive amounts of anthropogenically fixed N or industrially manufactured N fertilizer to cropping systems disrupts the natural global N cycle (Socolow, 1999). Therefore, it is paramount to explore alternative viable N fixation strategies to fill N demand in the global agriculture sector. Exploring potential solutions from the soil itself is important, as it would promote sustainable agriculture systems and reduce the environmental impact and social cost involved with N.

1.2 Soil biological nitrogen fixation

Soil biological nitrogen fixation (BNF) is the reduction of atmospheric di-nitrogen (N_2) to ammonium (NH_4^+) under localized anaerobic conditions by specific soil microbes called diazotrophs (Zerkle and Mikhail, 2017). They are a phylogenetically diverse microbial group, including bacteria and archaea, in different environmental conditions (Vitousek et al., 2013). Further, N fixers can belong to obligate aerobes or obligate anaerobes communities (Boyd and Peters, 2013). BNF can be categorized as symbiotic N fixers, non-symbiotic N fixers, associative and free-living N fixers. Free-living N fixers occurred 1.5 to 2.2 billion years ago, and symbiotic N fixation occurred 59 million years ago. Free-living N fixers occurred in more diverse and variable conditions over symbiotic N fixers (Smercina et al., 2019). The mutualistic relationship between rhizobia and legume is a form of symbiotic N fixation, which has been extensively studied. The breaking of N triple bonds is a highly energy-demanding task that requires 16 ATP to fix one molecule of N_2 (Zerkle and Mikhail, 2017; Roley et al., 2019), as shown below.

$$8H^+ + 8e^- + N_2 + 16ATP = 2NH_3 + H_2 + 16ATP + 16P_i$$

The overall N fixation process is carried out by the nitrogenase enzyme, which involves multiple genes for the entire N fixation process (Bothe et al., 2010). Three different forms of nitrogenase enzyme are based on the enzyme structure's metallic components. Molybdenum (Mo), Vanadium (V), and Iron (Fe) are the three metals involved in the catalytic site of the nitrogenase enzyme (Einsle and Rees, 2020). Many factors affect BNF, including the availability of N_r in the environment, level of oxygen (O_2), substrate availability, and the availability of other micronutrients (Mo, V, Fe) for enzymatic activity (Rucker and Kaçar, 2024). Nitrogenase denatures when it is exposed to aerobic conditions. Therefore, many O_2-protecting mechanisms were inherited by N-fixing microbes (Vitousek et al., 2013).

Symbiotic biological N fixation (BNF) has been widely studied in cropping systems, with soybean-related N fixation being prominent in crop rotations; however, other N-fixing and cover crops have also been extensively studied (Peoples et al.,1995). Associative N-fixing bacteria are associated with the roots where root priming or root exudates are used for their energy (Dommelen and Vanderleyden, 2007; Roley et al., 2019). Still, free-living N fixers can also live in the rhizosphere and use available carbon substrates for energy. The use of associative N fixers as biofertilizers is a trend in the present agriculture sector and it will be discussed in the later part of this chapter.

1.3 Nitrous Oxide Emissions

The highly potent greenhouse gas, nitrous oxide (N_2O), has increased in concentration in the atmosphere since the beginning of the industrial era and is a critical component of climate change (Jones et al., 2023). In the United States, total N_2O emissions accounted for ~ 6.2% of the total GHG budget in 2021 while agriculture contributed ~75% of total N_2O emissions, which was ~ 294 MMT CO_2 eq (USEPA 2023). The global warming potential of N_2O is approximately ~300 times that of atmospheric CO_2 over a 100-year time frame (Vallero, 2019). Further, N_2O

gas contributes to ozone depletion in the stratosphere (Crutzen, 1970; Crutzen and Oppenheimer, 2008). The global warming potential of N_2O is higher than CO_2 due to the longer atmospheric resident time of N_2O (~114 years) compared to atmospheric CO_2 (100 years) and stronger ozone depletion gas (Aneja et al., 2019). The troposphere accumulation of N_2O gas is transparent to the incoming short-wave radiation, but it absorbs outgoing long-wave terrestrial radiation, which leads to a rise in the temperature of the earth's surface (Braker and Conrad, 2011). Therefore, discussing the N_2O production process in the terrestrial soil N cycle is important.

Soil microbes mediate most of the soil N_2O production through denitrification and nitrification processes(Braker and Conrad, 2011; Cameron et al., 2013). Firestone and Davidson, (1989) discussed the conceptual model of the N gas leaky pipe in the N cycle during nitrification and denitrification. Apart from that, dissimilatory nitrate reduction to ammonium potentially produces N_2O (Braker and Conrad, 2011).

Nitrification is an oxidation reaction that converts NH_3 to NO_3^- in aerobic conditions by nitrifying microbial groups (Firestone and Davidson, 1989) in a two-step process known as nitritation and nitratation. Nitritation is the oxidation of NH_3 to NO_2^- and nitratation is the oxidization of NO_2^- to NO_3^- (Ayiti and Babalola, 2022). In the agricultural systems the nitrification process results in approximately 50% N loss as NO_3^- is more prone to physical loss from soil systems through leaching and denitrification. The factors affecting nitrification are salinity, organic matter concentration, substrate, temperature, pH, C: N ratio, and oxygen levels in the system (Ward, 2008). This process is governed biologically by ammonia-oxidizing bacteria, ammonia-oxidizing archaea, and nitrite-oxidizing bacteria (Beeckman et al., 2018). Further, complete ammonia oxidation (comammox) involves the direct, one-step conversion of ammonium to nitrate by the Comammox organism. Nitrospira bacteria, or complete ammonia-oxidizing bacteria, have been studied by many researchers (Zhu et al., 2022). Due to higher potential fertilizer loss during the nitrification process, the use of nitrification inhibitors was suggested by many studies (Beeckman et al., 2018; Ayiti and Babalola, 2022) .

Nitrous oxide has been identified as a significant contributor to the increase in global mean surface temperature, as highlighted in the IPCC's Sixth Assessment Report (IPCC, 2023), alongside CO_2 and CH_4, which play a critical role in driving the observed warming. The IPCC report suggested that the estimated range of total human-induced global surface temperature increase during the periods of 1850 to 1900 and 2010 to 2019 was likely between 0.8°C and 1.3°C. Global N_2O emissions were calculated mainly through bottom-up and top-down approaches, and many uncertainties were recorded. Emission factor (EF) is a standard bottom-up method that estimates the N_2O emission based on total N inputs (Walling and Vaneeckhaute, 2020). Various emission factors have been introduced, and according to IPCC guidelines for national greenhouse inventories, the default EF to estimate N_2O emission from managed soil is 1%, which can vary with different climatic regions, weather conditions, and site-specific characteristics (Liang and Noble, 2019).

1.4 Biological denitrification inhibition

During the biological denitrification inhibition, biological compounds limit the competition between denitrifying organisms and roots over available NO_3^- in the system (Galland et al., 2019). Certain flavonoids, like procyanidin, inhibit the denitrifying bacterial activity in the rhizosphere; thus, root availability of NO_3^- in the rhizosphere increases and assimilates accordingly. Lack of NO_3^- eventually reduces the denitrification process and reduces the N_2O flux during the process. Most of the time, soil microbes outcompete plant roots to assimilate N, at least in the short term. However, some plant species can gain a higher portion of plant available N (mostly NH_4^+) by developing secondary metabolites and taking control of available N in the rhizosphere (Wang et al., 2021b) . Some *Brachiaria* species' exudate secondary metabolites to inhibit nitrification to gain an advantage over the highly competitive environment in the rhizosphere (Bardon et al., 2014; Galland et al., 2020). Microbes drive the denitrification process, which is aided by transmembrane transport. During this process, periplasmic nitrate reductase enzymes reduce nitrate (NO_3^-) within the system. Procyanidins inhibit membrane-bound nitrate reductase which leads to lower N_2O production in the process. Bardon et al., (2014) suggested that the invasive species *Fallopia spp* reduces N_2O emissions by 95% in the soil.

1.5 The fate of nitrogen in terrestrial ecosystems

The enormous production of synthetic fertilizer to keep up with the global population demand for food has massively changed the availability of N_r on earth and perturbated both the C and N cycles (Gruber & Galloway, 2008). Disrupting natural nutrient cycles can lead to unprecedented, costly outcomes in the future. Plant N uptake occurs through both active enzyme-driven mechanisms and passive processes like mass flow, where dissolved nutrients move to plant roots as they absorb water, as well as through diffusion (Adhikari et al., 2023). Maize is a highly N-dependent crop and it uses higher N fertilizer than most cereal crops. Therefore, the maize cropping system is prone to higher N losses if it is not managed productively (Quan et al., 2021a).

Plants take up N as ammonium (NH_4^+), nitrates (NO_3^-), or as lower-weight amino acids or proteins (Masclaux-Daubresse et al., 2010; Paungfoo-Lonhienne et al., 2008; Rentsch et al., 2007). Plants prefer NH_4^+ over NO_3^- due to the energy cost involved in the assimilation of the N compound to the plant tissues under N deficiency condition (Zayed et al., 2023). A balanced N supply is critical for optimal root development in plants, as both excessive and deficient levels can adversely affect root branching. Ammonium (NH_4^+) is often the preferred form of N, capable of stimulating lateral root branching. However, elevated concentrations of NH_4^+ can inhibit the elongation of primary and lateral roots. Similarly, nitrate (NO_3^-) concentrations in the soil can either promote or suppress the growth of lateral and primary roots, depending on their levels (Jia and von Wirén, 2020). Thus, the availability and composition of N forms in the soil are key regulators of root architecture, with significant implications for overall plant productivity.

Given the critical role of N in plant growth, global N inputs intro agricultural systems have increased substantially over the past decades. The chemical transport model simulation by Ackerman et al., (2019a) indicated an 8% increase of inorganic N globally by 86.6 to 93.6 Tg N Yr^{-1} from 1984 to 2016. Total cropping system N inputs including synthetic fertilizer, organic fertilizer, biological N fixation, and atmospheric N deposition were approximately four times higher in 2010 (161 Tg N Yr^{-1}) than in 1960 (40 Tg N Yr^{-1}). Further, synthetic N fertilizer input increased from 57% (48 Tg N Yr^{-1}) in 2000 to 2015 globally. Nitrogen surplus, which is the difference between N inputs and harvested output, increased by 16 to 86 Tg N Yr^{-1} from 1961 to 2010 (Zhang et al., 2021).

US Midwest maize cropping systems can cause significant health and environmental costs due to N fertilizer management practices. These costs were considered 6 times greater than the profit earned by the farmers (Goodkind et al., 2023). US Midwest maize production accounts for 20% of global maize production which is 60% of the US total maize production (Goodkind et al., 2023). Schlesinger (2009) has shown the various losses associated with the anthropogenically altered N cycle and its potential higher impact on the environment and lives. Goodkind et al. (2023) calculated that the pollution cost coefficient of N_2O was \$66.81/kg N_2O emission and \$0.22 /kg NO_3^- for NO_3^- leaching.

There are higher uncertainties that occur in the evaluation of the negative impacts and costs involved with N fertilizers. The societal impacts and economic burdens due to N_r vary spatially, and therefore, it is important to evaluate based on the site-specific conditions (Keeler et al., 2016).Any stage of a cropping system involves energy costs and GHG emissions associated with the processes. A life cycle assessment of N fertilizer production and utilization in cropping systems involves many GHG emissions throughout the processes. Gao and Cabrera Serrenho (2023) evaluated the GHG gas emission from synthetic N fertilizer and manure production. They assessed that 2.6 Gt CO_2 -equivalent is associated with the production and use of N synthetic fertilizer and Manure (Gao and Serrenho, 2023)

Strategic scenarios were proposed to overcome the challenges involved with N use in cropping systems. Houlton and coworkers highlighted five-pronged strategies: Rapid improvement of N use efficiency, getting N to where it is needed, removing N pollution from the system, reducing food waste, and low-carbon food print diets to reduce the GHG emissions (Houlton et al., 2019). Nitrogen use efficiencies can be increased with many strategies if the N losses are mitigated with different N application scenarios while adopting novel techniques such as soil microbial inoculants and other related products of management techniques (Anas et al., 2020,Maitra et al., 2022).

1.6 Bioinoculants: potential benefits and ecological concerns

Bio-inoculants are defined as living or dormant microorganism strains mainly from bacteria, fungi, algae, or a combination of them directly or indirectly stimulating the soil nutrient cycles for the benefit of plant growth (Shahwar et al., 2023). The use of microbial-mediated bioinoculants in the agriculture sector started in the 19th century, and has increased immensely in the past decade and is about to gain more market presence in the future (Massa et al., 2022; Montoya-Martínez et al., 2022). The bioinoculants or plant growth-promoting organisms (PGPMs) or root colonizing rhizobacteria (PGPR) are considered as an alternative or addition to synthetic fertilizers (Maitra et al., 2022b; O'Callaghan et al., 2022; Olanrewaju et al., 2017). Recently, besides having single strains, using a consortium of strains for the cropping systems was also suggested (Canfora et al., 2021; Seenivasagan and Babalola, 2021). Using such bioinoculants for global crop production systems sounds sustainable and a promising alternative for mitigating environmental effects. Therefore, the availability of microbial-mediated bioinoculants in agriculture will increase with time.

Genetically modified or engineered soil microbial products are available for various agricultural applications. The agronomic and economic benefits of using microbial bioinoculants in crop production have been discussed extensively (Maitra et al., 2022b; O'Callaghan et al., 2022). The soil is a highly heterogeneous medium, and a foreign microorganism's survival is challenging due to the competition for resources and predators. However, the impact of such bioinoculants on native or resident soil microbial communities and their functions has not been discussed comprehensively. Long and short-term temporal effects on native microbial composition and their functions need to be investigated comprehensively for regulatory compliance and to test the overall efficacy of sustainable ecosystems 'services and functions.

Recently introduced N-fixing bacterial strains were genetically engineered and have a competitive advantage over the native soil microbes. *Klebsiella varicella* strains available in the market can fix atmospheric N with or without exogenous N content availability in the soil systems(Wen et al., 2021). Nitrogen-fixing bacteria typically alter their N fixation process when there is an excess amount of N in the soil. When developing these strains, the regulatory genes were removed and took the competitive advantage over the Indigenous N-fixing diazotrophs in the systems (Wen et al., 2021). Such developed bacterial strains have the capability of harnessing more resources temporally or spatially over the native microbes. Anthropologically induced microbial inoculants might be able to out-compete some taxa and use existing resources to spread and grow (Mawarda et al., 2020). If the invader has specific traits, it gives them access to more resources and may promote soil nutrient changes. Using the same bacterial variant seasonally for a longer period for crop production might create a higher competitive environment and alter native microbial diversity. Failure of such invasion by microbial invaders also can cause impact soil microbial communities by shifting the niches and larger shifts can occur with less diversified communities (Mallon et al., 2018). Jack et al. (2021) suggested three ways microbial inoculants may cause unintended consequences; adopting a parasitic/pathogenic lifestyle, escaping to natural habitats other than the applied fields, and disrupting ecosystem services. Further, they suggested that human-mediated actions might amplify microbial invasion, such as repeated application of inoculants.

Extended use of microbial inoculants may change or alter the soil microbial composition, eventually pushing toward alternative stable states in particular ecosystems (Beisner et al., 2003). Further, the resilience of such ecosystems is at stake if the system's diversity gets diluted when considering the “diversity-stability hypothesis”. A study on the impact and temporal succession trajectories of native soil communities with varying levels of diversity, following invasion by *Bacillus mycoides* (BM) and *Bacillus pumilus* (BP), showed that the invasion could affect the resident community regardless of its success. However, a stronger impact was observed when the invader successfully established itself in the community (Mawarda et al., 2022). Further, they observed the Alpha diversity, richness, evenness, and phylogenetic diversity in the invaded soil microbiomes by BM were significantly different between treatments.

The introduction of a commercially available AMF inoculant in pea cultivation survived and altered the relative abundance of eight native AMF taxa, which was site-specific (Islam et al., 2021). Further, they suggested that competing with residence taxa depends on climate conditions. A bioinculant for managing the soil-born fungus disease in banana cultivation induced general pathogenic suppression (Fu et al., 2017). Moreover, the inoculant created a broader microbial shift in the soil mesosphere. They suggested that bioinoculants were the primary driver in reducing fungal abundance, causing general disease suppression (Fu et al., 2017). Wang et al., (2021)evaluated the impact of microbial inoculants on native microbial communities and functions at different time points. Periodic inoculation affected the succession of resident bacterial communities in bulk soil, with abiotic factors such as soil temperature, moisture, and pH playing a role in shaping these changes. Furthermore, the native microbiome exhibited temporary resistance to bioinoculant applications, but its resilience was disrupted by repeated inoculations. They suggested that the second inoculation made noticeable changes in the native microbial community structure. The periodic use of bioinoculants and their persistence in the soil are discussed in the agriculture sector to gain more agronomic benefits Wang et al., (2021c).

The novel-developed bioinoculants challenge the dynamics of the soil microbial communities and their functions. Therefore, it is necessary to investigate the direct and indirect effects of bioinoculants on the soil ecosystem to establish a sustainable ecosystem. Microbial invasion frameworks that are linked to microbial bioinoculants have been discussed (Mawarda et al., 2020). Ecological secondary succession could be possible after the long-term use of bioinoculants in the agroecosystems, potentially removing keystone species and leading to an ecological tipping point

Disturbance of native soil microbial communities can occur in response to the introduction of alien species that has the potential to invade the native community or shift the diversity. In contrast, the native community may be highly resistant to such invasion, specifically in the bulk soil systems. Soils with more diverse native communities have a greater ability to exploit and compete for available resources. However, if the alien species can overcome the native biotic resistance, the displacement of the non-resilient microbiome is a possibility. Previous studies have shown that invasive species can leave a footprint in new environments and have a legacy effect on native communities. A microcosm study inoculated with *E. coli* strains was monitored for 120 days and the native communities were not resistant to invasion and native bacterial diversity declined (Xing et al., 2021).

Cornell et al. (2021) found that inoculation significantly affected soil microbial diversity, and those effects were mediated by inoculant type, diversity, and disturbance regime. Fungal diversity was not affected by the inoculation, but the bacterial communities changed. Mawarda et al. (2020) reported that 86% of their studies in a meta-analysis suggested that inoculants modified soil microbial communities in agricultural systems. Moreover, regarding the long-term effects of these inoculants, 80% of studies reported that the native microbial communities failed to revert to their original state following inoculation (Mawarda et al., 2020).

Several conceptual models have been proposed to elaborate on the potential negative microbial effect in native soil microbial communities. Mawarda et al. (2020) proposed four mechanisms that changed the soil microbial dynamic after inoculation. During the initial phase of inoculation, a new microbial strain with an initially higher population may compete with the native taxa for resources and might stimulate unrelated taxa multiplication. Non-established bioinoculants may leave lasting changes and unoccupied niches in the systems. Further, antagonism and synergism between bioinoculants and native soil microbial communities can occur. Moreover, bioinoculants can affect native communities by modifying the rate and composition of exudates released by plant roots (Dos Santos and Olivares, 2022). It is indeed a challenge to evaluate the inoculation-induced changes in any ecosystem. Investigating the long-term persistence of microbial inoculants, their impacts, or their footprints in the ecosystems and understanding the cascading effect would be a demanding task for researchers in the future.

A comprehensive evaluation of soil N dynamics is essential for advancing our understanding of soil N dynamics. Enhanced empirical research endeavors are pivotal in unraveling the complexities of soil N dynamics, thereby facilitating the development of more accurate predictive mechanisms for future cropping system scenarios. This chapter delves into the fundamental biological concepts underlying the soil N cycle and underscores their significance in comprehending N cycle dynamics. Additionally, key management strategies are proposed, alongside an examination of the latest advancements in novel N-fixing microbial inoculants. This discussion not only explores the potential benefits but also addresses ecological concerns associated with using bioinoculants, thereby contributing to establishing a theoretical framework for their application and evaluating the N dynamics in the corn cropping system.

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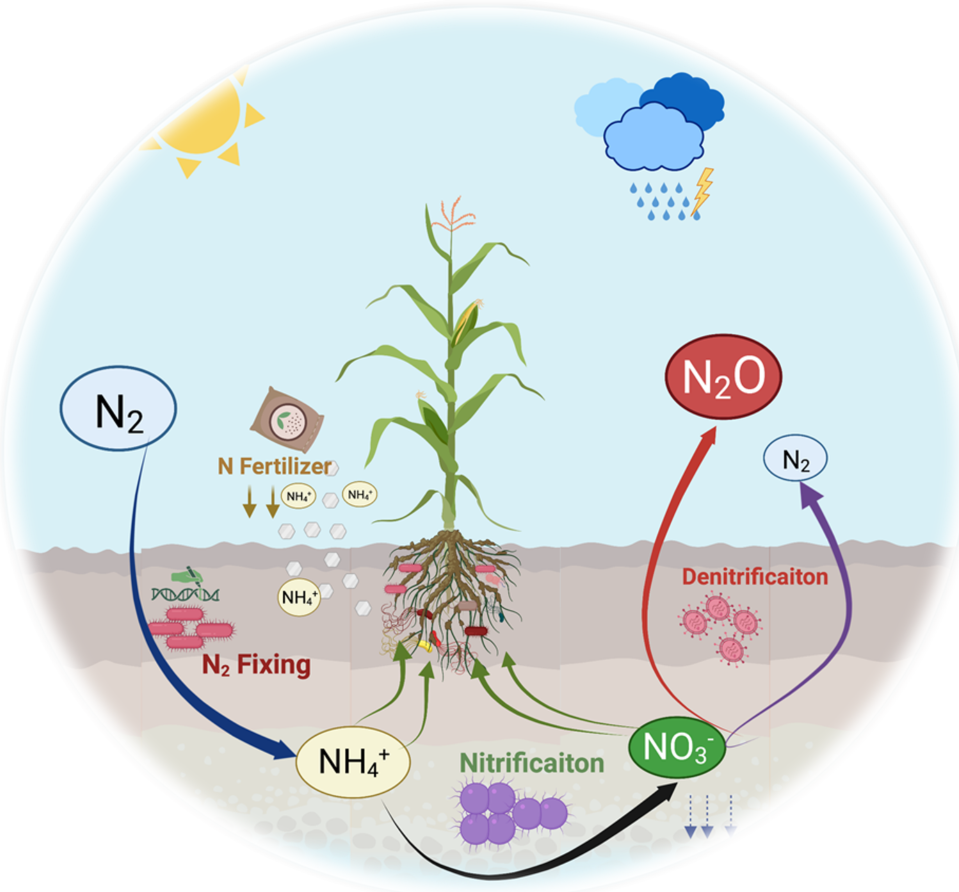

Figure 1.1. The nitrogen cycle in agricultural systems.

This illustrates the nitrogen (N) cycle within an agricultural corn cropping system. The figure highlights the interconnected pathways of nitrogen movement, showing beneficial plant uptake and N losses. Plant essential ammonium (NH_4^+) is supplied as N fertilizer (Urea). In addition to N fertilizer application, atmospheric nitrogen (N_2) is converted into ammonium (NH_4^+) through biological nitrogen fixation, specifically by a novel-developed N-fixing microbial inoculant. The (NH_4^+) is then subject to nitrification, a two-step microbial process that oxidizes NH_4^+ to nitrate (NO_3^-). Plants assimilate NH_4^+ and NO_3^-. Nitrates are susceptible to loss due to leaching. Additionally, denitrification occurs under anaerobic conditions, where NO_3^- is reduced to gaseous forms such as nitrous oxide (N_2O) and dinitrogen (N_2), contributing to atmospheric greenhouse gas emissions.

Chapter 2 - Nitrous oxide (N_2O) flux in maize cropping systems with a nitrogen-fixing microbial inoculant

Abstract

Nitrous oxide (N_2O) is a potent greenhouse gas and a major pathway for nitrogen (N) loss in agricultural systems, making mitigation crucial for reducing agriculture's environmental impact. This study examines the effect of a N-fixing bacterial bioinoculant on N_2O emissions and N dynamics in corn cropping systems over the 2022 and 2023 growing seasons at the North Farm in Manhattan, KS, managed by the Kansas State University Agronomy Department. A randomized complete block design was employed, with N fertilizer rates of 0, 56, 112, and 168 kg N ha^{-1}, applied with and without the bioinoculant across six replicates. Nitrous oxide fluxes were measured bi-weekly using a static chamber technique, and emission factors (EF%) were calculated to evaluate the proportion of applied N lost as N_2O. N_2O emissions increased significantly with higher N fertilizer rates in both years. In 2022, the highest daily N_2O flux was 0.151 kg N_2O-N ha^{-1} day^{-1}, observed in plots treated with 168 kg N ha^{-1} and bioinoculant, with cumulative emissions at 1.13 kg N_2O-N ha^{-1}. The lowest cumulative emission was 0.075 kg N_2O-N ha^{-1} in the fertilizer control plots treated with bioinoculant. In 2023, N_2O fluxes were more evenly distributed, with a peak flux of 0.077 kg N_2O-N ha^{-1} day^{-1} at the 112 kg N ha^{-1} with bioinoculant and cumulative emissions reaching 0.8 kg N_2O-N ha^{-1} at the highest N rate. While bioinoculant-treated plots consistently showed lower N_2O emissions than untreated plots, these reductions were not statistically significant. All treatments' emission factors (EF%) remained below 1% for both years, indicating a low contribution of fertilizer-induced N_2O emissions. Specifically, in 2022, EF% was 0.38% for bioinoculant-treated plots compared to 0.51% in

untreated plots, whereas in 2023, bioinoculant-treated plots exhibited an EF% of 0.56% compared to 0.35% in untreated plots. The N_2O-yield linear relationship for both years showed a contrasting trend. Plots without the N-fixing bioinoculant depicted a clear significant linear trend for both years for the N_2O-yield curve, but the effect was unclear with bioinoculant. These findings indicate the significant role of N fertilizer rates in driving N_2O emissions and the uncertainties of using N-fixing microbial application in continuous corn cropping systems to evaluate N_2O. The study highlights the need for further research to optimize bioinoculant application strategies, aiming to improve N use efficiency and reduce the environmental footprint of corn production systems.

2.1 Introduction

Global atmospheric nitrous oxide (N_2O) emissions have increased significantly in the last four decades, and the atmospheric concentration of N_2O has increased from an average of 315.8 ppb in 2000 to 335.9 ppb in 2022 (Tian et al., 2024). Nitrous oxide is one of the most potent greenhouse gases with significant stratospheric ozone-depleting capabilities (Crutzen, 1970; Ravishankara et al., 2009). Nitrous oxide has a long atmospheric lifespan of about ~ 110 years and a global warming potential approximately 300 times greater than carbon dioxide (CO_2). Fertilizer-induced direct N_2O emissions are a significant portion of global N_2O emissions. Global N_2O emissions increased by 180% from 1961 to 2014 in croplands, and synthetic N applications accounted for ~ 70% of total emissions from 2000-2020 (Xu et al., 2020). In the United States, agriculture accounts for 74.6% of total N_2O emissions, which is 4.6% of total gross GHG emissions in the US (U.S. Environmental Protection Agency, 2024).

In the U.S. Corn Belt, N_2O emissions were estimated to be significant due to high N fertilizer use. From 1987 to 2012, N fertilizer use in the US Corn Belt averaged 6.2 ± 0.9 Tg N per year, with an annual increase of 0.08 Tg N. Simultaneously, biological N fixation increased due to expanded soybean production, contributing to an estimated rise in N_2O emissions of at least 4 Gg N_2O-N per year (Griffis et al., 2017). Further, a six-year analysis of hourly N_2O mixing ratios in the US corn belt region by Griffis et al., (2017) revealed significant interannual variability in emissions, ranging from 316 to 585 Gg N_2O-N per year. Regional emission factors were highly sensitive to climate, with the warmest year (2012) showing an emission factor of 7.5%, nearly double the previous estimates. Indirect emissions from runoff and leaching primarily contributed to this variability. Under current climate and N use trends, N_2O emissions in the region are projected to exceed 600 Gg N_2O-N annually by 2050, indicating a potential increase in emissions from other agricultural regions (Griffis et al., 2017). The nitrogen-related health and environmental costs of maize production are six times greater than the profits earned by farmers in the Midwest of the US. As food demand grows, agriculture's environmental and human health impacts will likely increase, resulting in higher social costs. New agricultural practices may be required to achieve significant societal benefits while maintaining profits and mitigating negative impacts (Goodkind et al., 2023).

Best management practices for fertilizer N applications in agroecosystems are vital to reduce environmental impacts. Adopting the 4R principles of nutrient management, selecting the right source, rate, time, and place is crucial in improving crop N uptake and reducing N losses through leaching, runoff, and gaseous emissions such as N_2O and ammonia (Snyder et al., 2009). In rainfed agricultural systems, achieving significant reductions in N_2O emissions may require more comprehensive strategies that simultaneously adjust several 4R components. By fine-tuning the timing and type of N application, it is possible to moderately lower N rates while maintaining crop yields and decreasing emissions. However, relying on a single modification, like delayed or split applications or utilizing specialized N fertilizers with inhibitors, has proven unreliable in consistently reducing N_2O emissions. Consequently, an integrated approach that incorporates multiple 4R strategies is necessary for more effective and consistent emission reductions (Snyder et al., 2009; Venterea et al., 2016; Banger et al., 2020a).

The adoption of microbial inoculants in agriculture and the latest technological developments is crucial for addressing future scenarios. Nitrous oxide-respiring bacteria and other microbial consortia have been a focal point for many researchers (O'Callaghan et al., 2022; Hiis et al., 2024). Implementing N_2O reduction strategies can improve soil and environmental quality while preserving atmospheric conditions, thus contributing to sustainable development.

Evaluating different management techniques to mitigate N_2O emissions is crucial in the agricultural sector, aiding in better management decisions regarding N fertilizer use for future scenarios. The social cost of N_2O is significantly higher than most key greenhouse gases (Serrao-Neumann et al., 2021). Implementing N_2O reduction strategies could qualify farmers for N or carbon credit programs, ultimately helping to achieve better atmospheric quality for present and future generations.

Given these findings, it is crucial to investigate the potential factors influencing N_2O emissions and develop strategies for effective mitigation. Implementing improved management practices can significantly reduce agricultural N_2O emissions, helping address environmental and economic challenges. Nitrification and denitrification processes in the soil N cycle lead to N_2O production (Kox and Jetten, 2015). Most N_2O is released from the soil under anaerobic conditions, where soil nitrate is denitrified by heterotrophic soil microbes (Schlesinger, 2009). When soil water-filled pore space reaches 70%, anaerobic conditions favor N_2O production (Linn and Doran, 1984). During anaerobic conditions, denitrification is triggered as a function of soil nitrate availability, soil temperature, and low soil O_2 content. Apart from anaerobic conditions, other factors such as pH, substrate availability, and moisture content (which drives soil oxygen concentration) also influence N_2O production (Xu et al., 2020; Wang et al., 2021a; Pan et al., 2022). Soil nitrifying bacteria also emit low amounts of N_2O (Ward, 2008).

While nitrification and denitrification processes contribute to N_2O emissions, biological N fixation (BNF) offers a natural and sustainable method to mitigate these emissions. BNF, performed by specific microorganisms, converts atmospheric N into a form that plants can utilize, significantly reducing the need for synthetic N fertilizers. This process supports plant growth and enhances soil health by maintaining ecosystem N balance. As agriculture seeks to minimize its environmental footprint, optimizing BNF becomes increasingly essential for sustainable crop production and effective N_2O emission reduction. As described by Arora et al., (2016) BNFs can reduce the use of N fertilizers by approximately 0.160 billion tons per year, equating to a reduction of 0.270 billion tons of coal consumed in the industrial production process. The symbiotic relationship between legumes and rhizobium bacteria is crucial in symbiotic N fixation. Inoculating legume crops with rhizobia has become one of agriculture's most successful applications of biofertilizers. The positive effects of diazotrophic microorganisms on crop production have significantly expanded the biofertilizer market (Soumare et al., 2020).

Biofertilizer use in agriculture has increased immensely since the beginning of the 21st century, and the use of soil-mediated microbial strains and the development of such applications gained popularity with the development of sustainable agriculture (Daniel et al., 2022; Ammar et al., 2023). Alternatives to synthetic fertilizers are a trending topic due to their environmental impacts. Among microbial inoculants, *Bacillus* spp. are widely used and commercially available as biofertilizers (Ammar et al., 2023).

The use of biofertilizers and microbial inoculants to reduce N_2O emissions has garnered interest from many stakeholders (Samantaray et al., 2024). Various bioinoculant products with different microbial strains have been implemented in recent years (Maitra et al., 2022b). Research on microbial inoculants and their impact on N_2O emissions is crucial for developing sustainable agricultural practices that benefit both the environment and agricultural productivity.

Recently, many microbial bioinoculants have been introduced to enhance crop yields. One such novel N-fixing diazotrophic bioinoculant, commercialized by Pivot Bio as Proven 40, contains genetically edited strains of *Klebsiella variicola* and *Kosakonia sacchari* (Franzen et al.,2023; Wen et al., 2021). These associative N-fixing bacterial strains are promising to replace up to ~ 45 kg N ha^{-1} of N fertilizer in corn cropping systems. However, Pivotbio™ Proven 40 has not been extensively evaluated for its impact on N_2O emissions in corn cropping systems as a novel N-fixing biological inoculant. Targeted field studies with in-depth analyses of N_2O flux and N dynamics under the influence of novel N-fixing microbial inoculants are crucial for future sustainable agriculture. Understanding how these bioinoculants affect N_2O emissions can inform better management practices and help mitigate the environmental impact of agricultural activities.

Therefore, the objectives of this study were (i) to measure the N_2O emissions from fields inoculated with N-fixing microbial applications at different N fertilizer rates and (ii) to evaluate the emission factors and N_2O-yield curve associated with using N-fixing bioinoculants in continuous corn cropping systems. This research aimed to provide insights into the possible environmental benefits by evaluating N_2O emissions in continuous corn cropping systems for sustainable agriculture practice.

2.2 Materials and methods

2.2.1 Study site

This experiment was conducted during the growing season from 2021 to 2023 at the North research farm managed by the Kansas State University Agronomy department (39°12' N, 96°35' W). The soil types at the experimental site were Ivan and Kennebec silt loams (*fine-silty, mixed, super-active, mesic Cumulic Hapludolls*). Corn (*Zea mays* L.) was planted with a N-fixing bioinoculant (PivotBio® Proven 40, Pivot Bio Inc., Berkeley, CA) on June 4, 2021, June

19, 2022, and April 27, 2023. Continuous corn was cultivated in 2021, 2022, and 2023 with four N fertilization rates (0, 56, 112, and 168 kg N ha^{-1}) using broadcast-applied urea, both with and without the bioinoculant. The bioinoculant was applied in-furrow at a rate of 1.90 liters per hectare (25.6 fl. oz per acre) with a carrier volume of 46.8 liters per hectare (5 gallons per acre). The study employed no-till management and was arranged as a randomized complete block design (RCBD) with six replications, using a split-plot design to accommodate the presence and absence of bioinoculant. The bioinoculant was applied according to the product's recommended formulation.

Above-ground biomass was harvested at the V8, VT, and R6 corn developmental stages, with grain harvested in September and October each year. All above-ground biomass samples were dried in a forced air oven at 50°C, ground to pass through a 2 mm sieve, and analyzed for total nitrogen (N) and total carbon (C).

Soil samples were collected at each growth stage, including baseline soil samples taken before planting each year. Soil samples were collected from depths of 0-5, 5-15, and 15-30 cm in each plot and analyzed for total C and N using standard soil analysis, and the bulk density of each soil depth was taken once a year. Biomass and soil samples, including grain samples, were analyzed using a LECO C and N automated analyzer (St. Joseph, Michigan, USA), employing combustion methods. Soil inorganic N was extracted by mixing moist soil with 1 M KCl at a 1:4 ratio in an Erlenmeyer flask and shaking for 1 hour. The supernatant was decanted and filtered through the Whatman No. 42 paper, then analyzed for NO_3^- and NH_4^+ using a continuous flow colorimetric analyzer by the Soil testing lab at Kansas State University. Soil moisture was determined by drying 10 g of soil at 105°C until a constant weight was achieved.

2.2.2 Nitrous oxide measurements

The growing season N_2O measurements were taken from April through October, corresponding to the planting and harvest dates, which varied yearly. The frequency of measurements was determined by fertilizer application and rainfall events: twice a week from planting to the VT stage and once a week from the VT stage to harvest. Gas samples were collected the following day after significant rainfall events (>10mm). Soil moisture and temperature at a depth of 5 cm were recorded during each gas sampling event. Daily precipitation was obtained from a weather station (Mesonet) close to the research plots managed by K-State Agronomy. In addition, soil moisture and temperature at 5-10 cm were collected using a HydroSense handheld soil moisture sensor.

Rectangular-shaped aluminum box chamber techniques were used for gas sampling. Gas chambers (0.5 x 0.29 x 0.9 m deep) were constructed from 20-gauge stainless steel steam pans, with the bottom removed to allow insertion into the soil so the flange was seated with the soil surface. These anchors were installed 24 hours before the first gas sampling event and remained in place until harvest. The chambers were insulated, vented, and equipped with a flange with rubber weatherproof stripping liners and a gas sampling port as per the guidelines by Clough et al. (2020). The sampling port had a rubber septum on one end and a manifold on the other, with four fluorinated ethylene propylene tubes branching out to each quadrant of the chamber to ensure sample homogeneity.

Samples were collected between 0900 and 1200 local time. The chamber tops were secured to the anchors on each sampling day using binder clips. Gas samples of approximately 25 mL were collected at 0, 20, and 40 minutes after deployment of the chamber cover, using a

30-mL medial-grade syringe and needle. Collected gas samples were transferred to pre-evacuated vials. The concentrations of N_2O were determined using gas automated chromatography (GC) (Brucker) equipped with a ^{63}Ni electron capture detector. The instrument was calibrated daily before analysis using four analytical-grade standards levels (0.2, 0.51, 3.5, and 15 ppm). The daily N_2O-N flux calculations methodology was performed as described by Mosier and Hutchinson, (1981) and Arango and Rice, (2021).

When the chamber's gas concentration decreased steadily with time points (0, 20, and 40 minutes) ($C_0<C_1$, $C1>C2$) in a non-linear trend, then the flux was calculated following Hutchinson and Mosier's (1981) equation:

$$f_0 = kd\left[\frac{273}{T}\right]\frac{V(C_1-C_0)^2}{At_1(2C_1-C_2-C_0)}\ \ln\left[\frac{C_1-C_0}{C_2-C_1}\right], if\ \ \ln\left[\frac{C_1-C_0}{C_2-C_1}\right] > 1$$

$$f_0 = kd\left[\frac{273}{T}\right]\frac{V}{A}\left[\frac{\Delta C}{\Delta t}\right], \text{if } \ln\left[\frac{C_1-C_0}{C_2-C_1}\right] \leq 1$$

Where;

f_0 = the flux rate (g ha^{-1} day^{-1})

k = 144000 (the unit of conversion)

d = 1.25 x10-3 g cm^{-3} (gas density at 273.15 K and 0.101 MPa pressure)

T = the air temperature (K)

V = the volume within the chamber (cm^3)

A = The area occupied by the chamber is (cm^2)

C = the gas concentration in ppm [v/v]

$\Delta C/\Delta t$ = gas concentration change

After measuring and calculating the daily N_2O fluxes, cumulative N_2O fluxes were calculated using trapezoidal integration of daily fluxes and time.

The N_2O emission factor (EF%) was calculated by dividing the cumulative N_2O flux difference between treatment and control by the N fertilizer rate. For this study, I calculated the N fertilizer-induced emission factor only. Further, the N_2O emission-yield curve was assessed to identify the direction and magnitude of the curve change by adopting an N-fixing bioinoculant. The relationship between N_2O emission and the yield was assessed using a linear plot. Cumulative N_2O emission and yield curve indicated the relationship between N_2O emissions and yield.

2.2.3 Statistical analysis

Statistical analyses were performed using Python's `Statsmodels` library. A general linear model was applied to analyze variance (ANOVA) to evaluate each response variable. For the cumulative N_2O flux with N rates and the N_2O flux-yield model, a significance level of $\alpha = 0.05$ was used. Post-hoc mean comparisons were conducted using the Least Significant Difference (LSD) test to identify specific means that were significantly different after significant ANOVA results were obtained. The LSD test provided detailed insights into the differences between treatment groups, facilitating a comprehensive understanding of the effects of N rates and bioinoculant application on N_2O flux.

2.3 Results

2.3.4 Growing season precipitation and water-filled pore spaces (WFPS%)

Total precipitation for the 2022 and 2023 growing seasons was 311 mm and 346 mm, respectively (Figures 2.1 and 2.2). During 2022, there were 27 precipitation events, with only 8 exceeding 10 mm. Notably, the first 40 days after planting had 219 mm of precipitation, with 91 mm falling within the first ten days across four events in 2022. In contrast, there were 36 precipitation events, 12 surpassing 10 mm, in 2023. During the first 40 days after planting in 2023, 147 mm of precipitation was recorded, and within the first ten days, there was only one rain event of 12.95 mm. Growing season precipitation in 2021, 2022, and 2023 (Table A2.1) was below average compared to most previous years, and total annual precipitation was below the 30-year average for Manhattan, Kansas, USA. The 2021 data, included in the appendix (Figure A2.1), are not discussed in detail here due to the significantly drier conditions in that year compared to 2022 and 2023. During the 2021 growing season, total precipitation was 299 mm across 22 precipitation events. In the first 40 days after planting, 11 precipitation events occurred, totaling 98 mm, with only 5.08 mm falling within the first ten days.

In 2022, the water-filled pore spaces (WFPS) % at the beginning of the growing season ranged between 60-70%. Higher WFPS% was associated with higher precipitation events at the beginning of the season, and lower precipitation resulted in lower WFPS% potential during the remainder of the growing season in 2022. After the first 40 days of planting, WFPS% fluctuated between ~20-60% till the end of the growing season (Figure 2.9). In 2023, higher WFPS% was also recorded at the beginning of the growing season, associated with higher precipitation events (Figure 2.10). However, later in the season, the WFPS% dropped due to the drier condition of the soil. During the later period of the growing season, WFPS% ranged from ~20-60%.

2.3.5 Daily N_2O flux for the growing season, 2022-2023

During the first 20 days of the growing season, higher N_2O fluxes were observed in 2022 than in 2023 (Figures 2.3 and 2.4). The highest flux during this period was 0.151 kg N_2O-N ha^{-1} day^{-1}, which occurred with 168 kg N ha^{-1} combined with the bioinoculant. The highest average emission for the first 40 days was 0.032 kg N_2O-N ha^{-1} day^{-1} with the 168 kg N ha^{-1} application rate without bioinoculant. The lowest emission during the growing season 2022 was 0.0017 kg N_2O-N ha^{-1} day^{-1}, recorded with 0 kg N ha^{-1} with bioinoculant.

Throughout the 2022 growing season, the majority of the N_2O emissions occurred within the first 40 days after planting (Figure 2.3). The lowest emissions were observed after 60 days of planting. However, later in the season, relatively higher emissions were noted in the 0 kg N ha^{-1} associated with bioinoculant compared to those without the bioinoculant.

During the first 20 days of the growing season, there was a notable increase in N_2O fluxes (Figure 2.4) in 2023. The highest flux during this period was 0.077 kg N_2O-N ha^{-1} day^{-1}, which occurred with 112 kg N ha^{-1} combined with the bioinoculant. Over the first 40 days of the 2023 growing season, the average N_2O emissions were lower than the first 40 days of 2022. The highest average emission during this period was 0.014 kg N_2O-N ha^{-1} day^{-1}, observed with 168 kg N ha^{-1}. Conversely, the lowest average emission over the same period was 0.0043 kg N_2O-N ha^{-1} day^{-1}, recorded with 0 kg N ha^{-1}).

The N_2O fluxes were more evenly spread throughout the 2023 growing season (Figure 2.4) than in 2022. The fluxes decreased later in the growing season than in the earlier stages of the growing season. N_2O flux intensities were higher in 2022 than in 2023. Further higher emissions were observed in the first 20 days after planting.

2.3.6 Cumulative N_2O emissions

The summary of cumulative values for years 2022 and 2023 with their mean difference is shown in Table 2.2. For the 2022 growing season, the highest cumulative emission was 1.13 kg N_2O-N ha^{-1} with 168 kg N ha^{-1}, while the lowest cumulative emission was 0.070 kg N_2O-N ha^{-1} with 0 kg N ha^{-1} (Figure 2.5). The highest N rate (168 kg N ha^{-1}) inoculated with bacteria was 1.03 kg N_2O-N ha^{-1} cumulated N_2O emission. At the 112 kg N ha^{-1} with and without the bioinoculant, the cumulative N_2O flux was 0.76 and 0.64 kg N_2O-N ha^{-1}, respectively. Cumulative N_2O emissions at 56 kg N ha^{-1} with and without bioinoculant were 0.28 and 0.24 kg N_2O-N ha^{-1}. The cumulative N_2O flux was 0.075 kg N_2O-N ha^{-1} in the control plot with the bioinoculant application. Without application, it was 0.70 kg N_2O-N ha^{-1} for the N_2O reduction using bioinoculant, this reduction was not significant.

The higher cumulative values were statistically significant ($p<0.001$) (Table 2.1) when comparing the lower N application rates of 56 kg N ha^{-1} and the control plot to the 168 kg N ha^{-1}. The overall N_2O cumulative emission increased with the N fertilizer application rate. The highest cumulative N_2O flux was ~ 167 % higher than the control and 64% higher than the 56 and 112 kg N ha^{-1}, respectively. Bioinoculant reduced the overall N_2O flux by 10-20% across all N application rates, except for the control, where relatively lower N_2O fluxes were observed with the bioinoculant.

Table 2.1. Analysis of variance (ANOVA) results of cumulative N_2O flux measurement for 2023.

Source of variation	----------P values--------- 2022	2023
N-rate	**<0.001**	**<0.001**
Bioinoculant	0.603	0.275
N-Rate * Bioinoculant	0.982	0.074

Analysis of variance (ANOVA) for cumulative N_2O flux measurements in 2022/ 2023 examined the effect of different Nitrogen rates (N-Rate: 0, 56, 112, and 168 kg N ha^{-1}) as urea fertilizer and bioinoculant application. Bioinoculants were applied in-furrow at planting. (α=0.05)

Table 2.2. Cumulative N_2O flux measurement for 2023.

N Rate	Cumulative flux 2022				2023			
	With bio		Without bio		With bio		Without bio	
kg N ha^{-1}	------------------------*kg N_2O-N ha^{-1}*						--------------------	
0	0.08	***b***	0.07	***b***	0.13	***b***	0.08	***b***
56	0.22	***b***	0.27	***b***	0.63	***a***	0.28	***ab***
112	0.53	***ab***	0.62	***ab***	0.52	***a***	0.37	***ab***
168	1.03	***a***	1.13	***a***	0.58	***a***	0.79	***a***

Nitrogen rates (N Rate) are 0,56, 112, and 168 kg N ha^{-1} as urea fertilizer. Cumulative flux in kg N-N_2O ha^{-1} in mean values. The same lower-case letters are not significantly different at α=0.05

In the 2023 growing season, the highest cumulative emission was 0.8 kg N_2O-N ha^{-1} with an application of 168 kg N ha^{-1}, while the lowest cumulative mean emission was 0.079 kg N_2O-N ha^{-1} with no N application (0 kg N ha^{-1}) (Figure 2.6). No significant difference was observed with the bioinoculant. Cumulative N_2O fluxes were significantly higher with N fertilizer rates than the control (0 kg N ha^{-1}). The bioinoculant at the highest N rate (168 kg N ha^{-1}) resulted in relatively lower cumulative N_2O fluxes than without the bioinoculant.

2.3.7 Emission factor

Emission factors (EF) were calculated for treatments with and without bioinoculant over two growing seasons, 2022 and 2023 (Figure 2.10). In 2022, the EF% of plots treated with bioinoculant was 0.38%, while the untreated plots depicted 0.51%. The lower EF% indicated a lower N_2O emission associated with the bioinoculant treatment during the 2022 growing season. However, the trend was changed in 2023. Higher EF% was recorded for the bioinoculant-treated plots, which was 0.56% compared to 0.35% for the untreated plots. Direct fertilizer-induced EF% was below 1% for both years. Despite year-to-year dynamics, EF% remained consistently below 1% across all treatments in both years, indicating a relatively low contribution of fertilizer-derived N to N_2O emissions for the study period.

2.3.8 N_2O emissions-yield curve

Figures 2.12 and 2.13 illustrate the linear relationship of N_2O flux (kg N_2O-N ha^{-1}) with yield (kg ha^{-1}) for 2022 and 2023. In 2022, slope, R^2, and p values of the linear curve associated with bioinoculant were 6.2952×10^{-5}, 0.14, and 0.07, respectively. The slope and R^2 values with no bioinoculant application were 1.58×10^{-4} and 0.53, respectively. The linear curve depicted a significant ($P<0.001$) trend with no bioinoculant application. Root means square error (RMSE) values with and without bioinoculant were 0.35 and 0.34, respectively. For 2023, the curve slope associated with bioinoculant was $6.7\text{x}10^{-5,}$ with an R^2 value of 0.71. With bioinoculant, the slope was $3.5\text{x}10^{-5}$, and the R^2 value was 0.30. Both curves were significant at a=0.5 where without bioinoculant was $p<0.001$ and with bioinoculant $p=0.026$. The quadratic trend can fit well with N rates, and the bioinoculant trend is unclear (Figures 2.14 and 2.15). Root means square error (RMSE) values in 2022 and 2023 without bioinoculant application were 0.31 and 0.09.

2.4 Discussion

Daily N_2O emissions were higher at the beginning of the 2022 growing season. Out of total growing season precipitation, ~70% of precipitation occurred in the first 40 days after fertilization. Significant precipitation events occurred within the first 10 days following fertilization with urea (broadcast application), leading to high-intensity fluxes across all treatments in the corn cropping systems. This early spike in emissions can be attributed to the increased soil moisture levels from precipitation, which promote greater water-filled pore spaces (WFPS%) that favor the microbial-mediated denitrification processes that produce N_2O from the soil (Linn and Doran, 1984; Song et al., 2019). Water-filled pores spaces (WFPS%) did not exceed 70% across both years (Figures 2.8a and 2.8b). Most of the N_2O fluxes were observed with 60% - 70% of WFPS in 2022 and 2023. As described by Schlesinger, (2009), the denitrification process primarily governs N_2O fluxes. Further, higher emissions could occur during higher precipitation events if the oxidized form of N is available in the soil.

Cumulative N_2O flux in 2022 was ~43% greater than in 2023, with the highest N rate. In contrast to 2022, only ~42% of total precipitation occurred in the first 40 days after fertilization in the 2023 growing season. The first 10 days after fertilization in 2023 received only 12.5 mm of precipitation from a single event, which was significantly lower than the 91 mm of rain observed during the same period in 2022. Consequently, we did not observe higher fluxes in the first 10 days of fertilization in 2023, as precipitation is a crucial driver of N_2O emissions. Furthermore, in 2023, the fluxes were spread throughout the season, but the overall intensity of all the flux regimes was lower compared to 2022.

Most fluxes in 2022 were observed early in the planting season. The highest N_2O fluxes were observed in the first 10 days after corn planting and fertilizer application. N_2O emissions were decreased after 40 days. Many other studies have reported higher flux regimes earlier in the growing season. Research conducted by McGowan et al., (2019; Arango and Rice, (2021) noted that N_2O emissions tend to be higher during the early stages of crop growth and then decline in the latter phase of the growing season. During the latter period of the growing season, the lower denitrification occurred mainly due to less availability of the reduced form of N in the soil as N was assimilated by the plants and loss by leaching (Govindasamy et al., 2023).

The highest cumulative N_2O emission was recorded under the highest N application rate, amounting to 1.13 kg N_2O-N ha^{-1}. This value, although significant, was lower than the emissions reported by McGowan et al., (2019) and Arango and Rice, (2021) for the continuous corn with no-till operations on a silt -loam soil. The discrepancy may be due to the lower average growing season precipitation events in 2022 compared to previous years, which could have caused more N to be leached away or utilized by plants, thereby reducing the amount available for microbial processes that produce N_2O.

fertilizer N application rate significantly affected ($P<0.001$) cumulative flux during the 2022 and 2023 growing seasons. An increase in fertilizer rate corresponds with higher cumulative N_2O emissions (Roy et al.,2014). Furthermore, Roy et al. (2014) reported that higher N inputs lead to increased N_2O emissions.

The corn plots treated with N-fixing microbial inoculant had a lower trend in cumulative N_2O flux in 2022, although the effect was not statistically significant. Some plant-growth-promoting rhizobacteria products or mixtures of microbial strains (bioinoculant) have shown lower N_2O emissions associated with different fertilizer types in corn (Calvo et al., 2013, 2016). Calvo et al. (2016) further argued that using such microbial-mediated bioinoculants with urea did not significantly impact cumulative N_2O emissions in greenhouse experiments. Using N-fixing microbes might increase below-ground root growth, leading to higher inorganic N consumption, eventually lowering N_2O emissions due to reduced nitrification potential. Research conducted by Wang, (2022) on invasive plant species and biological N-fixing strains showed higher root mass and growth in poor N soils. In corn cropping systems in Illinois, USA, unpublished data showed higher below-ground biomass with the application of the same nitrogen-fixing microbial inoculant, suggesting that the bioinoculant may enhance nitrogen recovery efficiency (Woodward, unpublished data, 2023).

In contrast, the cumulative N_2O flux in 2023 did not show a consistent trend with soil microbial inoculant application (Figures 2.7a and 2.7b). Only the highest fertilizer rate with inoculants showed a decrease in N_2O flux, which was not statistically significant. Higher fluxes were recorded in inoculated plots where N-fixing microbial application increased the cumulative flux in the 112 and 56 kg N ha^{-1} plots. The bioinoculant activity can be governed by many constraints, which might explain the vague behavior of fluxes with inoculant application. Symbiotic N-fixing bioinoculants can influence below-ground biomass and eventually change N efficiency. Still, targeted experiments are needed to assess the effect of associative N-fixing microbial inoculants in agroecosystems, since there is a lack of findings with associative microbial inoculants.

Souza et al. (2023) found that the addition of N-fixing microbial inoculants with nitrification inhibition resulted in lower N_2O emissions in corn cropping systems. Further, higher soil nitrate leaching was discovered using N-fixing microbes (Souza et al., 2023). In contrast, both N_2O emissions and NO_3^- leaching were greater with N-fixing microbial products associated with irrigated potato cropping systems (Souza et al., 2019).

The effect of N-fixing inoculants on daily N_2O fluxes in our study was inconsistent. The data from two years showed different fluxes, possibly due to seasonal variations in the rhizosphere or micro-scale environment associated with the N cycle. In-depth targeted experiments should be required to comprehend the microscale and macroscale ecological behavior of novel bioinoculants, specifically the rhizosphere dynamics in corn with newly developed bioinoculants.

Evaluating and implementing emission factors associated with bioinoculant application in corn cropping systems is crucial for future scenarios and revision of IPCC default EF values. The Intergovernmental Panel on Climate Change (IPCC) has provided guidelines for estimating greenhouse gas (GHG) emissions and associated emission factors (EF%) under various management scenarios. The default Tier 1 EF% for direct N_2O emissions has undergone significant revisions over the decades. In 1996, the IPCC proposed an EF% of 1.25% (0.25-2.25%), which was later adjusted to 1% (0.1-1.8%) in the 2019 report (IPCC, 2019), reflecting efforts to reduce uncertainty (Mathivanan et al., 2021).

Our study highlights that all treatments' fertilizer-induced emission factors (EF%) were consistently below 1% across both years. This finding is particularly noteworthy, as the recorded EF% values are lower than those typically observed in many corn cropping systems. This reduced EF% can likely be attributed to implementing no-till practices, careful soil and crop management, and less intensive precipitation events during the growing seasons. These results highlight the importance of region-specific practices, suggesting that traditional EF% values might overestimate emissions in similar contexts.

The lower EF% observed in our study contrasts with other findings, such as those from the U.S. Corn Belt, where bottom-up N_2O emission inventories interpreted through an inverse transport model suggested indirect emissions that were 1.9-4.6 times higher than IPCC default values (Chen et al., 2016). Similarly, studies from Ontario, Canada, in cold climate conditions, reported higher growing season mean EF% values of 0.84% and annual EF% values of 1.69% for N_2O in corn and soybean systems (Baral et al., 2022). These comparisons emphasize the variability of EF% across different regions and the need for more localized emission factor estimates.

Corn inoculated with N-fixing microbes had the lowest EF% for 2022. Reduction of N_2O emission suggests better N use efficiency with bioinoculant application in 2022. However, in 2023, the highest EF% occurred in bioinoculant-treated plots, indicating that biotic and abiotic factors might influence higher N_2O flux with bioinoculant application with lower N rates. Soil health conditions and rhizosphere microbial activity with seasonal precipitation conditions might influence those variabilities. These variabilities in EF% indicate the challenges of managing N_2O emissions in cropping systems. However, N-fixing bioinoculants had a positive effect on reducing N_2O emissions in the 2022 growing season. Their effectiveness can vary due to soil biotic and abiotic factors.

In order to identify the management effect on N_2O emission during the growing season and grain yield, researchers proposed N_2O emission-yield curves (Venterea et al., 2011; Kim et al., 2023). As suggested by Kim et al. (2023), the direction and magnitude of shifting the curve could help to recognize the yield and N_2O emissions in a given cropping system. The quadratic trend can fit well only with N rates without bioinoculant application. The quadratic trends of N_2O emission yield are similar to those Kim et al. (2023) suggested. When considering the slope of N_2O-yield curves, the bioinoculant-associated curve had N_2O emissions that were lower with a higher yield. The curve slope was 6.4×10^{-5} with bioinoculant and 1.6×10^{-6} without bioinoculant, indicating an increased slope trend associated with bioinoculant application. In

2023, the same trend was also depicted, where higher yields tended to have lower N_2O emissions. However, with lower yields, bioinoculants showed higher N_2O emission trends. Both years' R^2 values without bioinoculant application were 0.53 and 0.71, which described a stronger correlation than with bioinoculant in both years. Both years' R^2 values were 0.14 and 0.30 with the bioinoculant application. Further, P values showed significant solid values ($p<0.001$) without bioinoculant application, suggesting a strong relationship between N_2O flux and yield.

In our research, yield-scaled N_2O emissions were 28.6, 57, 97.7 g N_2O-N Mg^{-1} yield for 2022 and 39, 36.4, and 57.5 g N_2O-N Mg^{-1} yield for 2023 at N rates of 56, 112, and 168 kg N ha^{-1}, respectively. With the application of bioinoculants, these yield-scaled emissions were changed to 23.1, 51, 68.6 g N_2O-N Mg^{-1} yield in 2022 and 94.6, 48.9, and 41.6 g N_2O-N Mg^{-1} yield in 2023 for the same N rates. A similar trend occurred when comparing our findings to the study conducted in a maize cropping system in Indiana, USA, associated with nitrification inhibitors with varying rates of N fertilizer (0, 90, and 180 kg N ha^{-1}) (Burzaco et al., 2013). Yield-scaled N_2O-N emissions were reduced when using the inhibitor, with values of 174, 211, and 312 g N_2O-N Mg^{-1} yield at the respective N rates, and further reduced to 157, 135, and 268 g N_2O-N Mg^{-1} yield with the inhibitor application. Though the overall yield-scaled emissions were higher due to higher cumulative N_2O emissions and lower yields, this aligns with the concept illustrated by N_2O emission-yield curves. Furthermore, when compared to the study by McGowan et al., (2019)where biomass-scaled emissions ranged from 114 to 344 g N_2O-N Mg^{-1} due to higher cumulative N_2O emissions, our results indicate that the use of bioinoculants and other management practices can effectively shift the N_2O emission-yield curve downward, reducing emissions while sustaining or improving yields. These findings highlight the importance of integrating N_2O emission-yield relationships into future agricultural management practices. By focusing on strategies that reduce emissions while maintaining yields, we can contribute to developing more sustainable agricultural systems that support global efforts to mitigate greenhouse gas emissions.

This discussion aimed to explore these dynamics comprehensively, providing insights that could lead to more sustainable agriculture practices. These observations highlight the complexity of N dynamics in agricultural systems and emphasize the need for further research into the interactions between microbial inoculants, soil chemistry, and crop management practices.

2.5 Conclusion

The study revealed significant variability in N_2O emissions between the 2022 and 2023 growing seasons, driven primarily by differences in precipitation patterns. Nitrogen fertilizer application rates significantly affected the cumulative N_2O emissions. In 2022, higher precipitation within the first 40 days post-fertilization increased soil moisture, promoting conditions favorable to denitrification and elevated N_2O emissions. In contrast, 2023 experienced lower early-season precipitation, which resulted in reduced N_2O emissions. The highest cumulative N_2O emissions were recorded with the highest N application rate, underscoring the importance of managing fertilizer use to mitigate N_2O emissions.

The overall crop management operation's EF was below the IPCC guidelines. Bioinoculants demonstrated year-to-year variability, with lower EF% observed in 2022 and higher EF% observed in 2023. This highlights the need for further research to optimize bioinoculant use and understand their interactions with environmental variables. While bioinoculants show potential for improving N use efficiency and reducing N_2O emissions, their effectiveness can vary significantly based on the environmental conditions.

Overall, our findings emphasize the complexity of N_2O emission dynamics in agricultural systems and the critical need for tailored management practices to achieve sustainable crop production. Continued research is essential to refine bioinoculant application strategies, enhance N management, and reduce agriculture's environmental impact.

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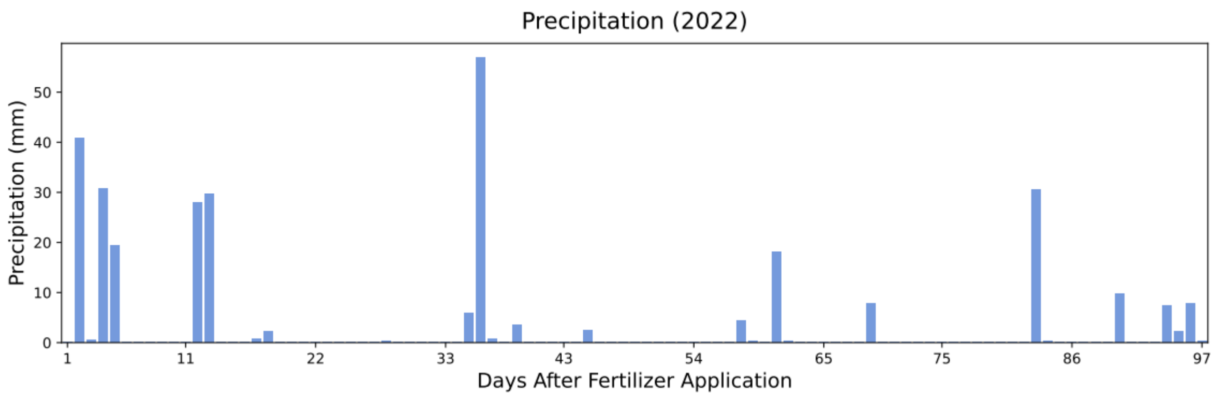

Figure 2.1. Precipitation (2022). Daily precipitation totals were measured from the Mesonet tower managed by the Department of Agronomy at Kansas State University. Corn planting and fertilizer application on June 19, 2022.

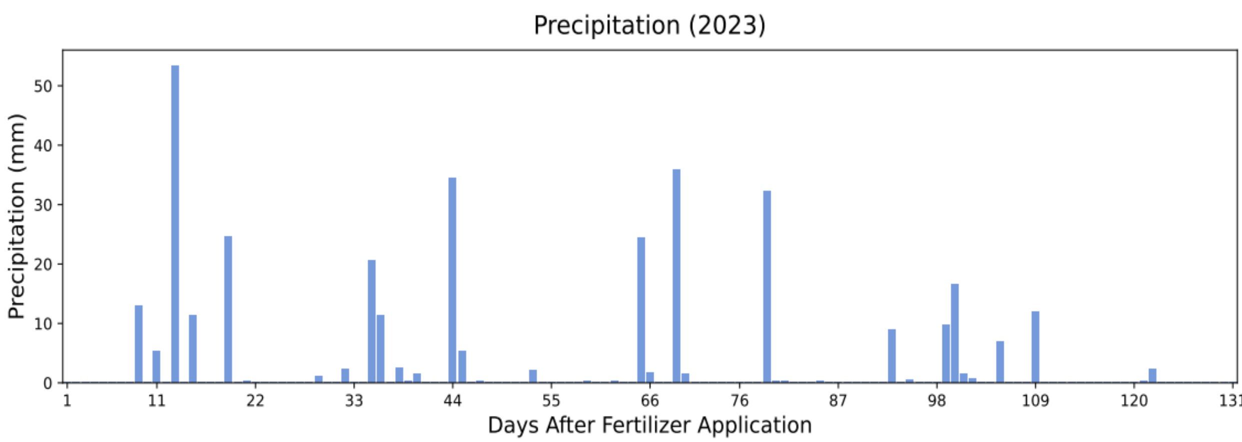

Figure 2.2. Precipitation (2023). Daily precipitation totals were measured from the Mesonet tower managed by the Department of Agronomy at Kansas State University. Corn planting and fertilizer application on April 26, 2022.

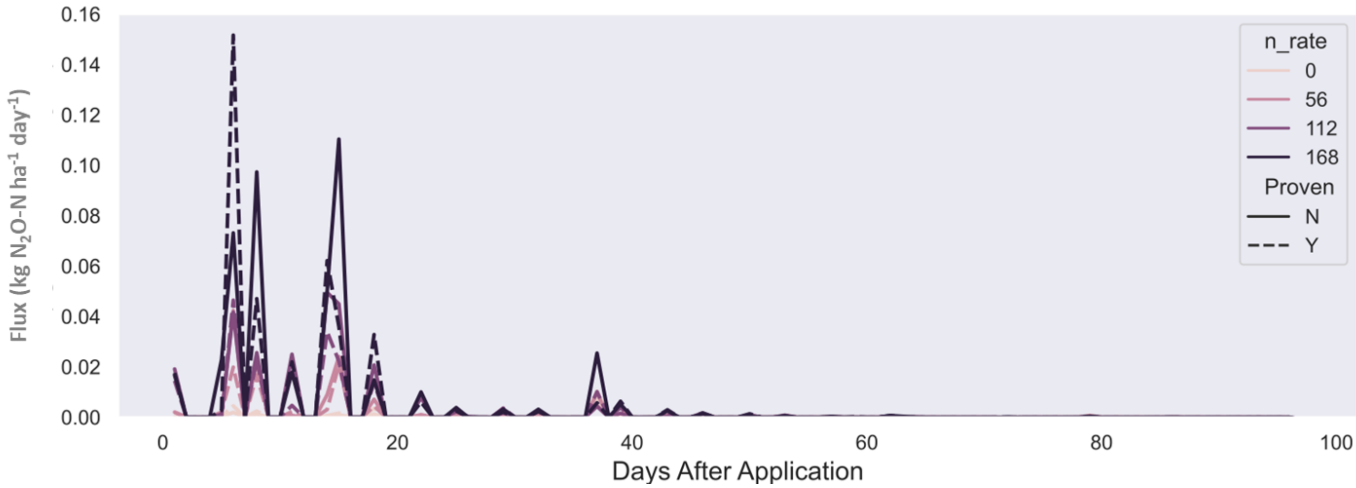

Figure 2.3. Daily N_2O flux measurements in kg N_2O-N ha^{-1} for the 2022 growing season.

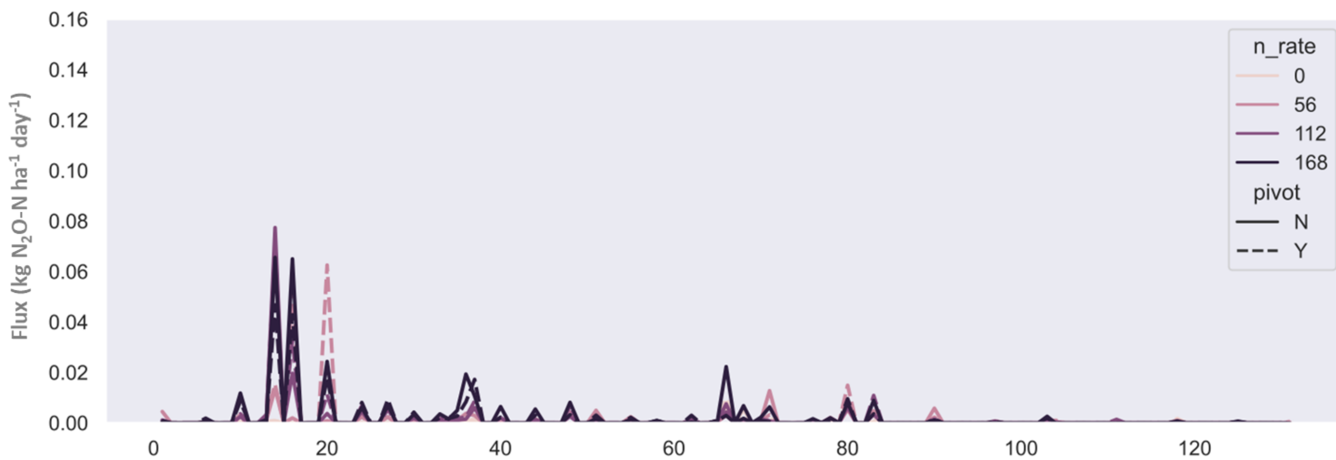

Figure 2.4. Daily N_2O flux measurements in kg N_2O-N ha^{-1} for the 2023 growing season.

Figures 2.3 and 2.4 display daily N_2O-N flux over time during the 2022/2023 growing season, measured in kg N_2O ha^{-1} day^{-1}, across various nitrogen fertilizer application rates (0, 56, 112, and 168 kg N ha^{-1}). The x-axis represents the days after nitrogen fertilizer (urea) application, while the y-axis shows the N_2O-N flux in kg N_2O-N $ha^{-1}day^{-1}$. Solid lines represent treatments without the nitrogen-fixing bioinoculant, while dashed lines represent treatments with the bioinoculant.

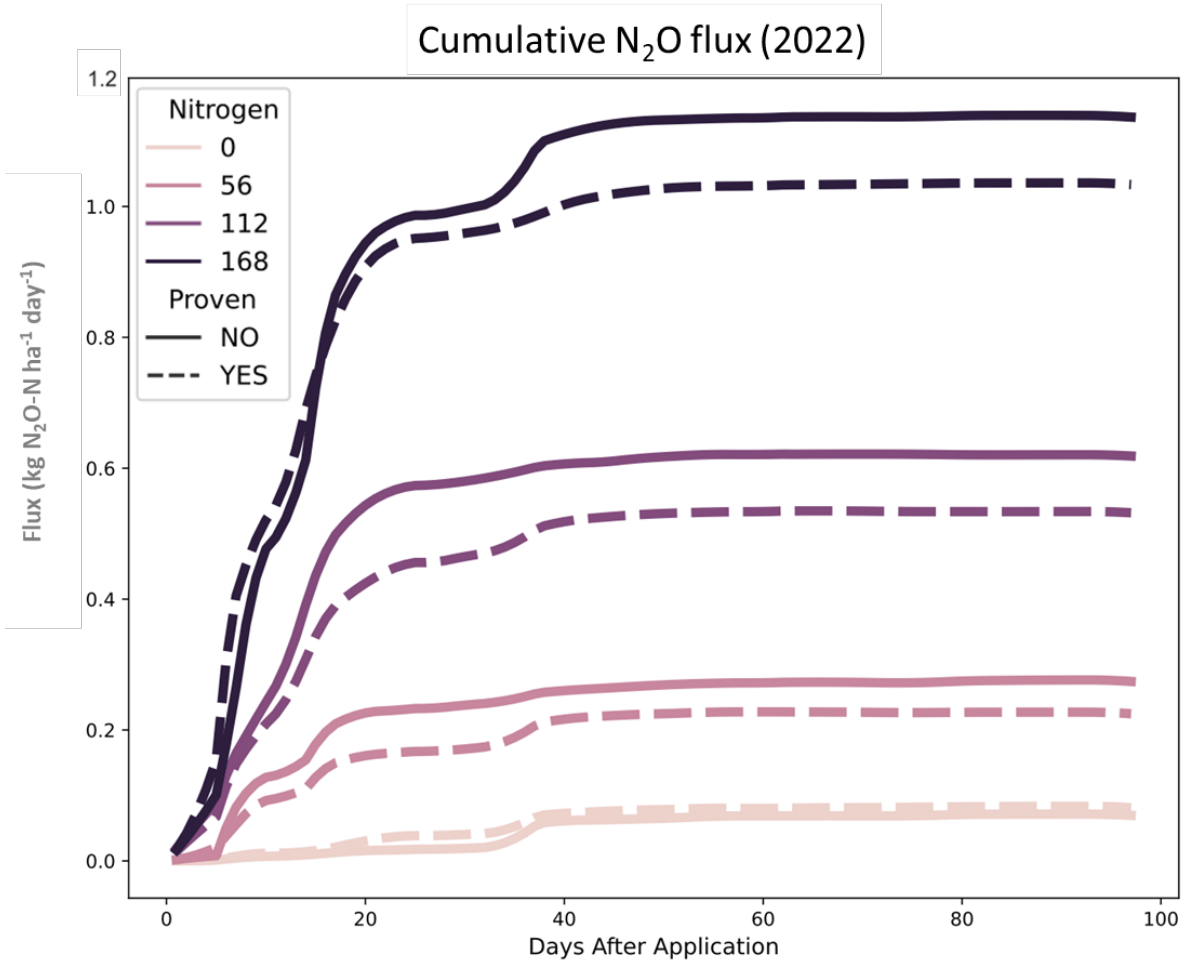

Figure 2.5. Cumulative N_2O flux measurement 2022.
The figure illustrates the cumulative N_2O emissions in 2022 over time, measured in kg N_2O -N ha^{-1}, across different nitrogen fertilizer application rates (0, 56, 112, and 168 kg N ha^{-1}). Solid lines represent plots without the nitrogen-fixing bioinoculant, while dashed lines represent plots treated with the bioinoculant. The x-axis represents the days after nitrogen application, and the y-axis shows the cumulative N_2O emissions in kg N_2O -N ha^{-1}.

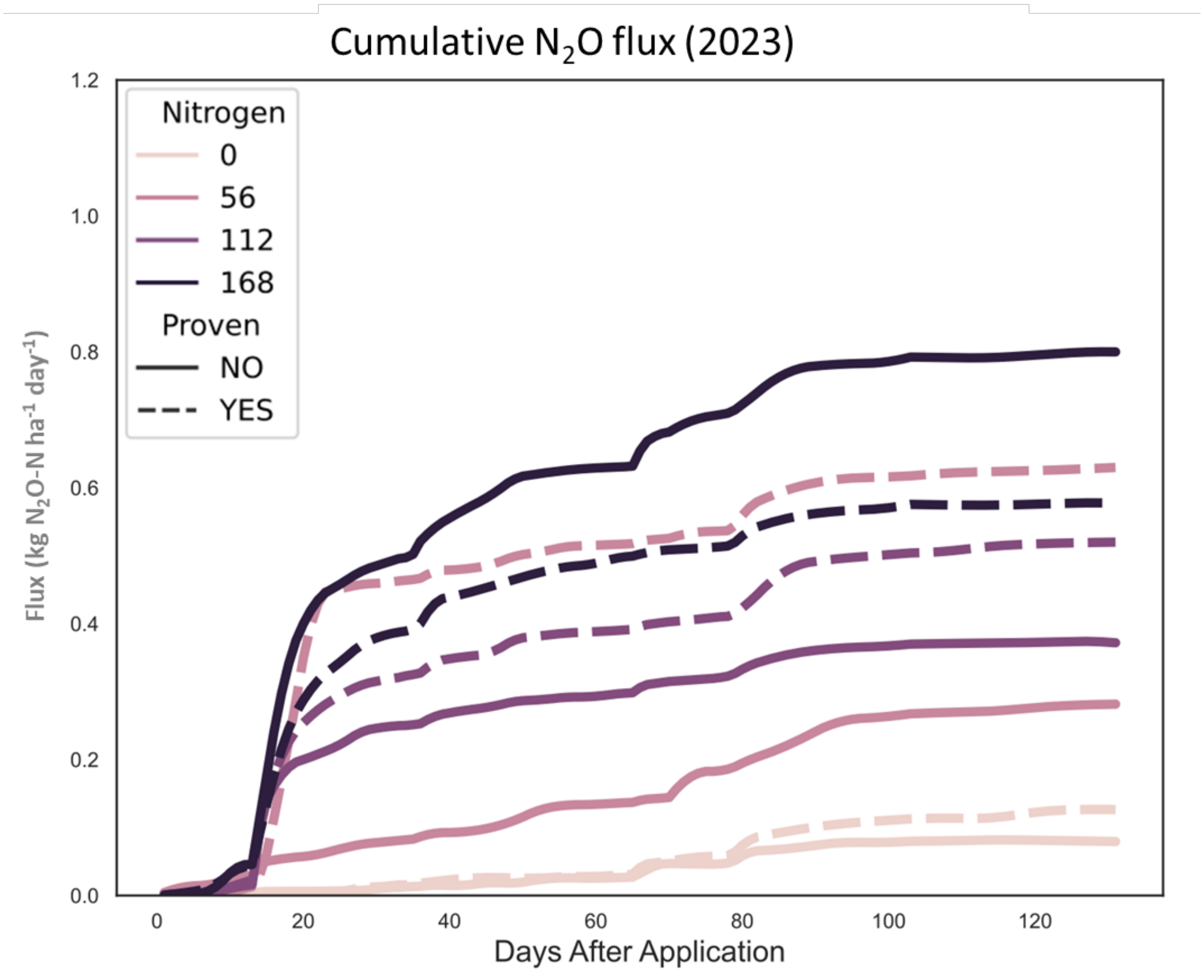

Figure 2.6. Cumulative N_2O flux measurement 2023

The figure illustrates the cumulative N_2O emissions in 2023 over time, measured in kg N_2O-N ha^{-1}, across different nitrogen fertilizer application rates (0, 56, 112, and 168 kg N ha^{-1}). Solid lines represent plots without the nitrogen-fixing bioinoculant, while dashed lines represent plots treated with the bioinoculant. The x-axis represents the days after nitrogen application, and the y-axis shows the cumulative N_2O emissions in kg N_2O-N ha^{-1}.

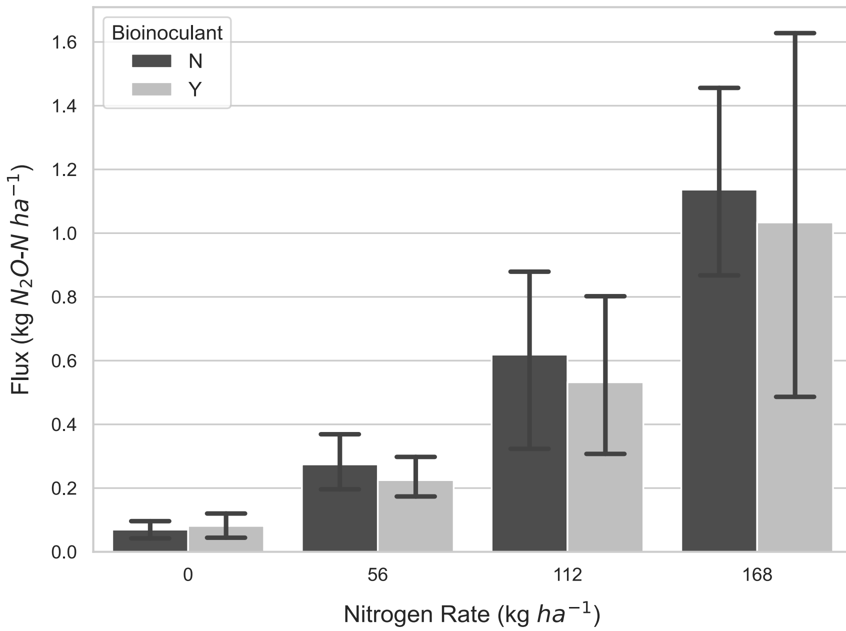

Figure 2.7. Cumulative N_2O flux measurements for 2022.

Error bars represent the standard errors of cumulative N_2O flux values.

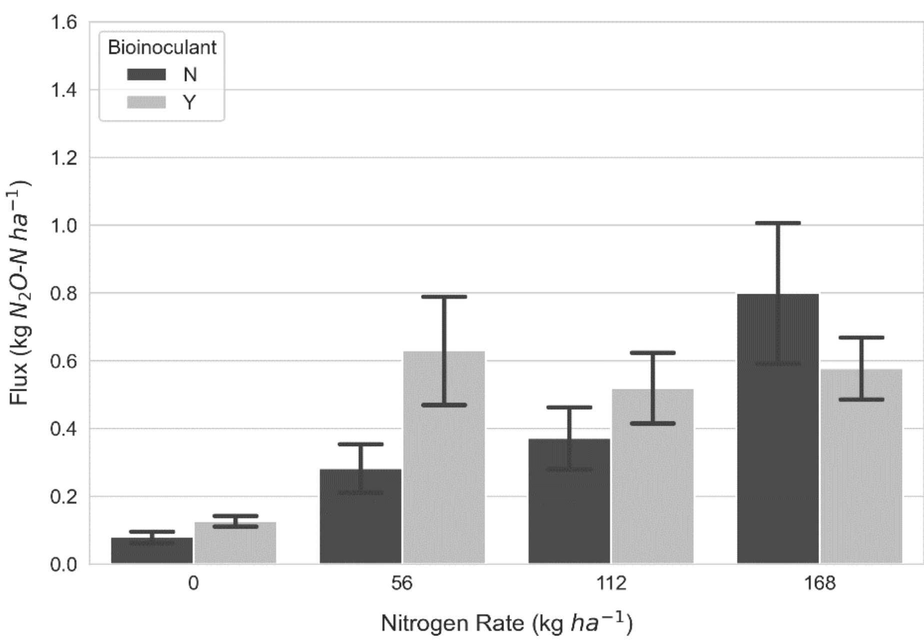

Figure 2.8. Cumulative N_2O flux measurements for 2023.

Error bars represent the standard errors of cumulative N_2O flux values.

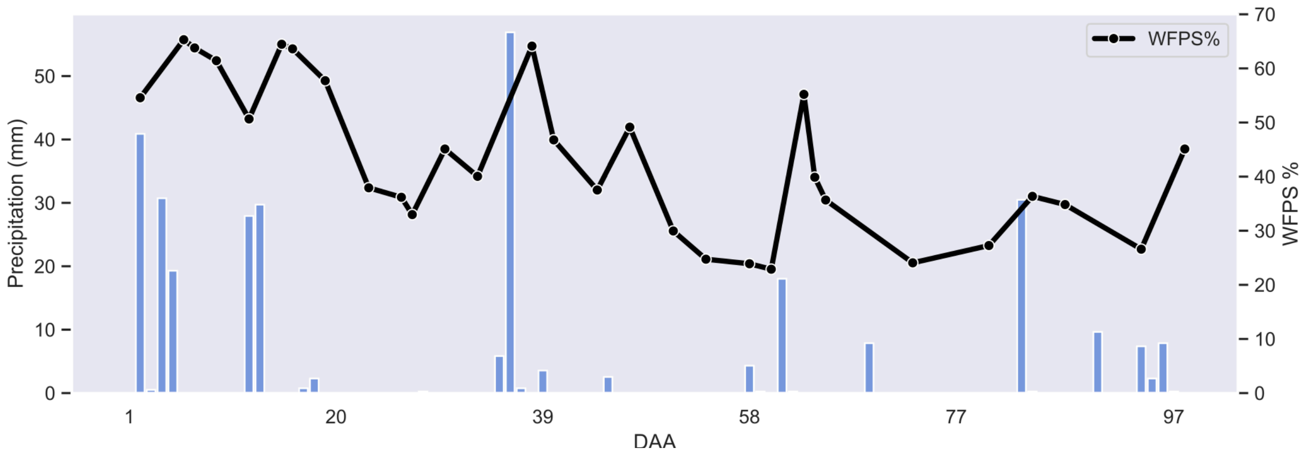

Figure 2.9. Water-filled pore spaces percnt for 2022

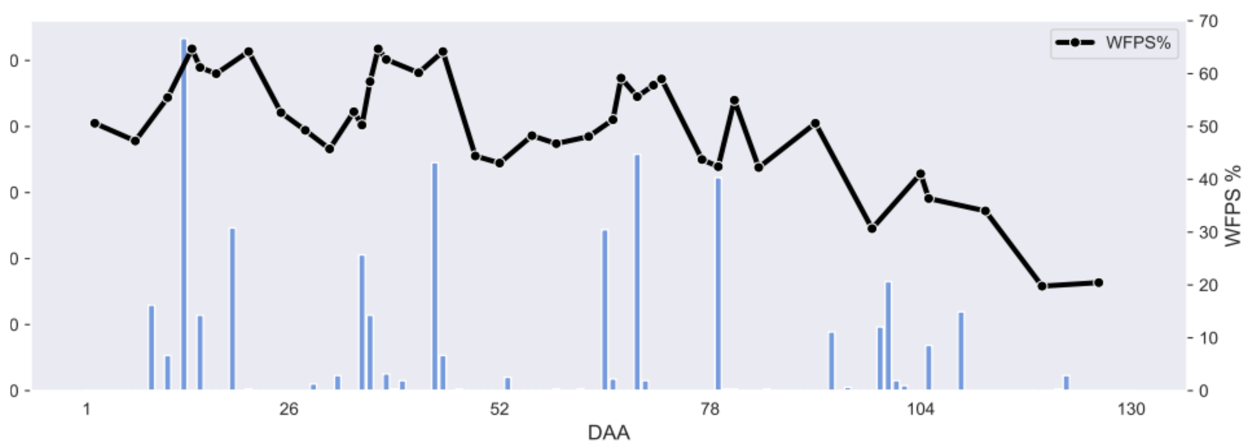

Figure 2.10. Water-filled pore spaces percent for 2023

Figures 2.11 and 2.12 display the growing seasons' precipitation (mm) and water-filled pore spaces (%) of 2022 and 2023 years. DAA (Days after application of fertilizer)

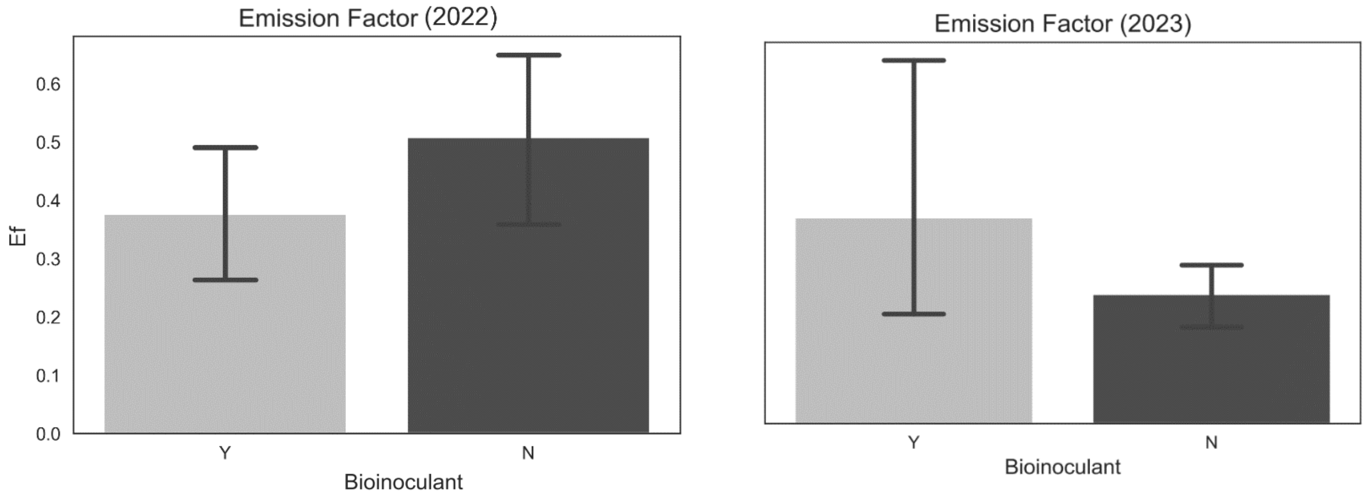

Figure 2.11. Emission factors for 2022 and 2023

Error bars represent the standard errors of N_2O EF% values.

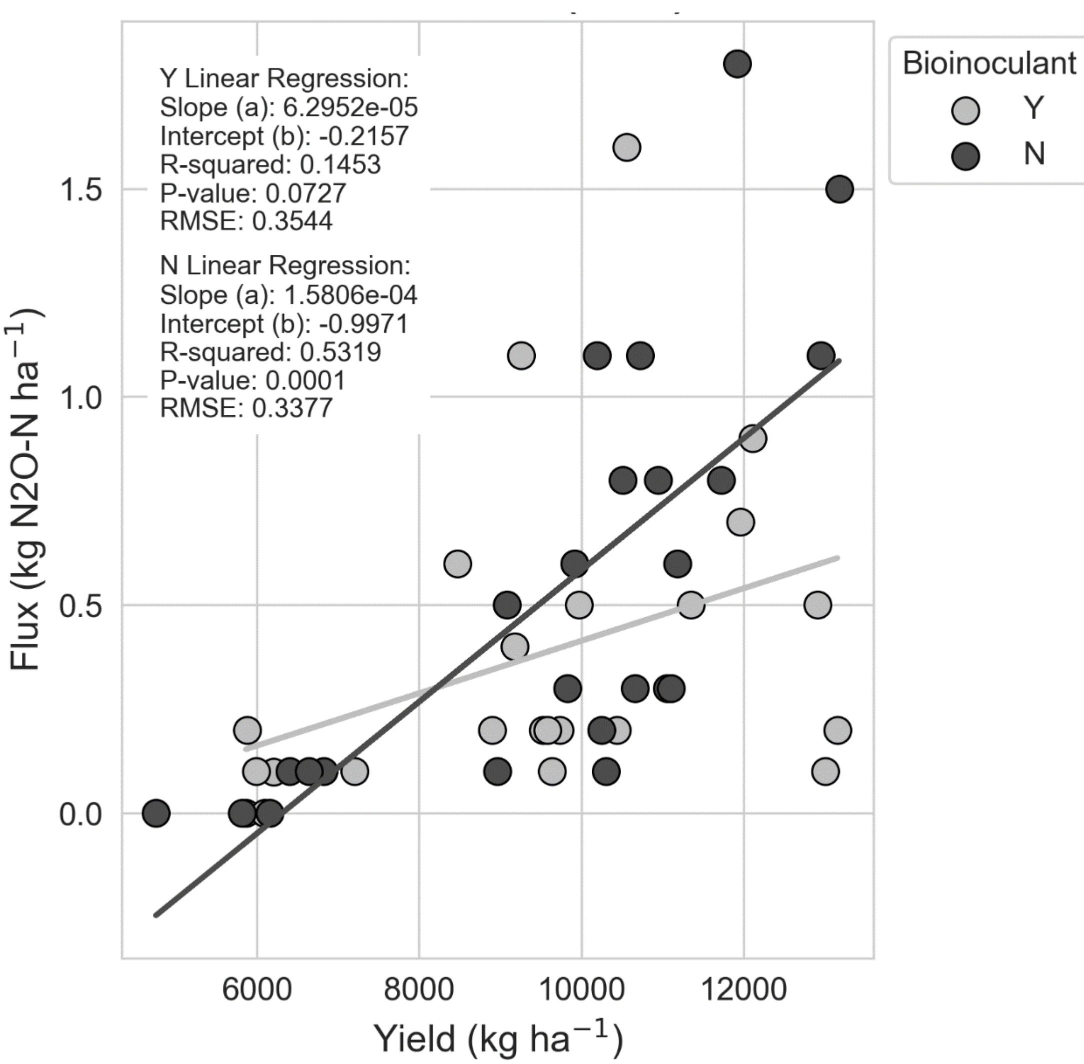

Figure 2.12. N_2O emission-yield curve -2022 (Linear)

Figure 2.9 illustrates the linear relationship of N_2O flux (kg N_2O-N ha^{-1}) with yield (kg ha-1) for 2022. The figure displays data with and without bioinoculant, and each group was fitted with a linear regression model ((α=0.05)

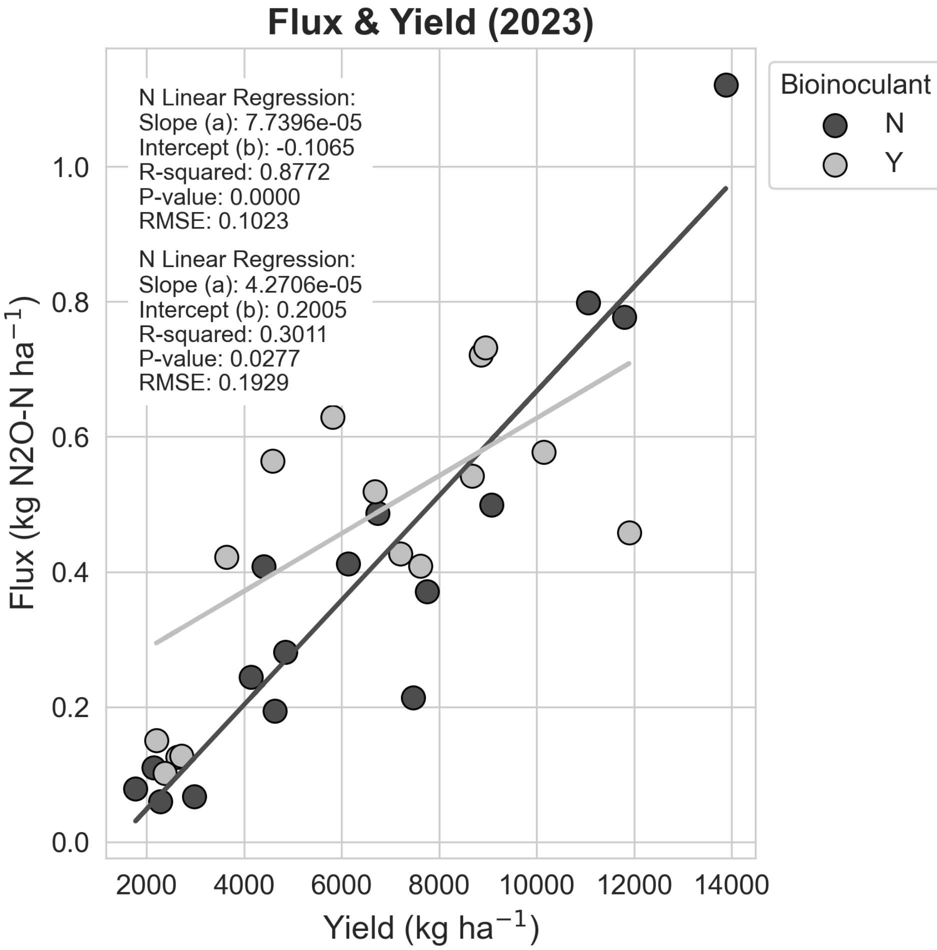

Figure 2.13. N_2O emission-yield curve – 2023 (Linear)

Figure 2.9 illustrates the linear relationship of N_2O flux (kg N_2O-N ha^{-1}) with yield (kg ha-1) for 2023. The figure displays data with and without bioinoculant, and each group was fitted with a linear regression model ((α=0.05)

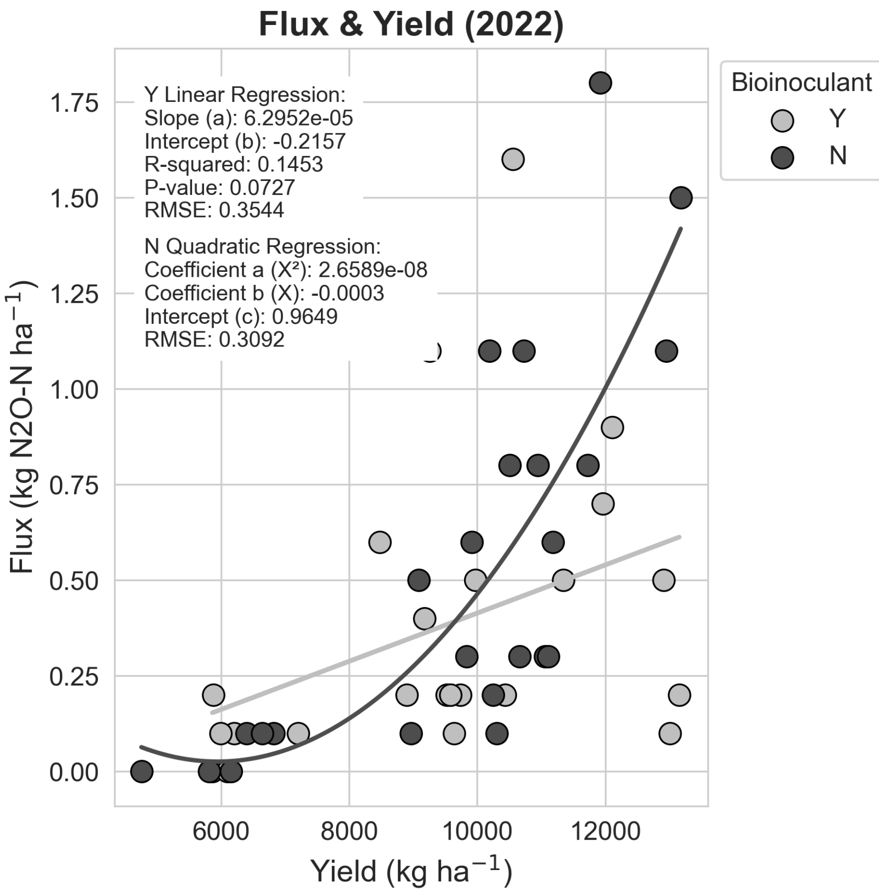

Figure 2.14. N_2O emission-yield curve -2022 (quadratic and linear)

The quadratic and the linear relationship of N_2O flux (kg N_2O-N ha^{-1}) with yield (kg ha-1) for 2022. The figure displays data with and without bioinoculant, and each group was fitted with a linear regression model ((α=0.05)

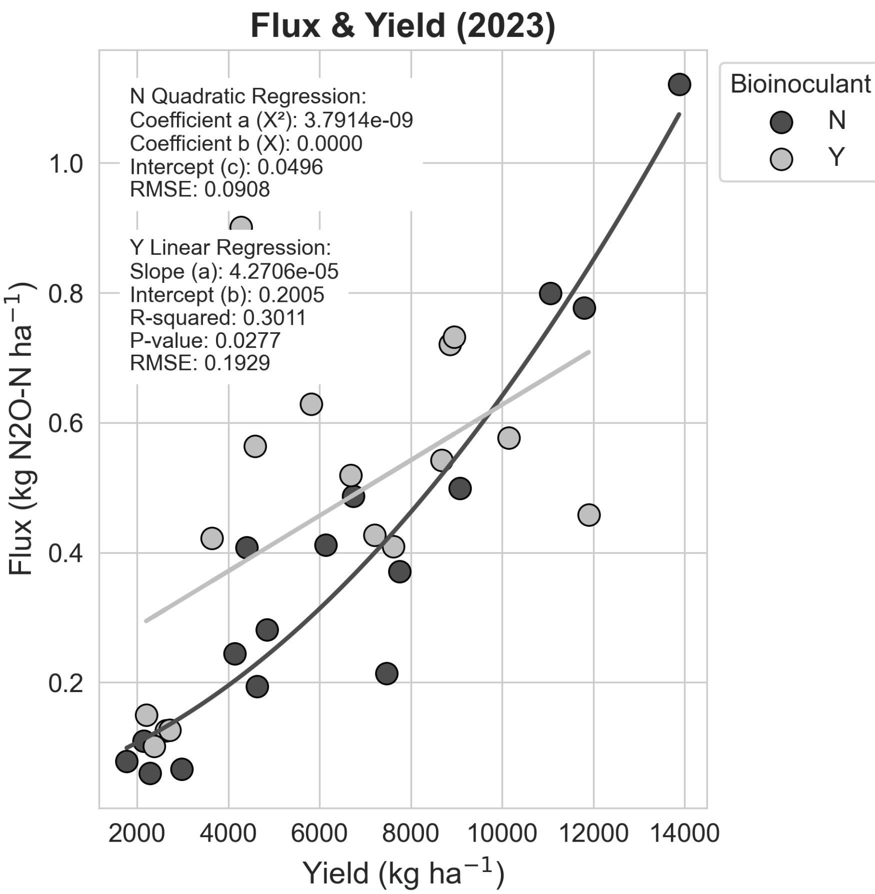

Figure 2.15. N_2O emission-yield curve 2023 (quadratic and linear)

The quadratic and the linear relationship of N_2O flux (kg N_2O-N ha^{-1}) with yield (kg ha-1) for 2023. The figure displays data with and without bioinoculant, and each group was fitted with a linear regression model ((α=0.05)

Chapter 3 - Denitrification and decomposition model simulation for continuous corn inoculated with a nitrogen-fixing microbial inoculant

Abstract

The Denitrification-Decomposition (DNDC) model is a process-based tool widely employed to predict and quantify N_2O emissions across diverse agricultural practices. This study assesses the DNDC model's effectiveness in predicting N_2O fluxes from a continuous no-till corn cropping system with varying nitrogen (N) rates and a N-fixing microbial inoculant in Manhattan, Kansas. Integrating these microbial inoculants into corn systems holds promise for reducing synthetic fertilizer use, enhancing N efficiency, and lowering N_2O emissions. However, their effectiveness and environmental impacts require thorough evaluation. The DNDC model is pivotal in this context, offering predictions that can guide best management practices and inform policies for sustainable agriculture. Our study demonstrates the model's accuracy in simulating N_2O emissions post-calibration, particularly at higher N fertilization rates, though some refinement is needed at lower rates. Incorporating precipitation data validated the model's sensitivity to environmental variations, underscoring its applicability in varied agricultural settings. Both the measured and the simulated N_2O emissions were below the IPCC Tier 1 emission factor of 1%, which lowers the estimated Greenhouse gas (GHG) impact for corn production systems in this climatic region. Future enhancements should include microbial-related parameters to simulate better novel agricultural practices and their impacts on greenhouse gas emissions. Continuous evaluation and recalibration of the model are crucial to maintaining precision and ensuring its utility in both research and practical applications.

3.1 Introduction

Global atmospheric nitrous oxide (N_2O) levels have increased from 270 ppb to 336 ppb from 1750 to 2012 (Tian et al., 2024). Among natural sources, soils are the largest contributors, emitting 3.9–8.6 Tg-N of N_2O annually from 2010–2019, followed by oceanic emissions at 2.5–4.7 Tg-N annually. Natural sources like lightning, oceanic shelves, inland waters, estuaries, and coastal vegetation also contribute a substantial amount of N_2O to the atmosphere. Global agriculture accounts for a significant portion of N_2O emissions, contributing substantially to the environmental footprint associated with agriculture (Menegat et al., 2022; Tian et al., 2024). Direct agriculture accounts for 56% (2.7–4.8 Tg N yr^{-1}) of total global anthropogenic N_2O emissions, followed by fossil fuel, industry, and biomass burning. Additionally, ammonia (NH_3) emissions and their subsequent deposition indirectly contribute to agricultural N_2O emissions. The rapid pace of climate change is increasingly affecting global food production, with agricultural practices contributing to and being impacted by these changes (Ivanovich et al., 2023). Further, climate change-driven N_2O emissions account for 07 (0.2–1.2) Tg N yr^{-1}(Tian et al., 2024).

Implementing the 4R principles, right source, rate, time, and place has proven essential for optimizing crop N uptake and mitigating environmental impacts, particularly in reducing N losses through leaching, runoff, and emissions of N_2O and ammonia (Snyder et al., 2009). However, to achieve more significant and lasting reductions in N_2O emissions, it is crucial to implement to innovative approaches. Cover crops, fertilizer inhibitors, and alternative fertilizer application and management methods can significantly enhance N use efficiency, thereby further reducing N_2O emissions (Venterea et al., 2016). Integrating these novel strategies with the 4R framework is vital for advancing sustainable agriculture, ensuring both the protection of environmental health and the long-term viability of agricultural systems. By combining traditional practices with emerging techniques, we can more effectively address the challenges of reducing greenhouse gas emissions while maintaining productive and sustainable agroecosystems.

Novel biological products and technologies, such as N-fixing microbes, promise to improve food production while mitigating environmental impacts (Ahmad et al., 2011; Montoya-Martínez et al., 2022). Modeling these outputs specifically GHG and predicting their impacts is crucial for better decision-making for all the stakeholders, including producers' consumers, and policymakers at both site-specific and regional levels (X. Li & Gao, 2023; Weiskopf et al., 2022; Zhu et al., 2024).

The environmental benefits of using recently developed biological fertilizer products has not been assessed extensively. Reduced GHG emissions under novel biological fertilizers might advance sustainable agricultural goals; however, the lack of data and high costs associated with obtaining reliable data present significant challenges. Real-time *in situ* measurement of N_2O emissions from agriculture is crucial for understanding soil, crop, and climate processes. Still, practical and economic limitations restrict the extent of these measurements. A simulation tool for predicting future and current N_2O flux through modeling is essential in such scenarios.

Predicting N_2O emissions from the agricultural sector was guided by IPCC's Tier 1, Tier 2, and Tier 3 methodologies. Tier 1 estimates agricultural direct N_2O emissions by applying a default factor of 1% (0.01) of the applied N fertilizer (or other N sources). The tier 2 approach is based on regression models and integrated to estimate annual N_2O emission available data (Obi-Njoku et al., 2024a). However, this approach carries significant uncertainties, ranging from 0.002 to 0.018 (de Klein et al., 2020). Tier 3 involves a more detailed process, incorporating multiple variables to predict emissions more accurately. This approach accounts for various management and ecological factors, including climate, land use, and specific management practices. By integrating these diverse elements, environmental models can more effectively predict N_2O emissions from different cropping systems, considering the comprehensive influence of climate and land management factors.

Many environmental modeling techniques have been used in the recent past to predict N_2O emissions from cropping systems. Models such as DayCent, DNDC, and EPIC, as well as gap-filling and machine-learning models, have received significant interest from researchers for predicting N_2O emissions in land-specific management or on a regional scale (Dorich et al., 2020; Giltrap et al., 2020; Saha et al., 2021).

Of the many environmental models, one of the most promising approaches to understanding and predicting the impact of agricultural practices on N_2O emissions is the Denitrification-Decomposition (DNDC) model. The DNDC model is a process-based computational simulation software designed to simulate carbon and N dynamics in agroecosystems (Li et al., 1992a; b). Later, the DNDC model evolved and developed immensely for better prediction performance while adhering to many climate and regional practices with more input parameters. Further, it has been modified by many researchers based on regional prediction requirements (Gilhespy et al., 2014).

This model framework comprises four primary sub-modules: crop vegetation, soil/climate, decomposition, and denitrification/nitrification (Figure 3.1A, 3.1B). Researchers have extensively applied the DNDC model to estimate greenhouse gas emissions, soil temperature, water dynamics, soil carbon, and N cycling, and crop growth across various ecosystems, including cropland, grassland, and forest systems (Gilhespy et al., 2014; DNDC

Scientific Basis and Processes, 2017). Additionally, it has proven valuable in assessing the effect of current and future climate scenarios under different crop management methods (Deng et al., 2020; Jiang et al., 2023; Yao et al., 2023)

The DNDC model has been used to predict N_2O emissions in various cropping systems and with various ecosystems and management regimes. The comparison of IPCC, tier 1, 2, and 3 methods for N_2O emissions from biosolids by Obi-Njoku et al. (2024b) indicated that the tier 3 DNDC model was the most accurate for estimating N_2O emissions. The DNDC model was more accurate than tier 1 and tier 2 methods. However, the DNDC model faces challenges in predicting N_2O fluxes in different climatic, regional, and management conditions. In such a scenario, the root mean square error (RMSE) parameter can be used to evaluate the prediction component (Zhao et al., 2023).

The DNDC model evaluation by Macharia et al. (2021) in corn cropping systems under different fertilizer regimes in East Africa (Kenya) suggested that DNDC simulated yield scale emission and emission factor values provided similar predictions as field-measured data. Different simulation model comparisons were conducted by Gaillard et al., (2018), who found that DNDC underestimated N_2O flux prediction compared to DayCent and EPIC models. This suggests that uncertainty in predicting GHG is due to the heterogeneity of soil systems and weather patterns.

Injecting N fertilizer at planting or side-dressing decreases NH_3 loss but increases nitrate leaching and N_2O emissions (Banger et al., 2020b). Adjusting N rates and adding inhibitors, as proposed by the DNDC model, mitigated N_2O emissions by 42–57% and resulted in 3–4% higher yields compared to the baseline scenario in Ontario corn production (Banger et al., 2020b).

Corn yield and soil N_2O emissions study in Tennessee exhibited different responses to precipitation changes, which the DNDC model validated (Kaur et al., 2023). Corn yields increased linearly with more precipitation in dry years while unchanged in average years and yields decreased in wet years. Soil N_2O emissions responded linearly to precipitation (Kaur et al., 2023.The DNDC model over and underestimated cumulative N_2O flux in the maize in Colorado and Minnesota regions compared with actual measured data (Foltz et al., 2019). The DNDC model underestimated the measured N_2O flux for all counties in Iowa (Jarecki et al., 2015).

The foundation of the DNDC model relies on microbial processes, which are governed by many soil chemical factors, including pH, which can significantly influence microbial activity under anaerobic conditions. Understanding these basic principles is crucial for effectively using the DNDC model. When evaluating the DNDC model, it is essential to compare the modeling input based on real-time empirical data. In agroecosystems, yield data combined with N_2O emissions and other land-scale baseline parameters are important for predicting N_2O emissions. Using high-quality empirical data is the foundation of robust and accurate predictions through the model.

Integrating novel N-fixing microbial inoculants into corn cropping systems offers a promising approach to reducing synthetic fertilizer use, enhancing N use efficiency, and lowering N_2O emissions (Wen et al., 2021). However, these bioinoculants' effectiveness and environmental benefits must be thoroughly evaluated to ensure these benefits are realized in practice. The DNDC model can play a crucial role in this evaluation by predicting the outcomes of bioinoculant applications, guiding best management practices, and informing policy decisions promoting sustainable agriculture. This research aims to use the DNDC model to predict N_2O

emissions in corn cropping systems inoculated with novel associative N-fixing microbes. By comparing model predictions with field observations, we seek to validate the model's accuracy and reliability in capturing bioinoculant effects on N_2O fluxes.

3.2 Methodology

This experiment was conducted from 2021 to 2023 at the Kansas State University Agronomy Research Farm in Manhattan, Kansas (39°12' N, 96°59' W). The soil types at the experimental site were Ivan and Kennebec silt loams (fine-silty, mixed, super-active, mesic Cumulic Hapludolls). Continuous corn (*Zea mays* L.) was planted in furrows in 2021, 2022, and 2023 with four different urea fertilization rates (0, 56, 112, and 168 kg N ha^{-1}), both with and without N-fixing bioinoculants. The study employed a no-till management approach and was arranged as a randomized complete block design (RCBD) with six replications, using a split-plot design to accommodate the presence and absence of bioinoculants. The bioinoculant was applied according to the product's recommended formulation, produced and marketed by Pivot Bio Inc., Berkeley, CA, under the Proven40 trade name. Corn was planted with bioinoculant in-furrow application on June 19 and April 26, in 2022 and 2023. The simulation model was calibrated for the 2022 growing season cumulative N_2O flux without the bioinoculant and validated using the 2023 growing season cumulative N_2O flux.

Daily N_2O flux was measured as described in Chapter 2 using a rectangular-shaped aluminum static chamber deploying one chamber per treatment plot. All measurements were analyzed using a model described by Mosier and Hutchinson (1981) and Arango et al. (2023).

The DNDC model (version 9.5) was used to estimate daily and growing season cumulative N_2O emissions with measured field N_2O fluxes. The DNDC model's primary inputs include daily weather data, soil profile information, field management practices, and crop parameters. Daily weather data for both years were obtained from the *Mesonet* Manhattan, Kansas, weather station, managed by the Department of Agronomy at Kansas State University. Weather input parameters included maximum and minimum air temperatures (°C) and daily cumulative precipitation (cm). For both years, soil profile parameters included bulk density, pH, and other chemical properties (N, C). Baseline data were collected before planting each year and after harvest. Most crop parameters were used as default in the cropping parameter to avoid uncertainties in modeling. Apart from the field measurement data, some data were obtained from the web soil survey for the respective research plot area to get default data and compare measured data.

For this study, the model outputs of interest were growing season daily N_2O flux and growing season cumulative N_2O emission under different fertilizer rates.

3.3 Calibration and validation

The DNDC model was calibrated for 2022 growing season N_2O fluxes with different N rates. Further, model calibration was performed to simulate the effect of bioinoculant on the cropping system. However, the calibration effort failed due to higher uncertainties of the measured N_2O flux with the bioinoculant, which did not show a significant effect in both years, 2022 and 2023, and a lack of parameter optimization options, where the N fixation option was not defined for the associative N fixation.

As described by Chen et al. (2020), seasonal mean air temperature, soil pH, soil organic C, and N fertilizer rate are specific factors when modeling N_2O emission in different straw retention depth experiments. A set of the most sensitive parameters was selected based on similar research by Foltz et al. (2023). Model performance was calculated for each parameter value. Model optimum parameters were identified during the model calibration for different parameters, as indicated in Table 3.1. Soil pH and soil organic carbon (SOC) were assigned based on real field measurements. Soil pH values ranged in field scale as 6.3-7.8 and SOC ranged from 0.012-0.02 kg C kg $^{-1}$ soil. Field soil bulk density values were taken at 0-5cm and 5-15 cm. Average 0-15 cm values were taken based on field BD values as the DNDC model required 0-10cm bd values as inputs.

Table 3.1. Default parameters and tested parameter ranges that were used to optimize the DNDC model.

Parameter	**Tested**	**Default**
pH	6.3-7.8	7.0
Clay fraction (%)	14-18	14
SOC (kg C kg $^{-1}$ soil)	0.012-0.02	0.02
Field Capacity (%)	30-45	40
Rainwater index	0.95-1.0	1.0
Bulk Density Units (g cm^{-3})	0.9-1.3	1.1

3.4 Model performance metrics

Model performance evaluation provides crucial insights into model prediction accuracy, reliability, and potential biases. To quantitatively assess how well the DNDC model simulates N_2O emissions, several metrics were used, including the mean absolute error (MAE), Root Mean squared error (RMSE), and mean bias error (MBE), as described by Abdalla et al., (2011; Foltz et al., (2019 and Macharia et al., (2021). The MAE measures the average magnitude of prediction errors, indicating how far predictions deviate from actual values, and is valued for its simplicity and interpretability(Willmott and Matsuura, 2005). The RMSE, sensitive to outliers, assesses the square root of the average squared differences between predicted and observed values, providing insight into the error magnitude and facilitating model comparison. The MBE, reflecting average bias, highlights whether a model tends to underpredict or overpredict, aiding in identifying systematic errors.

Mean Absolute Error (MAE)

$$MAE = \frac{1}{n}\sum_{i-1}^{n} |yi - yi|$$

Root Mean Squared Error (RMSE)

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(yi - yi)\text{^}2}$$

Mean Bias Error (MBE)

$$MBE = \frac{1}{n}\sum_{i-1}^{n}(yi - yi)\text{^}2$$

Python packages such as Pandas, NumPy, and Matplotlib were utilized for calculating and visualizing flux parameters. Python kScikit-learn was used to compute RMSE and MBE for the model comparison evaluations. All statistical analyses of field flux data and evaluation model parameters were conducted using Python stat-model libraries.

3.5 Results

Daily-scaled N_2O flux predictions were simulated from the beginning of the 2022 growing season to the end of the 2023 growing season. Figure 3.3 depicts the daily measured and simulated N_2O fluxes alongside precipitation and illustrates notable flux peaks during major precipitation events following significant N applications in both years.

The simulated model had higher fluxes at the beginning of the growing season, just after the fertilizer application for both years. Further, higher fluxes were observed at the end of the growing season in 2022 (October -November) after higher precipitation (>50mm) events. Additionally, unexpected fluxes during the winter of 2023 (January-February) were observed for both N-applied and control plots despite the absence of significant precipitation events. This suggests an uncertainty in simulating early spring fluxes under colder environment conditions, indicating areas for further model improvement.

To evaluate the cumulative N_2O flux predictions, measured cumulative values and modeled cumulative values were compared using zero-intercept linear regression plots, as shown in Figures 3.4 and 3.5.

Table 3.2 presents the cumulative N_2O emissions (kg N ha^{-1}) for different N rates in 2022, compared with the default, calibrated, and validated (2023) model simulations. The default model values, derived from the Web Soil Survey and the model's built-in data, showed significant discrepancies from the measured values across all N rates, generally overestimating emissions. For instance, at 168 kg N ha^{-1}, the default model predicted 1.40 kg N_2O-N ha^{-1}, while the measured value was 1.14 kg N_2O-N ha^{-1}. Similar patterns were observed at other N rates, with the default model consistently overestimating emissions.

Table 3.2 Model parameter metrics in models evaluated with default, calibrated, and validated values.

Parameters	Default	Calibrated	Validated
MAE	0.255	0.016	0.056
RMSE	0.275	0.023	0.067
R^2	0.54	0.997	0.934

The table represents model performance metrics at each simulated phase. The mean absolute error (MAE), Root mean square error (RMSE), and squared (R^2) values of each phase were measured.

Default models were calibrated based on field soil measurement data, as shown in Table 3.1. Default input values in the DNDC model were adjusted according to soil field data. Specifically, pH, SOC, and BD values were adjusted based on field data. Upon calibration using the measured data in 2022, the model's predictions aligned much more closely with the observed values. The calibrated model predictions for 168 kg N ha^{-1} were 1.18 kg N ha^{-1}, closely matching the measured 1.14 kg N ha^{-1}. This pattern of improved accuracy was consistent across other N rates. The calibration process significantly enhanced the model performance, as reflected in the improved performance metrics: MAE decreased from 0.225 to 0.016, RMSE from 0.275 to 0.023, and R^2 increased from 0.54 to 0.997.

The validation phase further tested the model's accuracy using 2023 data. The validated model agreed well with measured values, particularly at higher N rates. For example, at 168 kg N ha^{-1}, the validated model predicted 0.72 kg N_2O-N ha^{-1} compared to the measured 0.79 kg N_2O-N ha^{-1}. However, there was some underestimation at lower N rates, such as at 56 kg N ha^{-1}, where the model predicted 0.18 kg N_2O-N ha^{-1} compared to the measured 0.28 kg N_2O-N ha^{-1}. Despite these discrepancies, the validation metrics remained strong with a MAE of 0.056, RMSE of 0.067, and R^2 of 0.934, indicating substantial explanatory power and reliability.

Table 3.3 Comparison of measured and simulated N_2O fluxes at different nitrogen application rates.

N-rate (kg N ha^{-1})	Measured (2022)	Default	Calibrated	Measured (2023)	Simulated (validation)
	----------kg N_2O-N ha^{-1}----------				
168	1.14	1.4	1.18	0.79	0.72
112	0.62	1.01	0.63	0.37	0.38
56	0.27	0.53	0.28	0.28	0.18
0	0.07	0.17	0.08	0.08	0.04

N_2O simulation for 4 N rates (0, 56, 112, and 168 kg N ha^{-1}). Cumulative N_2O emissions were calibrated based on measured cumulative N_2O data in 2022. The model was validated through 2023 N_2O cumulative flux data.

The calibrated and validated models significantly improved accuracy over the default model, particularly at higher N rates. However, the underestimation at lower N rates highlights the necessity for ongoing model refinement to enhance prediction accuracy across different N application rates and conditions. The performance metrics (Table 3.3) underscore the effectiveness of the calibration and validation processes, reinforcing the DNDC model's potential for reliable N_2O emission predictions under various agricultural management practices.

The initial DNDC model simulations used default values derived from the Web Soil Survey and the model's built-in data. These default cumulative flux measurements for different N rates were compared with the measured values (Table 3.1). The comparison revealed poor agreement, with R^2 values of 0.54 and RMSE and MAE values of 0.275 and 0.255, respectively.

The model showed significant improvement after calibration using the 2022 cumulative flux measurements collected during the growing season. The calibrated model achieved an R^2 of 0.997, with MAE and RMSE values reduced to 0.016 and 0.023, respectively. Subsequently, the calibrated model was validated using the 2023 growing season's measured data. The validation results showed an R^2 of 0.934, with MAE and RMSE values of 0.056 and 0.067, respectively.

Daily measured and simulated N_2O fluxes for both years were graphed alongside precipitation (Figure 3.3). Interestingly, the measured fluxes for both growing seasons exhibited peaks during major precipitation events following significant N applications. Additionally, notable fluxes were observed during the winter of 2023 (January- February) for both N-applied and control plots. These winter fluxes occurred despite the absence of significant precipitation events during that period, indicating uncertainty in simulating such peaks under those conditions.

The performance of the DNDC model in simulating cumulative N_2O emissions under bioinoculant conditions showed notable discrepancies between the calibration (2022) and validation (2023) years (Figure 3.6). In 2022, when the model was calibrated using microbial activity index parameter, it demonstrated high accuracy (MAE= 0.0095, RMSE =0.018, R^2= 0.99), reflecting the model's ability to explain nearly all the variance in the observed emissions. However, when the model was applied to the 2023 validation data, including bioinoculant treatments, performance declined significantly (MAE = 0.1241, RMSE= 0.184, R^2= 0.39), indicating larger discrepancies between the predicted and actual values. Additionally, it showed that the model tended to underestimate N_2O emissions consistently. Discussion

We employed the Denitrification-Decomposition (DNDC) model to simulate cumulative N_2O emissions under continuous corn no-till management with/without an N_2-fixing bioinoculant. The successful calibration using 2022 data and subsequent validation with 2023 data provide confidence in the DNDC model's application for predicting N_2O emissions in similar agricultural systems. Compared to the IPCC Tier 1 emission factor (EF) of 1%, the default values generated by the DNDC model (Tier 3) for our study were consistently lower, even under higher N application rates. For instance, the default EF (1%) value at 168 kg N ha^{-1} was considerably below the expected 1.68% threshold (the DNDC default model estimated 0.8%, which is 1.4 kg N_2O-N ha^{-1}). This suggests that the DNDC model estimation of cumulative N_2O emissions for corn cropping systems, particularly under no-till management, is quite similar to field-measured data. Furthermore, at lower N application rates, the default emission values remained below the IPCC's proposed emission factor (Tier 1), further highlighting the model's estimation of N_2O flux in these systems. This data suggests that corn systems in this region have a much lower GHG impact than estimated by IPCC Tier 1.

Several studies have successfully calibrated and predicted N_2O flux across various cropping systems with different management practices, which aligns with the success we observed in our study using similar models(Abdalla et al., 2009; Deng et al., 2016 : Macharia et al., 2021 ; Jiang et al., 2023). Cumulative N_2O fluxes were simulated well with DNDC; however, daily fluxes did not fit with observed values (Gaillard et al., 2018b). Precipitation significantly influenced N_2O emissions, with the model accurately reflecting increased emissions during high precipitation periods and reduced emissions during drier periods (Macharia et al., 2021). Notably, higher emissions were observed during precipitation exceeding 10 mm in both years. In the spring season, even before fertilizer application, the simulated data showed higher peaks of N_2O flux, indicating the model's sensitivity to precipitation. Early spring N_2O fluxes were produced by the DNDC model simulation in a study by Foltz et al. (2019, 2021). The comparative analysis of the two seasons underscores the model's sensitivity to annual environmental variations and the need for continuous evaluation and recalibration. These findings validate the DNDC model's reliability in predicting N_2O emissions with different N rates and emphasize the crucial role of precipitation. The model also accurately predicted late-season fluxes, corresponding with measured fluxes, suggesting potential late-season emissions as more mineralized N becomes available for denitrification, especially with high precipitation events in the third year of no-till application.

All major process-based models like DAYCENT, DSSAT, and EPIC simulated N_2O fluxes using nitrification- and denitrification-based mathematical equations (Gabbrielli et al., 2024). The DNDC model incorporates microbial interactions and activities through algorithms to predict N_2O fluxes, and it has also been developed using other hydrological measurements (Smith et al., 2020; Deng et al., 2021). While numerous studies have utilized the model to predict N_2O fluxes in legume-based systems, the novel integration of associative microbial-related conditions has yet to be evaluated.

These results indicate the effect of bioinoculants in 2023 introduced variability and uncertainties that the DNDC model has difficulty capturing despite limited parameters available for adjustment. These findings highlight the need for additional model refinement or alternative calibration strategies when predicting emissions under bioinoculant scenarios. To accurately evaluate N_2O emissions in cropping systems with microbial applications, it is essential to emphasize the use of different parameters, such as the microbial activity index. This index may capture the dynamic interactions and temporal shifts in microbial activity mediating the N cycle's critical components. By incorporating the microbial activity index into the DNDC model calibration process, researchers can better account for microbial influences' complex and transient nature on N_2O emissions. This approach enhances the model's predictive accuracy and provides a more comprehensive understanding of microbial-mediated N dynamics in agricultural systems.

3.5.1 Use of microbial activity index as a calibration optimization tool in DNDC model

Incorporating novel microbial inoculants into agricultural practices has provided a competitive edge over many previously available biofertilizers, enhancing the effectiveness of crop management strategies (Maitra et al., 2022a; Olmo et al., 2022). When calibrating the DNDC model to include these microbial inoculants, using a microbial activity index as an optimization tool offers a promising approach. Traditionally, the DNDC model was developed focusing on symbiotic N fixation, particularly in crop rotation with corn-soybean, where N fixation parameters were explicitly assessed. However, integrating new N-fixation products may result in temporal enhancements or increments in N availability to plants, which could be challenging to detect due to poor or insufficient field data quality. This underscores the importance of improving our field data quality to harness the potential of these new agricultural technologies.

Numerous researchers have demonstrated that biofertilizers or specific biological strains can induce temporal changes in microbial diversity and activity within the rhizosphere (Jack et al., 2021; Cornell et al., 2021). These changes are often reflected in the microbial activity index, which captures the temporary shifts in microbial communities thriving under such conditions. I successfully calibrated the DNDC model using the microbial activity index parameter (Figure 4.6). However, the validation using field-measured data was less successful due to uncertainties in N_2O cumulative flux with bioinoculant. Therefore, developing a rhizospheres-related process-based model for evaluating new microbial-related scenarios is of utmost importance. Kuppe et al., (2022) discussed the foundational concepts of rhizosphere modeling provide a critical framework for integrating trait discovery and applying them to plant-soil ecosystems. By addressing key gaps, such as linking soil ecology, physics, and chemistry, and considering the role of microorganisms we can understand and make effective rhizosphere models that can bridge experimental and theoretical approaches.

The added microbial inoculum can temporarily enhance ecosystem functions, benefiting plant growth until maturity. At this point, the microbes may lose their competitive advantage due to a reduction in root-exuded carbohydrates necessary for survival, particularly in the bulk soil where microbial competition is intense (Mawarda et al., 2020).

Although microbial activity indexes have not been well-assessed with the DNDC model, the development and application of such parameter's present challenges due to various factors (Chandel et al., 2023; Manzoni and Schimel, 2024). Microbial activity or behavior, temporal diversity, and abundance can be influenced by many factors beyond introducing developed microbial strains to the system. When implementing such parameters into the model, energy acquisition and transfer, availability of substrates, and temporal uncertainties of rhizosphere ecology should be considered (Kuppe et al., 2022).

The accuracy achieved during calibration underscores the model's potential for use in research and practical applications, such as assessing greenhouse gas emissions and developing mitigation strategies. However, the comparative analysis of performance metrics between 2022 and 2023 highlights the necessity for continuous model evaluation and recalibration to maintain high predictive accuracy across different growing seasons. As described by Arango et al. (2023), known and unknown uncertainties govern modeling dynamics. Such uncertainties needed to be addressed through targeted experiments.

3.6 Conclusion

In conclusion, our study demonstrates the reasonable accuracy of the DNDC model in simulating N_2O emissions through calibration and validation using field data for N rates. The significant improvements in performance metrics post-calibration underscore the model's robustness, particularly at higher N rates, while highlighting areas for refinement at lower rates. The incorporation of precipitation data further demonstrated the model's sensitivity to environmental variations, reinforcing its applicability in diverse agricultural systems. Future integration of microbial-related parameters would allow one to simulate novel agriculture management practices and the impact of such practices on environmental GHG emissions. Incorporating microbial activity indexes in the calibration process presents a promising avenue for capturing dynamic microbial influences on N cycles, enhancing predictive accuracy.

Continuous model evaluation and recalibration remain essential to maintain high precision across varying conditions, ensuring the DNDC model's utility in research and practical applications.

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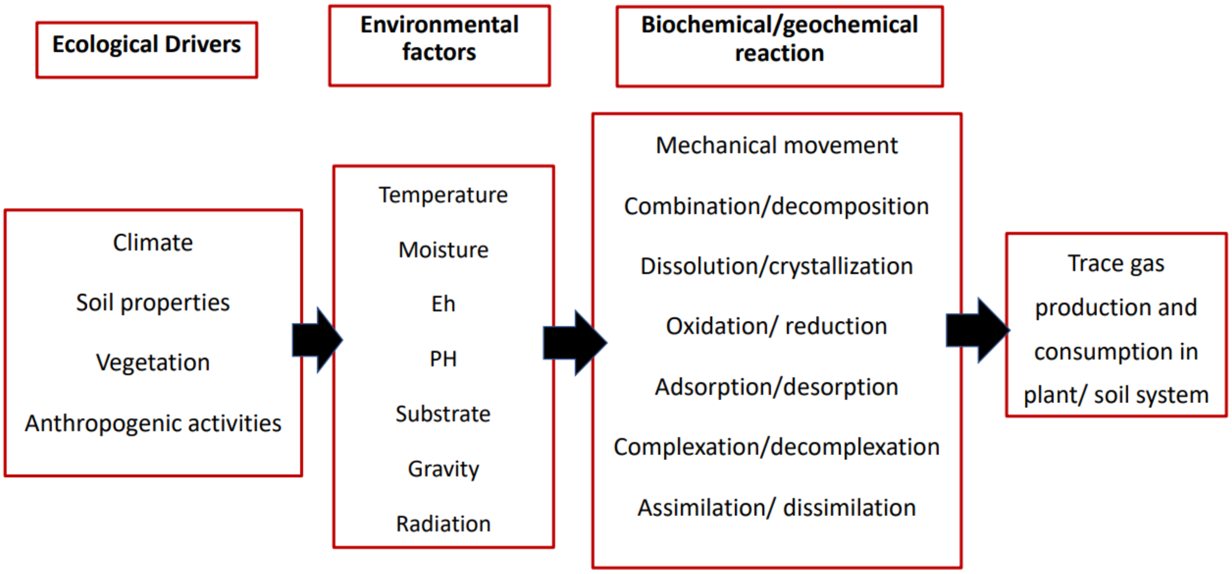

Figure 3.1. DNDC model key parameter flow framework (DNDC 9.5 Scientific basis and process)

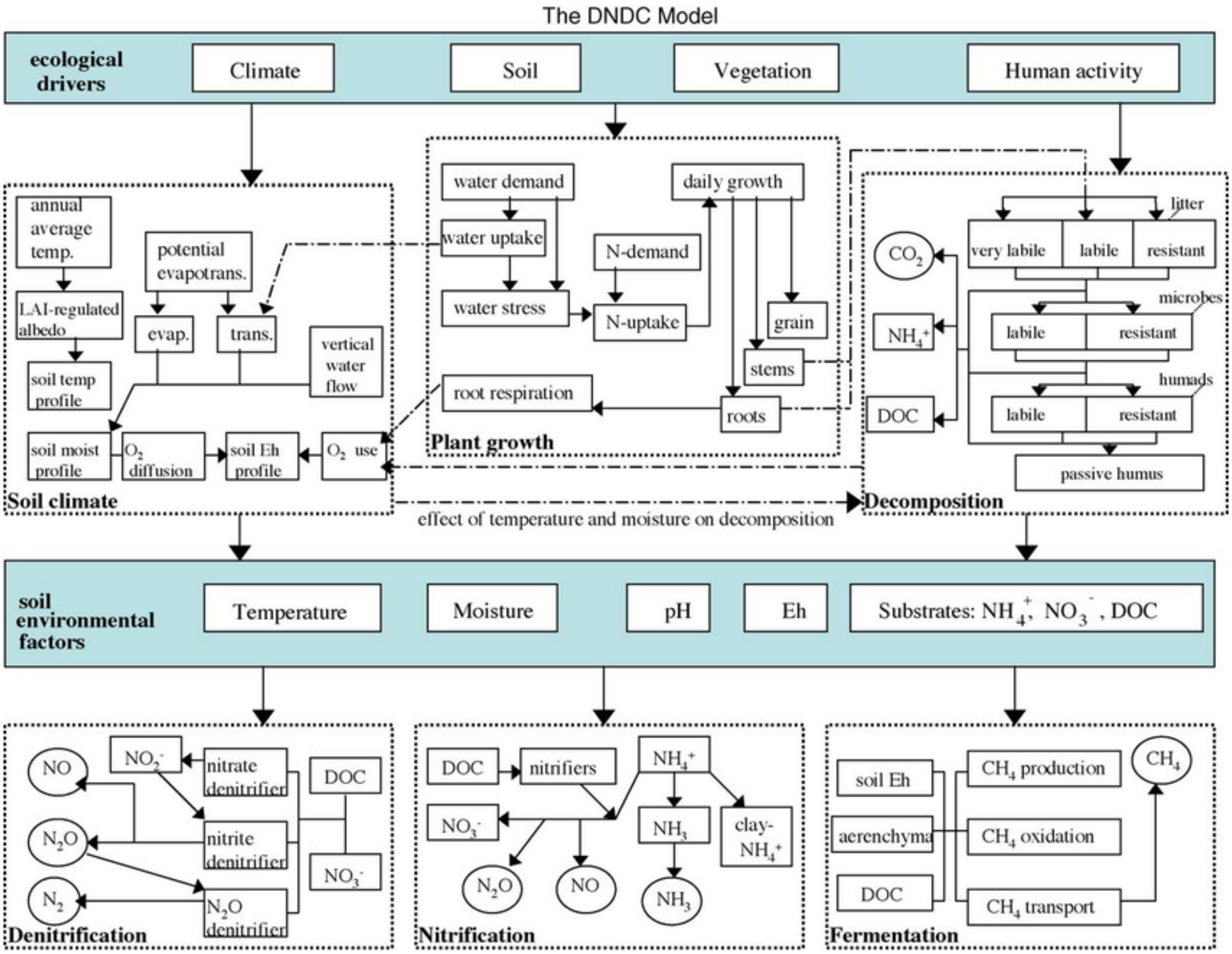

Figure 3.2. Denitrification and decomposition model (Li et al., 2006)

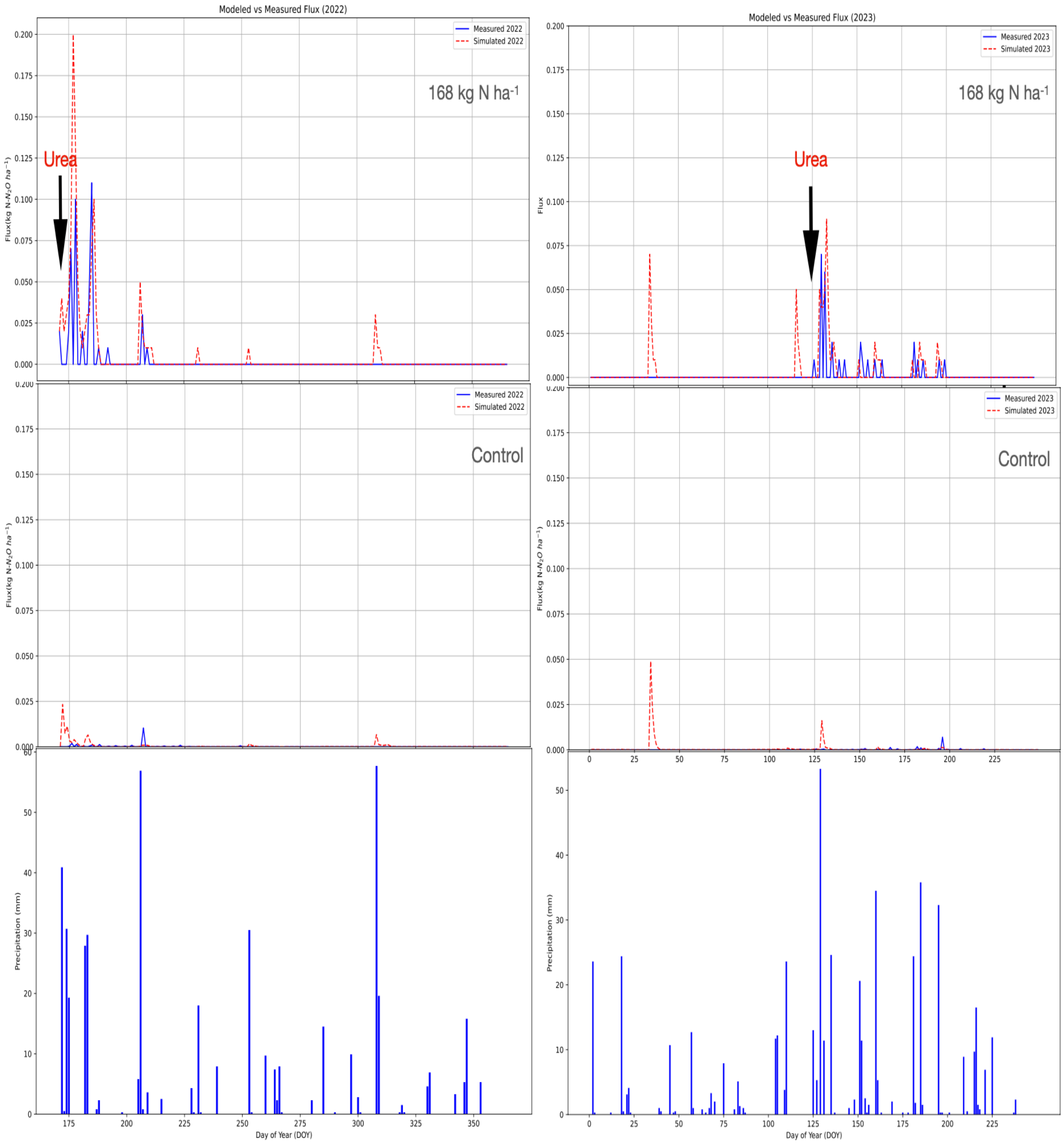

Figure 3.3. Growing season precipitation and N_2O flux measured and simulated in June 2022 to September 2023. Daily-scale modeled and measured N_2O fluxes (kg N_2O-N ha $^{-1}$) (A, B, C, D) and precipitation (mm) (E, F) for the years 2022 and 2023 continuous no-tilled corn. Panel (A, B) shows the modeled and measured daily flux at 168 N rate, where arrows indicate fertilization dates. Panel (C, D) shows the modeled and measured daily flux at 0 N rate or control plots. Panel (E, F) shows the precipitation from the growing season on June 20th, 2022, to the end of the growing season on September 4th, 2023.

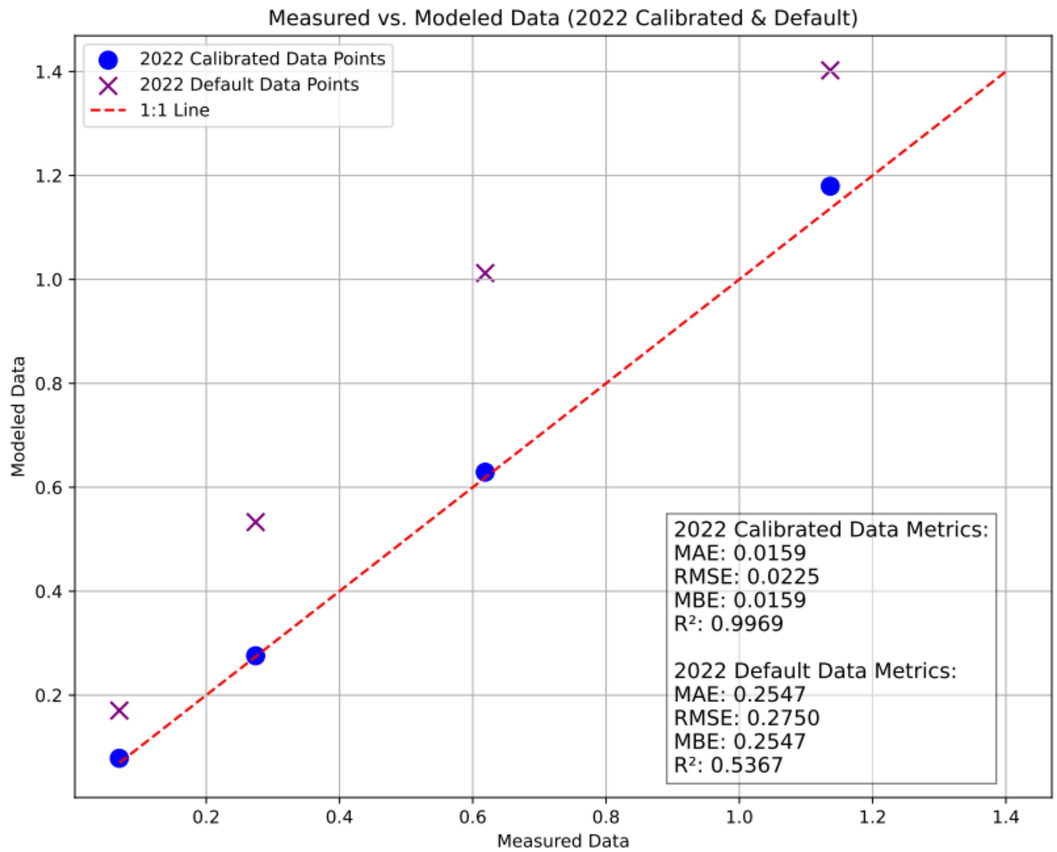

Figure 3.4 Measured vs. modeled data (2022 calibrated & default), The scatter plot compares measured data with modeled data for the year 2022 using both calibrated and default model settings. Blue circles represent the calibrated model data points, while green crosses represent the default model data points. The red dashed line is the 1:1 line, indicating perfect agreement between measured and modeled values. the metrics inside the figure provide quantitative measures of the model performance.

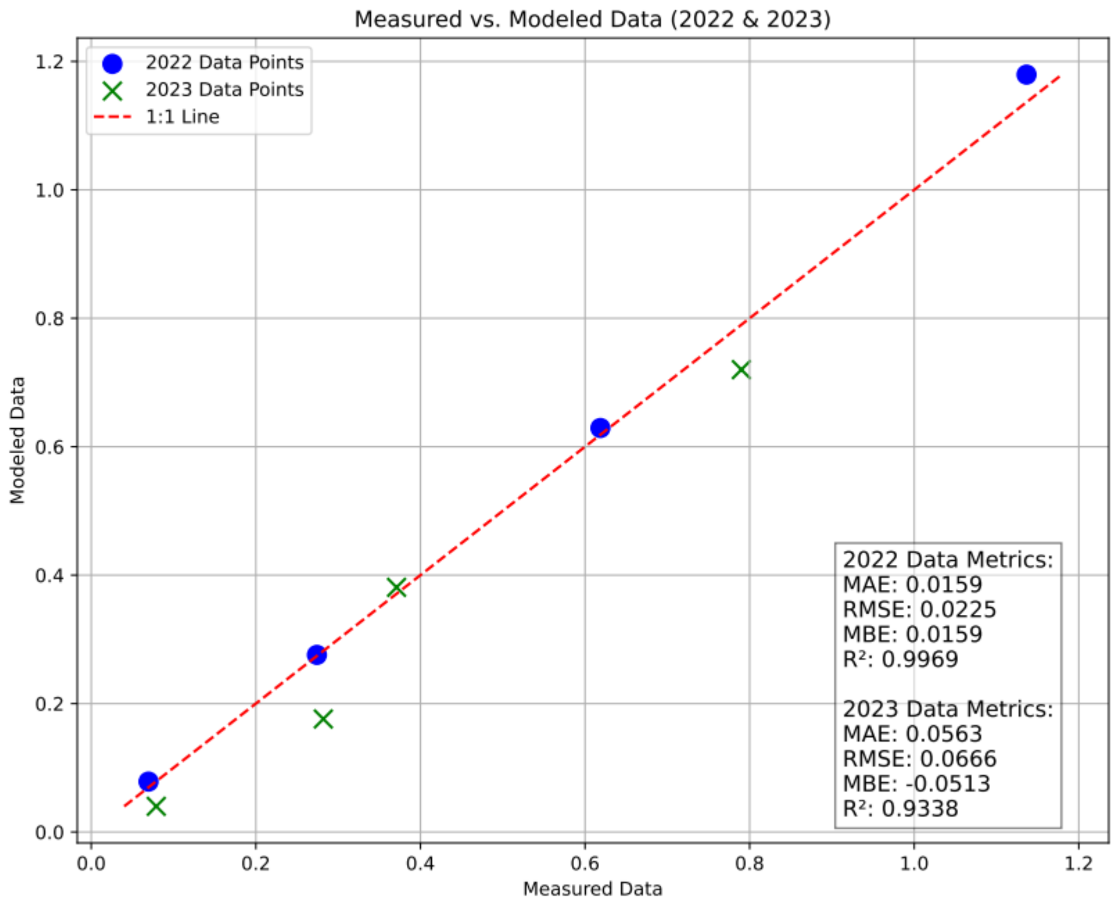

Figure 3.5 Measured vs. modeled data validation using 2023 measured data. The scatter plot compares measured data with modeled data for the year 2023. Using both calibrated and validated model settings. Blue circles represent the calibrated model data points, while green crosses represent the model data points. The red dashed line is the 1:1 line. The metrics inside the figure provide quantitative measures of the model performance.

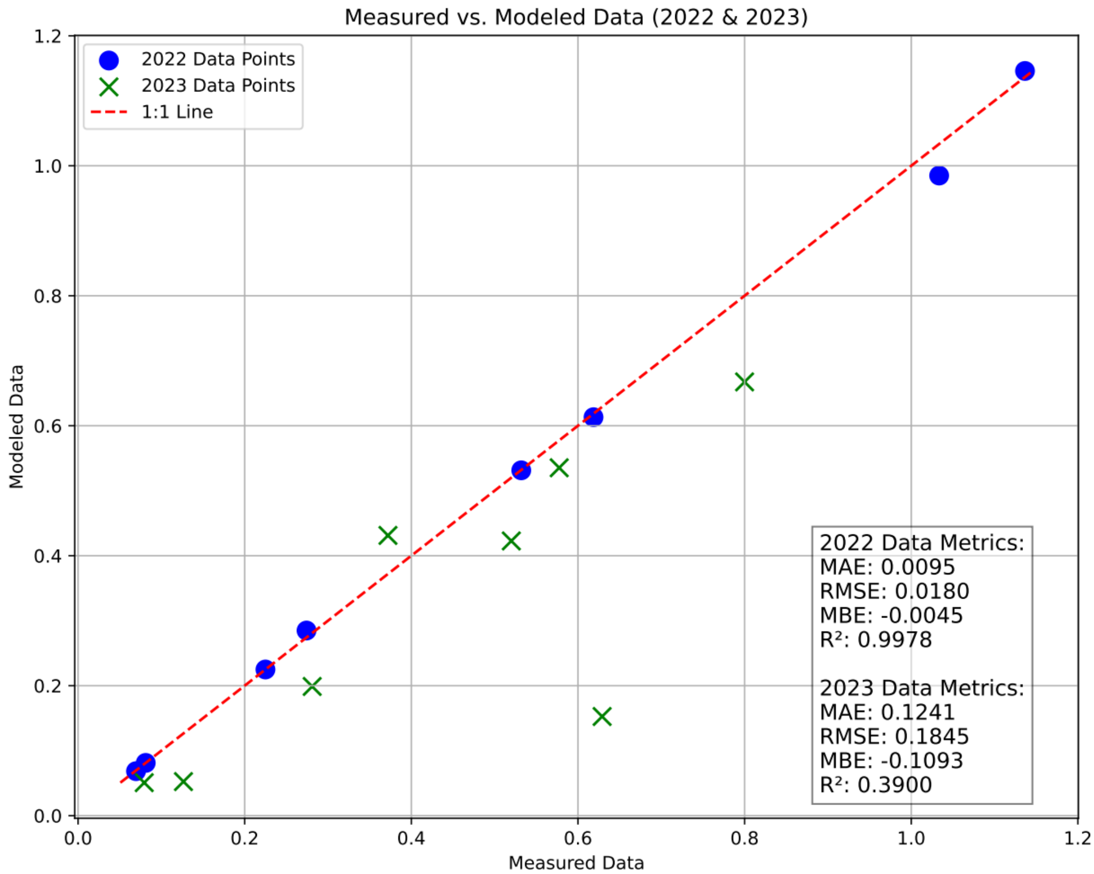

Figure 3.6 Measured vs. modeled data validation using 2023 measured data including bioinoculant-treated plots. The scatter plot compares measured data with modeled data for the year 2023. Using both calibrated and validated model settings. Blue circles represent the calibrated model data points (including bioinoculant) while green crosses represent the model data points. The red dashed line is the 1:1 line. The metrics inside the figure provide quantitative measures of the model performance.

Chapter 4 - Nitrogen fertilizer in corn cropping system inoculated with N-fixing microbial bacteria: ^{15}N isotope fertilizer study

Abstract

Nitrogen (N) is a vital nutrient for plant growth, essential for synthesizing amino acids, proteins, and nucleic acids, influencing crop yield and quality. However, industrial N fertilizers pose environmental challenges, necessitating sustainable management practices. This study explores the effects of a N-fixing microbial bioinoculant and different N application rates (56, 112, and 168 kg N ha^{-1}) on fertilizer N recovery in above-ground biomass and soil inorganic and organic N in a continuous corn not-till cropping system across different growth stages (V6, VT, harvest). The study used a stable isotope ^{15}N tracer approach to quantify N recovery. The experiment was conducted at the North Farm research facility, managed by the Department of Agronomy, Kansas State University. A randomized complete block design with six replications and a split-plot design to accommodate bioinoculant treatments in the study. In 2022, N recovery in above-ground biomass was lower, ranging from 6.7% to 11.62%, compared to 2023. Recovery in above-ground plant biomass in 2023, ranged from 17% to 30.9% across all N rates. The lower N recovery in 2022 might be due to higher early-season precipitation, which can potentially increase N losses through leaching and denitrification. Well-distributed precipitation in 2023 facilitated better N recovery in above-ground biomass. N immobilization was greater at deeper soil layers (15-30 cm), especially at lower N rates. Fertilizer N recovery as soil organic N ranged from 8.7% to 28.3% at the harvest stage in 2023. At 56 kg N ha^{-1} without bioinoculant, 28 % of N was recovered as soil organic N at harvest in 2023. Nitrogen rate significantly affected

fertilizer N recovery as inorganic N across all treatments in 2022 and 2023. Higher N recovery (12.4%) as inorganic soil N was observed in 168 kg N ha^{-1} without bioinoculant application, which was significantly higher than other treatments. There was no effect of N-fixing bioinoculant on N recovery. Long-term field trials are needed to investigate the impact of N fixing microbial inoculant on N recovery by corn and soil.

4.1 Introduction

Nitrogen (N) is a fundamental nutrient essential for plant growth, playing a critical role in forming key biological components like DNA, amino acids, and proteins, which are vital for all living organisms and for plant development and productivity (Anas et al., 2020b; Martínez-Dalmau et al., 2021). Nitrogen's importance in agriculture cannot be overstated, as it significantly influences crop yield and quality.

Since the early 20th century, industrial N fertilizers have revolutionized agriculture, enabling greater crop production to meet the demands of a growing global population. This shift has been particularly pronounced in the cultivation of N-intensive crops (Lu et al., 2019; Govindasamy et al., 2023). Despite these benefits, extensive use of N fertilizers raises significant environmental concerns. The overapplication of N fertilizers contributes to reactive N emissions, which can degrade air and water quality and disrupt natural ecosystems (Schlesinger, 2009). This dual impact of N fertilizers increasing agricultural productivity while posing environmental risks makes it a critical area of study.

Industrial N fertilizers have been indispensable, particularly for cereal production. A comprehensive global N budget from 1961 to 2010 revealed that cereals harvested 1551 Tg of N, with 48% derived from fertilizers and 4% from net soil depletion. As described by Ladha et al.

(2016), non-symbiotic N2 fixation significantly contributes to the N pool in the agriculture sector, which was 370 Tg (24%) of the total N in crops. Manure and atmospheric deposition supply 14% and 6% of the N, respectively, while crop residues and seeds contribute minimally (Ladha et al., 2016). These findings underscore the multifaceted N in agriculture and the reliance on synthetic fertilizers to meet crop demands.

The efficient use of N fertilizers in agriculture is crucial for achieving high crop yields while mitigating adverse environmental impacts. Studies indicate that over 50% of applied N in cropping systems is lost through various pathways, including gaseous emissions such as nitrous oxide (N_2O) and leaching as nitrate (NO_3^-) (Govindasamy et al., 2023). Nitrous oxide is a potent greenhouse gas contributing to global climate change and stratospheric ozone depletion. Additionally, nitrate leaching contaminates groundwater, leading to eutrophication of aquatic ecosystems and posing severe risks to water quality and human health (Robertson et al., 2013). These challenges highlight the need for improved N management strategies.

Improving fertilizer use efficiency within the soil-crop system can mitigate the negative impacts of N fertilizer use. Enhanced N recovery ensures that a larger portion of applied N ends up in marketable products, such as corn grain, or is stabilized into soil organic matter, thereby increasing soil fertility (Govindasamy et al., 2023). Nitrogen losses represent a substantial economic burden for farmers and impose significant social costs due to environmental degradation (Keeler et al., 2016; Goodkind et al., 2023). Therefore, there is an urgent need for sustainable N management practices that enhance fertilizer use efficiency, reduce environmental pollution, and ensure the economic viability of agricultural producers (Zhang et al., 2015; Ekins and Zenghelis, 2021).

Corn cropping systems, particularly in the Midwest's Corn Belt in the U.S., are among the highest consumers of N fertilizers, with this region accounting for approximately 33% of global corn production (Wang et al., 2020). Thus, effective N management strategies in this region are crucial for productivity and environmental sustainability. Factors influencing N use efficiency include physical and chemical characteristics of soil, such as soil texture, pH, and organic matter content; field management practices; fertilizer rate, source, and timing; and external factors, such as precipitation patterns, disease, or pest pressure (Govindasamy et al., 2023).

Addressing these factors comprehensively can lead to more efficient N use and reduced environmental impacts. Innovative approaches to N management are being explored to address these challenges. One promising strategy is the integration of N-fixing microbial inoculants into corn cropping systems (Arif et al., 2020; Olmo et al., 2022; Klimasmith and Kent, 2022). These bioinoculants can improve N use efficiency by promoting biological N fixation, reducing the need for synthetic fertilizers. Precision agriculture techniques, such as soil testing and variable rate application, can optimize N use by aligning application rates with crop needs and soil nutrient levels. By adopting these innovative strategies, it is possible to enhance N use efficiency, reduce environmental impacts, and support sustainable agricultural practices that meet the demands of a growing population while protecting natural ecosystems.

Isotopic experiments have gained significant popularity in research for identifying the fate of N in agroecosystems. Non-radioactive ^{15}N isotope tracer techniques are among the leading methods for evaluating N circulation within agroecosystems (Fry, 2006). Enriched or depleted ^{15}N fertilizers are used in these experiments; however, the high 99% ^{15}N enrichment cost limits its widespread use. Instead, lower ^{15}N enrichment is commonly employed in agroecosystem research. Various approaches to using ^{15}N isotope fertilizers in research include applying the isotope directly to the root rhizosphere through targeted injection, which minimizes N losses and maximizes uptake by the plants and using a microplot within a larger plot, where selected rows of plants are treated with ^{15}N isotope (Clode et al., 2009; Scheer and Rütting, 2023). This approach ensures a more controlled and efficient use of the isotope, allowing for precise measurement of N uptake and movement within the system.

Fertilizer recovery efficiency can be estimated using two primary methods: the traditional non ^{15}N approach, which measures the net effect of fertilization on N recovery in the crop, and the stable isotope ^{15}N tracer method, which differentiates between fertilizer-derived N and soil-derived N (Warembourg, 1992). Studies employing the labeled ^{15}N-enriched fertilizer have successfully quantified fertilizer-derived N (FDN) recovered in aboveground biomass and soil and as environmental loss across various cropping systems.

A comprehensive meta-analysis by (Quan et al., 2021c) reviewed 366 field ^{15}N tracer observations from 74 publications worldwide, revealing significant regional differences in N uptake efficiency ($NUE^{15}N$) in corn. The study found that the proportion of ^{15}N absorbed by aboveground biomass was notably lower in China (33%) compared to North America (42%) and the European Union (54%). These findings highlight the variability in NUE ^{15}N and highlight the role of environmental factors, such as soil organic carbon and management practices, in influencing the efficiency of N use in agriculture. Rainfall variability, soil texture, and no-till or conventional tillage can affect NO_3^- loss in an agroecosystem. Higher coarse textured sandy soil potential drains more water, leading to higher N leaching. Further, with no-till systems, N leaching potential is due to higher infiltration from the soil organic matter content (Daryanto et al., 2017).

Complimenting this analysis, another study involving ^{15}N tracer experiments conducted at four sites in Northeast China, encompassing 23 site-year field experiments, found that, on average, 34% of the applied N (222 kg N ha^{-1}) was accumulated in the aboveground biomass. Additionally, 35% of the N was retained in the soil, and 31% was lost to the environment. These results provide a detailed overview of N utilization and losses in corn agriculture within China, illustrating the diverse fates of applied N in different regions and the impact of environmental conditions and management strategies on N use efficiency(Quan et al., 2020). Together, these studies offer valuable insights into the dynamics of N utilization in corn cropping systems. They emphasize the need for tailored N management practices to enhance NUE and minimize environmental impacts across different geographical regions.

This study aims to explore the fate of N management strategies in corn cropping systems, focusing on management that incorporates N-fixing microbial inoculants. The study used the stable isotope ^{15}N tracer methods to evaluate the N recovery in the above-ground biomass and the distribution of N in the soil profile. The findings contribute to developing sustainable N management practices that enhance agricultural productivity while minimizing environmental harm.

4.2 Methodology

4.2.1 Study site and experimental design

The experiment was conducted from 2021 to 2023 at the Kansas State University North Agronomy Research Farm in Manhattan, Kansas (39°12' N, 96°35' W). The soil at the site consisted of Ivan and Kennebec silt loams (fine-silty, mixed, super-active, mesic Cumulic Hapludolls). Corn (Zea mays L.) was planted with a N-fixing bioinoculant (PivotBio® Proven

40, Pivot Bio Inc., Berkeley, CA) on June 4, 2021, June 19, 2022, and April 27, 2023. Continuous corn was cultivated in 2021, 2022, and 2023 with four N fertilization rates (0, 56, 112, and 168 kg N ha^{-1}) using broadcast-applied urea, both with and without the bioinoculant. The bioinoculant was applied in-furrow at a rate of 1.90 liters per hectare (25.6 fl. oz per acre) with a carrier volume of 46.8 liters per hectare (5 gallons per acre). A no-till management approach was employed, and the experiment was arranged as a randomized complete block design (RCBD) with six replications. A split-plot design accommodated the bioinoculant treatment. To address the variability and challenges encountered in the first year of our study (2021), we have chosen to present data exclusively from the 2022 and 2023 growing seasons. The 2021 data were excluded due to unusual weather resulting in lower yields.

4.2.2 Microplot establishment and ^{15}N labeling

Microplots (2.4 m^2) were established within each main plot (Figure 4.1) at different locations each year, as described by Omay et al., (1998). In these microplots, 2% enriched ^{15}N labeled urea ($[^{15}NH_2]C([^{15}NH_2])=O$, Sigma-Aldrich) was applied according to the designated urea fertilization rates. The labeled urea was dissolved in water and uniformly sprayed on the corn plots post-planting.

4.2.3 Plant and soil sampling

Soil samples were collected at three depths (0-5cm, 5-15cm, 15-30cm) at V8, VTR1 and after harvest. Every year after harvest, the bulk density of each depth was taken using a Giddings probe. Plant samples were collected at the V8, VTR1, and R6 stages of corn growth. At R6, the grain was separated from vegetative biomass and analyzed separately. Four plants from each plot were harvested at each stage and grain was dried and ground at harvest and sent to the IT^2 Isotope Tracer Technologies in Waterloo, Canada, for ^{15}N isotopic analysis. Soil samples were taken at three depths (0-5 cm, 5-15 cm, and 15-30 cm) during each growth stage to evaluate inorganic ^{15}N using standard KCl extraction methods and diffusion methods described by Brooks et al. (1989).

4.2.4 ^{15}N enrichment analysis

For the ^{15}N enriched soil samples, KCl extraction was performed three times. From the first extraction, 40 ml was used for the ammonium diffusion. Devarda's alloy (~0.4 g) and MgO (~0.4 g) were added to the KCl extract to reduce nitrate (NO_3^-) and nitrite (NO_2^-) to ammonium (NH_4^+). The released NH_3 was trapped using acidified filter paper (2.5 M K_2HSO_4) placed on top of the specimen cup, following the procedure shown in Figure 4.2. The solution was well-mixed using glass beads until it became cloudy, ensuring no contamination of the filter paper. The setup was left for 6 days. Post-incubation, the filter papers were retrieved, placed on a foam platform (Figure 4.3), and stored in an acidified desiccator to dry. Once dried, the filter papers were sent to IT2 Isotope Tracer Technologies in Waterloo, Canada, for the isotope analysis.

The following equation calculated the percent recovery of fertilizer isotopic 15N:

$$pecent\ fertilizer\ recovery = \frac{(NF)\ x\ (C - B)}{R\ (D - B)}\ x100$$

Where *NF= total N uptake in corn from fertilizer plots*

C= atom % ^{15}N of plant tissue from N fertilized plots

B= atom % ^{15}N from unfertilized plots

D= atom % ^{15}N in applied fertilizer

R= rate of applied ^{15}N-labeled fertilizer

4.2.5 Data and statistical analysis

Soil N content data were analyzed using linear mixed models (LMM) and analysis of variance (ANOVA) to evaluate the effects of various factors, including N application rates, bioinoculant presence, and soil depth, across different crop growth stages. The LMM analysis was performed using the `MixedLM` function from the `statsmodels` library in Python. ANOVA was used to determine the significance of the fixed effects, focusing on the factors' main effects and interactions on fertilizer N recovery content. Tukey's honestly significant difference (HSD) test evaluated the mean differences with a significance level set at $\alpha = 0.05$.

4.3 Results

4.3.1 Nitrogen fertilizer recovery in above-ground biomass

The highest N fertilizer recovery in above-ground biomass in 2022 was observed at the VTR1 growth stage (Table 4.1, Figure 4.6). The V8 and harvest recovery (including grain and stover) were lower than the recovery at the VTR1. Across all the treatments, fertilizer N recovery in above-ground plant biomass ranged from 0.6 -1.17% at the V8, while it was 12.7-19.8% in the VTR1 and 6.71-11.6% at harvest. The highest fertilizer N recovery percentage was 19.8% from 168 kg N ha^{-1} with bioinoculant. Fertilizer N recoveries did not significantly differ among treatments in 2022 (Table 4.1).

The highest fertilizer N recovery in 2023 was at R6 (including grain and harvest plant stover) growth stage, which ranged from 17.0%- to 30.9% across all the treatments. However, the values were not significantly different among treatments. The highest fertilizer recovery was with 168 kg N ha^{-1} with bioinoculant, and the lower fertilizer recovered with 112 kg N ha^{-1} without bioinoculant application. The fertilizer recovery percentage significantly differed at the VTR1 stage. The highest recovery was with 168 kg N ha^{-1} without the bioinoculant, which was 23. 8%. The lowest recovery was recorded as 11. 6 with bioinoculant in 56 kg N ha^{-1}. Nitrogen rate significantly affected fertilizer N recovery at VTR1 ($p=0.002$).

Table 4.1 Fertilizer N recovery percentage in above-ground biomass at each stage in 2022 and 2023 and analysis of variance (ANOVA).

N_ rate kg N ha^{-1}	Bioinoculant	2022 V8	2022 VTR1	2022 R6	2023 V8	2023 VTR1	2023 R6
		Fertilizer N recovery %					
56	N	0.62	12.7	8.22	8.41	14.6 *b*	20.8
56	Y	0.76	19.2	8.18	9.04	11.6 *b*	23.3
112	N	0.46	18.4	6.71	8.32	14.3 *b*	17.0
112	Y	0.60	18.3	11.6	11.6	18.8 *b*	19.6
168	N	0.76	15.5	7.94	6.7	23.8 *a*	29.7
168	Y	1.17	19.8	8.95	10.5	18.2 *b*	30.9

N_ rate	0.851	0.523	0.645	0.851	**0.002**	0.087
Bioinoculant	0.786	0.193	0.984	0.786	0.274	0.668
N_ rate x Bioinoculant	0.915	0.628	0.112	0.915	**0.035**	0.981

Different letters (a, ab, b) within a column indicate significant differences between means as determined by Tukey's HSD test ($p < 0.05$). The Tuckey HSD test was evaluated separately for each stage, and if there was no significant difference amount, each application did not use a letter. Values represent the mean percentage of fertilizer N recovery. The table presents the p-values from two-way ANOVA analyzing the effects of nitrogen rate and bioinoculant on fertilizer nitrogen recovery % across the V8, VT, and R6 stages ($\alpha < 0.05$).

The effect of the bioinoculant was not significant (P=0.274). However, N rate and bioinoculant interaction had a significant effect (p=0.035) on fertilizer N recovery at the VTR1 stage in 2023. Comparing fertilizer N recoveries in 2022 and 2023, 2023 had higher N recovery than 2022. Further, N fertilizer recovery decreased after VTR1 in 2022 in contrast to 2023, which increased. There was a tendency for higher recovery with the bioinoculant but this trend was not significant.

4.3.2 Fertilizer N recovery (kg N ha^{-1}) in the soil profile as organic N

The amount of fertilizer N recovered (kg N ha^{-1}) as soil organic N at each crop development stage by depth in the soil profile for both years is presented in Table 4.2 and Figure 4.7. Organic N was greater in 2023 compared to 2022.

Table 4.2 Distribution of fertilizer N (kg N ha^{-1}) in the soil as organic N in each stage/depth in 2022/2023 and analysis of variance (ANOVA) to a depth of 0-30 cm.

			-----------2022----------			----------2023----------		
N_ rate	Bio	depth	V8	VTR1	R6	V8	VTR1	R6
Kg ha^{-1}			--------------------------kg N ha^{-1}			-------------------------------		
56	N	0-5	2.68	2.89	2.59	4.4	5.03	4.63
		5-10	2.13	3.71	3.67	3.33	3.41	4.94
		15-30	4.46	3.45	6.01	6.7	6.16	6.29
56	Y	0-5	2.42	1.85	3.45	4.06	5.4	4.07
		5-10	2.03	2.98	3.34	3.38	3.54	4.34
		15-30	4.72	4.00	6.33	5.45	6.06	4.61
112	N	0-5	3.58	2.73	3.25	7.82	8.26	5.24
		5-10	2.33	4.77	4.11	3.58	5.46	3.88
		15-30	5.16	3.25	5.86	11.13	6.78	4.95
112	Y	0-5	4.34	3.35	4.8	6.02	6.27	6.84
		5-10	2.36	4.1	4.56	3.73	5.16	11.07
		15-30	4.83	3.84	6.59	6.47	7.2	7.37
168	N	0-5	4.03	5.2	3.54	7.25	7.99	4.81
		5-10	2.79	4.96	5.6	7.31	4.83	4.58
		15-30	4.27	5.05	7.22	8.3	6.94	5.21
168	Y	0-5	3.32	3.39	5.08	8.77	10.58	6.95
		5-10	3.46	3.63	5.22	4.99	5.97	7.63
		15-30	4.6	3.93	6.28	7.07	7.29	7.88
			-------------------------kg N ha^{-1}	-----(0-30cm)		----------------		
56	N		9.27	10.05	12.26	14.44	14.6	15.87
	Y		9.17	8.83	13.13	12.88	14.99	13.02
112	N		11.07	10.77	13.22	22.53	20.48	14.07
	Y		11.52	11.29	15.92	16.22	18.65	25.27
168	N		11.06	15.2	16.38	22.85	19.8	14.64
	Y		11.37	10.95	16.58	20.83	23.83	22.46
					p-value			
N_ rate			0.358	0.031	0.031	0.001	**<0.001**	0.229
Bioinoculant			0.87	0.132	0.264	0.061	0.402	**0.043**
N_ rate x Bioinoculant			0.984	0.198	0.635	0.459	0.075	0.08

The table represents the mean N recovered as soil organic N in kg N ha^{-1} in all the depths and 0-30 cm in the soil profile. The p-values from ANOVA analyzing the effects of nitrogen rate and bioinoculant on fertilizer nitrogen recovery (kg N ha^{-1}) across the V8, VT, and R6 stages at 0-30 cm depth ($\alpha < 0.05$)

The effect of soil depth, alongside N rate and bioinoculant application, on organic N recoveries is presented in Table 4.3.

Table 4.3 Fertilizer N recovery in the soil as organic N in each stage/ depth in 2022 and 2023 and ANOVA table.

N_rate Kg ha^{-1}	Bioino	depth	2022 V8	2022 VTR1	2022 R6	2023 V8	2023 VTR1	2023 R6
			Fertilizer N recovery %					
56	N	0-5	4.79	5.16	4.62	7.86	8.98	8.28
		5-15	3.81	6.62	6.55	5.95	6.09	8.82
		15-30	7.96	6.17	10.7	11.9	11	11.2
56	Y	0-5	4.32	3.3	6.17	7.24	9.64	7.26
		5-15	3.63	5.31	5.97	6.04	6.32	7.75
		15-30	8.42	7.14	11.3	9.73	10.82	8.23
112	N	0-5	3.19	2.44	2.91	6.98	7.37	4.68
		5-15	2.08	4.26	3.67	3.2	4.88	3.46
		15-30	4.61	2.9	5.23	9.94	6.05	4.42
112	Y	0-5	3.88	2.99	4.28	5.37	5.6	6.11
		5-15	2.1	3.66	4.07	3.33	4.61	9.88
		15-30	4.31	3.42	5.88	5.77	6.43	6.58
168	N	0-5	2.4	3.09	2.11	4.31	4.75	2.86
		5-15	1.66	2.95	3.33	4.35	2.88	2.73
		15-30	2.54	3.01	4.3	4.94	4.13	3.1
168	Y	0-5	1.98	2.02	3.03	5.22	6.3	4.14
		5-15	2.06	2.16	3.11	2.97	3.55	4.54
		15-30	2.74	2.34	3.74	4.21	4.34	4.69

	P-value					
N_ rate	0.101	**0.028**	**0.023**	0.078	**<0.001**	**0.026**
Bioinoculant	0.677	0.077	0.096	0.704	0.505	0.612
Depth	**0.001**	0.362	**<0.001**	**0.001**	**<0.001**	0.29
N_ rate x Bioinoculant	0.715	0.254	0.882	0.541	**0.054**	0.626
N_ rate x Depth	0.265	0.678	**0.015**	0.063	**0.036**	0.8
Bioinoculant x Depth	0.834	0.131	0.267	0.583	0.835	0.723
N_ rate x Bioino x Depth	0.908	0.614	0.929	0.596	0.441	0.676

Mean values of fertilizer N recovery percentage (%) at three depths (0-5 cm, 5-15cm, 15-30cm) by each stage (V8, VTR1, harvest) for years 2022 and 2023.The p-values from ANOVA analyzing the effects of nitrogen rate, bioinoculant, and soil depth on fertilizer nitrogen recovery across the V8, VT, and R6 stages ($\alpha < 0.05$).

In 2023, the highest organic N recovered (23.8 kg N ha$^{-1)}$ was associated with the 168 kg N ha^{-1} application with bioinoculant at VTR1 stage. At harvest, the fertilizer-derived N as organic N amount was 22.6 and 14.6 kg N ha^{-1} associated with 168 kg ha^{-1} plots with and without bioinoculant application, respectively. In both the 2022 and 2023 years at the harvest stage, bioinoculant application increased the recovery of fertilizer as organic N retained in the soil. Relatively higher organic N derived from fertilizer N was present in the 15-30 cm depths than in the shallower depths 0-5 cm and 5-15cm.

In 2022, depth was significant for fertilizer N recovery as organic N at V8 and harvest ($p = 0.001$). At V8, the 15-30 cm recovery was higher than 0-5 cm and 5-15 cm depths. The highest N recovery was 11.3% at harvest for 56 kg N ha^{-1} with bioinoculant ($p=0.23$) and N rate by depth interaction ($P<0.001$) had a significant effect on fertilizer N recovery as organic N at harvest in 2022.

Table 4.4 and Figure 4.8 depict fertilizer N recovery in the soil profile (0-30 cm) as organic N. N rate significantly affected N recovery at all stages ($P<0.001$). At 56 kg N ha^{-1}, fertilizer N recovery in the soil as organic N was significantly higher than all the other N rates.

In 2022, the highest recoveries were 23.5% for 56 kg N ha^{-1} with bioinoculant and 21.9% without the bioinoculant. The lowest N recoveries were with 168 kg N ha^{-1}. Across all the stages, fertilizer N recovery at 168 kg N ha^{-1} was significantly lower than 56 kg Nha^{-1}.

The N application rate significantly affected fertilizer N recovery at all stages ($p < 0.001$) in 2022 and 2023. Higher N recoveries were with 56 kg N ha^{-1} across the stages. The lowest N recoveries were associated with 168 kg N ha^{-1}. The bioinoculants did not significantly impact N recoveries as organic N in the soil. However, the interaction of N rates with bioinoculant had an N recovery effect at the harvest stage 2023 ($P=0.06$).

In contrast to 2022, N rate significantly affected fertilizer N recovery as soil organic N at both VTR1 and harvest in 2023. Higher N recoveries were observed at 15-30 cm with 56 kg N

ha^{-1} with and without bioinoculant application. Further, depth ($p<0.001$) and N rate by depth interaction ($P=0.036$) significantly affected N recovery at VTR1.

Table 4.4 Fertilizer N recovery in the soil as organic N in each stage in 2022 and 2023 and analysis of variance (ANOVA) to a depth of 0-30 cm.

N_ rate kg N ha^{-1}	Bioinoculant	V8	VTR1	R6	V8	VTR1	R6
		------------2022----------			----------2023--------		
		------------------------Fertilizer N recovery % ---------------------					
56	N	16.6 a	18.0 a	21.9 *a*	25.8 *c*	26.1 *a*	28.3 *a*
56	Y	16.4 a	15.8 *ab*	23.5 *a*	23.0 *c*	26.8 *a*	23.3 *ab*
112	N	9.9 *a*	9.6 *b*	11.8 *b*	20.1 *a*	18.3 b	12.6 *b*
112	Y	10.3 *a*	10.1 *b*	14.2 *b*	14.5 ab	16.7 *b*	22.6 b
168	N	6.6 *b*	9.1 *b*	9.8 *b*	13.6 ab	11.8 *c*	8.7 a
168	Y	6.8 *b*	6.5 *b*	9.9 *b*	12.4 *a*	14.2 c	13.4 a

N_ rate	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Bioinoculant	0.93	0.23	0.42	0.27	0.68	0.26
N_ rate x Bioinoculant	0.98	0.39	0.69	0.44	0.24	**0.06**

Different letters (a, ab, b) within a column indicate significant differences between means as determined by Tukey's HSD test ($p < 0.05$). The Tuckey HSD test was evaluated separately for each stage.

Table 4.5 Tuckey HSD post-hoc test for N recovery percentage as organic N across different soil depths.

Depth comparisons		2022	2023
		------p-value-----	
0-5 cm	5-15 cm	0.760	**0.027**
0-5 cm	15-30 cm	**<0.0001**	0.186
5-15 cm	15-30cm	**<0.0001**	**0.0001**

The Tukey HSD post-hoc test was performed to compare the mean across different N fertilizer recovery across soil depth across all the stages. The p-value indicates the significance ($p < 0.05$).

The Tukey HSD tests across all stages for all the depths 0-5, 5-15 and 15-30 cm in 2022 and 2023 is presented in Table 4.5. For 2022, the deepest layer (15-30 cm) had significantly higher N recovery compared to shallower depths ($p < 0.0001$). For 2023, similar patterns were observed, with significant differences in N recovery between the 0-5 cm and the 15-30 cm depth ($p < 0.0001$).

4.3.3 Fertilizer N recovery in the soil profile as inorganic N

Fertilizer N recovery as soil inorganic N in the soil profile is provided in Table 4.6 and as percent recovery in Table 4.7. Neither N rate nor bioinoculant significantly affected inorganic N recovery at any stage in 2022. The highest N recovery was with 112 kg N ha^{-1} with bioinoculant, which was 1.2%. All other N recoveries were below 1% in 2022 across all the stages (Table 4.6, Figure 4.10).

Table 4.6 Fertilizer N recovery in kg N ha^{-1} (0-30cm) in the soil as inorganic N at each stage in 2022 and 2023 and analysis of variance (ANOVA).

		2022			2023		
N rates		V8	VTR1	R6	V8	VTR1	R6
kg N ha^{-1}	Bio	kg N ha^{-1}					
56	N	0.33	0.10	0.31	0.80	0.15	0.15
56	Y	0.22	0.08	0.48	0.65	0.20	0.12
112	N	0.93	0.37	0.82	6.79	0.30	0.17
112	Y	1.34	0.35	0.58	2.39	0.43	0.37
168	N	1.54	0.56	1.26	20.9	0.84	0.36
168	Y	1.37	0.62	1.01	12.9	0.70	0.36

	p-value					
N_rate	**0.048**	**0.046**	0.064	**<0.001**	**0.002**	**<0.001**
Bioinoculant	0.904	0.968	0.663	0.149	0.923	0.194
N_ rate x Bioinoculant	0.798	0.974	0.734	0.533	0.684	0.081

The table represents the mean N recovered in kg N ha^{-1} in 0-30 cm in the soil profile. The p-values from ANOVA analyzing the effects of N rate and bioinoculant on fertilizer nitrogen recovery (kg N ha^{-1}) across the V8, VT, and harvest stages at 0-30 cm depth ($\alpha < 0.05$).

Table 4.7 Fertilizer N recovery percentage in the soil as inorganic N in each stage in 2022 and 2023 and analysis of variance (ANOVA).

		-----------2022-----------			------------2023-----------		
N_ rate	Bioinoculant	V8	VTR1	R6	V8	VTR1	R6
Kg ha^{-1}		------------------------Fertilizer N recovery % ------------------					
56	N	0.58	0.18	0.55	1.43 *c*	0.27	0.27
56	Y	0.4	0.15	0.85	1.17 *c*	0.35	0.22
112	N	0.83	0.33	0.73	6.07 *b*	0.27	0.15
112	Y	1.20	0.32	0.52	2.13 *b*	0.38	0.33
168	N	0.92	0.33	0.75	12.4 *a*	0.50	0.22
168	Y	0.82	0.37	0.60	7.70 *b*	0.42	0.22

N_rate	0.799	0.633	0.805	**0.009**	0.236	0.251
Bioinoculant	0.725	0.854	0.378	0.937	0.594	0.473
N_rate x Bioinoculant	0.722	0.964	0.504	0.607	0.624	0.055

Different letters (a, ab, b) within a column indicate significant differences between means as determined by Tukey's HSD test ($p < 0.05$). The Tuckey HSD test was evaluated separately for each stage, and if there was no significant difference amount, each application did not use a letter. Values represent the soil profile's mean percentage of fertilizer N recovery (0-30cm).

Inorganic N recovery in 2022 was not significantly affected by N rate, bioinoculant, or depth (Table 4.9). Low N recovery was observed at all depths. However, depth significantly affected fertilizer N recovery as inorganic N at R6 ($p = 0.017$) in 2023. Higher N recovery was observed in shallow (0-5 cm) and deeper (15-30 cm) layers with the 168 kg N ha^{-1} treatment.

Table 4.8 Fertilizer N recovery percentage in soil as inorganic N in each stage/depth in 2022 and 2023 and analysis of variance (ANOVA).

			--------2022-------			---------2023---------		
N_rate kg N ha^{-1}	Bioinoculant	depth	V8	VTR1	R6	V8	VTR1	R6
			-------------------------Fertilizer N recovery % ---------------------					
56	N	5	0.20	0.10	0.08	0.37	0.12	0.13
		15	0.32	0.04	0.15	0.33	0.11	0.12
		30	0.07	0.04	0.33	0.75	0.04	0.05
56	Y	5	0.14	0.06	0.17	0.32	0.17	0.09
		15	0.20	0.04	0.23	0.33	0.14	0.08
		30	0.06	0.05	0.47	0.53	0.04	0.05
112	N	5	0.39	0.15	0.09	4.51	0.15	0.07
		15	0.29	0.08	0.21	0.37	0.09	0.05
		30	0.16	0.09	0.46	1.21	0.03	0.02
112	Y	5	0.25	0.13	0.16	0.87	0.16	0.08
		15	0.67	0.10	0.19	0.41	0.16	0.15
		30	0.30	0.08	0.18	0.87	0.06	0.10
168	N	5	0.24	0.13	0.05	5.49	0.39	0.07
		15	0.40	0.10	0.45	1.77	0.03	0.11
		30	0.27	0.10	0.26	5.19	0.10	0.04
168	Y	5	0.35	0.09	0.14	5.42	0.33	0.10
		15	0.15	0.05	0.31	0.86	0.04	0.10
		30	0.32	0.23	0.14	1.42	0.06	0.03

	P-value					
N_rate	0.697	0.801	0.972	**0.014**	**0.002**	0.092
Bioinoculant	0.794	0.605	0.608	0.980	0.552	0.276
Depth	0.559	0.722	0.353	0.969	0.591	**0.017**
N_rate x Bioinoculant	0.741	0.987	0.994	0.279	0.631	0.354
N_rate x Depth	0.900	0.999	0.412	0.331	0.031	0.563
Bioinoculant x Depth	0.940	0.891	0.966	0.996	0.917	0.464
N_rate x Bioino x Depth	0.450	0.801	0.765	0.404	0.984	0.239

The table shows the fertilizer nitrogen recovery (%) at three soil depths (5 cm, 15 cm, and 30 cm) during the V8, VT, and Harvest stages for N rates of 56, 112, and 168 kg N ha^{-1} in 2022 and 2023.The table presents the p-values from ANOVA analyzing the effects of nitrogen rate, bioinoculant, and soil depth on fertilizer N recovery across the V8, VT, and harvest stages ($\alpha < 0.05$).

The N rate significantly impacted fertilizer N recovery as inorganic N in the soil profile at V8 ($p = 0.009$) in 2023. Higher N rates lead to greater N recovery. The highest N recovery was 12.4% with 168 kg N ha^{-1} without bioinoculant, significantly higher than all other N recovery values in the V8 stage with other treatments. The lowest N recovery was 1.17% with 56 kg N ha^{-1} with bioinoculant.

Table 4.9 Tuckey HSD post-hoc test for N recovery percentage as inorganic N across different soil depths.

Depth comparisons		2022	2023
		-------p-value-------	
0-5 cm	5-15 cm	ns	**0.025**
0-5 cm	15-30 cm	ns	0.251
5-15 cm	15-30cm	ns	0.557

The Tukey HSD post-hoc test was performed to compare the mean across different N fertilizer recovery across soil depth across all the stages. The p-value indicates the significance ($\alpha < 0.05$).

The Tukey HSD technique was used to analyze the fertilizer N recovery as inorganic N by depth across all the years and stages (Table 4.9). In 2022, depth did not significantly affect fertilizer N recovery as inorganic N. However, the top layer significantly affected N recovery across all treatments as inorganic N in 2023.

The average grain yield data in Table 4.10 depicted a higher overall grain yield in 2022 than in 2023. At the highest rate, 168 kg N ha^{-1}, grain yield without bioinoculant was 11,648 kg ha^{-1} and 10,384 kg ha^{-1} in 2022 and 2023, respectively. In both years with bioinoculant, grain yield was lower than without the application. The lowest grain yield was observed in 2023 with 0 kg N ha^{-1}.

Table 4.10 Grain yield for the years 2022 and 2023.

N_ rate Kg ha^{-1}	Bioinoculant	Grain Yield 2022	Grain Yield 2023
		kg ha^{-1}	
0	N	6097	2143
	Y	6205	2382
56	N	9984	4919
	Y	9428	4576
112	N	10789	6481
	Y	11024	7075
168	N	11648	10384
	Y	10859	9630

The mean values of grain yield in kg ha^{-1} for 2022 and 2023.

4.4 Discussion

The objective of this study was to investigate the effects of N fertilizer rate and N-fixing microbial bioinoculant on fertilizer N recovery in both above-ground biomass and the soil at various growth stages of corn. Fertilizer N recovery in biomass at harvest in 2022 and 2023 was lower than N recoveries measured using different methods. However, the year 2023 biomass recovery data aligned with Omay et al. (1998) . Other ^{15}N fertilizer recovery studies in continuous corn cropping systems reported N recovery of 29.8% and 25.6% in Crete silt loam and Eudora loam soils under continuous corn in KS with a similar N application rate of 168 kg N ha^{-1} (Omay et al., 1998).

Griesheim et al. (2023) found significant differences in ^{15}N fertilizer recovery with broadcast applications. At a rate of 90 kg N ha^{-1}, the plant recovered only 12% and 6.1% at harvest. With broadcast application, fertilizer uptake efficiency was between 7% to 34% (Griesheim et al., 2023). However, the decrease in N recovery at R6 compared to VTR1 in 2020 was perplexing. This indicates that ^{15}N isotope N values were diluted after VTR1. This may be attributed to the enhanced uptake of N from native soil sources rather than from fertilizer-derived N, particularly after the VTR1 growth stage.

Higher below-ground biomass was observed in corn cropping systems with the same N-N-fixing microbial inoculant in Illinois, USA, suggesting that the bioinoculant could recover N more efficiently (Woodward, 2023). Our research results also had higher N recovery with bioinoculant application in above-ground biomass. Investigating below-ground biomass would be important for a more comprehensive understanding of the plant's N recovery under novel N-fixing microbial inoculant.

Applying these bioinoculants can lead to more extensive and deeper root systems (Woodward, 2023), enhancing the plant's ability to absorb N, including ^{15}N-labeled fertilizer, from a larger soil volume. This improved root growth can result in a higher apparent uptake of fertilizer-derived N. However, a better root structure can also assimilate mineralized soil N due to the enhanced overall nutrient acquisition. In such a scenario, ^{15}N depletion could occur in biomass due to the higher acquisition of mineralized soil N from the bulk soil system. In addition to that ^{15}N depletion could occur with greater N fixation through the bioinoculant. Our research suggests higher N recovery in soil with bioinoculant at R6 in 2023, specifically with 112 -168 kg N ha^{-1} rates. This may be due to higher overall favored soil health conditions and biological activity in the late season of the soil profile due to higher moisture retention in the no-till system. The use of multiple N fixing microbes, *Azospirillum brasilense*, and *Bacillus subtilis*, combined with N application rates on corn enhanced the overall N recovery in soil (Galindo et al., 2024). Straw addition in corn cropping systems enhanced the molecular copy numbers of N-fixing microbes in the soil and enhanced overall N recoveries (Duan et al., 2023). These also suggest that N-fixing microbial applications could enhance N recovery in soil with no-till and favorable climate conditions for plant growth.

In a study by De Oliveira et al. (2018), early fertilizer N applications in corn with cover crop had higher recovery of fertilizer N in topsoil due to greater microbial activity with greater immobilization in no-till systems. A global meta-analysis on the fate of N recovery in above-ground biomass in the North American region revealed that maize uptake was 42.4 % ± 13 % at harvest. Further, soil retention was recorded at harvest as 27.8 % ± 11.8%, and recovered accounted for about 30.5%±17.2% in northern American regions (Quan et al., 2021b).

The differences in precipitation patterns between 2022 and 2023 emphasize the importance of consistent soil moisture for optimal N use efficiency (Figure 4.12). A relatively higher N_2O flux was observed in the 2022 than the 2023 growing seasons (Chapter 2). In addition to precipitation, soil NO_3- was lower in 2022 than in 2023 (Figure 4.11). Particularly in well-drained soils, reducing N availability for plants during critical growth stages would potentially lower N recovery. This indicated that the precipitation pattern likely contributed to increased N_2O emission and leaching of fertilizer N.

Table 4.11 Precipitation summary for the growing season of 2022 and 2023.

	2022	2023
Total (mm)	311	346
Total events	27	36
>10mm	8	12
Highest(mm)	57	53
First 40 days	219	147
Events	13	13
>10mm	6	6
Highest (mm)	57	53
First 10 days	91	13
Events	4	1
>10mm	3	1
Highest (mm)	41	13

In 2023, the more evenly distributed precipitation likely facilitated better conditions for both the mineralization of soil N and the uptake of fertilizer N. Studies have shown that well-distributed rainfall can significantly enhance N uptake efficiency and reduce losses through leaching and volatilization, thereby improving overall crop productivity and reducing environmental impact (Tremblay et al., 2012).

Compared to the V8 stage, fertilizer N recovery increased at R6, representing immobilization and mineralization dynamics throughout the growing season. Further higher fertilizer N recovery % in the 15-30 cm soil layer than the upper layers in 2022 might be due to higher precipitation and leaching potential in the early season after fertilization. Further, 2023 showed higher fertilizer N-derived organic N in the top layer of 0-5 cm and the deeper layer of 15-30 cm. No-till management might enhance microbial activity and more immobilization in the top layer, and leached inorganic N in the deeper layer might enhance immobilization and mineralization in the deeper layer due to higher microbial activity with substrate availability.

The combination of microbial inoculants and appropriate N application rates seems to improve fertilizer efficiency; however, it is unclear. This efficiency manifests in higher recovery of applied N in the biomass, particularly at higher application rates, such as 168 kg N ha^{-1}. The inoculated plants' ability to explore more of the soil likely allows them to access nutrients, thereby increasing the recovery of ^{15}N in above-ground biomass. This suggests that these plants are utilizing both applied fertilizer and naturally occurring soil N, including that fixed from the atmosphere.

These findings imply significant potential for optimizing N management in corn cropping systems. By integrating N-fixing microbial inoculants with precise N application strategies, it has the potential to enhance N use efficiency. This approach not only improves crop yields but also potentially reduces the overall dependency on synthetic fertilizers. Consequently, this can minimize environmental impacts such as nitrate leaching and reduce the associated economic costs. The synergistic effects of enhanced N fixation, improved root growth, and efficient nutrient uptake underscore the value of incorporating bioinoculants into modern agricultural practices for more sustainable and efficient crop production.

4.5 Conclusion

The study highlighted the complexity of fertilizer N recoveries in corn cropping systems inoculated with N-fixing microbes under different N rates. Fertilizer N recovery in biomass was lower in 2022. However, N recovery in 2023 aligned with previous ^{15}N-related studies in corn cropping systems. The lower N rates had consistently higher N fertilizer recovery as soil organic N. The study emphasizes the critical role of precipitation in influencing N recoveries. Heavy precipitation at the early stages of the growing season can increase losses through leaching and denitrification. Well-distributed precipitation patterns might support better N retention and uptake in 2023. The effect of N-fixing bioinoculant on fertilizer N recovery was not clear. Long-term field trials and more detailed investigations will be needed to evaluate the effect of the novel developed N-fixing microbial inoculants on N recovery in biomass and soil profiles.

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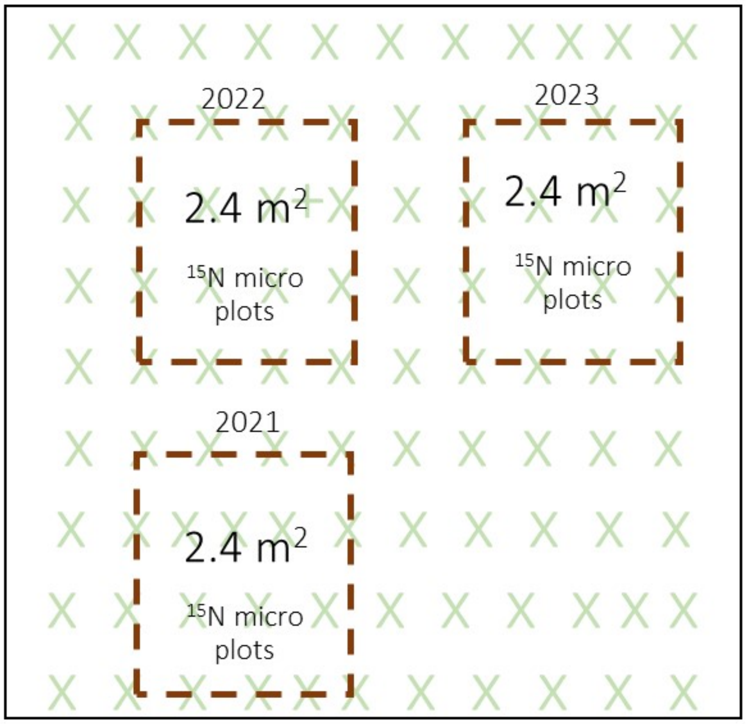


Figure 4.1. Microplot location in the whole plot. The microplot's area was 2.4m2, and its location changed each year, as described by the figure.

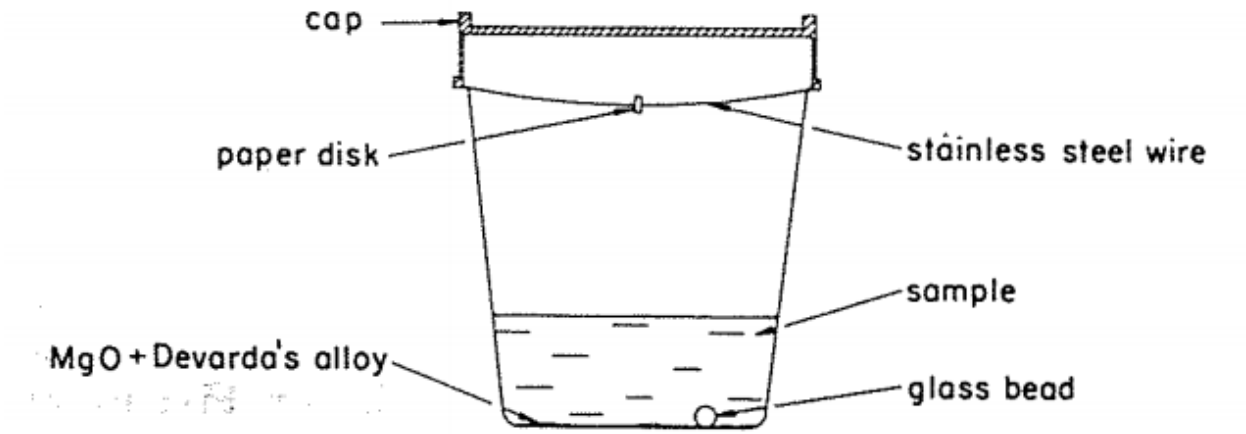


Figure 4.2. Setup for Trapping Released NH_3 Using Acidified Filter Paper**.** This figure shows the process of placing acidified filter paper on top of the specimen cup using SS wire and mixing the KCL solution with glass beads.

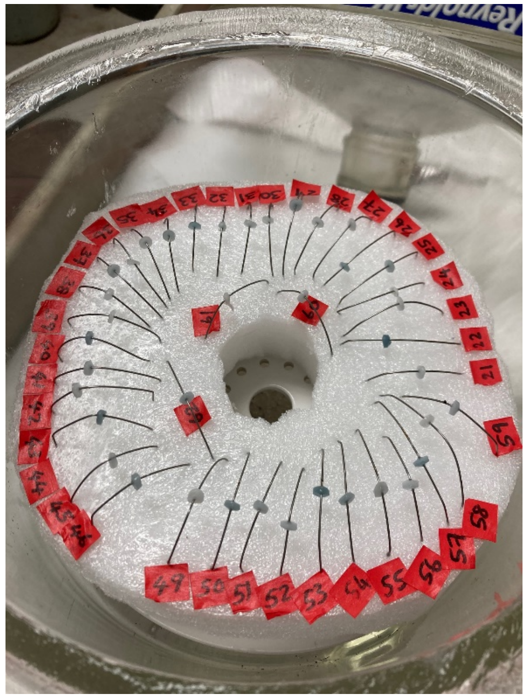


Figure 4.3. Post-incubation setup: Retrieved filter papers on foam platform.

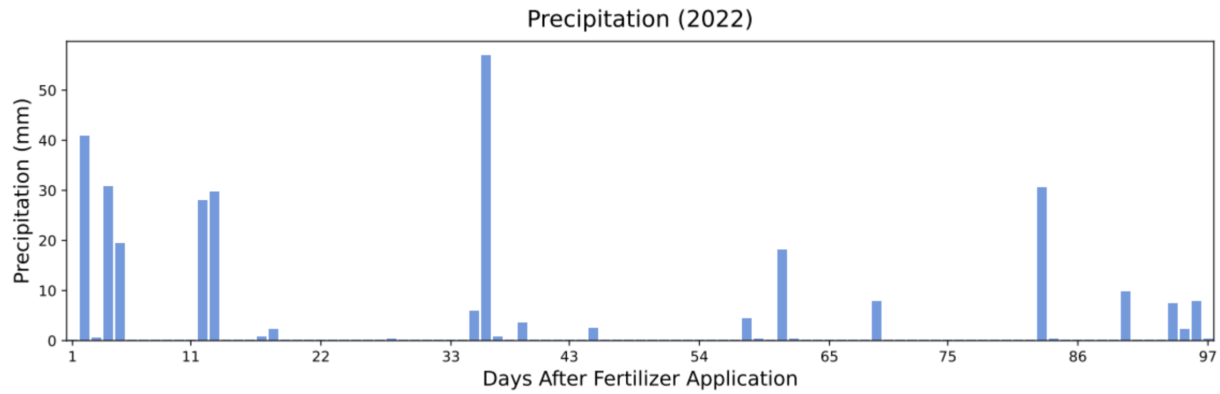


Figure 4.4. Precipitation (2022). Daily precipitation totals were measured from the Mesonet tower managed by the Department of Agronomy at Kansas State University. Corn planting and fertilizer application on June 19, 2022.

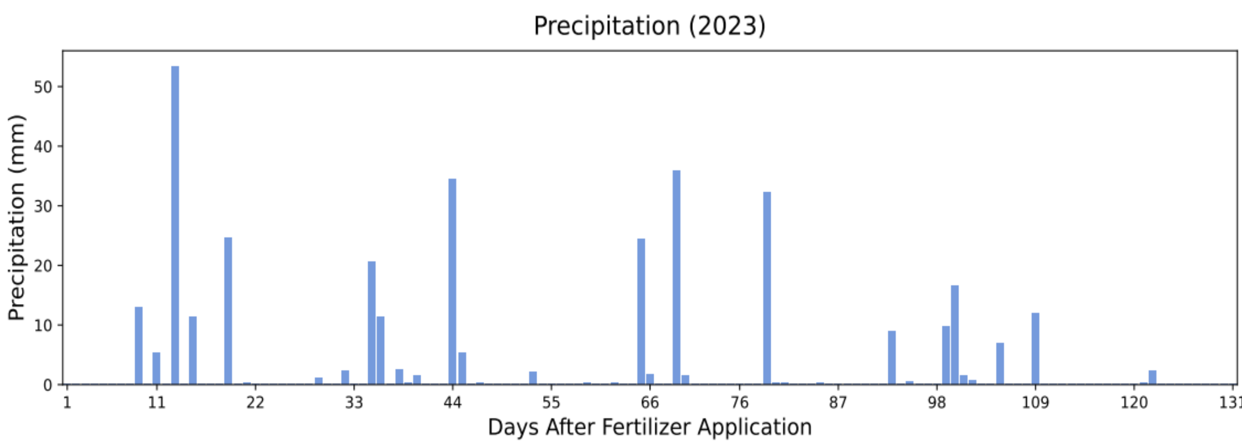


Figure 4.5. Precipitation (2023). Daily precipitation totals were measured from the Mesonet tower managed by the Department of Agronomy at Kansas State University. Corn planting and fertilizer application on April 26, 2023.

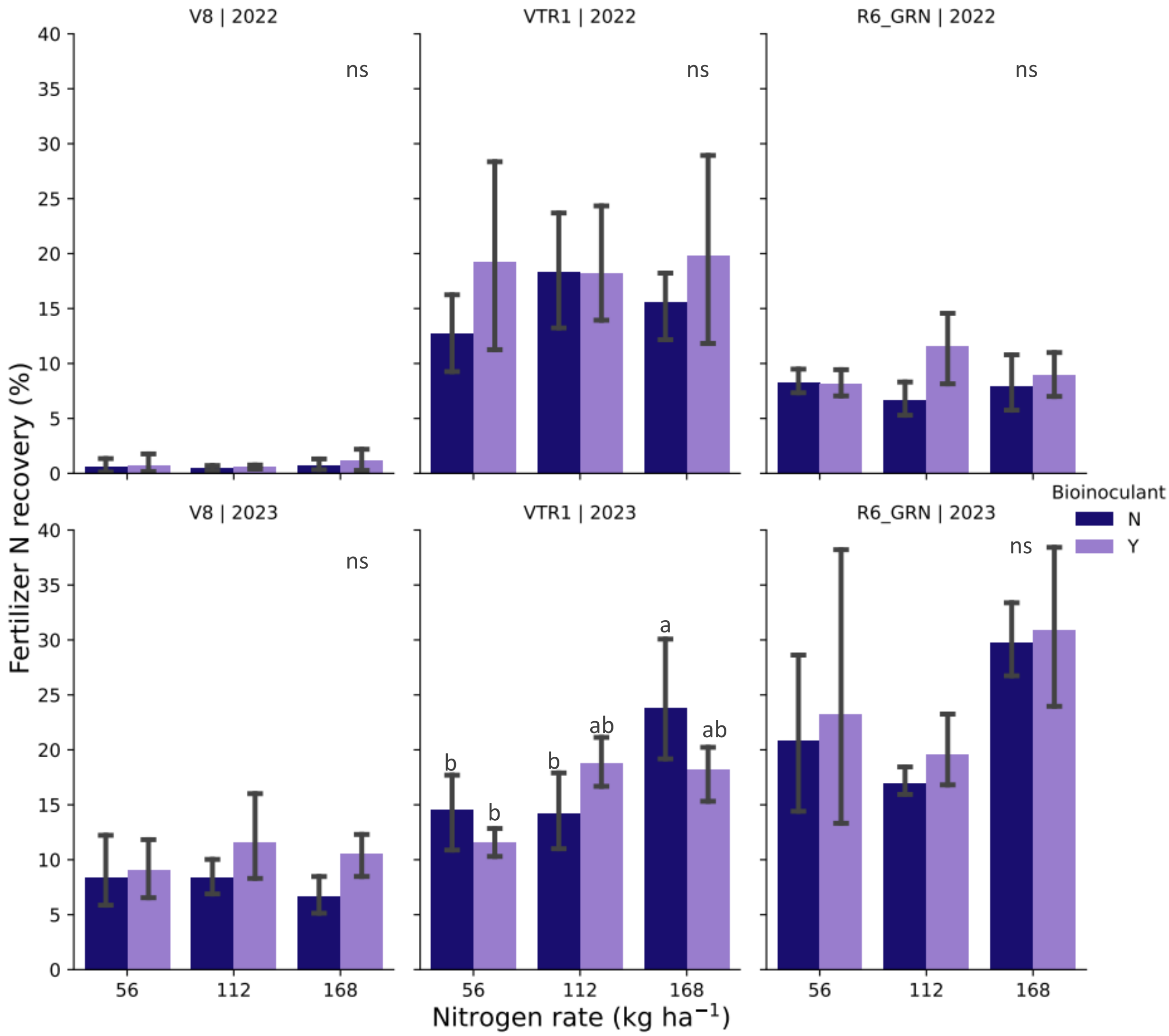


Figure 4.6. Fertilizer N recovery percentage (%) at V8, VTR1 and harvest (grain and plant stover) in above ground biomass for 2022 & 2023. Nitrogen rates are 0,56, 112, and 168 kg N ha^{-1} as urea fertilizer. The same lower-case letters are not significantly different at α=0.05.

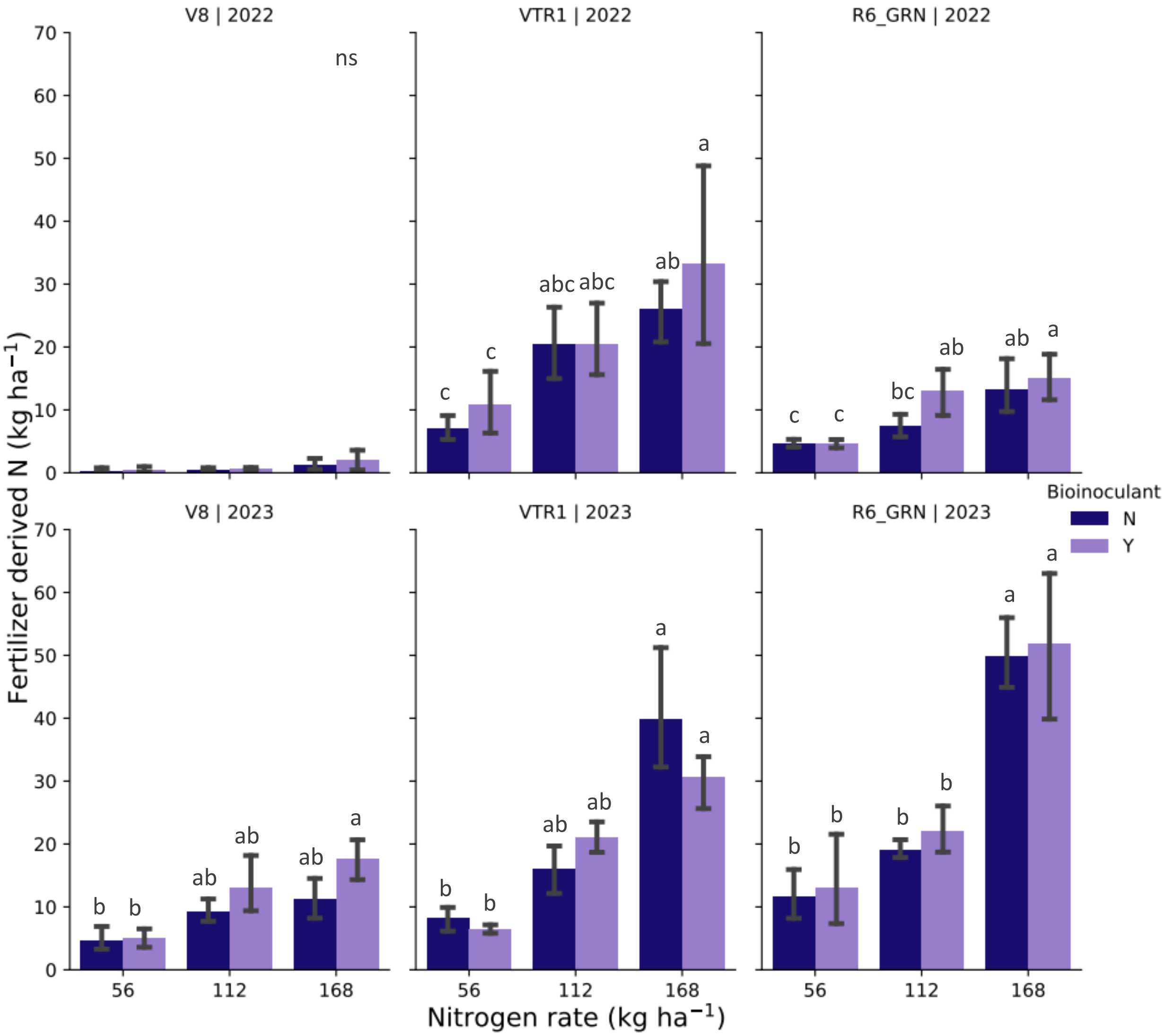


Figure 4.7. Fertilizer derived N (kg ha^{-1}) at V8, VTR1 and harvest (grain and plant stover) in above ground biomass for 2022 and 2023. Nitrogen rates are 0,56, 112, and 168 kg N ha^{-1} as urea fertilizer. The same lower-case letters are not significantly different at α=0.05.

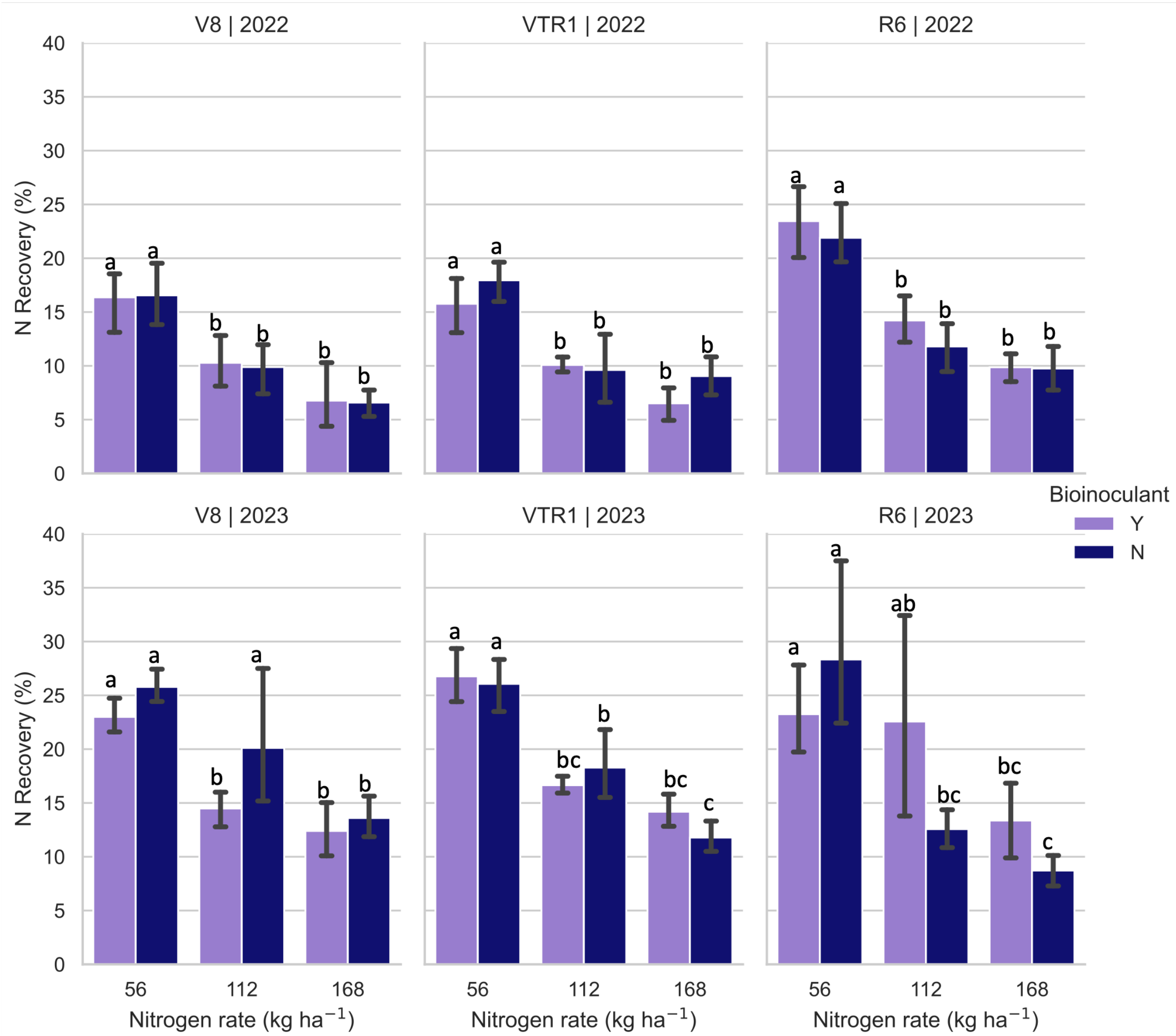


Figure 4.8. Fertilizer N recovery percentage in soil (0-30cm) at V8, VTR1, and R6 for 2022 & 2023. Nitrogen rates are 0,56, 112, and 168 kg N ha^{-1} as urea fertilizer. The same lower-case letters are not significantly different at α=0.05.

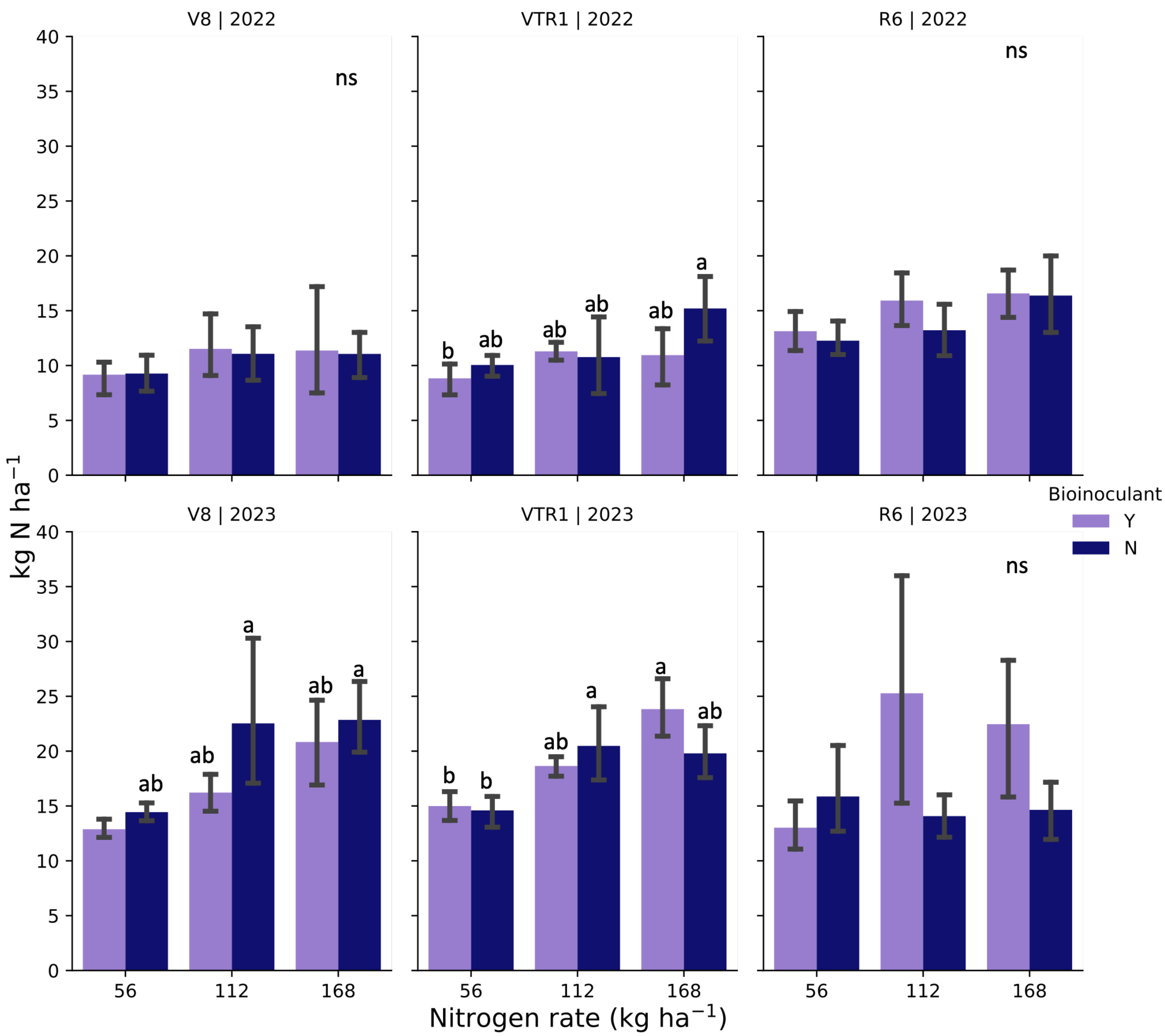


Figure 4.9. Fertilizer N recovery (kg N ha^{-1}) as organic N in soil (0-30cm) at V8, VTR1 and R6 for 2022 & 2023. Nitrogen rates are 0,56, 112, and 168 kg N ha^{-1} as urea fertilizer. The same lower-case letters are not significantly different at α=0.05.

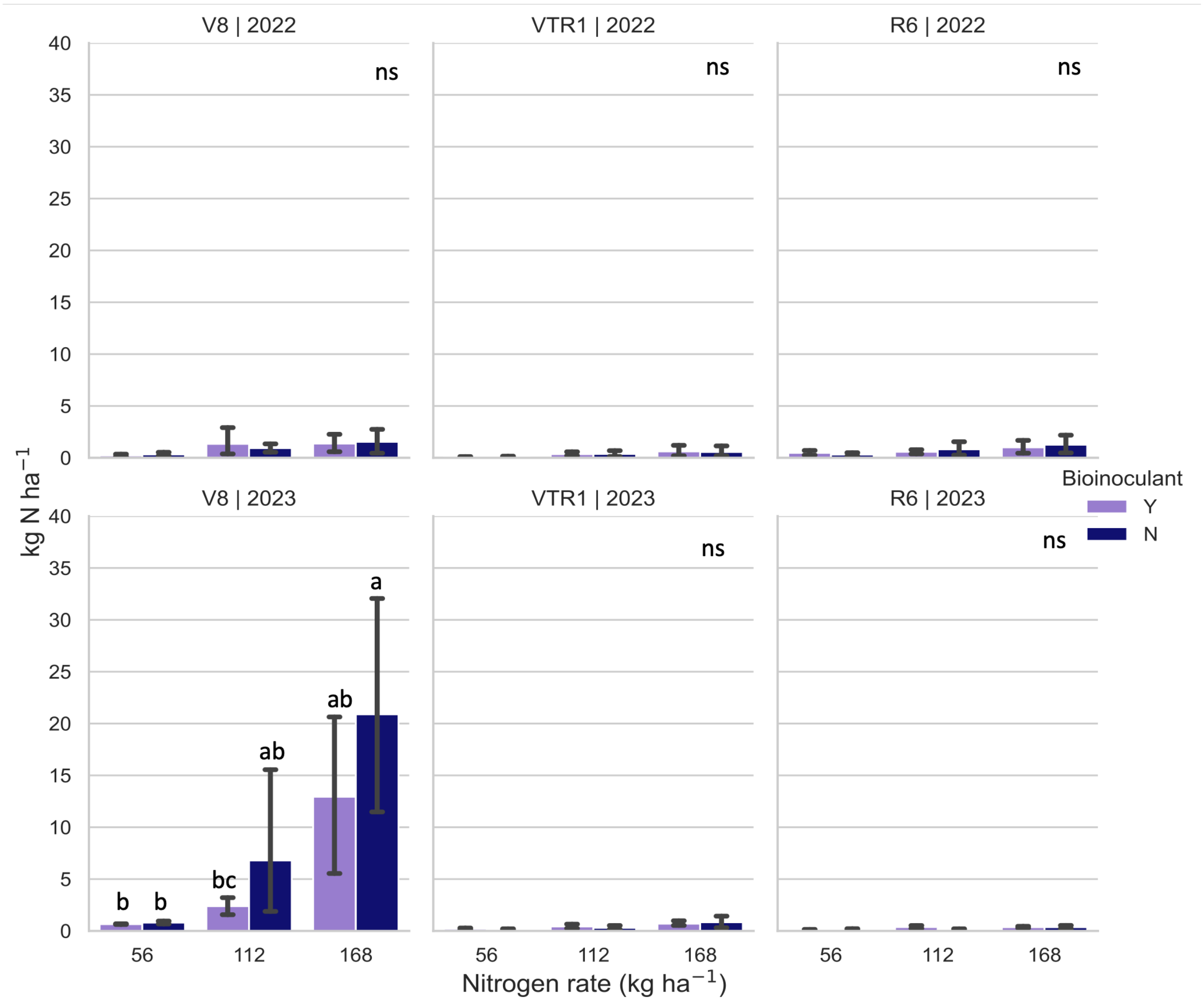


Figure 4.10. Soil nitrate ($^{15}NO_3^-$) kg N ha^{-1} at V8, VTR1, and harvest in 0-30 cm soil profile (^{15}N enriched plots). Nitrogen rates are 0,56, 112, and 168 kg N ha^{-1} as urea fertilizer. The same lower-case letters are not significantly different at α=0.05.

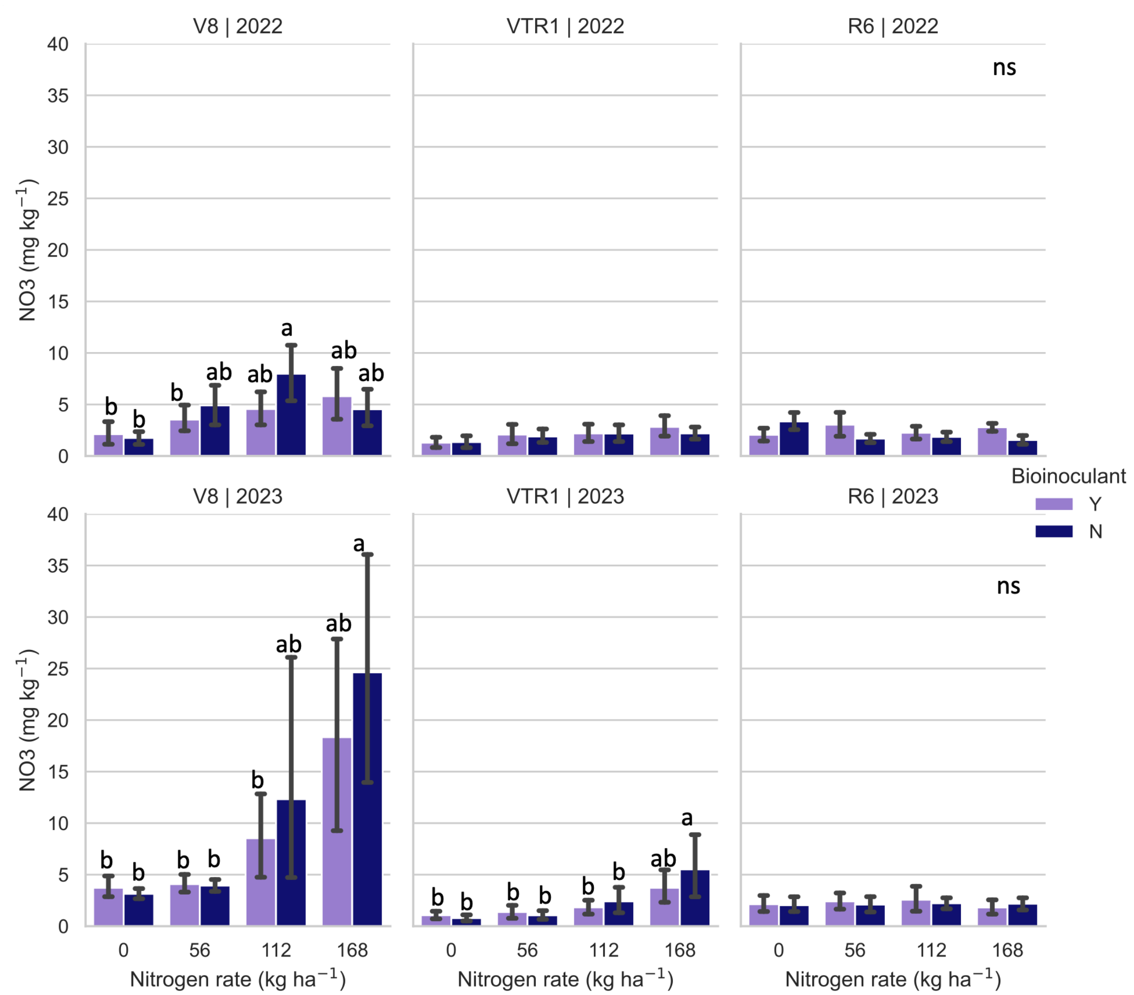


Figure 4.11. Soil nitrate (NO_3^-) mg NO_3^--N kg^{-1} in each stage for 2022 and 2023.Nitrogen rates are 0,56, 112, and 168 kg N ha^{-1} as urea fertilizer. The same lower-case letters are not significantly different at α=0.05.

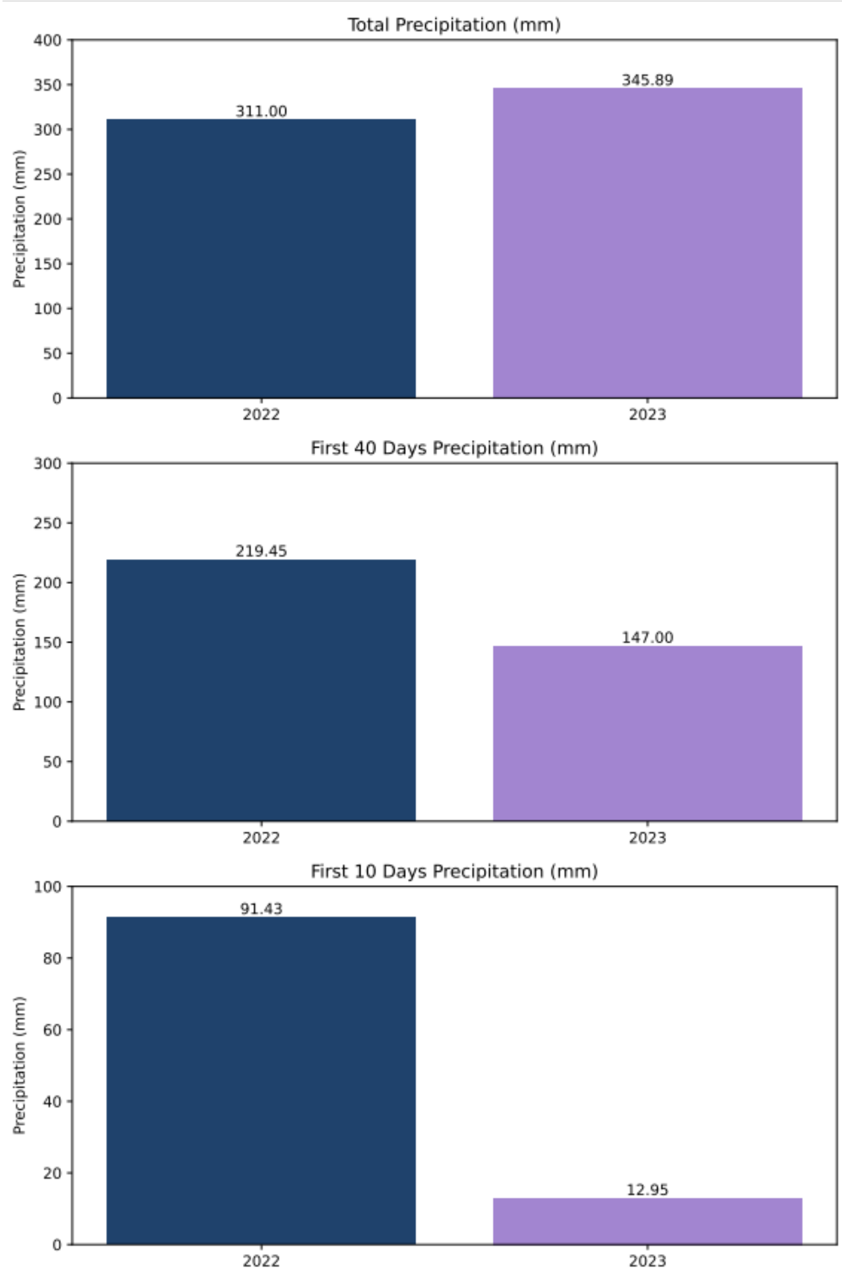


Figure 4.12. Total precipitation for the growing season, first 40 days, and first 10 days after planting and fertilization for 2022 and 2023.

Chapter 5 - Conclusions and Future Work

This dissertation has explored the multifaceted role of nitrogen (N) in agricultural systems, with a focus on a novel N-fixing microbial inoculant combined with different N fertilization rates in a no-till continuous corn cropping system. Nitrogen is an essential nutrient for plant growth, fundamental to synthesizing proteins, nucleic acids, and other critical biomolecules. However, the reliance on industrial N fertilizers in agriculture presents significant environmental challenges, including greenhouse gas emissions, soil degradation, and water pollution. This research examined the effects of N-fixing microbial inoculants and varying N application rates on N recovery and nitrous oxide (N_2O) emissions in no-till corn cropping systems. The research was conducted at North Farm, managed by the Kansas State University Department of Agronomy.

The findings indicate that N application rates are a significant driver of N_2O emissions, with higher rates leading to increased emissions. Higher N_2O daily fluxes during the growing season were correlated with higher precipitation, and this was reduced later during the growing season as nitrate was taken up by the plant. Overall, 2022 had higher cumulative N_2O emissions (1.13 kg N_2O-N ha^{-1}) than the year 2023 (0.79 kg N_2O-N ha^{-1}), with the highest N rates (168 kg N ha^{-1}). Higher fluxes were recorded due to the higher precipitation during the first 10 days after application. In 2022, 91 mm of precipitation was recorded in the first 10 days after fertilizer application, and in 2023, it was ~13mm. N-fixing bioinoculants showed potential for reducing N2O emissions in 2022, although the effects were not statistically significant for both years. Emission factor (EF) for both years were lower than the IPCC default value of 1%. In 2022, EF was 0.38% with plots treated with the bioinoculant, while the untreated plots depicted 0.51%. However, it was higher (0.56%) in 2023 with bioinoculant applications and 0.35% for the uninoculated plot. N_2O-emissions-yield curved had a significant linear trend for both years, but with bioinoculant, the trend was not clear.

The stable isotope ^{15}N tracer method provided insights into N recovery in above-ground biomass and soil profiles. Lower above-ground biomass fertilizer N recovery was recorded in 2022 compared to 2023. The highest above-ground biomass N recovery was observed, and the rates were higher in 2023. In 2022, fertilizer N recovery ranged from 6.7% to 11.62% at the R6 corn growth stage, with the bioinoculant having minimal impact. However, in 2023, N recovery in biomass increased significantly, ranging from 17% to 30.9%, with the most notable recovery observed at the 168 kg N ha^{-1} rate with bioinoculant application. Fertilizer N recovery as soil organic N was significantly related to N rates. Lower N rates resulted in higher N recovery in the soil as organic N. Soils at 15 to 30 cm depth had higher N recovery than shallower layers in 2022. N recovery was not significantly affected by the bioinoculant. Greater N leached potential due to higher rainfall in the early stages of the 2022 growing season. Greater N recovery was observed during 2023, which ranged from ~13-25 kg N ha^{-1} organic N in the soil profile with well-distributed rainfall. The effect of the bioinoculant in fertilizer N recovery was not clear. Overall, the N recovery of the systems was around 50%, lower than that of most of the ^{15}N isotopic-related research studies.

The Denitrification-Decomposition (DNDC) model, used to predict and quantify N_2O emissions across different management practices, was a critical tool in this research. The calibration and validation process demonstrated strong accuracy of model predictions, with a Mean Absolute Error (MAE) of 0.016, Root Mean Squared Error (RMSE) of 0.023, Mean Bias Error (MBE) of 0.016, and a Coefficient of Determination (R^2) of 0.997. Validation using 2023 data showed slightly lower accuracy, with MAE of 0.056, RMSE of 0.067, MBE of -0.051, and

R^2 of 0.934, indicating a minor underprediction bias. The model effectively captured the influence of precipitation on N_2O emissions, accurately reflecting emission peaks during periods of high rainfall. Incorporating environmental data, such as precipitation, further validated the model's sensitivity to external variables, emphasizing its utility in diverse agricultural settings. Model validation with bioinoculant was not successful due to a lack of parameter inputs in the DNDC model for associative N fixation. Integrating microbial-related parameters and using microbial indexes in the DNDC model calibration process emerged promising avenues for enhancing predictive accuracy, particularly in systems utilizing novel bioinoculants.

The research presented in this dissertation highlights the urgent need for sustainable N management practices in agriculture. The findings reinforce the importance of optimizing N application rates to balance crop productivity with environmental stewardship. The potential of N-fixing microbial bioinoculants to enhance N efficiency and reduce N_2O emissions presents a promising strategy for mitigating the environmental impact of N fertilizers. However, the variability in results across different growing seasons and the need for further long-term studies underscore the complexity of implementing these solutions at scale.

The DNDC model's demonstrated accuracy and reliability in simulating N_2O emissions across different management practices provide a valuable tool for researchers and policymakers. Its ability to integrate microbial parameters and environmental data makes it particularly relevant for developing and refining best management practices tailored to specific agricultural contexts. Continuous model evaluation and recalibration are essential to ensure its ongoing utility and accuracy in predicting the environmental impacts of agricultural practices.

5.1 Future research

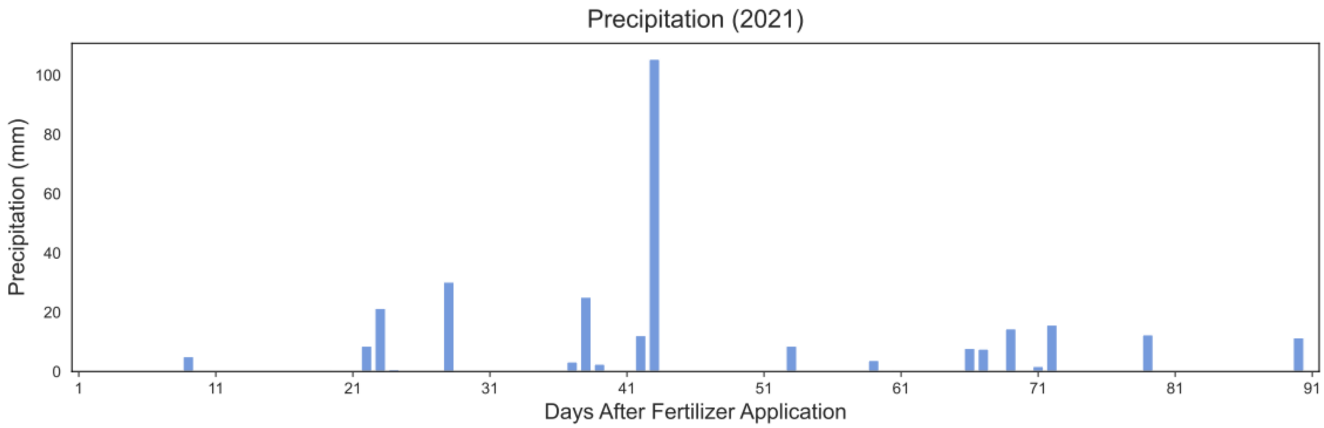
Future research should focus on long-term field trials and controlled environment research to comprehensively assess the effects of N-fixing bioinoculants on N_2O flux and fertilizer N recovery. Rhizosphere related analysis needs to be investigated with N-fixing microbial inoculation, which would help understand the effect of bioinoculants on the microscale. Molecular-level investigation (metagenomics) might be required to understand the behavior of N-fixing microbes in corn rhizosphere. Further, enzyme assays can be used to investigate the microbial activity in the rhizosphere soil.

Moreover, N_2O emissions vary with spatial variances due to heterogenic variability in the soil. Assigning more chambers or using automated smart chambers would help overcome the spatial variance obstacle in recording N_2O fluxes in research plots. Robust field scales and long-term N_2O flux data would be important for better process-based model implementation. Additionally, developing advanced microbial indexes and their integration into predictive models like DNDC will be crucial for accurately capturing the dynamic and transient nature of microbial activity in agricultural soils.

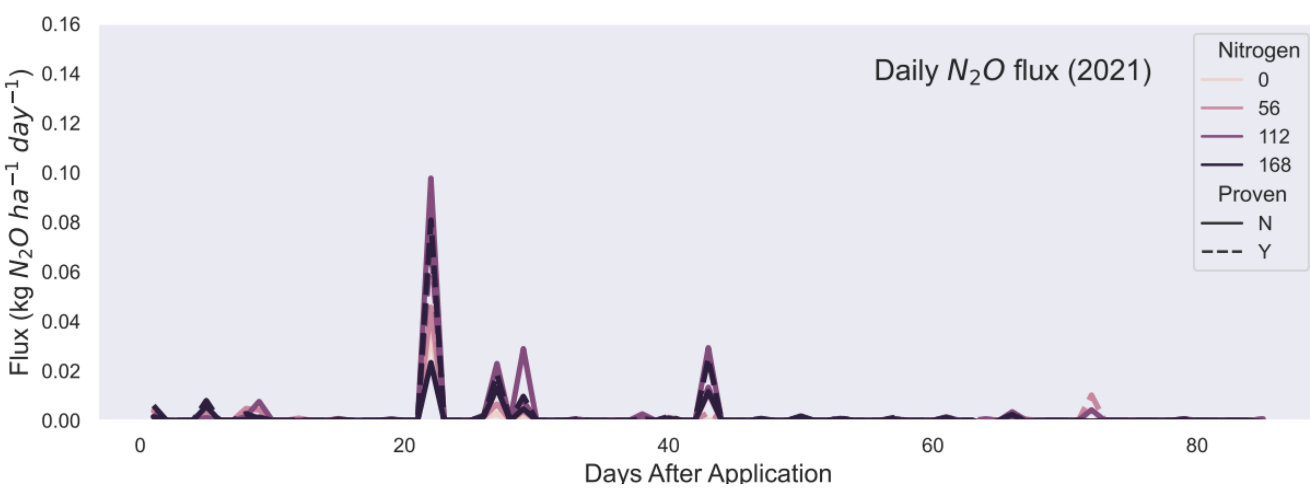
The long-term impact of novel N-fixing microbial inoculants on agroecosystems needs to be assessed for future policy and regulation implementation. Exploring the broader application of these findings across different crop systems and environmental conditions will also be important for generalizing the results and developing scalable solutions. As the global demand for food continues to rise, the need for sustainable agricultural practices that minimize environmental impact while maximizing productivity becomes increasingly critical. This research contributes to that goal by advancing our understanding of N dynamics in agriculture and offering practical strategies to improve N use efficiency and reduce greenhouse gas emissions.

By integrating N-fixing microbial bioinoculants, optimizing N application rates, and employing advanced models like DNDC, it is possible to enhance N efficiency, reduce environmental impacts, and support the long-term sustainability of agricultural production. The findings and evaluation of the process-based model in this research provide a foundation for future studies and practical applications aimed at improving sustainable crop production for the future.

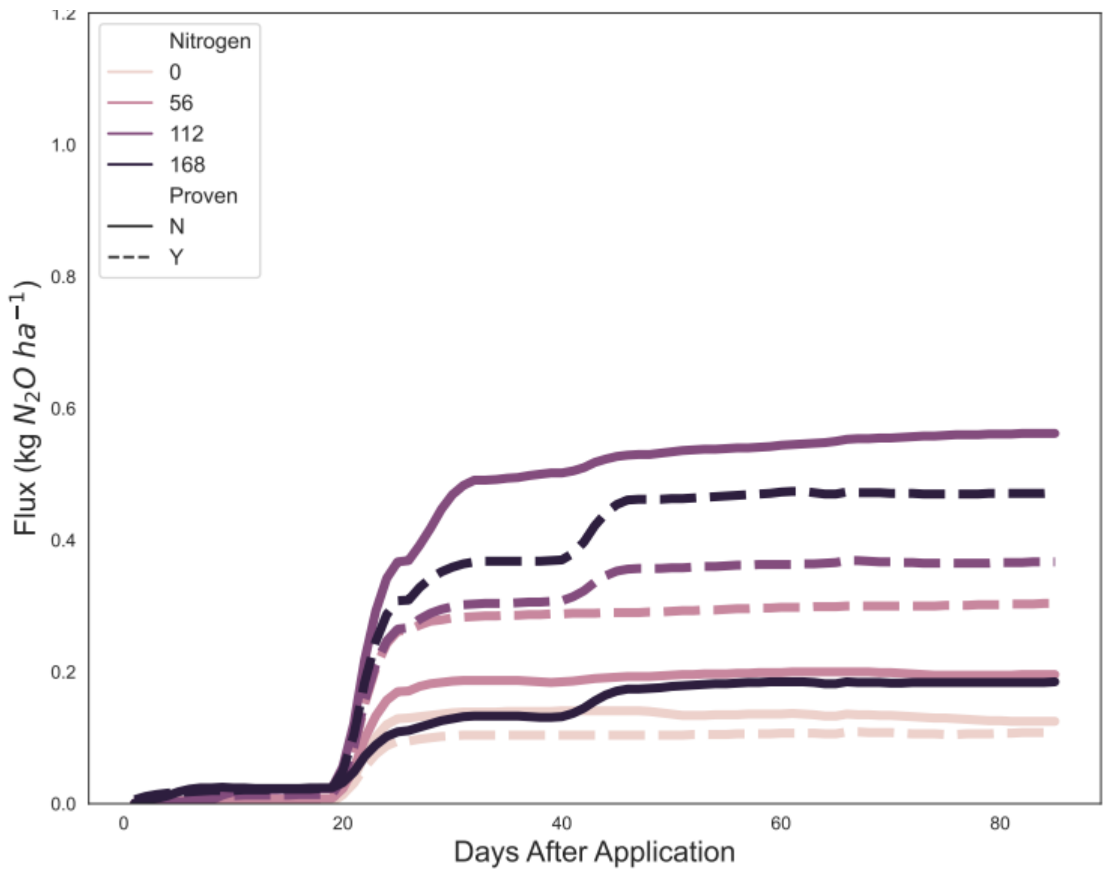
Appendix A - Chapter 2



Appendix Figure A.1.Precipitation (2021).
Daily precipitation fluxes were taken from the Mesonet tower managed by the Department of Agronomy at Kansas State University daily precipitation in mm.



Appendix Figure A.2. Daily N_2O flux measurements in kg N_2O-N ha^{-1} for the 2021 growing season.
Figures 2.3 and 2.4 display daily N_2O-N flux over time during the 2021 growing season, measured in kg N_2O ha^{-1} day^{-1}, across various nitrogen fertilizer application rates (0, 56, 112, and 168 kg N ha^{-1}). The x-axis represents the days after nitrogen fertilizer (urea) application, while the y-axis shows the N_2O-N flux in kg N_2O-N $ha^{-1}day^{-1}$. Solid lines represent treatments without the nitrogen-fixing bioinoculant, while dashed lines represent treatments with the bioinoculant.



Appendix Figure A.3 Cumulative N_2O flux measurement 2021.

The figure illustrates the cumulative N_2O emissions in 2021 over time, measured in kg N_2O-N ha^{-1}, across different nitrogen fertilizer application rates (0, 56, 112, and 168 kg N ha^{-1}). Solid lines represent plots without the nitrogen-fixing bioinoculant, while dashed lines represent plots treated with the bioinoculant. The x-axis represents the days after nitrogen application, and the y-axis shows the cumulative N_2O emissions in kg N_2O-N ha^{-1}.