2024 FINAL REPORT (project ongoing)

PROJECT TITLE: Effects of procyanidins on corn growth and nitrous oxide reduction

PROJECT NUMBER: 6116-24DD

PRINCIPAL INVESTIGATOR AND CO-INVESTIGATOR(S): Timothy Griffis and CheJen Hsiao

ABSTRACT

Provide a project summary describing an overview of the project including principal findings. Include a statement on how the project was of benefit to corn farmers.

Procyanidins have demonstrated strong potential as biological denitrification inhibitors for mitigating N_2O emissions in agricultural soils. However, their high cost has limited scalability. To address this barrier, we developed a low-cost synthesis method using catechin oligomerization catalyzed by horseradish peroxidase (HRP) and hydrogen peroxide. The objectives of this study is to optimize synthesis protocols and understand soil-specific responses, prompted by variability in N_2O inhibition performance of synthetic procyanidins. We tested multiple substrate concentrations, reaction times, and soil types (high and low activity soils), and identified factors influencing product efficacy. DEA (denitrification enzyme activity) and SIR (substrate-induced respiration) assays were refined to enhance detection sensitivity. Our results indicated that synthetic procyanidins reduced DEA by 30–60% in high-activity soils and were up to 2,000X more cost-effective than procyanidins from commercial grape seed extract. However, efficacy varied by soil type, emphasizing the need for tailored applications. These findings lay the foundation for future mesocosm trials and inform ongoing formulation improvements, positioning synthetic procyanidins as a promising biological denitrification inhibitor strategy for sustainable corn production.

INTRODUCTION

Provide background information related to the project including such item as the problem statement, knowledge gaps, and relevant previous work completed on this issue.

Procyanidins, naturally occurring polyphenolic compounds in berries, grapes, apples, and other fruits, have demonstrated strong potential as biological denitrification inhibitors for mitigating N_2O emissions in agricultural soils. Previous studies using grape seed extract procyanidins showed significant reductions in N_2O emissions, but their high cost has limited field-scale application. To address this barrier, we

collaborated with food nutrient scientists to develop a novel, cost-effective synthesis method using catechin oligomerization catalyzed by horseradish peroxidase (HRP) and hydrogen peroxide (H_2O_2) in phosphate buffer. The overarching goal of this study is to develop and validate synthetic procyanidins as scalable, economically viable biological denitrification inhibitors that can reduce N_2O emissions while maintaining or enhancing crop productivity and nitrogen use efficiency in agricultural systems.

While the synthetic procyanidins were significantly less expensive compared to commercial grape seed extract, initial microcosm experiments showed inconsistent N_2O inhibition efficiency. As a result, we focused on optimizing synthesis protocols, refining product consistency, and understanding soil-specific mechanisms of inhibition during the current reporting period. We adjusted substrate concentrations, HRP enzyme levels, and reaction times to improve product stability and performance. We also refined denitrification enzyme activity (DEA) analysis by optimizing pre-incubation conditions and introduced the DEA:SIR (substrate-induced respiration) ratio as a metric to better interpret results across soils with varying microbial communities. These advances support the development of procyanidins as a practical soil amendment for emission reduction and nitrogen management in cropping systems.

OBJECTIVE AND GOAL STATEMENTS

Objective: To optimize procyanidins synthesis and elucidate soil-specific responses, laying the groundwork for mesocosm trials in 2025.

While the original objective was to evaluate the impact of synthetic procyanidin products on soil N_2O emissions, corn growth, and soil nitrogen dynamics in field settings, early findings of inconsistencies in N_2O reduction prompted a pivot to prioritize optimizing synthesis methods, ensuring product reliability, and clarifying soil-specific denitrification inhibition mechanisms. These advancements will support the 2025 goal of evaluating synthetic procyanidins under mesocosm conditions.

Specific goals:

- Optimize procyanidins synthesis by varying substrate concentrations (catechin, HRP enzyme, H_2O_2) and reaction conditions (time, ratios).
- Refine analytical methods, including identification of optimal DEA assay parameters (pre-incubation period, DEA:SIR ratio).
- Assess inhibition efficacy across soil types (Waukegan silt loam, Klinger silty clay loam).
- Evaluate economic viability by comparing synthetic HRP to commercial grapeseed extracts.
- Prepare for scale-up through mesocosm trials.

MATERIALS AND METHODS

As appropriate, describe the site(s), experimental design, and other relevant methodology used in completing the project.

- Synthesis conditions: Varied catechin (0.17–1.72 mM), HRP enzyme (15–66.67 IU/mL), H_2O_2 (0.03–0.33 mM), and reaction times (1–10 min) to generate procyanidin products. Initial negative (urea and water) and positive controls (commercial procyanidins) .
- DEA and SIR assays: Soil samples pre-incubated for 2.5 hrs to 7 days; DEA measured as N_2O flux (ng N g^{-1} hr^{-1}), SIR as CO_2 flux. SIR reflects microbial biomass response to added carbon

substrates (e.g., glucose), and the DEA:SIR ratio was used to interpret relative microbial and denitrifier activity under procyanidin influence.

- Soil types: Experiments conducted using Waukegan silt loam (high denitrifier activity) and Klinger silty clay loam (lower activity) under urea amendment (100 µg N g^{-1} soil). Mesocosm soils were sourced from mesocosm facility.
- Analytical tools: High-performance liquid chromatography (HPLC/UPLC) characterized procyanidin monomers, dimers, trimers; gas chromatography measured N_2O and CO_2 fluxes.

RESULTS AND DISCUSSION

- Optimal DEA assay conditions: A 3-day pre-incubation maximized inhibition detection in high-activity Waukegan soils; 1-day pre-incubation was optimal for low-activity Klinger soil.

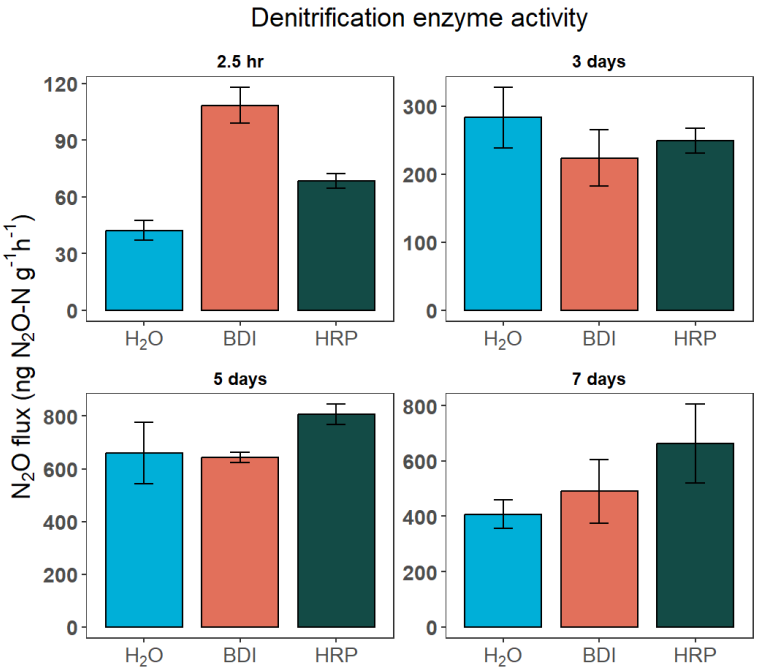

Figure 1. The denitrification enzyme activities of **Waukegan** soils under water addition as control (H_2O), commercial procyanidins (BDI) addition, and synthesized procyanidins addition (HRP) by different pre-incubation periods (2.5 hrs, 3 days, 5 days, and 7 days) (n = 3). Results indicate that after 3 days of pre-incubation, both BDI and HRP showed relatively lower N_2O flux compared to the control (H_2O). HRP resulted in higher N_2O flux during the other pre-incubation periods. These findings suggest that the effect of procyanidin addition varies with incubation time, with 3 days of pre-incubation leading to reduced N_2O flux in the HRP treatment compared to longer or shorter pre-incubation periods.

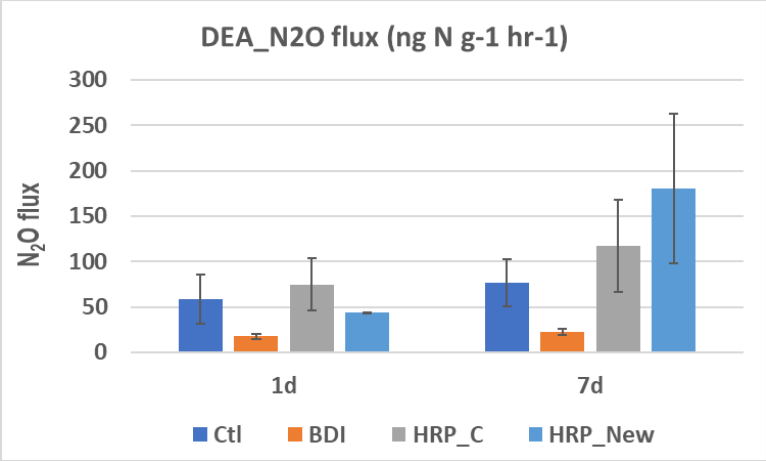

Figure 2. The denitrification enzyme activities of **Klinger** soils under urea, commercial procyanidins (BDI) addition, and synthesized procyanidins additions (HRP) after 1 and 7 days of pre-incubation (n = 3) using Klinger soils. The results indicated that 1-day pre-incubation yielded the most inhibition effect.

- Efficacy of synthetic procyanidins: We tested a range of reactant concentrations (0.1X, 0.25X, 0.5X, 1X, 2X, and 5X), using the 1X condition (Catechin 0.33 mM, HRP 66.67 IU/mL, H2O2 0.3 mM, 10-min reaction) as a baseline. As shown in Fig. 3, lower concentrations (0.1–0.5X) still significantly reduced N_2O fluxes compared to the urea-only control. We observed no differences among 0.1–0.5X concentrations, indicating potential for further cost reduction without compromising efficacy. We also tested a revised formulation (HRP_New: Catechin 0.33 mM, HRP 30 IU/mL, H_2O_2 0.3 mM) in both Waukegan and Klinger soils. The HRP_New formula maintained N_2O suppression but only needed half of the HRP enzyme than the previous approach, confirming it as a more cost-efficient yet effective candidate for future testing (Fig 2).

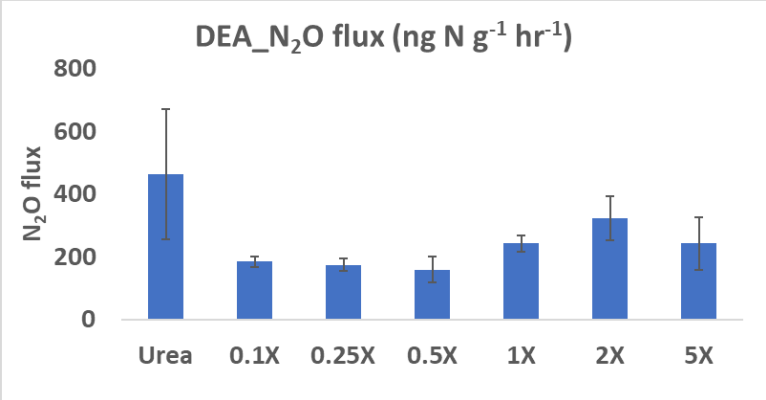

Figure 3. The denitrification enzyme activities of soils under urea and synthesized procyanidins addition (HRP) by different reactant conditions after 3 days of pre-incubation (n = 3). The results indicated HRP treatments reduced denitrification enzyme activity (DEA) by 30–60% compared to the control.

- SIR responses varied: Both HRP_C and HRP_H treatments increased SIR. Given the concurrent reduction in DEA, the relatively low SIR response suggests that procyanidins may selectively inhibit denitrifiers without broadly suppressing overall microbial activity.

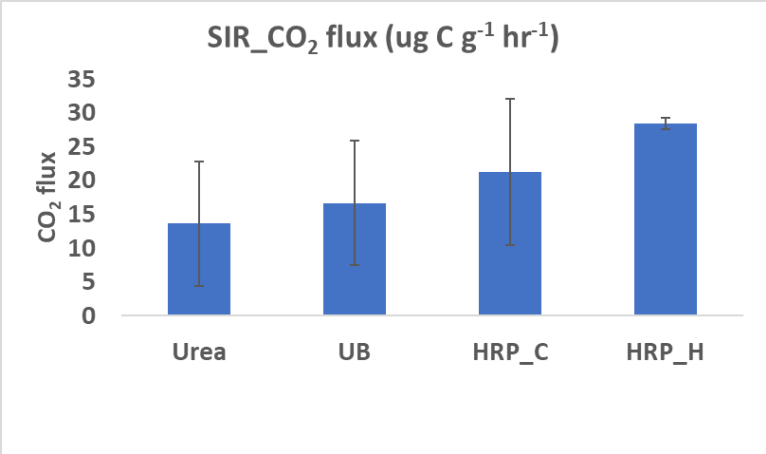

Figure 4. The substrate-induced respiration of soils under urea, commercial procyanidins (BDI) addition, and synthesized procyanidins addition (HRP) by different reactant conditions after 3 days of pre-incubation (n = 3). The results indicated HRP increases substrate-induced respiration by 50–100% compared to the control.

- Cost-effectiveness: Synthetic HRP_C at 0.5X concentrations ($125/500 g) was ~2,000× more cost-effective than commercial grape seed extract ($250/0.5 g).

- Soil-specific responses: Klinger soil exhibited lower baseline N_2O emissions and less response to procyanidins, potentially because that soil type, microbial community, and texture influence product performance.

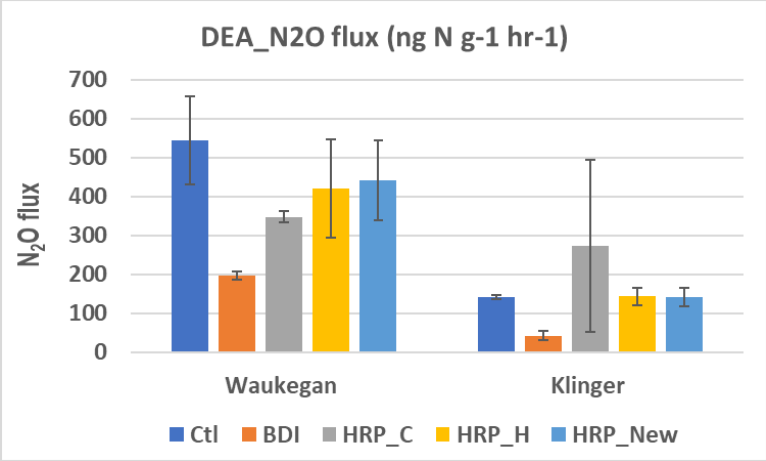

Figure 5. The denitrification enzyme activities of soils under urea, commercial procyanidins (BDI) addition, and synthesized procyanidins addition (HRP) by different reactant conditions (HRP_C: Catechin 0.33 mM, HRP 66.67 IU/mL, H_2O_2 0.3 mM; HRP_H: Catechin 1.72 mM, HRP 30 IU/mL, H_2O_2 0.03 mM; HRP-New: Catechin 0.33 mM, HRP 30 IU/mL, H_2O_2 0.3 mM) after 3 days of pre-incubation (n = 3). The results indicated Klinger soil exhibited lower N_2O emissions compared to Waukegan soil. For Klinger soil, there was no significant N_2O emission reduction in all HRP treatments.

- Synthesis Sensitivity: Reaction time affected product efficacy. One-minute reactions underperformed relative to 10-minute reactions. Catechins and HRP enzymes are moisture- and light-sensitive, indicating special storage and fresh procyanidins preparation for optimal results.

CONCLUSIONS

Synthetic procyanidin products show strong promise as biological denitrification inhibitors, with the potential to reduce N_2O emissions and improve nitrogen use efficiency in corn systems. Key advances included the identification of optimal synthesis conditions, cost-effective formulations, and improved understanding of soil-specific and microbial responses. The remaining challenges include variability in field conditions, product stability, and funding continuity. Next steps will focus on:

- Dose–response optimization of the HRP_New formulation,
- Expanded testing under mesocosm conditions,
- Preparation for publication and potential patent applications.

EDUCATION, OUTREACH, AND PUBLICATIONS

Identify conferences, workshops, field days etc. at which project results were presented. Include number of farmers estimated to be present. List articles and/or manuscripts in which project results were published.

Dr. Hsiao presented the project results at the Tri-Society Annual Meeting in San Antonio, held from November 9–15, 2024.

REFERENCES

NA