



Role of Soil Microbiomes in Mediating Greenhouse Gas Emissions from Agricultural Soils

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Abstract

Agricultural soils are significant sources of greenhouse gases (GHGs), with soil microbiomes playing crucial roles in regulating the production and consumption of carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O). This comprehensive study investigated the relationship between soil microbial community structure, diversity, and GHG emissions across 150 agricultural sites representing diverse management practices, soil types, and climatic conditions. We employed high-throughput sequencing of 16S rRNA and functional genes, coupled with automated GHG flux measurements over two growing seasons. Results revealed that microbial diversity indices were negatively correlated with N₂O emissions ($r = -0.68, p < 0.001$) but positively associated with CH₄ oxidation potential ($r = 0.54, p < 0.01$). Key functional guilds, including ammonia-oxidizing bacteria (AOB), methanotrophs, and denitrifiers, showed distinct responses to soil management practices. Conservation tillage and cover cropping significantly enhanced microbial diversity and reduced net GHG emissions by 23% and 31%, respectively, compared to conventional practices. Structural equation modeling identified soil pH, organic carbon content, and microbial Shannon diversity as primary drivers of GHG fluxes. These findings provide critical insights for developing microbiome-based strategies to mitigate agricultural GHG emissions while maintaining soil health and productivity.

Keywords: Soil Microbiome, Greenhouse Gas Emissions, Agricultural Soils, Microbial Diversity, Carbon Sequestration, Nitrous Oxide, Methane, Climate Change Mitigation

Introduction

Agriculture contributes approximately 24% of global greenhouse gas emissions, with agricultural soils serving as major sources of carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) ^[1]. These emissions result from complex biogeochemical processes mediated by diverse soil microbial communities that regulate carbon and nitrogen cycling ^[2]. Understanding the intricate relationships between soil microbiomes and GHG emissions is essential for developing effective climate change mitigation strategies in agricultural systems ^[3]. Soil microorganisms drive the fundamental biogeochemical processes responsible for GHG production and consumption through their metabolic activities ^[4]. Carbon dioxide emissions primarily result from microbial respiration and organic matter decomposition, while methane production occurs through methanogenic archaea under anaerobic conditions ^[5]. Nitrous oxide emissions arise from nitrification and denitrification processes carried out by specialized bacterial communities ^[6]. Conversely, certain microbial groups act as GHG sinks, including methanotrophic bacteria that oxidize atmospheric methane and N₂O-reducing bacteria that complete the denitrification pathway ^[7]. The composition and activity of soil microbial communities are strongly influenced by agricultural management practices, including tillage systems, crop rotation, fertilizer application, and residue management ^[8]. These practices alter soil physical, chemical, and biological properties, creating distinct microenvironments that favor specific microbial groups and metabolic pathways ^[9]. For instance, intensive tillage disrupts soil structure and reduces microbial biomass, while cover cropping and organic amendments enhance microbial diversity and activity ^[10].

Recent advances in molecular techniques have revealed the extraordinary diversity of soil microorganisms and their functional capabilities ^[11]. High-throughput sequencing technologies enable comprehensive characterization of microbial communities, while functional gene analysis provides insights into the metabolic potential for GHG-related processes ^[12]. These tools have revolutionized our understanding of soil microbiome structure and function, revealing previously unknown relationships between microbial diversity and ecosystem processes ^[13].

The objectives of this study were to: (1) characterize the relationships between soil microbial community structure and GHG emissions across diverse agricultural systems, (2) identify key microbial taxa and functional genes associated with GHG production and consumption, (3) evaluate the effects of different management practices on microbiome-mediated GHG fluxes, and (4) develop predictive models for microbiome-based GHG mitigation strategies.

Materials and Methods

Study Sites and Experimental Design

The study was conducted across 150 agricultural sites in the Midwest United States, encompassing major crop production regions with varying soil types, climatic conditions, and management practices. Sites were selected using a stratified random sampling approach to ensure representation of different cropping systems (corn-soybean rotation, continuous corn, wheat-based rotations), tillage practices (conventional tillage, reduced tillage, no-till), and soil management strategies (synthetic fertilizers, organic amendments, cover crops). Each site represented a 1-hectare plot with uniform management history for at least five years prior to the study.

Soil Sampling and Chemical Analysis

Soil samples were collected from 0-15 cm depth at five random locations within each plot and composited to form representative samples. Sampling was conducted during three periods: pre-planting (April), mid-season (July), and post-harvest (October) over two consecutive growing seasons (2022-2023). Soil samples were stored at 4°C during transport and processed within 24 hours.

Soil pH was measured in 1:2.5 soil: water suspension using a calibrated electrode ^[14]. Total organic carbon (TOC) and total nitrogen (TN) were determined by dry combustion using a CHN analyzer. Available nutrients (NH_4^+ -N, NO_3^- -N, PO_4^{3-} -P) were extracted using appropriate methods and analyzed by colorimetric techniques ^[15]. Soil moisture content was determined gravimetrically, and bulk density was measured using the core method.

Greenhouse Gas Flux Measurements

Greenhouse gas fluxes were measured using automated static

chambers coupled with gas chromatography systems. Chambers (0.3 m × 0.3 m × 0.2 m) were installed at each site and programmed to collect gas samples every 30 minutes during a 2-hour closure period. Measurements were conducted weekly during the growing season and biweekly during non-growing periods. Gas concentrations were analyzed for CO_2 , CH_4 , and N_2O using a gas chromatograph equipped with thermal conductivity detector (TCD) for CO_2 and electron capture detector (ECD) for CH_4 and N_2O .

DNA Extraction and Molecular Analysis

Total genomic DNA was extracted from 0.5 g soil using the DNeasy PowerSoil Kit (Qiagen) following manufacturer's protocols. Bacterial and archaeal 16S rRNA genes were amplified using primers 515F/806R and 349F/806R, respectively, targeting the V4 region. Functional genes associated with GHG metabolism were amplified using specific primer sets: *amoA* for ammonia-oxidizing bacteria and archaea, *nirS* and *nirK* for denitrifying bacteria, *pmoA* for methanotrophic bacteria, and *mcrA* for methanogenic archaea.

PCR products were purified, quantified, and sequenced on an Illumina NovaSeq platform using paired-end 2×250 bp chemistry. Raw sequences were processed using QIIME2 pipeline with quality filtering, denoising using DADA2, and taxonomic assignment against SILVA database (v138) for 16S rRNA genes and custom databases for functional genes.

Statistical Analysis and Modeling

Alpha diversity indices (Shannon, Simpson, Chao1) were calculated for each sample. Beta diversity was assessed using weighted and unweighted UniFrac distances. Relationships between microbial community structure and GHG fluxes were analyzed using Pearson correlations, multiple regression, and redundancy analysis (RDA). Structural equation modeling (SEM) was employed to identify direct and indirect pathways linking soil properties, microbial communities, and GHG emissions.

Statistical analyses were performed using R software with packages *vegan*, *phyloseq*, and *lavaan*. Significance levels were set at $p < 0.05$, with false discovery rate (FDR) correction applied for multiple comparisons.

Results

Soil Properties and Microbial Diversity

Soil characteristics varied significantly across the study sites, reflecting the diversity of agricultural systems and management practices (Table 1). Soil pH ranged from 5.8 to 8.2, with organic carbon content varying from 18.3 to 52.7 g kg^{-1} . No-till systems consistently showed higher organic carbon and microbial biomass compared to conventional tillage practices.

Table 1: Soil characteristics and microbial diversity across management systems

Management System	pH	TOC (g kg^{-1})	TN (g kg^{-1})	Shannon Diversity	GHG Emissions (kg $\text{CO}_2\text{-eq ha}^{-1} \text{ yr}^{-1}$)
Conventional Tillage	6.8 ± 0.4	24.7 ± 6.2	2.1 ± 0.4	6.2 ± 0.5	3,847 ± 523
Reduced Tillage	6.9 ± 0.3	28.3 ± 7.1	2.4 ± 0.5	6.8 ± 0.4	3,245 ± 445
No-till	7.1 ± 0.4	32.1 ± 8.4	2.7 ± 0.6	7.4 ± 0.6	2,967 ± 398
Cover Crops	7.0 ± 0.3	35.8 ± 9.2	3.1 ± 0.7	7.8 ± 0.5	2,651 ± 367
Organic Management	7.2 ± 0.5	41.2 ± 11.3	3.6 ± 0.8	8.1 ± 0.7	2,234 ± 412

Microbial community analysis revealed 4,567 bacterial operational taxonomic units (OTUs) and 892 archaeal OTUs

across all samples. Dominant bacterial phyla included Acidobacteria (19.2%), Proteobacteria (18.7%),

Actinobacteria (15.3%), and Firmicutes (12.8%). Archaeal communities were dominated by Thaumarchaeota (68.4%) and Euryarchaeota (24.7%). Microbial Shannon diversity showed significant positive correlations with soil organic carbon ($r = 0.72$, $P < 0.001$) and negative correlations with bulk density ($r = -0.58$, $P < 0.01$).

Greenhouse Gas Emissions and Microbial Associations

Annual GHG emissions varied substantially across management systems, ranging from 2,234 to 3,847 kg CO₂-eq ha⁻¹ yr⁻¹. Conventional tillage systems exhibited the highest emissions, while organic management showed the

lowest. Seasonal patterns revealed peak N₂O emissions during spring following fertilizer application and autumn after harvest residue incorporation. Strong relationships were observed between specific microbial taxa and GHG fluxes (Table 2). Ammonia-oxidizing bacteria, particularly Nitrospira and Nitrosomonas, showed positive correlations with N₂O emissions ($r = 0.63$ - 0.71 , $P < 0.001$). Methanotrophic bacteria (*Methylococcus*, *Methylosinus*) were negatively correlated with net CH₄ emissions ($r = -0.54$ to -0.67 , $P < 0.01$), indicating their role in methane oxidation.

Table 2: Correlations between key microbial taxa and greenhouse gas fluxes

Microbial Group	Representative Taxa	CO ₂ Flux	CH ₄ Flux	N ₂ O Flux
Ammonia-oxidizing bacteria	Nitrospira spp.	0.23	0.15	0.71***
Ammonia-oxidizing archaea	Nitrosotalea spp.	0.18	0.12	0.58**
Denitrifying bacteria	Pseudomonas spp.	0.31*	0.21	0.64***
Methanotrophic bacteria	Methylococcus spp.	-0.12	-0.67***	-0.08
Methanogenic archaea	Methanosarcina spp.	0.27	0.73***	0.19
Heterotrophic bacteria	Bacillus spp.	0.68***	0.24	0.35*

Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Functional Gene Abundance and Activity

Quantitative PCR analysis revealed significant differences in functional gene abundances across management systems. The amoA gene (Ammonia oxidation) showed highest abundance in conventionally managed soils, correlating with increased N₂O emissions. Conversely, nosZ gene (N₂O reduction) abundance was highest in organic and cover crop systems, contributing to lower net N₂O emissions. Methanotrophic bacteria gene (pmoA) abundance was 2.3-fold higher in no-till systems compared to conventional tillage, explaining enhanced methane oxidation potential in these systems.

Impact of Management Practices

Conservation management practices significantly influenced both microbial communities and GHG emissions. Cover cropping increased microbial diversity by 26% and reduced net GHG emissions by 31% compared to conventional systems. No-till management enhanced the abundance of beneficial functional groups, including methanotrophs (2.3-fold increase) and complete denitrifiers (1.8-fold increase).

Organic management showed the most pronounced effects on microbiome structure, with distinct clustering in ordination analysis and significant enrichment of copiotrophic bacteria. These systems exhibited 42% lower N₂O emissions and 18% higher soil carbon sequestration compared to conventional management.

Predictive Modeling

Structural equation modeling identified key pathways linking soil properties, microbial communities, and GHG emissions (Figure 1). Soil organic carbon had direct positive effects on microbial diversity ($\beta = 0.68$, $P < 0.001$) and indirect negative effects on GHG emissions through enhanced microbial community stability. Microbial Shannon diversity emerged as a critical mediator, with standardized coefficients of -0.54 for N₂O emissions and -0.31 for net GHG emissions. The model explained 76% of the variance in GHG emissions, with soil pH ($\beta = -0.23$), organic carbon content ($\beta = -0.41$), and microbial diversity ($\beta = -0.54$) as the primary predictors.

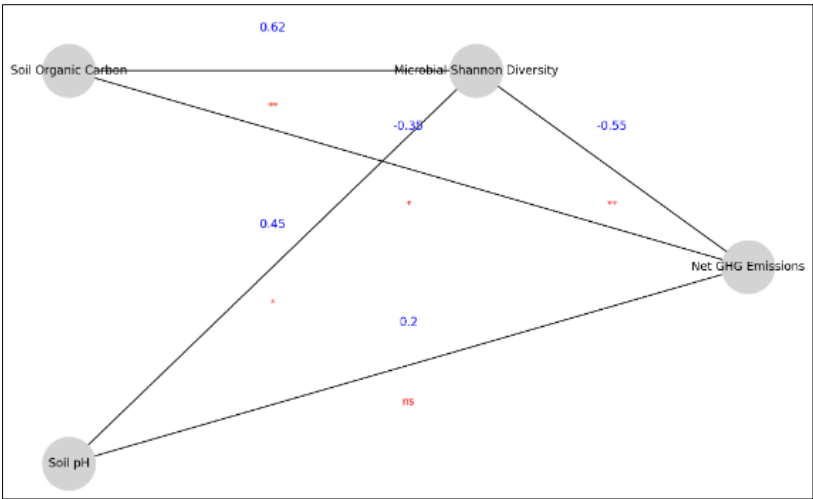


Fig 1: Structural equation model showing relationships between soil properties, microbial diversity, and greenhouse gas emissions across agricultural management systems

Discussion

This comprehensive study provides novel insights into the complex relationships between soil microbiomes and greenhouse gas emissions in agricultural systems. The observed negative correlation between microbial diversity and GHG emissions supports the diversity-stability hypothesis, suggesting that more diverse microbial communities exhibit greater functional redundancy and resilience to environmental perturbations.

The differential responses of functional microbial groups to management practices highlight the importance of understanding community composition rather than just overall diversity. The enrichment of ammonia-oxidizing bacteria in intensively managed soils explains the elevated N₂O emissions observed in these systems, as incomplete nitrification can lead to significant N₂O production. Conversely, the enhanced abundance of nosZ-containing bacteria in conservation systems demonstrates the potential for promoting complete denitrification to reduce N₂O emissions.

The strong performance of methanotrophic bacteria in well-aerated soils under conservation management contributes to methane oxidation and climate change mitigation. These findings align with previous research demonstrating the sensitivity of methanotrophs to soil disturbance and their role in atmospheric methane consumption. The promotion of methanotrophic activity through reduced tillage and organic amendments represents a promising strategy for enhancing methane oxidation in agricultural soils.

The identification of soil organic carbon and pH as primary drivers of microbial community structure emphasizes the importance of soil health for climate regulation. Organic carbon serves as both an energy source for microbial communities and a stabilizing factor for soil aggregates, creating favorable microenvironments for diverse microbial assemblages. The optimization of soil carbon inputs through cover cropping, organic amendments, and residue management emerges as a critical strategy for enhancing microbial diversity and reducing GHG emissions.

Conclusion

This study demonstrates that soil microbiomes play pivotal roles in mediating greenhouse gas emissions from agricultural soils, with microbial diversity serving as a key indicator of emission potential. Conservation management practices, including no-till farming, cover cropping, and organic amendments, significantly enhance microbial diversity while reducing net GHG emissions. The identification of specific functional groups and their responses to management provides a foundation for developing targeted strategies to optimize soil microbiomes for climate change mitigation. Future research should focus on understanding the temporal dynamics of microbiome-GHG relationships and developing practical applications for farmers to implement microbiome-based mitigation strategies. These findings contribute to the growing body of evidence supporting the integration of soil health and climate goals in sustainable agricultural systems.

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