FINAL REPORT

PROJECT TITLE: Reducing Reactive Nitrogen Losses from Corn Systems
PROJECT NUMBER: 6051-21DD
PRINCIPAL INVESTIGATOR AND CO-INVESTIGATOR(S): Tim Griffis, John Baker and Rod Venterea

ABSTRACT

This project evaluated the efficacy of Pivot Bio's designer microbe product (PROVEN®) in reducing reactive nitrogen losses from corn systems and its impact on overall plant health and productivity. Here, we tested nitrogen-fixing BI (PROVEN and PROVEN 40 , Pivot Bio) and evaluated its efficacy to produce a sustainable corn (*Zea mays L.*) yield over a range of fertilizer rates (FRs) and examined its impact on N_2O emissions. The experiments were conducted over seven seasons in an indoor mesocosm facility. The results indicate that the BI treatment did not have any significant effects on N_2O emissions, yield, leachate, soil or plant N concentrations, or nitrogen use efficiency. Fertilizer rate showed a significant relationship for increasing N_2O emissions, increasing soil N concentrations, and decreasing NUE. Nitrous oxide emissions increased linearly with increasing FR from 81 to 404 kg N ha^{-1}. Overall, the results indicate that BIs did not effectively mitigate N_2O emissions or improve yield.

INTRODUCTION

The global demand for synthetic nitrogen (N) fertilizer now exceeds 110 Tg (1 Tg = 10^{12} g) N and is steadily increasing (Griffis & Baker, 2020). Growing population and demand for food, fiber, and biofuel are accelerating the use of synthetic N (Erisman et al., 2008; Zhang et al., 2015). This massive increase has caused global emissions of ammonia (NH_3) and nitrous oxide (N_2O) to increase nearly 500% and 130%, respectively during the 20th century (Bouwman et al., 2013). These emissions are expected to accelerate through the 21st century (Ciais et al., 2013). The need to produce biomass, while reducing reactive nitrogen (N_r) represents a grand environmental challenge for society. Reactive nitrogen losses in the form of N_2O and NH_3 emissions and nitrate leaching in runoff are associated with a number of deleterious environmental impacts including stratospheric ozone depletion, climate warming, respiratory illness, and eutrophication.

There have been claims that "*designer*" microbial communities can be used in farm fields to help reduce the demand for synthetic N inputs and thereby reduce N_r losses. Here, we used the University of Minnesota Mescocosm facility, to evaluate the efficacy of using designer microbial communities (i.e. a commercially available bacterial inoculant (BI), PROVEN® (Pivot Bio, Berkeley, CA) in reducing N_2O emissions while maintaining plant health and high corn yields.

OBJECTIVE AND GOAL STATEMENTS

The goals of this study were to:

1. Lower N_r losses by using BI to enhance N availability;

2. Maintain plant health and crop yields by using BI to enhance N availability, while reducing synthetic N inputs;
3. Estimate N_r emission factors for the BI + Urea treatments to assess how designer microbial products could lower N_r for the entire US Corn Belt.

MATERIALS AND METHODS

The experiments were conducted over 7 seasons (years 2019-2023) in a climate-controlled greenhouse. We used 6 custom-designed standalone, insulated mesocosms (Groupe DHB, Quebec, Canada), 1.2 m tall with a surface area of 2 m^2. Each mesocosm contained approximately 4,100 kg of soil obtained from Goodhue County, MN that had previously been under a corn-soybean rotation for 25 years prior to extraction. Corn was grown in each mesocosm and hourly N_2O soil fluxes were measured with automated non-steady-state chambers. Additional information about the facility can be found in Miller et al. (2022).

The soil is a Kasson or Klinger Series silty clay with a 0.25-m topsoil, a clay horizon at 0.25 to 0.70 m, and a sand horizon at 0.70 m to 1.1 m. The surface layer (0 to 0.2 m) had an initial organic matter content of 2.4% and a pH of 6.8. A climate control system (Argus Classic Build 66g ver. 14.04) was used to maintain air flow, light level, and air temperatures. Photosynthetically active radiation (PAR) was provided for each mesocosm using KIND indoor LED grow lights (K5 Series XL1000 and K5 Series XL750, Kind LED Grow Lights). Lights were programmed to simulate typical daily PAR amounts for Saint Paul, Minnesota. Ceiling curtains were used to block insolation in the mesocosm facility to curtail high temperatures.

A commercially available bacterial inoculant (BI), PROVEN® (Pivot Bio, Berkeley, CA) was used to reduce synthetic nitrogen use and to improve crop growth and reduce N_2O emissions. The recommended amount of PROVEN® or PROVEN 40® was placed in a syringe and mixed into a graduated cylinder with 1 L of water. One cylinder per treated seed row was applied. We planted a corn hybrid (DKC-53-56RIB, Dekalb) with a 103-day relative maturity. The corn seeds were hand-planted to 0.05 m depth with each seed spaced 0.076 m apart in two rows. This amounts to 77,000 seeds ac^{-1}. The plants were thinned to 36,000 seeds ac^{-1} after the first month. A predetermined amount of stover was returned to each mesocosm from the previous season (none in Season 1).

Season 1 used 3 mesocosms with 100% N (202 kg-N ha^{-1}) fertilizer rate (FR) and 3 mesocosms with 75% N (152 kg-N ha^{-1}) FR with PROVEN® in one application after planting. In seasons 2-4, the treatments were rotated among mesocosms in which 2 mesocosms in each season were chosen for each treatment of 40% N (81 kg-N ha^{-1}), 60% N (121 kg-N ha^{-1}), and 80% N (161 kg-N ha^{-1}) using one application at the beginning of the season after planting. One of the mesocosms from each rate was treated with PROVEN® . In season 5, the treatments were 2 mesocosms for each treatment of 80% N (161 kg-N ha^{-1}), 120% N (242 kg-N ha^{-1}), and 160% N (322 kg-N ha^{-1}) using an evenly divided split application at the beginning of the season and mid-season. One of the mesocosms for each rate was treated with BI. In season 6, a 200% N (404 kg-N ha^{-1}) FR was applied on all mesocosms in an evenly divided split application at the beginning and middle of the season.

Season 7 used a 100% N (202 kg-N ha^{-1}) FR on all mesocosms in a single application after planting. The mesocosms treated with PROVEN® were chosen randomly for Season 1 and 2. The chosen treatments for mesocosms were rotated for seasons 3-5. The active BI ingredient was *Klebsiella variicola* and *Kosakonia sacchari*. The most current version of PROVENÒ that was available at the start of each season was utilized.

Flow-through automated non-steady-state chambers (Fassbinder et al., 2012, Miller et al., 2022) were used to measure hourly fluxes of CO_2 and N_2O in each mesocosm. Each chamber was sampled within a 60-minute cycle. Air inside each chamber was sampled for ten minutes by pulling air from the chamber to a closedpath LI-COR $CH_4/CO_2/H_2O$ Trace Gas Analyzer (LI-7810, LI-COR Biosciences, Lincoln, NB). The chamber air was taken through a desiccant column before an Ultra Sensitive Gas Filter Correlation N_2O Analyzer (Teledyne M320 EU, Teledyne Technologies International Corp, Thousand Oaks, CA). The air was then returned to the chamber. The sampling frequency was 1 Hz. A fan inside each of the chambers ensured a well-mixed volume, and the chambers were opened between sample intervals to allow ambient air inside. The procedures describing the chamber flux calculations have been described previously by Miller et al. (2022).

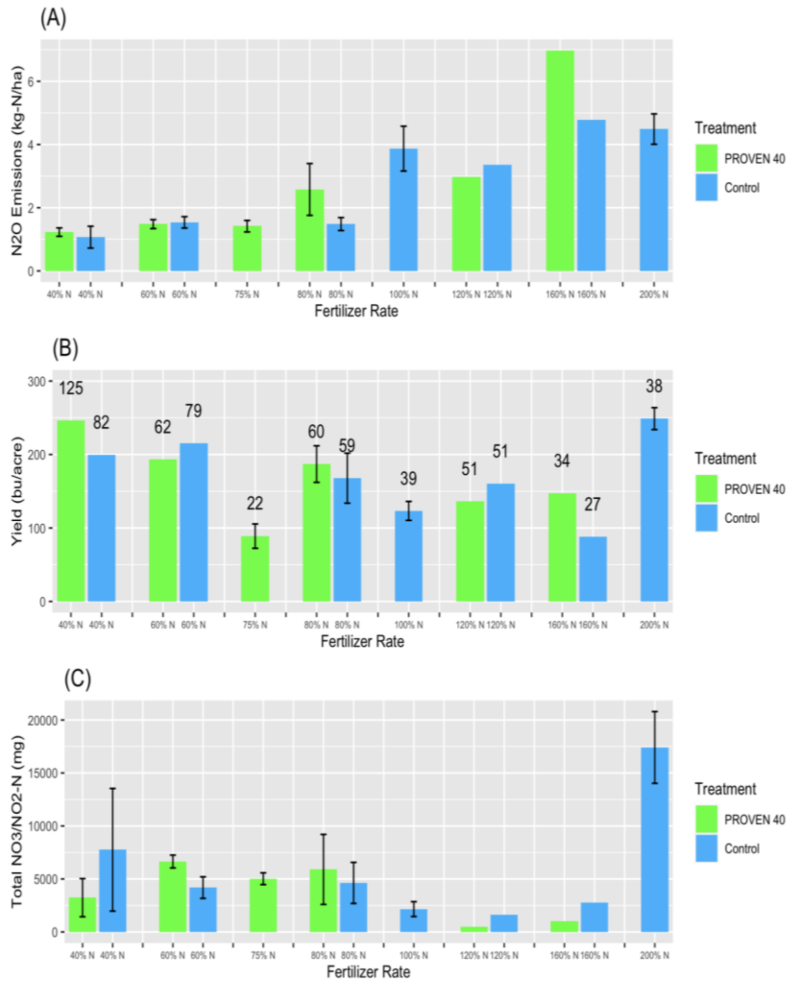

Figure 1. (A) Average normalized and integrated by 124 days cumulative nitrous oxide emissions for each treatment throughout all seasons. (B) Average yield for each treatment through all seasons. Average nitrogen use efficiency (%) for each treatment is

RESULTS AND DISCUSSION

Figure 1 shows the average cumulative N_2O emissions for all treatments and seasons (Bohnen, 2024). As expected, fertilizer rate had a significant positive influence on cumulative N_2O emissions ($p<0.05$) and crop yields ($p<0.05$). After gap-filling and seasonal integration, the cumulative mean (range) N_2O emission was 2.77 kg-N_2O-N ha^{-1} (0.53-7.93 kg N_2O-N ha^{-1}) over all seasons and treatments.

The mean (range) emissions factor (EF) was 1.40% (0.63-3.92%) over all seasons and treatments. The IPCC (2006) report recommends a fertilizer EF of 1% (0.3-3.0%), which is in good agreement with our conclusions. When considering all the experimental data (Figure 1), we concluded that the PROVEN BI did not significantly reduce N_2O emissions and did not significantly enhance crop yields. Our conclusions related to crop yield are supported by other recent field-based studies (Currie et al. 2020; Davis et al. 2020; and Steinkamp et al. 2023) who found that PROVEN® BI did not significantly enhance crop yields. We are not aware of any other published reports regarding the effects of PROVEN® BI on N_2O emissions. However, Dr. Rod Venterea (USDA-ARS & University of Minnesota) conducted similar experiments under field conditions and found similar results (personal communication).

CONCLUSIONS

Our data and analyses support the following conclusions:

1. An inoculant (PROVEN® BI) was tested with various fertilizer rates to assess if it could help mitigate N_2O emissions.
2. The BI treatment did not significantly influence N_2O emissions, crop yield, soil, water, or plant N concentrations.
3. As expected, nitrogen fertilizer rate had a significant relationship with N_2O emissions, yield, soil NO_3^- and NH_4^+ concentrations.

EDUCATION, OUTREACH, AND PUBLICATIONS

This research project provided training opportunities for 1 MS graduate student (Michelle Bohnen), 2 postdoctoral scholars (Dr. Jerry Hsiao and Dr. Alex Frie), and 2 research scientists (Matt Erickson and Sam Strack). Tim Griffis presented findings from this work at Cornell University, Purdue University, University of Innsbruck (Austria), the Tri-Societies Meetings, and USDA LTAR working group. Further, Michelle Bohnen presented findings from this research at the AgExpo Meeting on January 18, 2023 and the Department of Soil, Water, and Climate Seminar Series on March 1, 2023.

"Effects of climate warming and nitrogen fixing bacterial inoculants on nitrous oxide emissions", M. Bohnen, MS Thesis, University of Minnesota, Department of Soil, Water, and Climate, November 2024.

"Investigating the potential of designer microorganisms to reduce N_2O emissions from maize cultivation", A. Frie, T.J. Griffis, and L. Miller, American Geophysical Union (AGU), December 18, 2020, Virtual meeting due to the Covid-19 pandemic

"A regional perspective on reactive nitrogen and carbon cycling in the US Corn Belt", T.J. Griffis invited, Discovery Farms, January 27, 2021, Minnesota-Wisconsin, USA

"Reducing reactive nitrogen losses from agricultural ecosystems" T.J. Griffis invited, January 20, 2021, Minnesota Ag Expo, Video Presentation due to the Covid-19 pandemic

"Constraining regional N_2O and NH_3 emissions using tall tower observations and chemical transport modeling" T.J. Griffis, invited talk, Cornell University, October 28, 2021

"Constraining regional N_2O and NH_3 emissions using tall tower observations and chemical transport modeling" T.J. Griffis, invited talk, Purdue University, November 15, 2021

"Constraining regional ammonia and nitrous oxide emissions using tall tower observations and chemical transport modeling" T.J. Griffis, ASA invited talk, November 7-10, 2021

"Simulating N_2O Emission from Fertilized Mesocosm Using Knowledge Guided Machine Learning" J. Zhenong et al., American Geophysical Union (AGU), December 18, 2021.

"Constraining regional N_2O and NH_3 emissions using tall tower observations and chemical transport modeling" T.J. Griffis, invited talk, Department of Ecology, University of Innsbruck, May 23, 2022.

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