Project

Title: Long-term study on the impact of cover crop on soil microbiome function affecting C and N inputs in Corn Production Systems

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Project Summary (Issue/Response)

Soil microbial communities are pivotal players in nutrient cycling processes that substantially impact crop yield and soil vitality in agricultural systems. Despite the growing recognition of cover crops (CC's) as a promising avenue for enhancing soil health and shaping microbial composition, a comprehensive understanding of their synergistic effects on ecosystem services remains notably deficient. Addressing this significant knowledge gap, our three-year study employs a strip plot design across two distinct locations—Starkville and Newton—to rigorously assess the effects of various CC's (ryegrass, balansa, red clover, radish, and cover crop mixes) and nitrogen levels (0 lb. and 100 lb.) on soil microbial communities and carbon/nitrogen cycling within corn production systems. Both years (2022-2023) results shows significant disparities in microbial community structures across locations. While Shannon diversity index revealed no significant differentiation among cover crop treatments in Newton, significant differences were observed among cover crops in Starkville. Chao1 diversity index showed higher bacterial ASV richness for 2022 compared to 2023. Intriguingly, plots receiving 100 lbs. of N showed elevated Shannon diversity compared to 0 lbs. N plots. Moreover, enzymatic assays and active carbon analyses results show that soils from plots with rye grass and CC-mix2 had higher activity relative to other treatments. Abundance of N-cycling genes, specifically *amoA* and *nifH,* also exhibited significant differences across cover crop treatments. Overall, our results indicate that ryegrass and CC-mix2 (ryegrass + radish + red clover) markedly enhance bacterial richness, SDI, POXC, soil enzymatic activity, and abundance of microbial taxa integral to N-cycling.

Project Results/Outcomes

1. **Cover crops effect on soil parameters:** We have collected total 84 samples from both locations. These samples were analyzed for total nitrogen (%), total carbon (%), organic matter (%), active carbon (POXC-mg/kg), and glomalin (mg/g). While CC's did not significantly alter the overall soil physico-chemical properties, notable distinctions in POXC were observed among treatments ($p < 0.001$). We found plots with ryegrass had high active carbon (724.6± 9.60), followed by CC-mix2 (713.92 ± 8.32)).

2. **Cover crop effect on soil biological parameters:** Soil biological traits represents microbial activity in soils. In our study, we focused on quantifying soil enzyme activity to study functional differences in microbiota due to CC's. Specifically, we measured the activity of β-glucosidase and β-glucosaminidase, which are crucial indicators involved in degradation of cellulose and chitin respectively. These enzymes play crucial rule in C and N cycling in soils. ANOVA results revealed that plots with ryegrass (3.47±0.22) and CC-mix2(3.43±0.25) showed higher values for both the enzymes ($p < 0.0021$).

3. **Cover crop effect on microbial indicators:** We extracted soil genomic DNA from samples which stored in –80 freezer. We performed amplicon sequencing targeting 16S (bacterial) and ITS2 (fungal) communities to study the abundance,

Project Results

and diversity patterns across soil samples. Microbial relative abundance analysis revealed taxonomic changes across locations. The relative proportion of bacteria was analyzed at phylum level. In both years, *Proteobacteria* (35.7%) was dominant in Starkville, whereas in Newton *Actinobacteria* (33.74%) phyla was dominant.. *Firmcutes*, *Acidobacteria*, *Chloroflexi*, *Gemmatimonadetes*, and *Verrucomicrobia* are other dominant bacterial phyla in both locations. We also estimated alpha diversity (Shannon diversity index and Chao1), beta diversity of both bacterial and fungal communities. Beta diversity results revealed significant differences among communities in both locations (PERMANOVA; $p < 0.05$)

4. **Cover crop effect on abundance of N-cycling genes:** We have estimated abundance of microbial groups involved in N-cycling, specifically *amoA* (Ammonium monooxygenase) and *nifH* (Nitrogenase) genes through quantitative PCR method. Results revealed that for *nifH* gene treatments (Balansa, CC-mix2, red clover, and rye grass had higher gene copies compare to other treatments in both locations whereas for *amoA* gene, ryegrass, and CC-mix2 had higher gene copies compared to other treatments (Fig. 1). Presence of gene copies directly proportional to the abundance of microbial groups involved in these functions.

Overall, these two years results showed plots with ryegrass and CC-mix2 exhibited increased enzyme activities, elevated POXC levels, and higher presence of microbial groups dedicated to N-cycling.
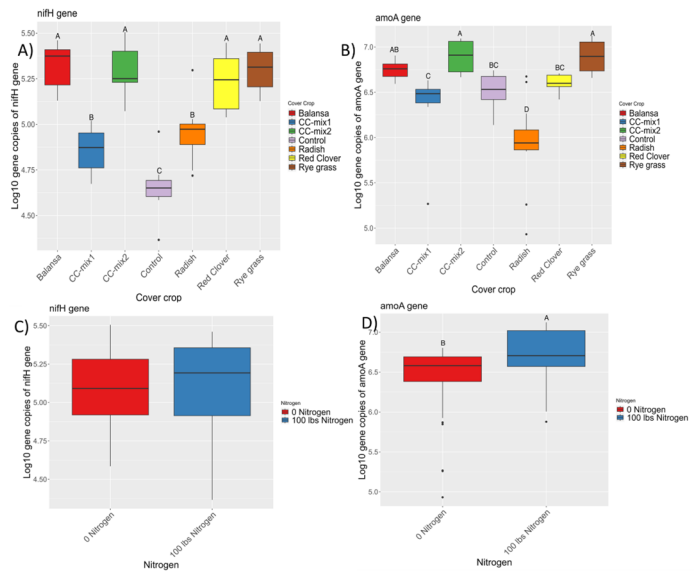

Figure 1. Cover crop (A and B) and Nitrogen fertilizer (C and D) influence on N-cycling genes (nifH and amoA); CC-mix1 = Oats + Radish; CC-mix2 = Radish + Ryegrass + Red clover

Project Impacts/Benefits

The comprehensive three-year study conducted across Starkville and Newton has elucidated the impactful role of various CC's and N levels on soil microbial communities and their functional attributes, particularly in corn production systems. The study's revelation of the substantial disparities in microbial community structures and the abundance of crucial N-cycling genes across different locations and treatments underscores the nuanced nature of microbial responses to agricultural interventions. By pinpointing the specific conditions that favor functional microbial diversity and activity, the research paves the way for tailored agricultural practices that optimize soil health and productivity. In practical terms, the project's outcomes advocate for the strategic use of cover crops and informed N management as vital tools for enhancing soil health and crop yield. This research not only fills a critical knowledge gap but also equips stakeholders with actionable strategies to harness the full potential of soil microbial communities in agriculture.

Project Deliverables

Presentations:

1. American Society of Agronomy, Crop Science Society of America, and Soil Science of America Conference meeting, at Saint Louis, MI, 2023.

2. Mississippi Academy of Sciences Annual Meeting at Biloxi, MS, 2023

3. Mississippi State University, Graduate School Symposiums -Spring 2023

4. Mississippi State University, Graduate School Symposiums -Fall 2023.