Nitrous oxide (N_2O) emissions from transitioning organic grain production systems: quantification and driving factors

A Dissertation Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

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December 2024

Acknowledgment

I express my deepest gratitude to my advisor, Dr. Debasish Saha, and co-advisor, Dr. Sindhu Jagadamma, for their unwavering support, guidance, and encouragement throughout my doctoral research. I am grateful for their insightful feedback, which has been crucial to the completion of my dissertation. I also want to extend my heartfelt thanks to my dissertation committee members, Dr. Jennifer DeBruyn and Dr. David Butler, for their thoughtful suggestions and support throughout this journey. Their input has significantly shaped my research work, and I appreciate their time and effort. I would like to acknowledge USDA NIFA for funding this project. I am especially thankful to the farm crew members at the East Tennessee Research and Education Center Research, Organic Crop Unit, for their invaluable assistance during my research. Additionally, I am deeply thankful to the undergraduate students who helped me through my research and to the faculty and staff of the BESS department for their support during my PhD journey. I am immensely grateful to all the lab members for their helpful suggestions and guidance, which contributed greatly to my research. To my friends, thank you for always being there for me when I needed support. I want to express my gratitude to all my family members for their love, patience, and unwavering belief in me. At this moment, I especially want to remember my mother, whose constant encouragement and sacrifices have made this achievement possible. I am forever indebted to you. Lastly, I would like to extend my deepest thanks to my wife, Puja, for her unconditional support and encouragement throughout this journey.

Abstract

Organic agriculture is growing worldwide due to its environmental benefits, economic potential, and positive implications for societal well-being. Organic agriculture practices through organic inputs can provide soil health benefits such as carbon (C) sequestration, improved soil biological activity, improved soil structure, and increased aggregate stability. However, uncertainty in N_2O emissions from organic agriculture systems may offset the C sequestration benefits. This study aimed to determine the cropping systems with N_2O emissions mitigation potential when transitioning to organic grain rotation systems in warm, humid climates. Four cropping systems with different tillage regimes, cover crop compositions, and PL application strategies were employed in a three-year soybean-wheat-corn rotation. From each cropping system, extensive soil sampling from 0–15 cm during the main crop growing season was done to measure the soil mineral nitrogen (N) (NH_4-N and NO_3-N). Similarly, gas samples from each system during the main crop season were collected using a static gas chamber to measure greenhouse gases [GHGs – carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O)]. High pulses of mineral N were observed following the application of organic inputs, with a high N surplus across all cropping systems. Peak N_2O fluxes were observed corresponding to high pulses of mineral N. Cumulative N_2O emissions were highest from the corn phase of rotation, likely attributed to the high rate of PL application and high tillage intensity. Despite similar N input in three of the four cropping systems, significantly low N_2O emissions from the cropping systems that received the least tillage intensity suggest that tillage reduction could potentially reduce N_2O emissions. While comparing the effect of cover crops and PL on N_2O emissions, we found that PL was the major contributor to N_2O emissions, with denitrification being the major microbial process controlling peak N_2O emissions. Global warming potential (GWP) was

calculated based on the change in soil organic C (SOC) stock, CH_4, and N_2O, and negative GWP was observed across all cropping systems. Negative GWP estimation across all cropping systems suggests that organic crop rotation with high organic inputs could potentially sequester C. However, a long-term study is essential to provide deep insight into the effect of organic crop rotation on C sequestration.

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Chapter 1: Introduction and dissertation overview

Organic agriculture is gaining popularity worldwide due to its environmental benefits, economic potential, and positive implications for societal well-being (Badgley et al., 2007; Meemken & Qaim, 2018; Reganold & Wachter, 2016; Seufert & Ramankutty, 2017). Therefore, the world's land coverage under organic agriculture reached 96.4 million hectares in 2022 (Willer & Travnicek, 2023). A similar trend was also observed in the United States, with land coverage under organic agriculture reaching 4.9 million hectares by 2021 (USDA-ERS, 2023). Organic agriculture, by eliminating the use of synthetic fertilizers and pesticides, holds significant potential to mitigate environmental risks and safeguard human health from the adverse effects of chemical inputs, however, meeting the growing demand for organic products presents challenges. Organic agriculture relies heavily on organic inputs for managing nutrients, pests, diseases, and weeds. Management practices such as tillage are often necessary to incorporate organic nutrient inputs and control weeds. These practices, while essential in organic agriculture systems, can lead to unintended negative environmental impacts such as soil degradation and N_2O emissions–a potent greenhouse gas (Lal, 2015; Gattinger et al., 2012).

Nitrous oxide is a significant contributor to global warming, with a warming potential 265 times greater than carbon dioxide (CO_2) over a 100-year time period (IPCC, 2014). This gas can remain in the atmosphere for up to 114 years without undergoing any chemical degradation and can deplete the stratospheric ozone layer (Ravishankara et al., 2009). Current atmospheric concentrations of N_2O have risen to 332 ppb, which is a 23% increase from pre-industrial time (1750) (IPCC, 2023). Agricultural activities are the largest source of anthropogenic N_2O emissions, accounting for approximately 66% of total N_2O emissions (Davidson & Kanter, 2014). In the United States, it is estimated that 74% of total N_2O emissions stem from agricultural systems (US EPA, 2015). While most of the N_2O emissions from agricultural systems are associated with the application of synthetic N fertilizer (Davidson, 2009; Smith et al., 2012), there remains a critical need to understand the role of organic farming systems in contributing to N_2O emissions.

In organic agriculture systems, N_2O emissions are primarily driven by soil and nutrient management practices. Unlike conventional agriculture systems, which rely on synthetic N fertilizer, organic systems rely on alternative N sources such as manure, cover crop residues, and biological N fixation from legumes (Drinkwater et al., 1998; Gaskell & Smith, 2007). The release of N from these organic inputs is controlled by microbial processes, which are influenced by various factors such as soil moisture, temperature, and the properties of the organic inputs, including the composition of cover crops (e.g., legume vs. grass) and manure type (e.g., slurry, biosolids) (Watson et al., 2002; Kuzyakov & Blagodatskaya, 2015). Additionally, tillage practices (e.g., conventional tillage, reduced tillage, no-tillage) also affect these microbial processes and subsequent N release (Petersen et al., 2011). However, N release from organic inputs may not always align with the demand for cash crops. This asynchrony between N supply and crop uptake can result in surplus N in the soil, which increases the potential for N_2O emissions (Cavigelli & Parkin, 2012). The complexity of agricultural management practices and N_2O emissions highlights the need to explore the microbial processes that drive these emissions, particularly within organic grain production systems. Understanding how various soil management strategies influence microbial activity and subsequent N_2O production is crucial for developing more sustainable agricultural practices.

1.1 Microbial processes for N_2O emissions

Several microbial processes contribute to N_2O emissions from agricultural soils including nitrification, denitrification, fermentative reduction of nitrite, and chemo-denitrification (Butterbach-Bahl et al., 2013; Thompson et al., 2012). However, nitrification and denitrification are the major microbial processes contributing to N_2O emissions. Nitrification, occurring primarily under aerobic conditions in the presence of ammonium (NH_4^+), leads to relatively steady background N_2O emissions. In contrast, denitrification, which occurs under anaerobic conditions when nitrate (NO_3^-) is present, results in episodic but often larger N_2O peaks. During denitrification, N_2O is an intermediate product, which may be further reduced to dinitrogen (N_2) under favorable conditions, such as when soil moisture is high (water-filled space > 90%) (Firestone & Davidson, 1989). Therefore, the availability of NH_4^+, NO_3^-, and soil moisture are the major regulator of N_2O emissions. Moreover, additional factors such as soil pH, temperature, the chemical composition of organic inputs, and their management practices also influence N_2O production in agricultural soils (Snyder et al., 2009). For example, the presence of NO_3^- in wet soils along with labile soil organic C may increase N_2O emissions through heterotrophic denitrification. Understanding these regulatory factors is essential for developing effective strategies to mitigate N_2O emissions. While there are several management and environmental factors influencing N_2O emissions, this section of the thesis focuses on the factors relevant to my research.

1.2 Soil environmental factors influencing N_2O emissions

Soil moisture and temperature are key drivers of N_2O emissions, as these soil parameters influence the microbial processes emitting N_2O. Soil environmental conditions, particularly soil moisture, control the N_2O emissions from nitrification and denitrification – dominant microbial processes contributing to N_2O emissions (Firestone & Davidson, 1989). Nitrification generally occurs in aerobic conditions, usually at 40–70% water-filled pore space (WFPS), contributing steady and background emissions. In contrast, denitrification occurs in anaerobic conditions, usually when WFPS exceeds 70%, contributing to episodic N_2O emissions (Butterbach-Bahl et al., 2013). However, increasing soil moisture beyond 90% (soil saturation), N_2O further reduces to N_2. In addition to soil moisture, soil temperature drives microbial processes responsible for N_2O emissions (Smith et al., 1998). Along with optimum soil moisture and temperature, substrate availability influences N_2O emissions.

1.3 Organic input (cover crop and manure) effect on N_2O emissions

Soil C and N are major substrates that drive N_2O emissions. Organic inputs such as cover crops and manure are the major sources of C and N in organic cropping systems. Surplus mineral N following the mineralization of organic inputs may result in N_2O emissions. The availability of both C and N from organic inputs may result in heterotrophic denitrification, one of the microbial processes that emit N_2O emissions (Huang et al., 2004; Saggar et al., 2013). However, N_2O emissions depend on the type of organic input type, composition, and management.

Cover crops offer numerous advantages, including soil moisture conservation, erosion reduction, and soil organic matter enhancement (Blanco-Canqui et al., 2015; Nouri et al., 2019). Additionally, legume cover crops host symbiotic diazotrophs that fix atmospheric nitrogen. However, the impact of cover crop residue on N_2O emissions remains unclear. The emissions depend on the quality and management of the cover crops. For instance, seasonal N_2O emissions increase as an influence of legume cover crops (Basche et al., 2014; Fiorini et al., 2020), while grass cover crops tend to reduce the emissions (Thomas et al., 2017; Guardia et al., 2016; Muhammad et al., 2019). The increase in N_2O emissions from legume cover crops is attributed to a low C:N ratio, whereas grass cover crops, with a high C:N ratio, result in reduced N_2O emissions (Huang et al., 2004). A mixture of legume and grass cover crops can decrease the mineralization rate (Poffenbarger et al., 2015), synchronizing mineral N availability and N uptake by crops, thus leading to reduced N_2O emissions (Basche et al., 2014; Rosecrance et al., 2000; Muhammad et al., 2019). Along with cover crops, supplementing N from manure with a lower C:N ratio relative to cover crops can further enhance N_2O emissions (Chang et al., 1998; Petersen, 1999).

In the context of the expanding poultry industry in the United States (Carlson et al., 2023), poultry litter (PL) can potentially serve as a valuable N source in organic cropping systems. However, due to its enrichment with both C and N (Alade et al., 2019), which are major substrates for microbial processes leading to N_2O emissions, it could significantly impact N_2O emissions. Previous studies have reported that PL applications in arable land contributed to N_2O emissions (Cabrera et al., 1994; Thornton et al., 1998; Watts et al., 2011; D'Amours et al., 2023). However, in a study of the effect of PL on cover crop decomposition and mineral N availability, Poffenbarger et al., 2015, found that subsurface banding of PL reduced the rate of cover crop decomposition and subsequent N release, potentially aligning N uptake by crops. The subsurface banded PL application could have reduced the microbial access to PL N, which could be utilized to decompose the surface cover residue. This might have resulted in slow N release from cover crops, enhancing synchronization between the N release from cover crop residue decomposition and crop N demand, eventually minimizing N loss as N_2O. This finding highlights the importance of management practices in controlling N loss from organic inputs like cover crops and PL.

1.4 Tillage effect on N_2O emissions

Tillage management of organic inputs can affect N_2O emissions through various mechanisms, including i) increasing microbial access to the C and N substrate, leading to higher mineralization rates and subsequent emissions, ii) promoting rapid drying of tilled soils compared to no-till (NT) soils, which can limit anoxia and reduce denitrification rates, iii) creating more macropores in the soil, potentially enhancing the escape of N_2O and iv) potentially increasing mineralization through microbial respiration, leading to oxygen depletion and creating optimal anoxic conditions for denitrification and N_2O emissions. Previous studies have consistently reported elevated N_2O emissions associated with increased tillage intensity and reduced N_2O emissions with reduced tillage intensity (Chatskikh & Olsen, 2007; Mutegi et al., 2010; Omonode et al, 2011; Petersen et al., 2011). However, there is evidence that reduced, or no tillage has contributed to elevated N_2O emissions (Aulakh et al, 1984; Baggs et al., 2000; Baggs et al., 2003; Rochette et al., 2008). The increase in N_2O emissions associated with reduced or no tillage was attributed to high soil moisture preserved under thick cover crop mulch (Rochette et al., 2008). There is also some evidence that there is no significant difference in N_2O emissions compared to conventional tillage and no-tillage practices (Elmi et al., 2003; Grandy et al., 2006). These contradictory results associated with tillage practices and N_2O emissions depict the importance of studying the impact of diverse tillage practices on N_2O emissions.

1.5 Objectives

This dissertation addresses the knowledge gap associated with uncertainty in N_2O emissions when employing comprehensive management practices, particularly while transitioning to organic grain rotation systems. During transitioning to organic crop rotation systems, tillage operations, especially to control weeds and incorporate organic inputs, such as cover crops and manure, to meet the nutrient needs of the crops, could potentially induce enhanced N_2O emissions. Therefore, the overarching goal of this dissertation was to understand the soil N_2O footprint from transitioning organic crop rotation systems in warm, humid climates of East Tennessee.

The specific objectives of this study were:

i) To determine the effect of different organic management practices on soil N availability during different phases of organic grain rotation systems and estimate the N budget using input and output information to understand N surplus at the end of a three-year rotation

ii) To identify organic cropping systems with the potential to mitigate N_2O emissions when employing comprehensive organic management practices

iii) To evaluate the effect of PL on cover crop decomposition and N_2O emissions and determine the key microbial processes driving N_2O emissions during progressive cover crop decomposition

iv) To understand the C sequestration potential of organic crop rotation systems when employing diverse tillage, cover crop, and PL management

1.6 Dissertation overview

The dissertation is divided into three main sections. i) introduction, which includes general introduction, knowledge gap, and the objective of the dissertation, ii) four research chapters in manuscript form, and iii) a summary that includes conclusions, implications, and future directions of the research

Chapter 2, titled "Soil nitrogen dynamics in response to diverse tillage, cover crop, and manure management in transitioning organic cropping systems," includes the estimation of the N input, output, and surplus N after three years of comprehensive organic crop management. Surplus N was estimated based on N input from legume cover crops and PL, and N output from yield. Soil inorganic N (SIN – the sum of NH_4-N and NO_3-N) for all crops (soybean, wheat, and corn) was measured to know the SIN availability for growing crops. Changes in SIN and total N concentrations from 2020 to 2023 were evaluated across soil profiles of 0–60cm soil.

Chapter 3, titled "Nitrous oxide (N_2O) emissions and its drivers in transitioning organic grain production systems employing diverse tillage, cover crop, and manure management" provides an insight into the impact of diverse tillage, cover crop management, and PL on N_2O emissions and understand the key drivers of N_2O emissions when employing comprehensive organic management systems. Nitrous oxide fluxes and associated variables such as soil moisture, temperature, and soil mineral N were extensively measured during the main crop phases of a three-year rotation. A random forest model was used to understand the key drivers of N_2O emissions.

Chapter 4, titled "Effect of poultry litter on soil nitrous oxide production processes and its dynamic controls during progressive cover crop residue decomposition in organic system," includes the study on the rate of cover crop decomposition, C and N release, and subsequent N_2O emissions. This study was conducted in 2022 and 2023 during the corn phase of rotation. Litter bags were used to study the rate of cover crop residue decomposition. To understand microbial processes for controlling N_2O emissions, I performed N_2O isotopomer analysis from the gas samples collected during peak N_2O emissions, and microbial gene abundance (for nitrifying and denitrifying bacteria) was analyzed from the soil samples.

Chapter 5, titled "Soil greenhouse gas budget in a three-year organic grain rotation system," estimates the greenhouse gas balance associated with three years of comprehensive organic management. The change in soil organic carbon (SOC) stock from 0 to 30 cm was calculated based on baseline soil sampling in 2020 and final soil sampling in 2023 and converted to CO_2

equivalent sequestration/emissions. Methane and N_2O emissions from three years of rotation were converted to the CO_2 equivalent emissions and the C sequestration from three years of organic rotation.

Chapter 6 summarizes and concludes my research and provides my perspectives on future research directions needed.

Chapter 2: Soil nitrogen dynamics in response to diverse tillage, cover crop, and manure management in transitioning organic cropping systems

2.1 Abstract

Organic rotation systems integrate ecological principles and system approaches to manage nitrogen (N) cycling. However, the availability of soil inorganic N (SIN) from organic inputs is complex due to microbial activity influenced by management practices and soil conditions (e.g., soil moisture and temperature). A three-year soybean (*Glycine max* L.)-winter wheat (*Trtiticum aestivum* L.)-corn (*Zea mays* L.) rotation system was established to understand the effect of organic management systems on SIN, estimate surplus N, and identify predictors of SIN. Surplus N was estimated by subtracting the N removal by grain yield from total N inputs [(N from legume cover crops and poultry litter (PL)]. We measured SIN and surplus N from four different organic cropping systems – i) Higher tillage system (HTS), ii) Moderate tillage system (MTS), iii) Least tillage system (LTS), and iv) Low input system (LIS). The mean SIN across three years and crop phases was high from HTS (15 mg SIN kg^{-1} dry soil) and LTS (15 mg SIN kg^{-1} dry soil) followed by MTS (12 mg SIN kg^{-1} dry soil) and LIS (4.6 mg SIN kg^{-1} dry soil). The surplus N was highest from LTS (558 kg N ha^{-1}), which is 66% greater than HTS (336 kg N ha^{-1}) and 26% greater than MTS (442 kg N ha^{-1}). Integrating organic inputs, especially legume cover crops and poultry litter (PL), along with tillage practices, elevated soil temperature, and soil moisture levels, collectively enhanced SIN. While surplus N in cropping systems is partially explained by SIN in topsoil and total N (TN) across deeper soil, negative SIN in deeper soil indicates that organic rotation systems might reduce SIN concentration in the deep soil profile.

2.2 Introduction

While conventional farming depends on simplified soil nitrogen (N) cycling mediated by abundant use of synthetic fertilizers readily supplying N, organic farming embraces a more complex route integrating a system approach and ecological principles of managing N cycling (Drinkwater, 2015). Organic agriculture avoids reliance on synthetic N fertilizer by recycling N from organic manure and often incorporating legume cover crops to introduce biologically fixed N. Unlike synthetic N fertilizer, N release from organic nutrient sources depends on coupled carbon (C) and N cycling via microbial mineralization and immobilization processes, which further depends on interactions with environmental (e.g., temperature, precipitation) and management (e.g., tillage, residue and manure management) factors (Blesh & Drinkwater, 2013; Finney et al., 2015). Therefore, managing N cycle in organic systems is complex to ensure timely crop N supply and avoiding N asynchrony that can cause environmental losses of N (Kramer et al., 2002). Given the upward trajectory of US organic acreages to meet the ever-increasing demand for organic products (OTA, 2023; Research Institute of Organic Agriculture, 2020), understanding complex soil N cycling processes is needed for the sustainable adoption of organic practices.

Tillage and cover crops are integral parts of organic cropping systems – often tactically integrated into the crop rotations as weed management and N provision tools. Intensive tillage – a strategy that involves soil health trade-offs – has traditionally been used in organic systems to control weeds and incorporate high biomass residues and organic fertility inputs (Barberi, 2006). Intensive tillage accelerates the decomposition of residue and organic fertilizer inputs and releases N into the soil. However, it also creates windows of high N availability beyond crop N need earlier in the growing season, which could lead to environmental N losses to limit sustained

N supply to crops throughout the growing season (Rice et al., 1987). As synthetic herbicides are prohibited, tillage is inevitable in organic cropping systems; however, innovative cover crop management strategies such as cover crop termination by roller-crimping and cover crop planting by interseeding into existing cash crop can limit the need for intensive tillage (Bavougian et al., 2019). While such modification in cover crop residue management techniques can enhance soil health and agronomic benefits, adds further complexity in predicting and controlling soil N dynamics (Liebman et al., 2018).

Winter cover crops are grown in between two cash crops and offer numerous advantages such as scavenging of surplus nutrients from soil during winter (Coombs et al., 2017), fixation of atmospheric N by the legume species, and soil and water conservation. Cover crops from grass family, generally possessing deep root systems, mitigate N loss via leaching (Kaspar & Singer, 2015). Whereas legume cover crops contribute N to the soil through biological N fixation, benefiting subsequent cash crops by enhanced N supply. The integration of diverse cover crop species and their mixture within a cropping system variably influenced soil N cycling (Kaye et al., 2019). The impact of cover crops on soil N dynamics is influenced by the quantity and quality of biomass produced (residue C:N ratio, lignin content, etc.), method and timing of cover crop termination, and co-application of other organic fertilizer. Narrow C:N ratio legume cover crop residues decompose rapidly, facilitating the rapid release of mineral N (Jani et al., 2015; O'Connell et al., 2015; Poffenbarger et al., 2015). Therefore, introducing legume cover crops into the cropping system increases soil mineral N (Kuo et al., 1997; Sainju et al., 2007). In contrast, grass cover crop residues have a higher C:N ratio, leading to a slower decomposition rate (O'Connell et al., 2015). Thus, a mixture of grass and legume cover crop may optimize the timing of residue N release and crop N uptake resulting in minimum environmental N losses

(Lawson et al., 2013; Sainju et al., 2007; Sainju & Singh, 2008; Shelton et al., 2018). Roller crimping, a high biomass cover crop termination method using no-till keeps season-long thick residue mulch that controls weeds and preserves soil moisture (Wagger & Mengel, 1988). While doing so, roller crimping offers slower rate of cover crop decomposition and N release (Coppens et al., 2007). Contrary to roller crimped cover crops, cover crops incorporation into the soil by tillage accelerates the rate of cover crop decomposition (Coppens et al., 2007). Incorporation of cover crops, particularly legume species with low C:N ratio may lead to asynchronization between N supply and uptake by crops (Poffenbarger et al., 2015). Additionally, application of N from organic manure accelerates the rate of cover crop residue decomposition and influences soil mineral dynamics.

In the context of growing organic poultry industry in the US (Carlson et al., 2023), poultry litter (PL) presents a promising organic manure source for organic cropping systems due to its high N content (Alade et al., 2019). The N release from organic manure like PL is influenced by its composition and the rate, timing, and method of application. Incorporating PL into the soil through tillage can minimize volatilization losses as ammonia – yet may increase N losses via leaching (Bierer et al., 2021). The application of PL also accelerates the rate of cover crop residue decomposition and affects soil mineral availability. Tillage operations that mix organic inputs (cover crop and PL) with soil enhances the rapid release of N which possibly leads to asynchrony between N release and N uptake by cash crops, resulting in excess soil N susceptible to environmental loss. The complexity of N availability from organic inputs (PL manure and cover crops) in organic cropping systems underscores the need for strategic management of both cover crop and PL manure to reduce environmental impacts.

Controlling the timing and magnitude of N availability to match with the crop demand by adjusting tillage, cover crop, and organic fertility inputs is a challenging task in organic systems. The limited knowledge and predictability of N availability from organic inputs necessitates a holistic approach to study the whole system and the emerging trends in response to integrated management components (such as tillage, cover cropping, manure addition) than their effects in isolation (Drinkwater, 2016). We studied four transitioning organic soybean (*Glycine max* L.)-winter wheat (*Triticum aestivum* L.)-corn (*Zea mays* L.) cropping systems employing diverse tillage, cover cropping, and PL management practices at different phases of the rotation to achieve diverse goals such as maximizing yield, enhancing soil health, and reducing external fertility input needs. Our objectives were to: 1) discern the effect of different organic management practices on soil inorganic nitrogen [SIN (NH_4-N + NO_3-N)] concentration during different phases of each rotation and identify key factors influencing soil N provisions, and 2) provide an estimate of surplus N using N input from legume cover crop and N output from grain yield by the end of a three-year rotation. We have hypothesized that SIN will be high in the cropping systems that received intensive tillage and N inputs from poultry litter (PL), and cover crops and surplus N will be high in the cropping systems with less N removal by yield.

2.3 Materials and Methods

2.3.1 Study site and experimental design

The study was conducted from October 2020 to October 2023 on a certified organic land in University of Tennessee - East Tennessee Research and Education Center's Organic Crop Unit (35°52′55.4″, -83°55′32″; 270 m above sea level) in Knoxville, TN. The soil at the research site is predominantly Decatur silt loam (thermic Rhodic Paleudults) (30% sand, 38% silt, and 32% clay). The research site has an mean annual temperature of 15.3°C and receives an annual precipitation of 1319 mm (National Oceanic and Atmospheric Administration, NOAA). Daily precipitation records during the growing season were sourced from the meteorological facility at the study site.

The experiment was conducted in a Randomized Complete Block Design (RCBD) with four replicated blocks over a three-year soybean-winter wheat-corn rotation. Each experimental block included three main plots that were randomly assigned to three cash crops as entry points (i.e., soybean, winter wheat, and corn), and rotated annually within these plots. For instance, a main plot with corn entry in 2021 transitioned to soybean in 2022, and to winter wheat in 2023. Therefore, there were three crop sequences in total: Soybean entry: soybean-winter wheat-corn; winter wheat entry: winter wheat-corn-soybean; and Corn entry: corn-soybean-winter wheat. Within each main plot, four cropping systems were randomly assigned as subplots (12 × 6 m), resulting in 48 experimental units. Unlike traditional approaches, where crops are mutually exclusive in space and time, our experimental design allows the presence of the three main crops for all three years.

2.3.2 Cropping systems descriptions and management goals

This study implemented four distinct cropping systems to evaluate their respective impacts on agroecological benefits. These cropping systems are: (i) Higher tillage system (HTS); (ii) Moderate tillage system (MTS); (iii) Least tillage system (LTS); and (iv) Low input system (LIS). Variations among these systems were attributed by tillage intensity (HTS > MTS > LTS > LIS), the composition and management of cover crops, and PL manure input (**Fig. 1**).

All plots received multiple passes of offset disking (OD) with soil disturbances up to a depth of 25 cm followed by pickup disking (PD) with soil disturbance up to a depth of 10 cm at the beginning of the experiment – in fall 2020 before planting cover crop (preceding soybean and corn entry) and winter wheat for the winter wheat entry plots. A description of system components and management strategies are listed in **Table 2** and briefly described as follows.

Higher tillage system (HTS): The objective for this cropping system was to maximize yield which was accomplished by inclusion of double crop soybean (DC-soybean) after winter wheat harvest and intensifying tillage for successful cover and cash crop establishment and weed control. With six primary tillage operations (three OD and three PD) in three years, HTS received the maximum tillage among the four cropping systems (**Fig. 1**). Hairy vetch (*Vicia villosa* R.) + winter pea (*Pisum sativum* L.) + radish (*Raphanus sativus* L.) + triticale (× *Triticosecale* Wittm.) cover crop mixture was grown before corn and terminated by flail mowing followed by incorporation with OD before corn planting in spring (**Fig. 1**). Triticale + radish cover crop mixture was planted after OD following corn harvest and terminated by flail mowing followed by incorporation with PD before full-season soybean planting in next spring. Winter wheat was planted on offset disked ground after soybean harvest in fall followed by pickup disk tilled DC-soybean planting after winter wheat harvest in next June. Corn and winter wheat phases in their respective growing season received PL applications at the rate of 14.8 Mg ha^{-1} (supplying 518 kg total N) and 4.9 Mg ha^{-1} (supplying 172 kg total N), respectively, applied in two splits (**Table 2**). Prior to planting, corn phase received 4.9 Mg ha^{-1} (supplying 172 kg total N ha^{-1}) followed by 9.8 Mg ha^{-1} (supplying 346 kg total N ha^{-1}) during the V5-V6 growth stage of the corn. Similarly, winter wheat received 2.45 Mg ha^{-1} (supplying 86 kg total N ha^{-1}) in fall before planting and the same amount of PL was applied to winter wheat in the following spring. Two to three inter-row cultivations were performed as needed to control in-season weeds in tilled corn and soybean crop phases.

Moderate tillage system (MTS): The overall cropping system goal was to increase organic grain yield by including DC-soybean as in HTS while reducing tillage relative to HTS (4 vs 6 primary tillage operations in three years). The cover crop mixture planted between cash crops was similar to HTS. Unlike HTS, full-season and DC-soybean were NT planted to reduce tillage. Tillage intensity was further reduced with PD for cover crop planting before soybean and winter wheat planting as compared to OD at these rotational phases in HTS. All other management strategies remained consistent with HTS including the rate and timing of PL application in corn and winter wheat.

Least tillage system (LTS): Driven by the broader goal of creating a sustainable organic system, this system's strategy was to further reduce tillage intensity to improve soil health. Additionally, in this system, yield maximization was not a goal, therefore, unlike HTS and MTS, DC-soybean was excluded in LTS. In this system, NT approach was implemented for planting cover crops before soybean and corn, planting of soybean, and planting of a summer cover crop (cowpea-*Vigna unguiculata* L.) after winter wheat. Unlike HTS and MTS, in LTS, cover crops (triticale and crimson clover (*Trifolium incarnatum* L.)) were planted before corn. Pickup disking was practiced only before winter wheat and corn planting. Before soybean planting, cover crops were NT terminated by roller crimping, whereas, before corn planting, the cover crops were first flail mowed and incorporated by PD. The rate and timing of PL application was consistent with HTS and MTS.

Low input system (LIS): The goal of this system was to reduce tillage as in LTS, while creating a closed-loop nutrient cycling by excluding any external nutrient inputs (PL) and enhancing cover crop N contributions. No tillage techniques were employed for planting cover crops, soybean, and spring cover crops (red clover - *Trifolium pratense* L. + timothy - *Phleum* *pratense* L.). Spring cover crops (red clover + timothy) were NT drilled into the existing winter wheat phase (frost seeding). This is done to allow longer red clover growing season to provide equivalent amount of N to the next corn crop and multiple other benefits over typically fall-planted hairy vetch-based cover crop (Snyder et al., 2016). Pickup disking was done before winter wheat, while OD was done before corn planting. Before soybean planting, cover crops were terminated by roller crimping, whereas in corn, cover crops were incorporated via OD.

In all cropping systems, the planting period for cover crops and winter wheat was generally set between mid-October and the first week of November, whereas corn and soybean planting occurred from mid-May to the first of June depending on the weather conditions. A comprehensive overview of the management practices, inclusive of varied planting and harvesting dates, is provided in **Table 2**.

2.3.3 Soil sampling and assessment of soil inorganic N

We began collecting soil samples in the summer of 2021. Therefore, we could not collect soil samples from the winter wheat phase in the first year of rotation. Soil samples were collected bi-weekly to monthly during the main crop phases over three growing seasons. From each plot, three soil samples were collected from a 0–15 cm depth using a soil probe (1.9 cm diameter) and homogenized. Following homogenization of soil samples from each plot, 10 g fresh soil samples were extracted with 40 ml of 2M KCl solution. After 1 hour of shaking at 200 rpm, the resultant solutions were filtered, and extracts were stored at –20°C until further analysis. Additionally, 10 g of soil samples were oven dried at 105°C for 48 hours and calculated the gravimetric water content. Extracts were colorimetrically analyzed for ammonium (NH_4-N) and nitrate (NO_3-N) concentrations using a Microplate reader (Bio Tek Instruments, Winooski, VT) (Doane & Horwáth, 2003; Verdouw et al., 1978). The sum of NH_4-N and NO_3-N was quantified and reported as soil inorganic N (SIN), mineral form of N readily available for plant uptake and potential environmental losses.

Furthermore, in order to quantify the changes in SIN in soil profile over the three-year rotation, deep soil cores (0–60 cm) were collected from each plot during the fall of 2020, prior to the experimental initiation, and 2023, at the completion of the third year of crop rotation. Two deep soil cores were extracted from each plot using a hydraulic probe (8 cm diameter) and samples were separated into four depths: 0–5, 5–15, 15–30, and 30–60 cm. The samples were air-dried, ground, and sieved through a 2-mm mesh. Five grams of dried soil samples were extracted with 25 ml of 2M KCl solution and measured for SIN as described earlier.

2.3.4 Assessment of N inputs, outputs, and surplus N

Total N input and output over the three-year rotation were quantified for each cropping system to estimate N surplus, an indicator of N use efficiency (Norton et al., 2015). Nitrogen input was determined by aggregating the N contributions from legume cover crops biomass (assuming biologically fixed N) and PL. To know the N input from legume cover crops, above ground cover crop biomass was collected before termination using 0.5 × 0.5 m quadrats (two from each plot). The quantity of different cover crops within each quadrat were separated and allowed to oven dry at 65°C for 48 hours. Oven dry mass of legume cover crop from each plot was used for the calculation of surplus N. Total N content in the legume cover crop residues and PL was quantified by grinding cover crop biomass and PL sample and analyzing it by dry combustion method (Elementar vario TOC cube, Hanau, Germany). Nitrogen input from the legumes was then calculated by multiplying the percentage of total N in the legume biomass by the dry mass. Similarly, the N contribution from PL was estimated by multiplying its N percentage by the applied quantity of PL. Nitrogen output via grain harvest was calculated by multiplying the N percentage in the grain by the total yield. The surplus N was subsequently determined by subtracting the total N output from the total N input, with positive values indicating N input in excess of grain N uptake. Of note, this N surplus estimate only accounts for inputs and outputs of the system and, hence, may not represent N transformations or retention within organic and inorganic pools in the system.

2.3.5 Statistical analysis

Statistical analysis was performed using the R software (version 4.3.1). The impact of cropping systems on surplus N was assessed using a linear mixed effect model, where the cropping system was treated as a fixed effect, and both the block and the crop entry (corn, soybean, or winter wheat) were treated as random effects. Due to the lack of homogeneity of variance, a rank transformation was applied before executing the analysis of variance (ANOVA) (Conover & Iman, 1982). The influence of the cropping system on the mean growing season SIN during each crop phase was analyzed using a linear mixed effect model, where the cropping system was a fixed effect, and the block was a random effect. Furthermore, the impact of cropping systems on SIN and total soil N (TN) within the soil profile was examined separately at each soil depth. To comply with ANOVA assumptions, data were transformed to obtain normal distribution, however non-transformed data is reported in the manuscript. Statistical significance was evaluated at $p \leq 0.05$.

A random forest regression model (Breiman, 2001) was used to determine the variables that predict the SIN in organic cropping systems. Random forest model operates by generating multiple decision trees using the training dataset through a method called bootstrapping, which involves sampling with replacement. In this process, two-thirds of the data is employed for training purposes, while the remaining one-third, known as out-of-bag (OOB) data, is utilized for prediction. The final predictions were obtained by averaging the outputs from the individual decision trees. Additionally, we used a built-in function in the "randomForest" package to measure the increase in mean square error of predictor variables. Two-dimensional partial dependence plots with color gradients were developed to see the correlation between two predictor variables and their influence on SIN. With an objective of identifying the drivers of SIN, total N input, soil temperature, soil moisture (VWC%), and two days precipitation were included as predictor variables and SIN was used as response variable.

2.4 Results

2.4.1 Weather conditions during the studied years

The study period experienced interannual variability in weather conditions (**Fig. 2**). Recorded cumulative annual precipitation was 2077 mm in 2021, 2019 mm in 2022, and 1112 mm in 2023. While 2021 had the highest annual cumulative precipitation among the three years, 2022 experienced relatively less but more evenly distributed precipitation throughout the year. Temperature patterns remained consistent across three years, although the summer months (May, June, and July) of 2022 experienced relatively higher temperatures compared to the same period in 2021 and 2023.

2.4.2 Soil inorganic N dynamics during corn phase

The magnitude and temporal pattern of SIN concentration during the corn phase varied across the growing seasons, with the highest seasonal mean value observed in 2022 (39.7 mg SIN kg^{-1} dry soil), approximately 32% and 262% higher than that in 2021 (30.2 mg SIN kg^{-1} dry soil) and 2023 (11 mg SIN kg^{-1} dry soil), respectively (**Table 1**). Mean SIN concentration across the three growing seasons followed the order HTS (35.3 mg SIN kg^{-1} dry soil) = LTS (33.8 mg SIN kg^{-1} dry soil) = MTS (31.8 mg SIN kg^{-1} dry soil) > LIS (6.7 mg SIN kg^{-1} dry soil). Soil inorganic N was dominated by NO_3–N across growing seasons and cropping systems. Notably, both NH_4–N and NO_3–N forms of soil mineral N, as well as SIN in LIS, receiving zero external N inputs, were significantly lower throughout the growing seasons than all other cropping systems receiving PL (HTS, MTS, and LTS) (**Table 1**). Across all cropping systems, peak SIN levels were noted following PL application, planting, and inter-row cultivation in three corn growing seasons (2021–2023) (**Fig. 3a, c, and e**). In 2021, HTS, MTS, and LTS showed peak SIN following the second PL application (range 68–76 mg SIN kg^{-1} dry soil), while LIS had the lowest (4.4 mg SIN kg^{-1} dry soil) SIN (**Fig. 3a**). By the end of the growing season, SIN concentrations dropped significantly across all treatments (range 2–9 mg SIN kg^{-1} dry soil). This pattern was consistent in the 2022 phase, with peak SIN occurring after three weeks of split PL application (**Fig. 3c**). In 2023, while the SIN peaks persisted, concentrations substantially decreased compared to 2021 and 2022.

2.4.3 Soil inorganic N dynamics during soybean phase

The soybean phase had much lower SIN concentrations than the corn phase and exhibited contrasting temporal trends across growing seasons and cropping systems (**Table 1; Fig. 3b, d, and f**). Across three growing seasons, tilled soybean in HTS showed significantly higher mean SIN (7.9 mg SIN kg^{-1} dry soil) than NT planted soybean, MTS (4.8 mg SIN kg^{-1} dry soil), and LIS (4 mg SIN kg^{-1} dry soil) (**Table 1**) ($p < 0.05$). Cover crop residue incorporation by PD under HTS resulted in early season peak SIN concentration in two of the three growing seasons, whereas the NT soybean system, particularly LTS, showed a slight increase in SIN later in the summer (August) (**Fig. 3b, d, and f**).

Cropping systems had no significant impact on SIN concentrations during DC-soybean phases after winter wheat in HTS and MTS (**Table 1**). However, tilled DC-soybean in HTS showed slightly higher SIN than NT planted DC-soybean in MTS (8.5 vs 6.5 mg SIN kg^{-1} dry soil, **Table 1**), more conspicuous during 2021 and 2023 growing seasons (**Fig. 4a, b, and c**). Mean SIN concentrations were comparable between full-season and DC-soybean, particularly in HTS (7.9 vs 8.6 mg SIN kg^{-1} dry soil) (**Table 1**).

2.4.4 Soil inorganic N dynamics during winter wheat phase

Winter wheat phase SIN levels remained low (<10 mg SIN kg^{-1} dry soil) in all cropping systems during early spring in both 2022 and 2023, despite PL application in all cropping systems except LIS at fall planting and top dressing in March (**Table 1, Fig. 5a and b**). However, an increase in SIN in all cropping systems was observed in June, correlating with the rising temperature. Mean SIN concentration across the 2022 and 2023 growing seasons was significantly higher under HTS ($p < 0.05$) than all other cropping systems (MTS, LTS, and LIS) (**Table 1**).

2.4.5 Surplus N across cropping systems

In the fertilized cropping systems (HTS, MTS, and LTS), N inputs from PL and legume cover crops exceeded the N outputs over the three-year rotation to result in a positive surplus N, whereas only LIS showed a negative surplus N (**Fig. 6**). Total N inputs were similar in the fertilized systems and dominated by N addition from PL application; however, total N output via crop harvest in LTS was 57% and 40% lower than HTS ($p < 0.05$) and MTS ($p < 0.05$) respectively, and similar to unfertilized LIS. As a result, LTS exhibited the highest surplus N over three years among the cropping systems (558 kg N ha^{-1}, $p < 0.05$). Among the fertilized systems, higher N output (428 kg N ha^{-1}) in HTS resulted in lower system surplus N (336 kg N ha^{-1}), which was also reflected in a negative relationship between total yield over three years and surplus N (**Fig. 10**).

2.4.6 Changes in profile soil N after three years of rotation

After three years of rotation, the surface soil layer (0–5 cm) showed an increase in SIN concentrations by 56% (calculating mean across cropping systems and crop entry points), with unfertilized system (LIS) had lowest increase in SIN than all other cropping systems (HTS, MTS, and LTS) (**Fig. 7a**). Similarly, TN concentration was also increased in the 0–5 cm layer but was not significantly different among the cropping systems (**Fig. 7b**). The SIN and TN concentration trends were contrasting across the deeper layers in the profile. While all cropping systems consistently showed depletion of SIN concentration in the deeper soil layers at the end of three-year rotation, TN generally increased across soil depths. Although the cropping systems effects on the changes in SIN and TN concentration in the deeper soil layers were not consistent and significant, HTS receiving the highest tillage operations showed the lowest increase in TN concentration in 5–15 and 15–30 cm soil layers (**Fig. 7b**).

2.4.7 Factors controlling soil inorganic N during the growing season

Variable importance plot by random forest model indicated that total N input was the most important variable determining mean growing season SIN level followed by environmental variables such as mean growing season soil temperature, soil moisture, and cumulative growing season precipitation (**Fig. 8**). Two-dimensional partial dependence plots (PDPs) revealed interactive management and environmental controls on growing season SIN availability (**Fig. 9**). For example, levels of SIN (~40 mg SIN kg^{-1} dry soil) was predicted during the growing season, when total N input (analysis i.e., N inputs from legume cover crop and PL) was >350 kg N ha^{-1} (typically observed during the corn phase) under wetter (mean growing season VWC >25%; cumulative growing season precipitation >600 mm; and warmer (soil temperature was >23°C) soils (**Fig. 9a, b, and c**).

2.5 Discussion

Nitrogen availability from organic fertility inputs, in synchrony with crop N demand, is a complex interplay between management practices and environmental conditions influencing microbial mineralization and immobilization processes to determine productivity and N surplus. We measured SIN availability during the growing seasons and estimated the N input, output, and surplus N in four soybean-winter wheat-corn transitioning organic cropping systems. Our findings indicate a substantial surplus N over three years in all PL fertilized cropping systems, i.e., N inputs through PL and legume cover crops exceeded N removal by grain harvest (**Fig. 6**). However, increased N output by higher crop yield in cropping systems that include DC-soybean (as in HTS and MTS) and frequent tillage (HTS > MTS) significantly lowered surplus N as compared to the reduced till system (LTS). Our findings also indicate that total N input followed by soil environmental variables, soil temperature, and moisture are the key drivers of SIN in organic cropping systems.

2.5.1 Soil inorganic N under different crop phases across cropping systems

Substantially higher SIN levels in corn than soybean and winter wheat phases of the rotations were attributed to the high rate of PL application and incorporation of legume containing cover crop residue by tillage (**Fig. 3a, c, and e**). Increase in SIN concentration in response to tillage was previously reported in cover crop and manure based organic feed and forage production systems (Finney et al., 2015). Relatively low SIN availability at the first soil sampling before cover crop termination preceding corn planting indicates cover crop effects in scavenging N from the preceding season (Wagger & Mengel, 1988; Tonitto et al., 2006; Blanco-Canqui et al., 2015). Subsequent sampling showed increased SIN concentrations, more conspicuously in the fertilized systems (HTS, MTS, and LTS), peaked after tillage and PL incorporation before corn planting and inter-row cultivation for split applied PL integration and in-season weed control. Tillage practices increase microbial access to organic inputs (cover crop residues and PL) as well as soil organic matter which accelerates the N mineralization (Finney et al., 2015, Franzluebbers et al., 1995; Mikha & Rice, 2004). A similar trend was observed after the second dose of PL application followed by inter row cultivation. This pattern was supported by Finney et al. (2015), who reported increased SIN with manure application and moldboard plowing, and Diaz et al., (2011), who observed elevated mineral N in June following corn planting. In contrast, the lowest SIN in corn under LIS was attributed to the exclusion of PL and limited cover crop (red clover) biomass N input due to poor cover crop establishment (**Table 1; Fig. 3 and Fig. 6**). Greater legume cover crop N inputs in other systems (HTS, MTS, LTS) although constitute a small fraction of the total N input (**Fig. 6**), may have also contributed to higher SIN availability than LIS shortly after their incorporation due to rapid decomposition of low C:N legume biomass (Poffenbarger et al., 2015). However, a relative increase in SIN concentration from the baseline was observed in LIS after tillage and in-row cultivations, much lower in magnitude than fertilized corn in other systems, which was perhaps attributed to the mineralization of soil organic matter. Therefore, the increase in SIN availability during the corn phase was due to the combined effect of a large amount of PL application, cover crop incorporation, and soil disturbances by tillage. Reduced SIN in all cropping systems at the end of corn growing seasons (except in 2022) was attributed to the crop N uptake (Franzluebbers et al., 1995). A key assumption of microbial and plant mediated N cycling in organic input based ecological systems is the continuous regeneration of a small SIN pool from organic N sources, leading to a tighter N cycling than synthetic N fertilized conventional systems (Drinkwater et al., 2008). However, our study highlights that the window of high SIN pulses (35–156 mg SIN kg^{-1}

soil) can occur quickly after organic input additions, which is contingent on management (tillage, organic input quality) and environmental conditions. The availability of SIN indicates that organic N inputs meet the critical mineral N requirement by corn (25 mg SIN kg^{-1} soil) (Sawyer, 2011), especially when the timing of N demand and supply are well-aligned. However, high mineral N can also lead to increased weed proliferation and heightened risk of environmental N losses, particularly under favorable conditions such as reduced plant uptake during drought or excessive moisture.

A consistent trend was observed across the soybean and winter wheat phase of rotation, however, with much lower SIN levels compared to the corn phase (**Table 1; Fig. 3b, d, and f**). The soybean phase did not receive PL and included triticale (dominant cover crop species) and hairy vetch cover crops in 2021, while only triticale in the subsequent years. The soil disturbance from PD in HTS likely led to higher SIN peaks early in the soybean phase in two (2021 and 2023) of the three growing seasons, whereas lack of sampling resolution during this period in 2022 limits data interpretation. The observed lower SIN levels earlier in the growing season and delay in SIN peaks in the NT soybean systems (MTS, LTS, LIS) could be attributed to N immobilization by the high C:N ratio triticale cover crop left unincorporated in soybean (Kuo & Sainju, 1998). However, the increase in SIN levels observed in the NT soybeans during the vegetative growth stage and towards the end of the growing season (2022) was attributed to slow N release from mulched cover crop residues, especially under well-distributed rainfall during the growing season in 2022.

Unlike other main crops, DC-soybean wheat was practiced only in HTS and MTS cropping systems after winter wheat to maximize grain production. Tilled DC-soybean (HTS) had slightly higher SIN across all growing seasons (**Fig. 4**), which could be attributed to the accelerated decomposition of incorporated winter wheat residue and N release to benefit DC-soybean establishment (Cordell et al., 2007).

The winter wheat phase of the rotation reflected a similar SIN trend, with cropping systems HTS, MTS, and LTS having higher SIN than LIS, likely due to the effect of PL applications. For winter wheat, the sole N source was PL, providing 172 kg N ha^{-1} per growing season. Among the fertilized systems, the higher SIN observed in HTS compared to other cropping systems is likely attributed to the increased soil disturbance from OD, as opposed to PD used in other cropping systems. Lower SIN availability during the spring months indicates limited N mineralization for PL under cooler temperature, and the availability increased with increasing temperature in the summer (Contosta et al., 2011; Thapa et al., 2021); this was also supported by random forest analysis (**Fig. 8 and 9**).

2.5.2 Inter-annual variability in soil inorganic N

Inter-annual variability in SIN availability was more pronounced in the corn than other crops in the rotations, which was largely driven by environmental factors, particularly soil moisture, and temperature, significantly influencing SIN due to their impact on microbial mineralization processes (Agehara & Warncke, 2005; Guntiñas et al., 2012; Sims, 1986; Zhang & Wienhold, 2002,). Relatively drier period earlier in the corn growing season in 2021 due to minimal precipitation between the end of May and mid-August (only 74 mm of cumulative precipitation during this period) lowered SIN availability from incorporated PL and cover crop residues (**Fig. 2b**). Sainju et al. (2007) found low level of NH_4-N in the soil when precipitation was very low, which was attributed to reduced mineralization in dry soils. The highest SIN peaks in the 2022 corn growing season were linked to the high and well-distributed precipitation throughout the growing season (**Fig. 2b**), particularly following PL application and inter-row cultivation. Consistent with our result, Mitchell & Tu (2006) found high NO_3-N in the year with higher precipitation while low concentrations in drier months and years. The highest SIN peak in August 2022 was associated with large precipitation events received around second PL application (237 mm precipitation in three weeks period following PL application) which possibly contributed to highest mean growing season SIN (**Table 1; Fig. 2b and Fig. 3**). Conversely, low SIN in 2023, likely due to lower precipitation following both first and second PL applications (**Fig. 2b**). This was further supported by random forest analysis which underscores the significant role of soil environment in regulating SIN, predicting sharp increase in SIN levels (>40 mg SIN kg^{-1}) with increasing temperature (>23 °C) and soil moisture (>25% VWC), especially when total N input exceeds 350 kg ha^{-1} (**Fig. 9a and c**). High soil temperature and moisture, along with the availability of the substrates, increase soil mineralization (Agehara & Warncke, 2005; Guntiñas et al., 2012). Soybean and winter wheat phases of the rotation displayed less annual SIN due to reduced N input to these crops that perhaps limited environmental effects on SIN availability (**Fig. 9a, b, and c**).

2.5.3 Cropping system surplus N partially accounted for the increase in soil N

Except for the unfertilized system (LIS), N input surpassed the N output in other cropping systems, leading to a surplus N after three years of rotation (**Fig. 6**). Poultry litter and cover crops were primary N sources in our cropping systems. Poultry litter and legume cover crops contributed 689 kg N ha^{-1} and 71 kg N ha^{-1} (calculating means across cropping systems) of total N across three crop entries (soybean, winter wheat, and corn) and three cropping systems (HTS, MTS, and LTS). The lowest N application from legume cover crops, 21 kg N ha^{-1} (5–52 kg N ha^{-1}), occurred in LIS, which did not receive N input from PL. The PL derived N application was consistent across cropping systems (except LIS which did not receive PL) and crop entries, suggesting that N input variability was due to legume cover crop incorporation. However, legume derived N input was relatively a small fraction of total N inputs across the cropping systems. Therefore, surplus N was determined by N output from the grain harvest, which was positively influenced by tillage intensity as indicated by higher N output in HTS receiving the maximum tillage intensity (**Fig. 6**). Generally, higher SIN availability in the tilled crop phases was observed and discussed above (**Table 1**), which perhaps contributed to better crop growth in the tilled organic crops (Isbell et al., 2021). Whereas NT crop phases such as soybean in MTS, LTS, and LIS suffered poor crop establishment under thick cover crop residue mulch in two of the three years. The negative correlation between crop yield and surplus N further indicates that surplus N disparities were linked to crop yield (**Fig. 10**), which was largely influenced by tillage. Reduced corn silage yield under NT was reported by Champagne et al. (2021) in the Northeastern US and attributed to poor crop establishment under thick hairy vetch cover crop residue, greater weed population, and reduced N availability. This further highlights the dilemma of reducing tillage in organic systems for soil health, while sustaining crop productivity and profitability (Champagne et al., 2021; Isbell et al., 2021). A negative surplus N in LIS was due to limited N input from PL, highlighting the role of PL in crop nourishment as well as surplus N in organic cropping systems. This aligns with findings by Zotarelli et al. (2012), who reported negative surplus N when excluding N fertilizer input. Consistent with our result, Isbell et al. (2021) and Musyoka et al. (2019) reported high N balance (66–1263 kg N ha^{-1}) in organic system that incorporates high organic inputs including animal manure as N source.

The observed changes in profile SIN and TN from baseline to the end of three-year rotation could partially account for the surplus N. Net changes in SIN and TN concentration over the three-year rotation showed contrasting trends across the soil profile (**Fig. 7, 11, and 12**). The topsoil layer (0–5 cm) consistently increased SIN and TN pools across the cropping systems, more so under fertilized systems (HTS, MTS, and LTS), indicating N accumulation from PL application and residue incorporation. This observation aligns with Adeli et al. (2008), who noted elevated nitrate concentrations in the top 15 cm soil after three years of broiler litter incorporation, while diminishing with depth. Tillage practices during crop rotation systems most likely induced N mineralization, thereby increasing SIN in shallow depths (Drinkwater et al., 2000). Soil inorganic N was depleted to <10 mg SIN kg^{-1} soil in the deeper soil layers (calculating SIN across 15–60 cm profile) after three years of rotation – a mean reduction of 60% from 2020, whereas TN showed a net mean increase of 12% in this layer. Reduction in SIN at depths can be linked to a three-year crop rotation, including organic inputs and diverse cover crops of contrasting rooting characteristics to scavenge residual SIN, especially nitrate, and subsequent recycling of N to the next crops (Hansen et al., 2000; Hirsh et al., 2021; Komatsuzaki, 2017; Thomsen, 2005; Tonitto et al., 2006; Wei et al., 2021). These findings suggest that the large amount of surplus N in fertilized systems may not increase SIN leaching losses, rather explains the possibility of decreasing SIN concentration across soil depths. Reduced nitrate leaching from organic vegetable was reported by Wang et al. (2024). In contrast, Isbell et al. (2021) observed higher SIN concentrations beyond 30 cm soil depth than the topsoil after three years of organic corn-soybean-spelt rotations, with more pronounced effect under intensive tillage. However, lack of baseline data on profile SIN in their study limits quantifying net change in SIN distribution over the rotational period. While our data suggests limited risks of SIN leaching in response to the cropping system management practices, the data should be interpreted with caution. For example, nitrate leaching substantially differed between different crop phases in a rotation (Musyoka et al., 2019). Large peaks in SIN availability after PL application in corn phase, especially in wetter years like 2022, could lead to temporal window of N leaching losses which was not captured due to limited sampling resolution in our study. Additionally, excess SIN in the topsoil (38 mg SIN kg^{-1} soil, **Fig. 11**) at the end of the rotation indicates a potential risk of N losses in subsequent seasons.

In contrast, increase in TN after three years, particularly at deeper layers (**Fig. 7b**) indicates enhanced N retention in the organic pool and is consistent with other studies. Schmer et al. (2020) reported increased TN at 0–15 cm soil after 4 years of crop rotation. Similarly, Van Eerd et al. (2014) suggested that soybean-wheat-corn rotation with NT system increased TN in 0–5 and 0–20 cm soil depths. Supporting our result, Sainju et al. (2017) reported an increase in TN at 0–5, 20–40, and 60–90 cm in wheat-pea rotation, emphasizing that crop rotation that includes cover crops and PL enhances soil N across soil depths. In our study, despite negative surplus N in LIS, observed positive TN across soil profile might be attributed to the decomposition of crop root residue. In three years of soybean-winter wheat-corn rotation, our research experienced different management practices including different tillage practices (NT, PD, OD), cover crop composition, and PL management which could have contributed to the TN in the soil profile.

2.6 Conclusion

Regardless of cropping systems, PL application in the summer enhances the SIN availability. The combination of PL, legume cover crops, and tillage increased SIN while lowering the surplus N, indicating better synchronization between N mineralization and crop N uptake. However, high SIN pulses quickly following organic input might result in asynchronization in mineralization and N uptake by crop, underscoring the risk of N loss to the environment. Soil inorganic N availability in organic cropping systems is further impacted by the changing environmental conditions, i.e., in the presence of organic N input, the increase in temperature and precipitation increases SIN availability. In our study, the presence of SIN in topsoil (0–5 cm) and TN concentration across soil profile after three years of crop rotation could partially explain the surplus N. After three years, the negative SIN in deep soil profile suggests reduced leaching, while positive SIN levels in topsoil highlight the potential for N loss under favorable conditions, such as nitrate leaching loss during high precipitation events. Despite the limited risk of SIN loss to deep soil profiles observed in our study, further high-resolution monitoring of SIN in deeper soil profiles is recommended to better understand the impact of crop rotation phases on SIN distribution and potential N losses from organic rotation systems.

Appendix

Table 1. Mean growing season soil mineral N (NH_4-N and NO_3-N) and soil inorganic N (SIN) concentration (mg kg^{-1} dry soil) in corn, soybean, and winter wheat phases from 2021 to 2023.

		2021			2022			2023			Mean		
		NH_4–N	NO_3–N	SIN	NH_4–N	NO_3–N	SIN	NH_4–N	NO_3–N	SIN	NH_4–N	NO_3–N	SIN
		----------	----------	----------	----------	----------	-mg N kg^{-1} dry soil-	----------	----------	----------	----------	----------	----------
Corn	HTS	14.15^{ab}	23.75^{a}	37.90^{a}	10.36^{a}	42.67^{a}	53.02^{a}	3.01^{a}	11.97^{a}	14.98^{a}	9.17^{a}	26.13^{a}	35.30^{a}
	MTS	14.37^{a}	23.31^{a}	37.69^{a}	8.82^{a}	35.18^{a}	44.00^{a}	2.44^{a}	11.29^{ab}	13.73^{ab}	8.55^{a}	23.26^{a}	31.81^{a}
	LTS	13.86^{ab}	25.99^{a}	39.85^{a}	13.53^{a}	39.13^{a}	52.66^{a}	1.79^{a}	7.29^{c}	9.08^{c}	9.73^{a}	24.14^{a}	33.86^{a}
	LIS	0.74^{b}	4.51^{b}	5.25^{b}	0.79^{b}	8.30^{b}	9.08^{b}	1.36^{a}	4.63^{c}	5.99^{c}	0.96^{b}	5.81^{b}	6.78^{b}
Soybean	HTS	1.32^{a}	8.39^{a}	9.71^{a}	0.67^{a}	5.17^{a}	5.84^{a}	2.08^{a}	6.05^{a}	8.14^{a}	1.36^{a}	6.53^{a}	7.90^{a}
	MTS	1.64^{a}	3.58^{b}	5.22^{ab}	0.77^{a}	4.18^{ab}	4.87^{a}	1.04^{a}	3.23^{b}	4.27^{b}	1.15^{a}	3.66^{bc}	4.79^{b}
	LTS	1.21^{a}	3.66^{b}	4.87^{b}	0.86^{a}	5.06^{ab}	5.92^{a}	2.06^{a}	4.66^{ab}	6.73^{ab}	1.38^{a}	4.46^{b}	5.84^{ab}
	LIS	1.31^{a}	4.87^{ab}	6.18^{ab}	0.74^{a}	2.42^{b}	3.22^{a}	1.95^{a}	0.90^{c}	2.85^{c}	1.34^{a}	2.73^{c}	4.08^{b}
DC-soybean	HTS	1.31^{a}	11.59^{a}	12.89^{a}	0.40^{a}	3.78^{a}	4.18^{a}	1.95^{a}	6.67^{a}	8.62^{a}	1.22^{a}	7.34^{a}	8.56^{a}
	MTS	0.59^{a}	8.98^{a}	9.57^{a}	0.41^{a}	3.66^{a}	4.06^{a}	1.78^{a}	4.34^{a}	6.12^{a}	0.93^{a}	5.66^{b}	6.59^{a}
Winter wheat	HTS				2.87^{ab}	4.22^{a}	7.09^{a}	2.19^{a}	5.93^{a}	8.11^{a}	2.53^{a}	5.07^{a}	7.60^{a}
	MTS				2.03^{ab}	1.45^{b}	3.48^{b}	1.88^{a}	4.40^{a}	6.28^{a}	1.96^{ab}	2.92^{b}	4.88^{b}
	LTS				2.95^{a}	2.42^{ab}	5.37^{ab}	1.41^{a}	3.56^{ab}	4.96^{ab}	2.18^{ab}	2.99^{b}	5.17^{b}
	LIS				1.42^{b}	0.89^{b}	2.31^{c}	1.43^{a}	2.30^{b}	3.73^{b}	1.42^{b}	1.60^{b}	3.02^{b}

Note: Different letters across cropping systems in each year and each N species indicate Tukey's honestly significant difference at $p < 0.05$. HTS: Higher tillage system, MTS: Moderate tillage system, LTS: Low tillage system, LIS: Low input system, DC-soybean: Double crop soybean.

Table 2. Field management practices for four cropping systems (HTS, MTS, LTS, and LIS).

Year	Date	Crop	Management practice			
			HTS	MTS	LTS	LIS
2020	Oct 20	Corn	CC planting	CC planting	CC planting	Inter seeded
	Oct 20	Soybean	CC planting	CC planting	CC planting	CC planting
	Oct 19	Wheat	PL application	PL application	PL application	
	Oct 20		Wheat planting	Wheat planting	Wheat planting	Wheat planting
2021	May 13	Corn	CC termination	CC termination	CC termination	CC termination
	May 25		PL application	PL application	PL application	
	May 26		Planting	Planting	Planting	Planting
	Jun 18		PL application	PL application	PL application	
	Sep 28		Harvest	Harvest	Harvest	Harvest
	May 13	Soybean	CC termination	CC termination	CC termination	CC termination
	May 26		Planting	Planting	Planting	Planting
	Nov 11		Harvest	Harvest	Harvest	Harvest
	Mar 9	Wheat	PL application	PL application	PL application	
	Jun 19		Harvest	Harvest	Harvest	Harvest
	Jul 6	DC Soy	Planting	Planting	Planting	Planting
			Harvest	Harvest	Harvest	Harvest
2022	Nov 11, 2021	Corn	CC planting	CC planting	CC planting	Inter seeded
	Nov 11, 2021	Soybean	CC planting	CC planting	CC planting	CC planting
	Nov 11, 2021	Wheat	PL application	PL application	PL application	
	Mar 21		Wheat planting	Wheat planting	Wheat planting	Wheat planting
	May 19	Corn	CC termination	CC termination	CC termination	CC termination
	Jun 5		PL application	PL application	PL application	
	Jun 6		Planting	Planting	Planting	Planting
	July 12		PL application	PL application	PL application	
	Sep 29		Harvest	Harvest	Harvest	Harvest
	May 19	Soybean	CC termination	CC termination	CC termination	CC termination
	Jun 6		Planting	Planting	Planting	Planting
	Oct 20		Harvest	Harvest	Harvest	Harvest
	Mar 21	Wheat	PL application	PL application	PL application	
	Jun 5		Harvest	Harvest	Harvest	Harvest

Table 2: Continued

	Jun 30	DC Soy	Planting	Planting	Planting	Planting
	Oct 20		Harvest	Harvest	Harvest	Harvest
2023	Oct 25, 2022	Corn	CC planting	CC planting	CC planting	Inter seeded
	Oct 25, 2022	Soybean	CC planting	CC planting	CC planting	CC planting
	Oct 25, 2022	Wheat	PL application	PL application	PL application	
	Oct 25, 2022		Wheat planting	Wheat planting	Wheat planting	Wheat planting
	May 12	Corn	CC termination	CC termination	CC termination	CC termination
	May 19		PL application	PL application	PL application	
	May 22		Planting	Planting	Planting	Planting
	July 22		PL application	PL application	PL application	
	Oct 2		Harvest	Harvest	Harvest	Harvest
	May 12	Soybean	CC termination	CC termination	CC termination	CC termination
	May 22		Planting	Planting	Planting	Planting
	Oct 3		Harvest	Harvest	Harvest	Harvest
	Jun 29	DC Soy	Planting	Planting	Planting	Planting
	Oct 3		Harvest	Harvest	Harvest	Harvest

HTS: Higher tillage system, MTS: Moderate tillage system, LTS: Low tillage system, LIS: Low input system, DC Soy: Double crop soybean

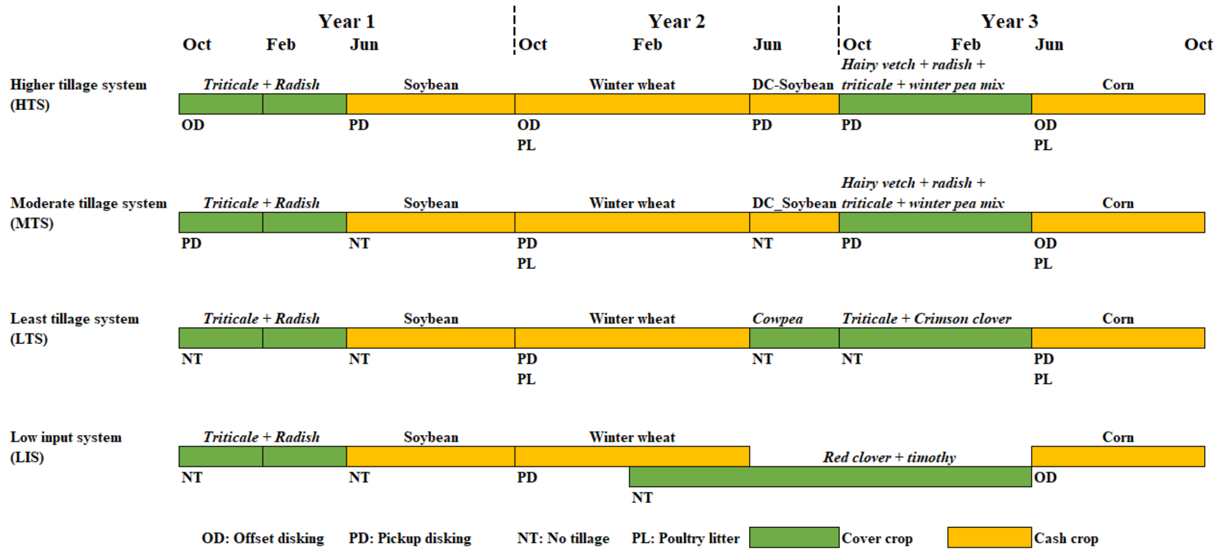

Figure 1. Three-year crop rotation with four different cropping systems (HTS, MTS, LTS, and LIS). Green color indicates the cover crop phase of rotation and yellow color indicates main crop phase of rotation. HTS: Higher tillage system, MTS: Moderate tillage system, LTS: Least tillage system, LIS: Low input system, OD: Offset disking, PD: Pickup disking, NT: No tillage, and PL: Poultry litter

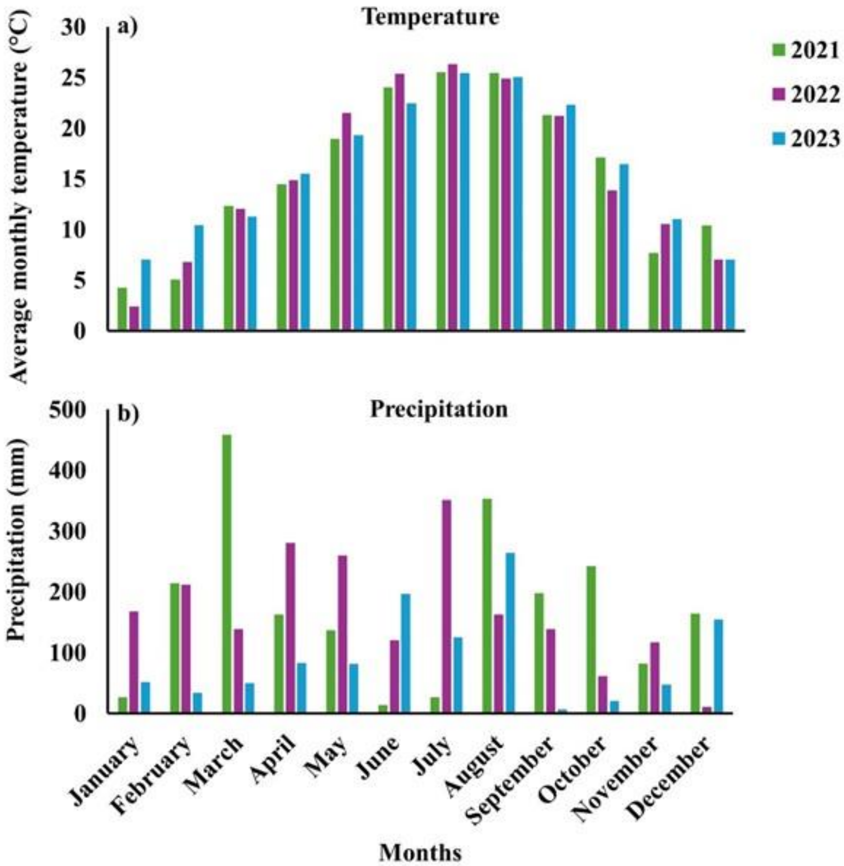

Figure 2. Temperature and precipitation during the study period. a) monthly mean temperature, and b) cumulative monthly precipitation.

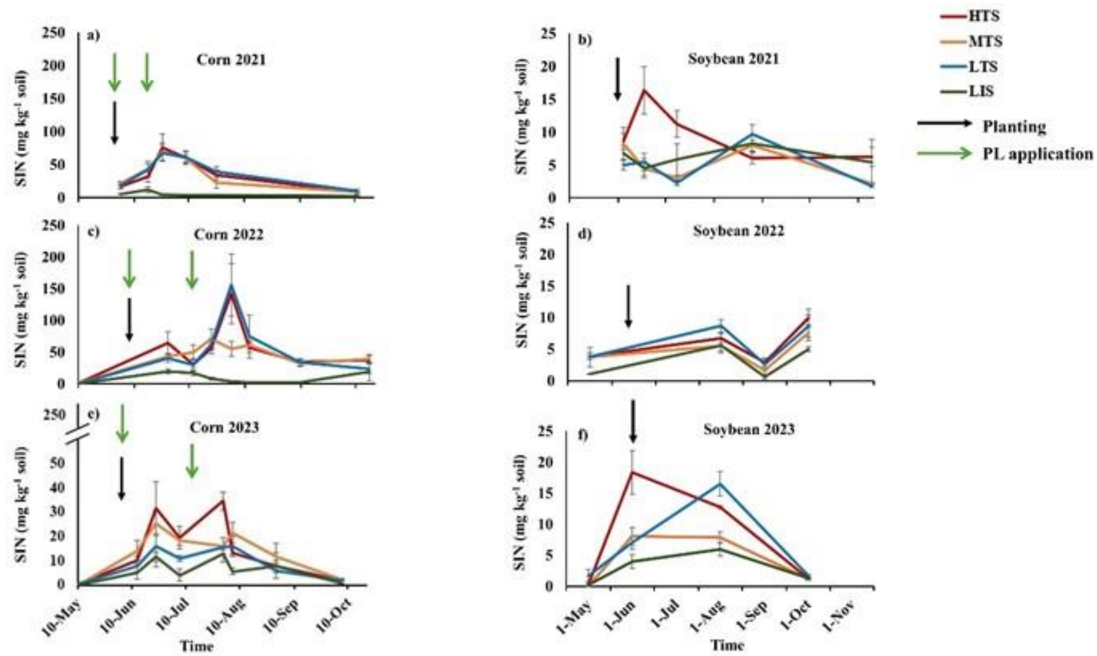

Figure 3. Soil inorganic nitrogen (SIN) availability during corn and full season soybean phase of organic grain rotation systems. a) Corn 2021, b) soybean 2021, c) corn 2022, d) soybean 2022, e) corn 2023, and f) soybean 2023. Systems vary in terms of cover crop and poultry litter (PL) management, and tillage intensities. Red, orange, blue, and dark green lines represent cropping systems; HTS, MTS, LTS, and LIS, respectively. The vertical black color arrow represents time of planting and vertical light green arrow represents time of PL application. HTS: Higher tillage system, MTS: Moderate tillage system, LTS: Least tillage system, LIS: Low input system. Note: The Y-axis upper limit is different for corn and soybean figures.

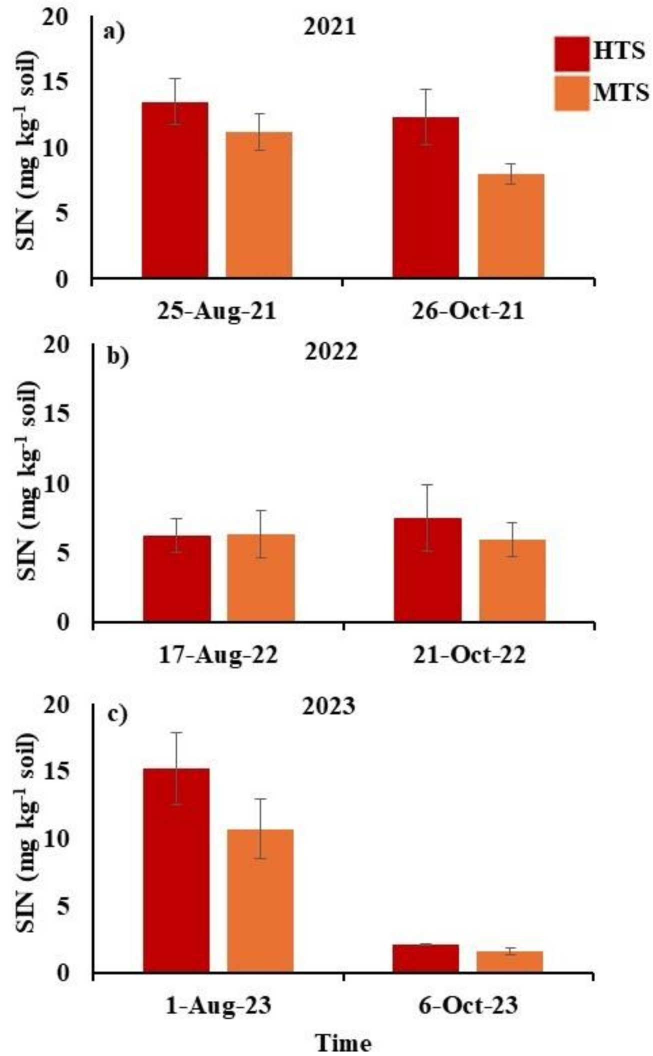

Figure 4. Soil inorganic nitrogen (SIN) in 0–15 cm profile depth in double crop soybean (DC-soybean) phase of organic grain rotation systems. a) 2021, b) 2022, and c) 2023. Systems vary in terms of tillage intensities, offset disking in higher tillage system (HTS) and no tillage in moderate tillage system (MTS).

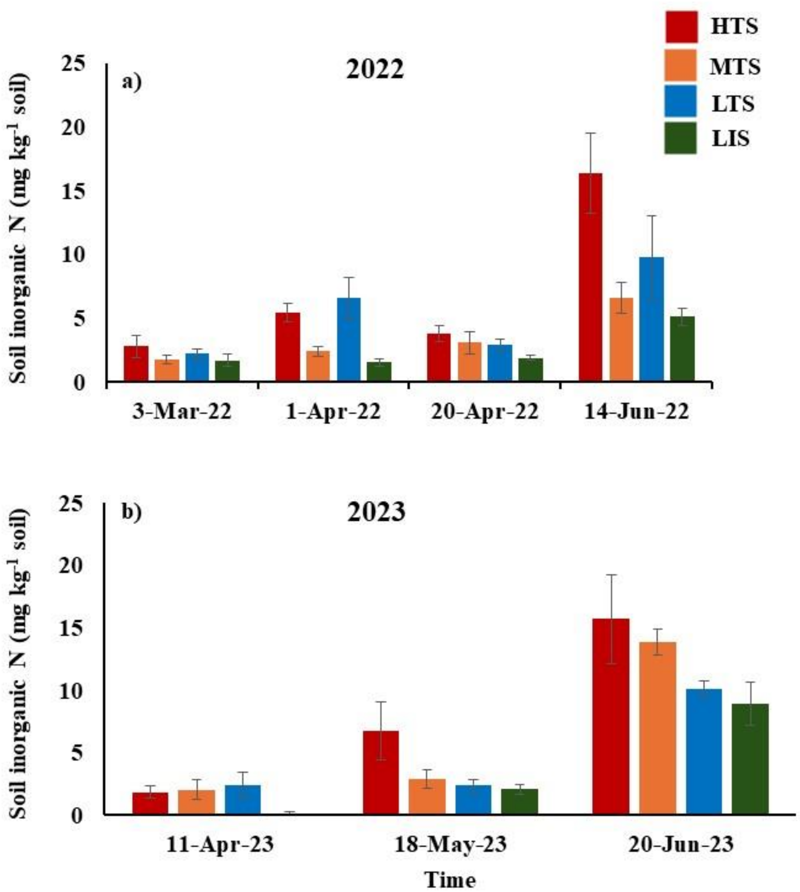

Figure 5. Soil inorganic N (SIN) 0–15 cm profile depth in winter wheat phase of organic grain rotation systems. a) 2022 and b) 2023. HTS: Higher tillage system, MTS: Moderate tillage system, LTS: Low tillage system, LIS: Low input system

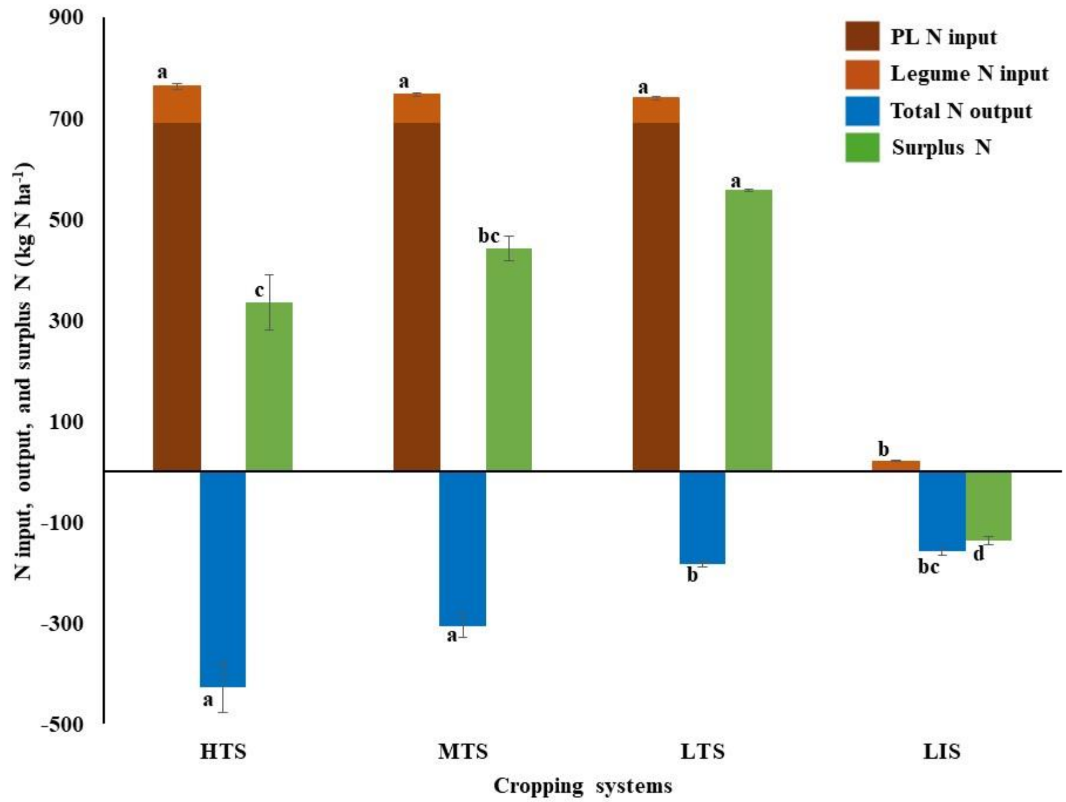

Figure 6. Cropping systems N input, output, and surplus N (kg N ha^{-1}) after three years of four organic soybean-winter wheat-corn rotation. Surplus N = N input (from legume cover crop + PL) - N output (from grain yield). PL N: Nitrogen input from poultry litter, Legume N: Nitrogen input from legume cover crop. HTS: Higher tillage system, MTS: Moderate tillage system, LTS: Low tillage system, LIS: Low input system. Negative values for total N output represent the total N removed from the grain harvest in three years of rotation.

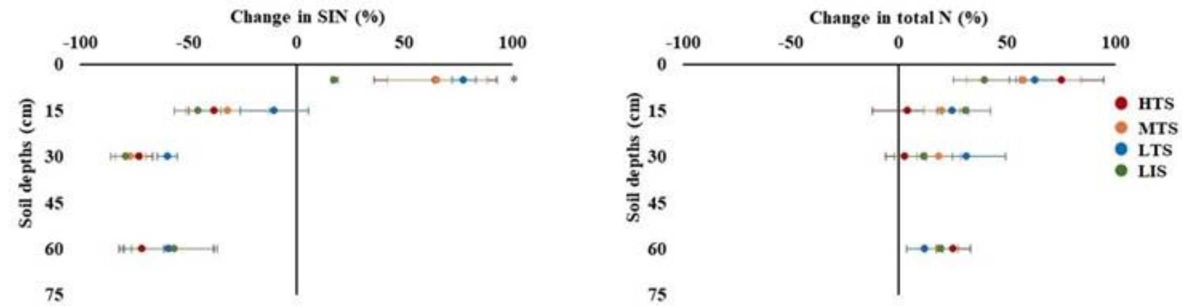

Figure 7. Percentage change in soil inorganic nitrogen (SIN) and total nitrogen (TN) after three-year soybean-winter wheat-corn rotation (October 2020 to October 2023). HTS: Higher tillage system, MTS: Moderate tillage system, LTS: Least tillage system, LIS: Low input system.

* Represents significant difference at least between two cropping systems at $p \leq 0.05$

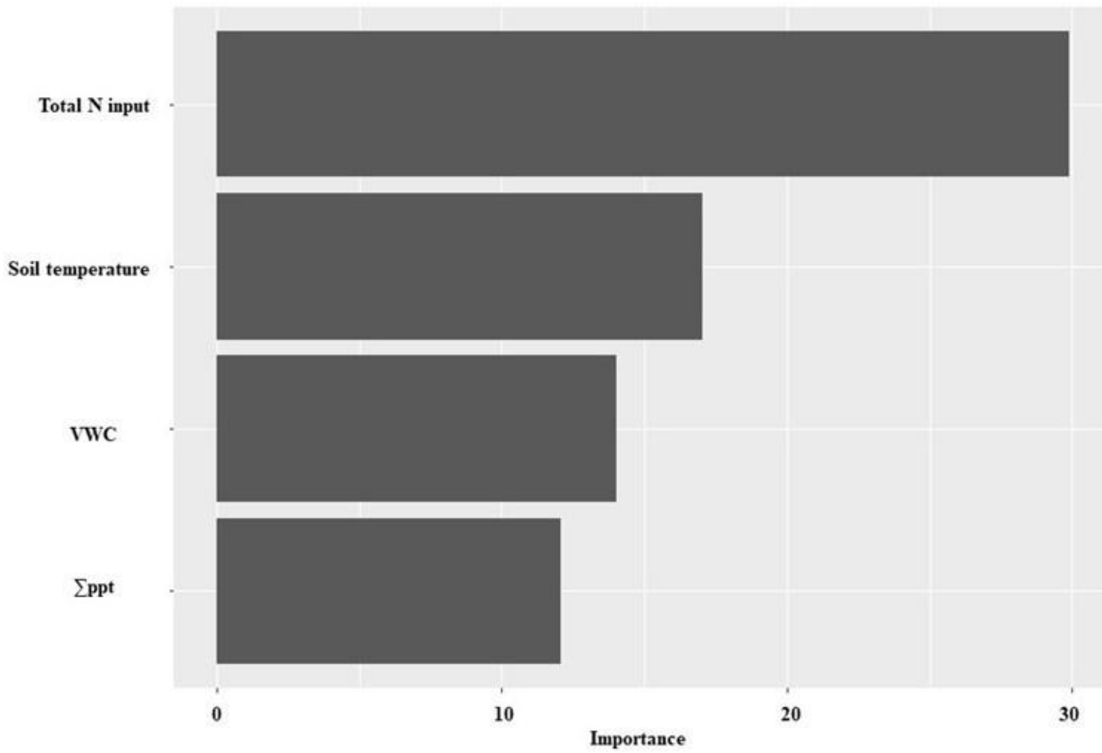

Figure 8. Variable importance plot based on a random forest model. Total N input: Total nitrogen applied (sum of mineral nitrogen from legume cover crop and poultry litter), Soil temperature: growing season mean soil temperature (°C), VWC: growing season mean soil volumetric water content (%), ∑ppt: growing season cumulative precipitation (mm).

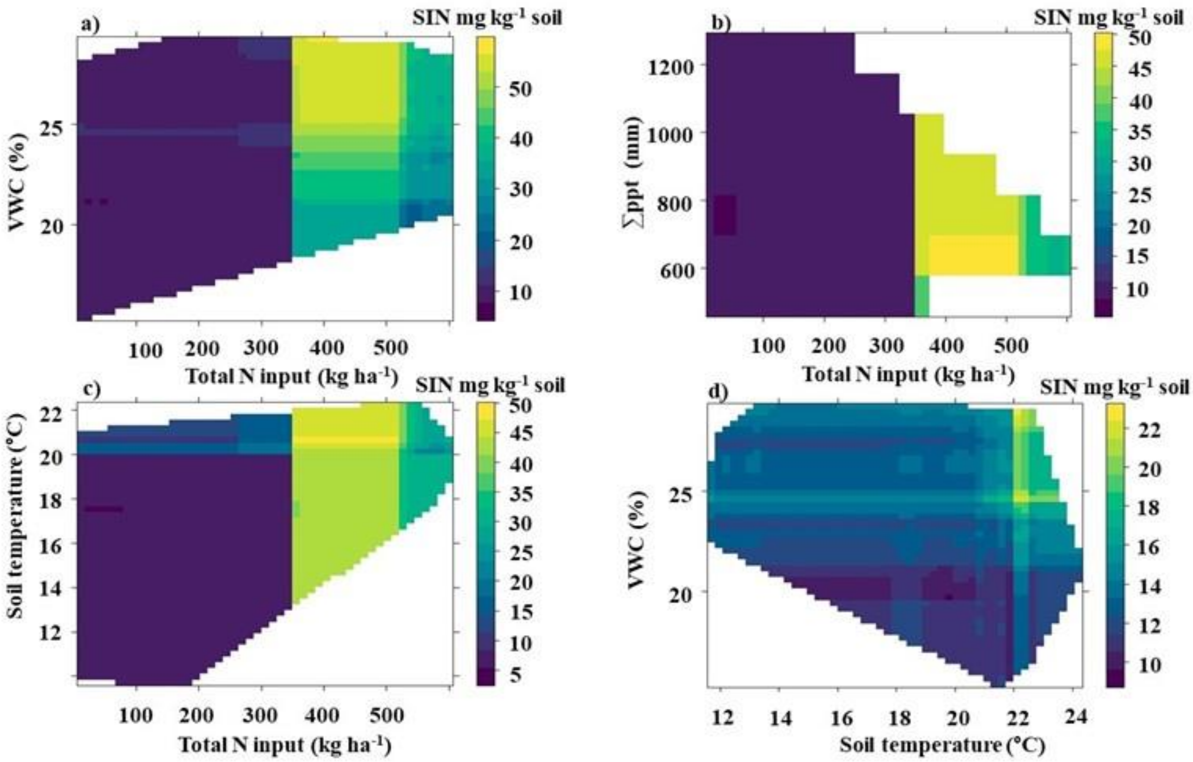

Figure 9. Two-dimensional partial dependence plot of predictor variables influencing soil inorganic nitrogen (SIN) as predicted by the random forest model. The color gradient from blue to yellow represents low to high SIN. Total N input: Total nitrogen applied (sum of mineral nitrogen from legume cover crop and poultry litter), Soil temperature: growing season mean soil temperature (°C), VWC: growing season mean soil volumetric water content (%), ∑ppt: growing season cumulative precipitation (mm).

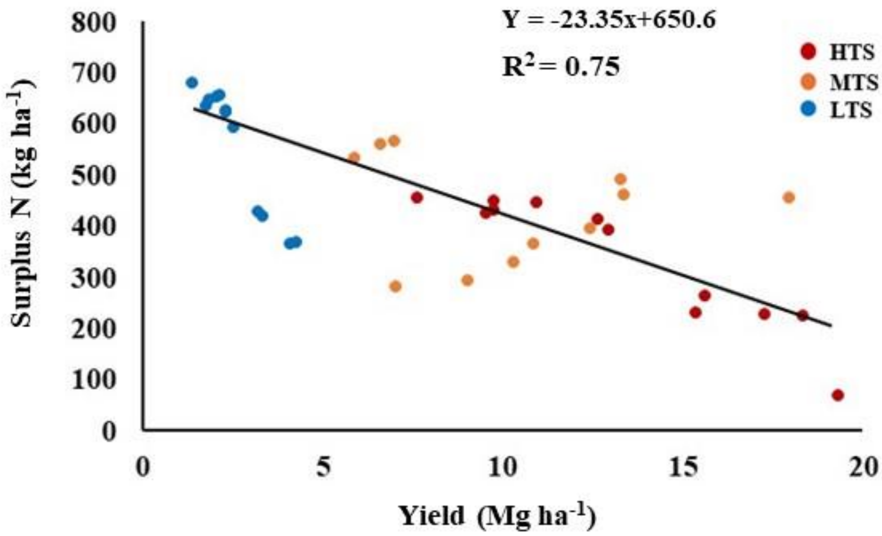

Figure 10. Correlation between crop yield and surplus N. HTS: Higher tillage system, MTS: Moderate tillage system, and LTS: Low tillage system.

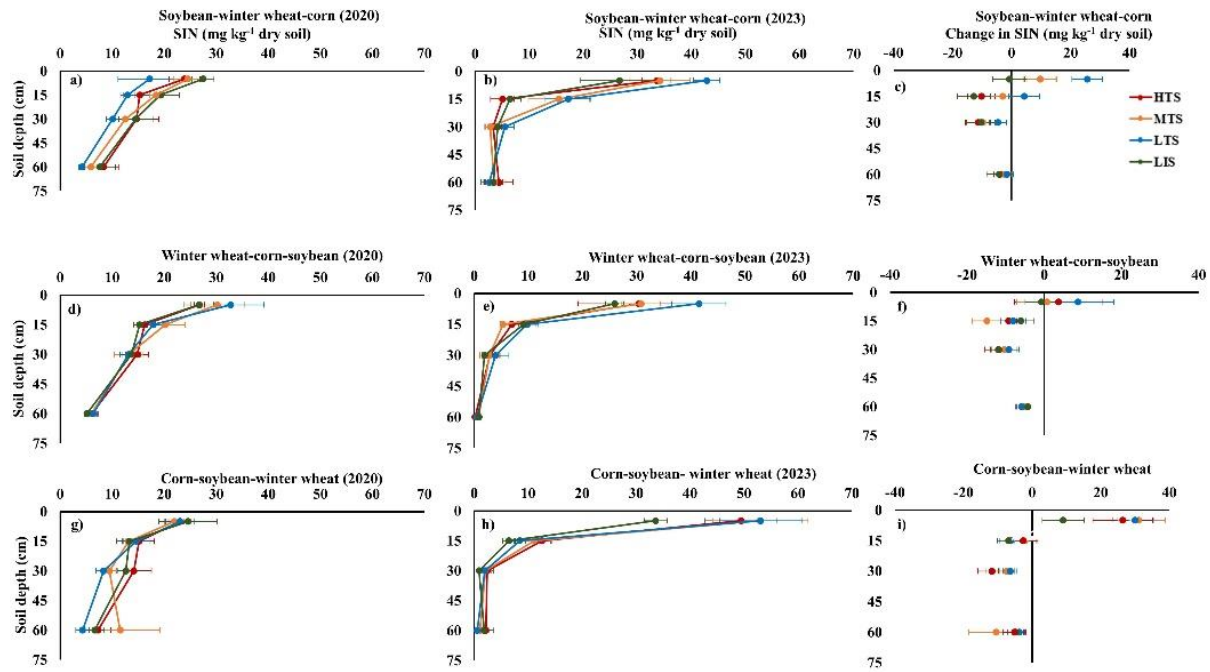

Figure 11. Soil inorganic nitrogen (SIN) across soil profile in three-year soybean-winter wheat-corn rotation. a) soybean entry (2020), d) winter wheat entry (2020), g) corn entry (2020), b) soybean entry (2023), e) winter wheat entry (2023), h) corn entry (2023), c) change in SIN (soybean entry), f) change in SIN (winter wheat entry), and i) change in SIN (corn entry). HTS: Higher tillage system, MTS: Moderate tillage system, LTS: Low tillage system, LIS: Low input system.

* Represents significant difference at least between two cropping systems at $p \leq 0.05$

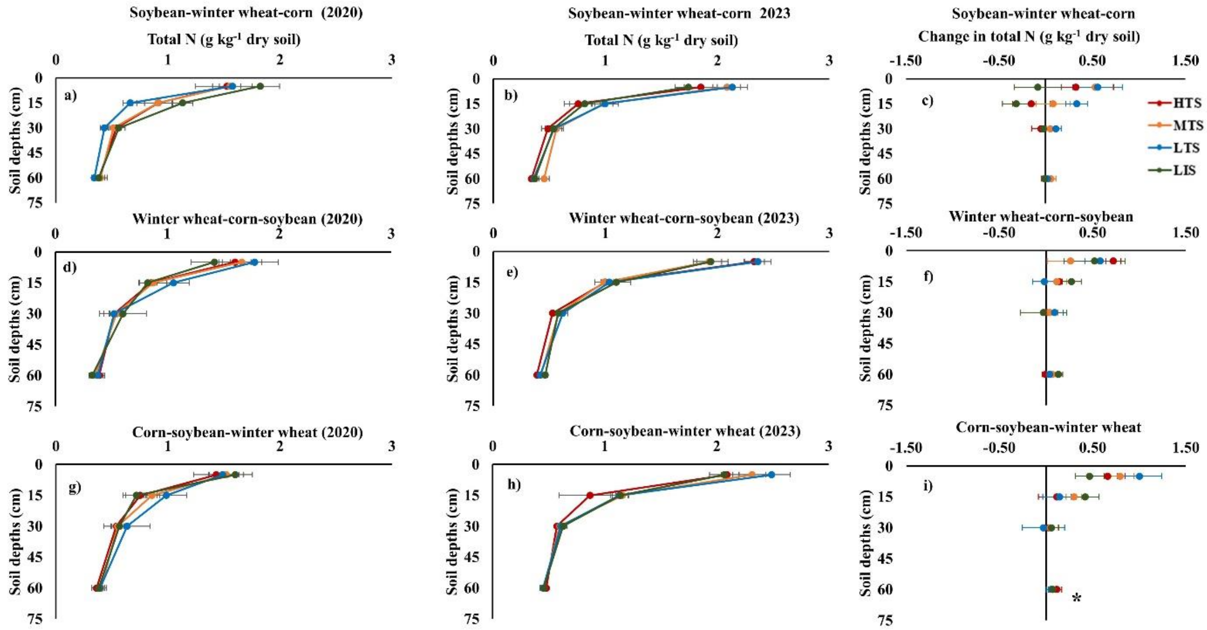

Figure 12. Total N (TN) concentration across soil profile in three-year soybean-winter wheat-corn rotation. a) soybean entry (2020), d) winter wheat entry (2020), g) corn entry (2020), b) soybean entry (2023), e) winter wheat entry (2023), h) corn entry (2023), c) change in total N concentration (soybean entry), f) change in total N concentration (winter wheat entry), and i) change in total N concentration (corn entry). HTS: Higher tillage system, MTS: Moderate tillage system, LTS: Low tillage system, LIS: Low input system

* Represents significant difference at least between two cropping systems at $p \leq 0.05$

Chapter 3. Nitrous oxide (N_2O) emissions and its drivers in transitioning organic grain production systems employing diverse tillage, cover crop, and fertility management

3.1 Abstract

The global shift towards organic agriculture is rising, but its effect on nitrous oxide (N_2O) emissions remains ambiguous. This uncertainty is largely attributed to the inconsistent mineral nitrogen (N) availability from organic inputs, which is influenced by environmental conditions (e.g., soil moisture, temperature), fertility sources, and management practices (e.g., tillage intensity). This study investigated the effects of organic management strategies, including diverse tillage, cover crops, and poultry litter (PL) application on N_2O emissions. Four organic cropping systems were evaluated: i) Higher tillage system (HTS), ii) Moderate tillage system (MTS), iii) Least tillage system (LTS), and iv) Low input system (LIS) over a three-year soybean (*Glycine max* L.)-wheat (*Triticum aestivum* L.)-corn (*Zea mays* L.) rotation. Results showed that emissions varied by crop phases, with the corn phase exhibiting the highest emissions (13 kg N_2O-N $ha^{-1}yr^{-1}$), followed by soybean (4.8 kg N_2O-N $ha^{-1}yr^{-1}$) and wheat (1.3 kg N_2O-N ha^{-1} yr^{-1}). After three years of rotation, LIS showed significantly lower N_2O emissions (6.0 kg N_2O-N ha^{-1}) compared to HTS (29.9 kg N_2O-N ha^{-1}), MTS (20.7 N_2O-N kg ha^{-1}), and LTS (20.1 kg N_2O-N ha^{-1}). Notably, LTS emitted significantly less N_2O than HTS, suggesting reduced tillage can mitigate emissions. The variation in N_2O emissions across crop phases and cropping systems highlights the influence of management practices (cover crop, tillage, PL) and soil environmental factors on N_2O emissions. Additionally, inter-annual variations with the highest emissions in 2021 followed by a declining trend in 2022–2023, highlights the influence of weather variability on N_2O emissions.

3.2 Introduction

Organic agriculture is gaining popularity worldwide due to its environmental benefits, economic potential, and positive implications for societal well-being (Badgley et al., 2007; Meemken & Qaim, 2018; Reganold & Wachter, 2016; Seufert and Ramankutty, 2017). The avoidance of synthetic inputs in agriculture potentially reduces the environmental risk associated with chemicals including inorganic nitrogen (N) fertilizer, which otherwise often contributes to environmental pollution (Robertson & Vitousek, 2009). Unlike synthetic fertilizer, the release of N from organic sources heavily depends on microbial processes (Braker & Conrad, 2011; Butterbach-Bahl et al., 2013), which are further influenced by soil environment (e.g. soil moisture, soil temperature) and management factors (e.g., tillage, crop rotation, residue management) (Parton et al., 1988; Whalen, 2014). The asynchronous nature of N release through microbial mineralization and N uptake by crops can lead to excess soil N, potentially resulting in environmental losses, including emissions of nitrous oxide (N_2O), a potent greenhouse gas.

Nitrous oxide is a significant contributor to global warming, with a warming potential 265 times greater than CO_2 in 100-year time scale (IPCC, 2014). It can persist in the atmosphere for approximately 114 years without undergoing any chemical degradation and has the potential to deplete the stratospheric ozone layer (Ravishankara et al., 2009). Agriculture contributes approximately 60% of global N_2O emissions (Chhabra et al., 2013). Agricultural soil management practices emit 73% of the total US N_2O (US EPA, 2015). Within organic systems, organic inputs such as cover crops and manure management play a crucial role in regulating soil biogeochemistry and microbial communities to affect N_2O emissions (Hartmann et al., 2015; Esperschütz et al., 2007; Tang et al., 2024).

Crop rotation with inclusion of cover crops between main crop season is common in organic production systems due to the numerous benefits associated with cover crops (Lotter, 2003; Sarrantonio & Gallandt, 2003; Wallander, et al., 2021). These benefits include the conservation of soil moisture through mulching effect, erosion control, and the enhancement of soil organic matter (Blanco-Canqui et al., 2015; Nouri et al., 2019). Furthermore, legume cover crops contribute to soil N enrichment by fixing atmospheric N and supplying N to the succeeding crops following termination. But the effect of cover crops on N_2O emissions is contentious (Basche et al., 2014; Mitchell et al., 2013; Saha et al., 2021). The N_2O emissions from cover crops largely depend on management practices (e.g., cover crop species, composition, termination method). For example, legume cover crops significantly contribute to N_2O emissions (Basche et al., 2014; Fiorini et al., 2020; Gomes et al., 2009; Guardia et al., 2016; Muhammad et al., 2019), whereas grass cover crops tend to reduce N_2O emissions (Gomes et al., 2009; Thomas et al., 2017; Guardia et al., 2016; Muhammad et al., 2019). The higher N_2O emissions from legume cover crops are attributed to their low C:N ratio, while reduced emissions from grasses are due to high C:N and lignin: N ratio (Gomes et al., 2009; Huang et al., 2004). Some studies reported reduction in N_2O emissions when legume and grass cover crop mixtures were employed (Basche et al., 2014; Rosecrance et al., 2000; Muhammad et al., 2019). This highlights the importance of biochemical composition of cover crop residues on N release and subsequent N_2O emissions. Moreover, cover crop management practices play a crucial role in determining decomposition dynamics, which in turn affects N release and potential N_2O emissions (Fiorini et al., 2020).

Tillage plays a critical role in organic systems by facilitating the incorporation of cover crop residues, manure, and controlling weeds. However, tillage accelerates the mineralization of

C and N from cover crop residues, potentially leading to increased N losses as N_2O emissions. The incorporation of cover crop residues through tillage enhances soil aeration and stimulates microbial activity, making the residues accessible to microorganisms. This increased microbial accessibility to residues results in higher mineralization rates and elevated N_2O emissions (Chatskikh & Olsen, 2007; Mutegi et al., 2010; Omonode et al, 2011; Petersen et al., 2011). Additionally, a temporary increase in soil macropores due to tillage allows the N_2O to escape from the soil surface (Dhaliwal et al., 2024a). Given these concerns, identifying strategies to reduce tillage is crucial for promoting soil health, nutrient cycling, and climate mitigation impacts while improving organic production systems. Although efforts to minimize tillage in organic systems, especially for high input demanding crops such as corn have shown many challenges including weed pressure and nutrient limitations (Buhler, 1995; Isbell et al., 2021), implementing reduced tillage practices across the entire rotation may help decrease overall cropping system level emissions (Mirsky et al., 2012).

The impact of tillage reduction on N_2O emissions is complex. In no tillage systems, thick cover crop residue mulch can potentially slow decomposition rates and mitigate N_2O emissions. However, some studies have reported high N_2O emissions from reduced or no tillage (Aulakh et al, 1984; Baggs et al., 2000; Baggs et al., 2003; Rochette, 2008). The elevated N_2O emissions from no tillage have been attributed to increased soil moisture content due to thick residue mulch and poor aeration (Rochette, 2008). Conversely, other studies reported minimal or no differences in N_2O emissions between conventional and no tillage practices (Elmi et al., 2003; Grandy et al., 2006). Additionally, the impact of tillage and cover crop residue on N_2O can become more complicated when manure is integrated – a common practice in organic systems to meet crop nutrient requirements. The addition of N from manure may accelerate the decomposition of cover crop residues, potentially creating a period where N supply exceeds crop uptake. This imbalance could result in episodic increase in mineral N availability and its losses as N_2O.

The contradictory result associated with cover crop residue and manure management through different tillage regimes depicts the uncertainties of N_2O emissions associated with organic cropping systems. Some studies reported high N_2O emissions, comparable with conventional farming systems (Davis et al., 2019; Saha et al., 2021), while others reported reduction in N_2O emissions associated with organic management practices (Hassan et al., 2022; Skinner et al., 2019). Elevated N_2O emissions from organic cropping systems have been linked to increased N availability resulting from the combined effect of legume cover crops, manure, and intensive tillage. Conversely, lower emissions from organic cropping systems have been attributed to excluding synthetic N inputs and improved soil quality, including increased organic C and enhanced microbial diversity through applying organic inputs (Skinner et al., 2019; De Gryze et al., 2009). The slow release of N from organic inputs could result in synchronization between N supply and the crop's demand, reducing potential loss as N_2O (De Gryze et al., 2009). These divergent findings underscore the complexity of managing N supply and demand in organic systems and the resulting uncertainty in N_2O emissions. The majority of research on N_2O emissions from organic cropping systems is concentrated in the temperate humid climate (for e.g., Saha et al., 2021; D'Amours et al., 2023), with limited knowledge regarding emissions existing in humid subtropical climates such as warm and humid southeastern region of the US. Such environmental conditions may accelerate the mineralization of organic inputs, further complicating the synchronization of N supply and demand.

Nonetheless, in the context of growing organic crop growers, transitioning to organic agriculture system presents significant challenges, particularly in managing weeds and ensuring

N availability for high N demand crops like corn. Addressing these challenges is crucial for developing a suite of organic management practices that promote a sustainable shift to organic cropping while mitigating N_2O emissions. This study aims to identify organic cropping systems that can reduce N_2O emissions when employing diverse tillage, cover crops, and manure and determine key managerial and environmental factors driving N_2O emissions within organic cropping systems. We have hypothesized that cropping systems with reduced tillage practices will lower N_2O emissions, and N inputs, mineral N, and soil conditions such as soil moisture and temperature will be the major drivers of N_2O emissions.

3.3. Materials and methods

3.3.1 Site description and experimental design

Research was conducted between October 2020 to October 2023, on organic certified land in University of Tennessee – East Tennessee Research and Education Center's Organic Crop Unit (35°52′55.4″, -83°55 ′ 32 ″; 270 m above sea level) in Knoxville, TN. The research site remained fallow for five years before the research began. The soil at the research site is classified as Decatur silt loam (thermic Rhodic Paleudults) with 29.2% sand, 36.2% clay, and 35% silt. The mean annual temperature and annual precipitation of the research site is 15.2 °C and 1319 mm, respectively (1991 – 2020 mean; NOAA).

The research was conducted with a three-year soybean (*Glycine max* L.) – winter wheat (*Triticum aestivum* L.) - corn (*Zea mays* L.) rotation in a randomized complete block design (RCBD) with four replications. In each block, three main plots (48 m × 6 m) were assigned for each crop start (soybean, wheat, and corn). Each crop start was planted with the next main crop in the subsequent year; for example, the main plot of soybean in the first year was planted with wheat in the second year and with corn in the third year. The sequence in a three-year rotation for each start was as follows: soybean start (soybean-wheat-corn), wheat start (wheat-corn-soybean), and corn start (corn-soybean-wheat). In each main plot (i.e., crop start), four cropping systems were randomly assigned in sub-plots (12 m × 6 m), resulting in 48 experimental units (3 crop start × 4 cropping systems × 4 replications). In this experimental design, all three main crops were grown for all three studied years to foster robust data collection and comparisons across crop phases in each year.

3.3.2 Description of cropping systems

For each main crop, four distinct organic cropping systems were assigned. These cropping systems are (i) Higher tillage system (HTS), (ii) Moderate tillage system (MTS), (iii) Least tillage system (LTS), and (iv) Low input system (LIS). The variation among cropping systems was based on cropping intensity (double cropping), tillage intensity, cover crop composition, cover crop termination method, manure application, and management (**Fig. 13**). Over the three years of rotation, these organic cropping systems were managed as follows.

Higher tillage system (HTS): In this cropping system, the main crops (soybean, winter wheat, DC-soybean, and corn) were grown over three years of rotation. This cropping system received the highest tillage intensity among the four cropping systems, three primary tillage (offset disking (OD) to a depth of 25 cm) and three secondary tillage (pickup disking (PD) to a depth of 10 cm) operations. Offset disking was done before planting cover crops, triticale (× *Triticosecale* Wittm.) + radish (*Raphanus sativus* L.), in the fall, which were terminated by flail mowing followed by incorporation via PD before soybean planting in the subsequent spring. After the soybean harvest, OD was done before planting wheat in the fall. Following wheat harvest, PD was performed before DC-soybean planting. Cover crops, hairy vetch (*Vicia villosa* R.) + radish + triticale + winter pea (*Pisum sativum* L.) was planted in the fall after PD, which were terminated by flail mowing and incorporated by OD before corn planting. In this cropping system, corn and wheat phase of the rotation received PL. During the growing season, corn was supplied with 14.8 Mg ha^{-1} of pelletized poultry litter (PL) (supplying 518 kg of total N) and wheat with 4.9 Mg ha^{-1} of PL (supplying 172 kg of total N), applied in two splits. For corn, 4.9 Mg ha^{-1} of PL was applied before planting, followed by 9.8 Mg ha^{-1} PL during the V5–V6 growth stage of corn. Similarly for wheat, 2.45 Mg ha^{-1} of PL was applied before planting in fall and again in the following spring (March).

Moderate tillage system (MTS): Similar to HTS, this cropping system included DC-soybean with an objective to increase the organic grain yield while reducing tillage. This cropping system received one primary tillage, three secondary tillage, and two no tillage (NT) over three years of the rotation. Pickup disking was practiced to plant cover crops (triticale and radish) and roller crimped before soybean planting. After soybean harvest, wheat was planted in pickup disked plots, but following wheat harvest, DC-soybean was planted as NT. Pickup disking was practiced to plant cover crops (hairy vetch + triticale + radish + winter pea) in fall, followed by flail mowing and incorporation to the soil by offset disking before corn planting in subsequent spring. The PL application in this system was consistent with HTS.

Least tillage system (LTS): To achieve the primary objective of developing a sustainable organic system, tillage intensity was further minimized in this system with only two secondary tillage. Since maximizing yield was not the primary goal of this system, DC-soybean was excluded from the rotation. This system received PD only before wheat and corn planting. In this system, cover crops and soybean were planted as NT. Before NT soybean planting, cover crop (triticale + radish) was roller crimped, while cover crops (triticale + crimson clover (*Trifolium incarnatum* L.)) were flail mowed and incorporated via PD before corn planting. Unlike HTS and MTS, cowpea (*Vigna unguiculata* L.) cover crop was planted in the summer, and terminated via flail mowing. Poultry litter application in this system was consistent with HTS and MTS.

Low input system (LIS): Unlike the other cropping systems (HTS, MTS, and LTS), this system excluded PL in both wheat and corn phases of rotation. This system received one primary tillage and one secondary tillage. In this cropping systems, spring cover crops (red clover (*Trifolium pratense* L.) + timothy (*Phleum pratense* L.) were inter-seeded between the wheat rows, which were terminated by flail mowing followed by incorporation via OD before corn planting in the subsequent year. Pickup disking was practiced before planting wheat, while cover crop (triticale + radish) and soybean were NT planted.

3.3.3 Measurement of N_2O and CO_2 fluxes

Soil gas flux measurements were conducted from May 2021 through September 2023 following the static closed chamber method (Parkin & Venterea, 2010). Fluxes from the first year of wheat and cover crop growing period (Fall 2020 to Spring 2021) were not accounted for. Gas samplings for quantification of N_2O emissions were done during the main crop growing season assuming minimal N_2O emissions during cover crop growing season. This assumption was made based on two factors: i) low temperatures in winter following cover crop planting, and ii) cover crop uptake of residual mineral N in the spring when temperature rises, which likely reduces N_2O emissions (Basche et al., 2014; Behnke & Villamil, 2019).

Gas samplings were conducted from main crop planting to harvest, typically from May to first week of October. However, in wheat, gas sampling was done following wheat planting in November through the April of following year. The frequency of sampling was based on the management practices and weather conditions. Sampling frequency was high (twice in a week), when we anticipated high N_2O flux following agronomical management practices (e.g., planting, PL application, inter row cultivation to manage weeds and incorporate PL) and weather conditions (e.g, precipitation). The sampling frequency was reduced to once every two to three weeks during periods when we anticipated minimal N_2O fluxes (e.g., drier period, minimal soil disturbances from agronomical practices).

A cylindrical, static, vented gas chamber (25 cm in diameter and 30 cm in height) made up of polyvinyl chloride (PVC) pipe was used to measure the N_2O flux from organic cropping systems. One chamber was randomly installed in each experimental unit following agronomical practices (e.g., tillage, planting, inter row cultivation) and remained undisturbed throughout the season, except during period of agronomic interventions (e.g., inter row cultivation). At each sampling event, the chambers were closed for one hour and headspace samples were collected at 0, 20, 40, and 60 minutes of chamber closure. Prior to each gas sample collection, the chamber headspace was mixed by performing 2-3 flushes using a 25 ml syringe. Gas samples were then collected with a 25 ml syringe inserted into the chamber through a rubber septum fitted in the chamber lid and transferred in high pressure (20 ml in 12 ml) pre-evacuated Labco exetainer vials (Labco Limited, United Kingdom). Nitrous oxide and CO_2 concentration in the stored gas samples were analyzed using a Gas Chromatography (GC) equipped with Electron Capture Detector (ECD) for N_2O and FID (Flame Ionization Detector) for CO_2 (Shimadzu GC – 2014, Japan).

3.3.4 Measurement of soil environment and mineral N

During each gas sampling, we measured soil moisture and temperature at a depth of 10 cm using a soil moisture sensor (HydroSense II handheld moisture sensor—Cambell Scientific) and a soil thermometer (Reotemp thermometer—Forestry suppliers), respectively. To quantify mineral N, soil samples were collected from each plot during the main crop growing season .

From each plot, three soil samples were collected from 0–15 cm soil depth using a soil probe (internal diameter 1.9 cm).

During the corn phase of the rotations, soil sampling frequency was higher than the soybean and winter wheat phases, as we expected higher soil N availability and N_2O emissions from corn receiving PL during the summer months. Similarly, temporal window periods following agronomic practices, for example, cover crop termination, tillage practices, PL application, and inter-row cultivation, were prioritized for soil samplings. Following sampling, subsamples from each plot were homogenized and analyzed for ammonium (NH_4-N) and nitrate (NO_3-N) concentrations. Ten g of fresh soil samples were extracted with 40 ml of 2M KCl solution. After 1 hour of shaking at 200 rpm, the resultant solutions were filtered using Fisherbrand^{TM} filter paper and extracts were stored at -20°C until further analysis. The concentration of NH_4-N and NO_3-N in the extract was quantified using a microplate reader (BioTek Instruments, Winooski, VT) using the methods described in (Doane & Horwáth, 2003; Verdouw et al., 1978). Additionally, 10 g of soil samples were oven-dried at 105°C for 48 hours to calculate the gravimetric water content.

3.3.5 Statistical analysis

Statistical analysis was done using R software (version 4.3.1). For the main crop phase of the rotations, we calculated the growing season N_2O emissions by linearly interpolating daily N_2O fluxes. To evaluate the effect of cropping systems on N_2O emissions, cumulative N_2O emissions from three years were averaged across three crop starts (*i.e.,* corn-soybean-wheat, soybean-wheat-corn, and wheat-corn-soybean) for each cropping system. Linear mixed effect model (lme4 package) with cropping system as fixed effect, and crop start and block as random effect were used to observe the statistical difference between cropping systems on cumulative

N_2O emissions after a three-year organic rotation. Likewise, to evaluate the effect of cropping systems on N_2O emissions from each crop, a linear mixed effect model was used separately for each crop, with the cropping system as a fixed effect and block and year as a random effect. We performed a log transformation of cumulative flux data to meet the assumption of normality and to perform an analysis of variance (ANOVA); however, the non transformed data was reported in the manuscript for graphical and tabular illustrations. Statistical significance was evaluated at $p \leq 0.05$.

A random forest regression model (Breiman, 2001) was used to determine the key management and environmental variables driving the daily N_2O fluxes. The random forest model works by creating multiple decision trees using the training dataset known as bootstrapping. During this process, two-thirds of the data was used for training purposes, while one-third (also known as out-of-bag) data was utilized for prediction. The final prediction was done by averaging the outputs from the individual decision trees. Our study used three years of data (785 observations) to run the random forest model. We used 70% of the observations to train the model, where hyperparameter tuning and cross-validation were performed before making predictions with 30% of the test data. The model performance was evaluated on the test data set based on root mean square error (RMSE), mean absolute error (MAE), and R^2 model metrics. Predictor variables used to train the model include daily CO_2 flux (kg ha^{-1} day^{-1}), total N input (kg ha^{-1}), NH_4-N (mg kg^{-1} dry soil), NO_3-N (mg kg^{-1} dry soil), soil volumetric water content (VWC%), soil temperature ($^{\circ}$C), two days cumulative precipitation (mm) prior to a sampling day. The “total N input” variable indicates the total N received during the main crop growing season from PL and legume cover crops for the corn phase, only PL in winter wheat, and no external N input for soybean. Additionally, soil sampling dates for mineral N (NH_4-N and NO_3-N) measurements did not always coincide with gas flux measurements. Therefore, selected flux measurement dates that coincided with or closely followed the soil sampling dates were used in the random forest analysis to match the temporal scales.

3.4 Results

3.4.1 Environmental conditions during the growing seasons

Monthly mean air temperatures during the growing seasons (May through September) were generally at or below the 30-year mean, except for slightly elevated temperatures during the summer months in 2022 (**Fig. 14a**). Cumulative monthly precipitation distribution showed variability across the growing seasons and was below 30-year mean (**Fig. 14b**). Annual precipitation was comparable between 2021 (2077 mm) and 2022 (2019 mm); however, it was notably lower in 2023 (1112 mm). Monthly precipitation distribution substantially varied between the years, with drier summer months in 2021 and relatively well-distributed precipitation in 2022 and 2023.

Soil moisture availability was not significantly impacted by cropping systems, but inter-annual precipitation variability largely influenced soil moisture dynamics (**Fig. 15a, b, c, and d**). During the summer drought of 2021, the soil volumetric water content (VWC) at a 10 cm depth dropped to as low as 6% in corn and 8% in soybean, while in 2022 and 2023, volumetric water content dropped below 10% only at the end of growing season. Soil temperature exhibited a similar trend to the daily mean air temperature throughout the three-year growing season, with higher soil temperatures observed from May to September and lowest temperatures from October to April (**Fig. 15a, b, c, and d**). Unlike soil moisture, soil temperature exhibited less inter-annual variation during the three-year rotation.

3.4.2 Soil mineral N

Corn phase across cropping systems and growing seasons showed greater NH_4-N and NO_3-N than soybean, wheat, and DC-soybean phases (**Fig. 16, 17, 22, and 23**). Within the corn phase, peak NH_4-N concentrations following one to three weeks of split PL application and inter-row cultivation were pronounced in 2021 (46 mg N kg^{-1} dry soil-mean value across HTS, MTS, and LTS) and 2022 (35 mg N kg^{-1} dry soil-mean value across HTS, MTS, and LTS) (**Fig. 16a and c**). Whereas peak NO_3-N concentration was only conspicuous in 2022 after three weeks of PL application and was higher in HTS (105 mg N kg^{-1} dry soil) and LTS (115 mg N kg^{-1} dry soil) than MTS (47 mg N kg^{-1} dry soil) and LIS (4 mg N kg^{-1} dry soil) (**Fig. 16d**). Late season split PL application in 2023 avoided peaks in soil mineral N availability. Over a three-year rotation, the highest mean NH_4-N concentration was recorded in the LTS cropping system for corn (10 mg N kg^{-1} dry soil), while the highest mean NO_3-N concentration was observed in the HTS cropping system for corn (26 mg N kg^{-1} dry soil) (see Chapter II). Three-year rotation did not impact NH_4-N in soybean but observed higher NO_3-N shortly after tillage in HTS as compared to other cropping systems (MTS, LTS, and LIS) (**Fig. 17**). In DC-soy, tillage did not significantly influence soil N availability, although slightly higher NO_3-N was observed in tilled (HTS) than NT (MTS) DC-soybean (22). A slight increase in NH_4-N was observed in HTS and LTS (5 and 6, respectively) of wheat in 2022 shortly after split PL application in spring, whereas NO_3-N availability increased in all the systems towards the end of the wheat growing season (23).

3.4.3 Temporal soil N_2O fluxes

Daily soil N_2O fluxes varied throughout the growing season, with the highest fluxes observed in the corn phase, averaging 105 g N_2O-N ha^{-1} day^{-1} across growing season and cropping systems, followed by DC-soybean (15 g N_2O-N ha^{-1} day^{-1}), soybean (14 g N_2O-N ha^{-1}

day^{-1}), and the lowest from wheat (5 g N_2O-N ha^{-1} day^{-1}) (**Fig 18a, b, and c; Table 3**). Temporal fluctuations in N_2O fluxes were associated with agronomic management practices and weather conditions, contributing to substantial interannual variability with mean (across cropping systems and crop phases) daily flux of 75, 44, and 16 g N_2O-N ha^{-1} day^{-1} in 2021, 2022, and 2023, respectively. Among crop phases, the corn phase exhibited high interannual variation with the highest flux recorded in 2021 (163 g N_2O-N ha^{-1} day^{-1}), followed by 123 g N_2O-N ha^{-1} day^{-1} and 28 g N_2O-N ha^{-1} day^{-1} in the subsequent years (**Table 3**). Within the corn phase, cropping systems that received PL (i.e., HTS, MTS, and LTS) exhibited the highest mean N_2O fluxes (131 g N_2O-N ha^{-1} day^{-1}), whereas the lowest flux (26 g N_2O-N ha^{-1} day^{-1}) was observed in PL excluded treatment (LIS). During corn phase in 2021, conspicuous peaks of 2243, 1183, and 1860 g N_2O-N ha^{-1} day^{-1} from HTS were observed 5, 7, and 12 days after PL application, respectively (**Fig. 18c**). Daily mean soil N_2O fluxes from soybean and wheat exhibited a similar trend to corn; however, at a much lower magnitude, with relatively higher fluxes from HTS, MTS, and LTS, and the lowest from LIS (**Table 3**).

3.4.4 Cumulative N_2O emissions

3.4.4.1 Rotational three-year cumulative emissions

The cumulative N_2O emissions after a three-year rotation period were significantly higher in HTS than LTS and LIS cropping systems ($p < 0.05$) (**Fig. 19**). While reduced till intensified grain production (with DC-soybean) led to a notable N_2O reduction in MTS as compared to HTS with similar cropping frequency at higher tillage intensity, this difference was not statistically significant at $p < 0.05$. Rotational N_2O emissions from HTS, MTS, LTS, and LIS were 29.9, 20.7, 20.1, and 6 kg N_2O-N ha^{-1}, respectively. The LTS system, characterized by reduced tillage, resulted in 32.5% reduction in N_2O emissions compared to HTS. The cropping system, LIS, with reduced tillage and PL exclusion, contributed the lowest N_2O emissions among all cropping systems (HTS, MTS, and LIS) ($p < 0.05$).

3.4.4.2 Annual cumulative emissions from each crop phase

The corn phase across the rotations exhibited the highest N_2O emissions with growing season cumulative N_2O emissions of 65.5% (13.1 kg N_2O-N ha^{-1} yr^{-1}), followed by soybean 23.9% (4.8 kg N_2O-N ha^{-1} yr^{-1}), wheat 6.3% (1.3 kg N_2O-N ha^{-1} yr^{-1}), and DC-soybean 4.3% (0.9 kg N_2O-N ha^{-1} yr^{-1}) (**Fig. 24**). While cumulative N_2O emissions from corn and soybean phases did not significantly vary between HTS, MTS, and LTS systems, the wheat phase in LTS showed significantly lower emissions than HTS and MTS ($p < 0.05$) (**Fig. 24**). Interannual variation in cumulative N_2O emissions was observed, with the highest emissions in 2021, followed by 2022 and 2023 (**Fig. 25**). Averaging across cropping systems and crops, cumulative N_2O emissions in 2021 (8 kg N_2O-N ha^{-1}) were 57.5% and 122.5% higher than 2022 (5 kg N_2O-N ha^{-1}) and 2023 (3.6 kg N_2O-N ha^{-1}), respectively. Across all cropping systems, N_2O emissions from corn phase were significantly ($p < 0.05$) lower in 2023 compared to 2021 and 2022 (**Fig. 25a**). The HTS in the wheat phase of rotation experienced significantly higher emissions in 2022 than in 2023 ($p < 0.05$) (**Fig. 25c**). In contrast to corn and wheat, soybean phase of rotation exhibited higher emissions in 2023 compared to 2021 and 2022 (**Fig. 25b**).

The yield scaled cumulative N_2O emissions were found to be higher in corn (mean value across cropping systems: 3.9 kg N_2O-N Mg^{-1} yield exceeding those in soybean, DC-soybean, and wheat by 1.3% (3.83kg N_2O-N Mg^{-1} yield), 84% (0.6 kg N_2O-N Mg^{-1} yield), and 87.6% (0.5 kg N_2O-N Mg^{-1} yield), respectively (**Table 4**). Within the corn phase of rotation, yield-scaled emissions in LIS were significantly lower than all other cropping systems, while in soybean, LIS was significantly lower than only HTS ($p < 0.05$) (**Table 4**).

3.4.5 Factors driving N_2O emissions

Random forest analysis, explaining 61% variability of N_2O emissions, indicated that soil variables were the most important predictors influencing daily N_2O emissions (**Fig. 20**). Daily CO_2 emission was the top variable followed by NO_3-N availability, volumetric water content, total N input, and NH_4-N availability. Soil temperature and two days cumulative precipitation had lower influence on N_2O emissions.

One dimensional partial dependence plot depicted that daily N_2O emission was increased with increased total N input beyond 200 kg total N ha^{-1} (**Fig. 21a**). The emissions further escalated to a magnitude of >100 g N_2O-N ha^{-1} day^{-1} when the total N input reaches > 500 kg total N ha^{-1}- a typical scenario during the corn phase of the rotations, except for LIS. In our analysis, corn phase of rotation only received a total N input > 200 kg total N ha^{-1}, suggesting that this phase in the organic crop rotation presents a higher risk for N_2O emissions. The partial dependence plot showed a sharp increase in N_2O emissions when soil NH_4-N and NO_3-N concentrations were 20 and 100 mg N kg^{-1} dry soil, respectively (**Fig. 21b and c**). Warmer (>23°C) and wetter (>26% volumetric moisture) soil conditions increased N_2O emissions (>100 g N_2O-N ha^{-1} day^{-1}) (**Fig. 21d and e**).

3.5 Discussion

Nitrous oxide emissions from organic grain rotation systems are influenced by a complex interplay of multiple factors such as tillage practices, cover crop quality, manure and their management, and soil conditions, including soil moisture and temperature. While organic systems enhance soil C sequestration through coupled C and N cycling, this can increase N_2O emissions – a significant tradeoff. Most studies quantifying N_2O emissions in organic systems are limited to temperate, humid regions (Saha et al., 2021; Davis et al., 2019; D’Amours et al., 2023), with few studies from warm, humid climates, especially during the transition to organic management practices. A three-year analysis of complete soybean-wheat-corn rotation showed elevated risks of annual N_2O emissions (5 kg N_2O-N ha^{-1} yr^{-1} across all crop phases and cropping systems). However, our findings also highlight the potential of reduced tillage in mitigating N_2O emissions during this critical transition phase.

3.5.1 Nitrous oxide emissions can be high from transitioning organic grain cropping systems

Corn phase receiving N inputs from PL and legume cover crops contributed the largest percentage to the total rotational N_2O emissions (65.5%, 13.1 kg N_2O-N $ha^{-1}yr^{-1}$-averaging across cropping systems) (**Fig. 24**). These findings are consistent with previous research conducted in the cool humid climate of the Mid-Atlantic United States reporting an annual emission of approximately 4 to 7 kg N_2O-N ha^{-1} across various corn-based organic cropping systems utilizing cover crops, PL, and liquid dairy manure (Davis et al., 2019; Saha et al., 2021). Nitrous oxide emissions from organic systems of these magnitudes align with emissions data from intensively managed conventional corn-soybean systems in the US Midwest (Parkin & Kaspar, 2006; Omonode et al., 2011). However, our estimates were higher than those reported by D'Amours et al. (2023) from organic cropping systems in cool humid climate, with a mean annual emission of 1.4 kg N_2O-N ha^{-1}. After three years of rotation, HTS cropping systems, employing PL application and intensive tillage, showed the highest cumulative rotational N_2O emissions of 30 kg N_2O-N ha^{-1} (**Fig. 19**). Such elevated N_2O emissions from managing these organic inputs could negate the soil C sequestration benefits (Dhaliwal et al., 2024b; Flessa et al., 2002**;** Grandy et al., 2006) and potentially shift agricultural systems from being net C sinks to sources. The high soil disturbances from initial land preparation, especially to mitigate the challenges such as crop establishment and weed control during transition to organic crop rotation perhaps resulted in large emissions during the initial years of transition. Additionally, large surplus N in cover crop and manure based organic rotations as reported in this study (see Chapter II) and Isbell et al. (2021) further underscores the challenges of synchronizing N supply and demand in organic crop rotations, which perhaps contributed to substantial N_2O emissions from intensive organic grain cropping systems.

3.5.2 Different crop phases exhibited different variability in N_2O emissions in response to inter-annual variability in weather conditions

Inter-annual variability in N_2O emissions across the growing seasons was highest in the corn phase, which received high N inputs from cover crops and PL, and generally showed a decreasing trend with years since transition (**Fig. 18; and Fig. 25a**). Averaged across the cropping systems, corn growing season N_2O emissions in 2022 and 2023 were 22.3% and 78.5% lower than those in 2021. Soil N_2O emissions are a complex process influenced by soil management and environmental factors such as temperature and precipitation (Baral et al., 2022; Laville et al., 2011; Novoa & Tejeda, 2006), contributing to the inter-annual variations in N_2O fluxes (Burchill et al., 2014; D'Amours et al., 2023). During our study period, annual cumulative precipitation was comparable in 2021 and 2022 (2077 mm and 2019 mm, respectively), but considerably lower in 2023 (1112 mm). While the summer of 2021 was dry, receiving only 60 mm of precipitation from July to mid-August, spring and early summer (March through June) experienced well-distributed precipitation (approximately 313 mm) (**Fig 15a**). The combination of favorable spring soil moisture conditions and intensive tillage practice in the preceding fall at the beginning of the rotations likely contributed to elevated N_2O fluxes in 2021 (**Fig. 15, 18 and Fig. 25**). In 2023, despite the well-distributed precipitation, the total cumulative precipitation was lower, which could have contributed to relatively less N_2O fluxes, while 2022 experiencing well-distributed precipitation contributing intermediate flux. This result is further supported by the random forest model, which predicted increased N_2O emissions under wetter soil conditions and abundant mineral N availability from organic N inputs (**Fig 20; Fig. 21a, b, c, and e**).

Winter wheat exhibited a similar trend to corn, with slightly lower emissions in 2023 than in 2022; however, this trend was not significant in all the cropping systems. In contrast to corn and wheat phases, increased N_2O emissions from soybean in 2023 than in 2022 and 2021 were consistent across cropping systems (**Fig. 25b**). This could be due to better soybean establishment in 2023 contributing to N_2O emissions from biological N fixation (Inaba et al., 2012). Additionally, C and N legacy from PL applications in preceding wheat (in 2021) and corn (in 2022) years of rotation may have contributed to greater residual soil N fertility to drive higher emissions from soybean in 2023. In contrast, inter-annual variation in N_2O emissions was minimal and inconsistent under DC-soybean, possibly due to limited N availability and drier soil conditions after late planting of DC-soybean. While our analysis did not account for crop start effect on rotational N_2O emissions, unusually high N_2O emissions from corn in 2021 (i.e., corn-soybean-wheat sequence) suggests that transitioning with high N demanding crops such as corn may lead to high N_2O emissions.

3.5.3 Effect of cropping system management on N_2O emissions: insights from tillage, PL, and cover crop effects

Cropping systems that received PL and highest tillage intensity emitted the highest N_2O (**Fig. 19**). In a three-year rotation, HTS received three primary and secondary tillage, while MTS received one primary and two secondary tillage, and LTS received only two secondary tillage. Despite similar N input through PL application in these three cropping systems (HTS, MTS, and

LTS), significantly higher N_2O emissions in HTS (than LTS, $p < 0.05$) revealed that reducing tillage intensity decreased N_2O emissions. A 31% reduction in N_2O emissions was observed with a 34% decrease in tillage intensity (HTS vs. MTS); however, this difference was not significant at $p < 0.05$. Accordingly, previous studies have also reported decreased N_2O emissions with reduced tillage as compared to conventional tillage (Dhaliwal et al., 2024b; Chatskikh & Olesen, 2007; Mutegi et al., 2010; Petersen et al., 2011). However, approximately 80% reduction in N_2O emissions from LIS compared to HTS was due to the combined effect of reduced tillage intensity by 54% and exclusion of N input through PL application and limited N input through legume crops in LIS, which also negatively impacted crop yield.

Tillage can impact N_2O emissions in multiple contrasting ways: 1) increased microbial access to C and N substrates can increase denitrification and N_2O emissions from tilled soils; 2) faster drying of tilled than NT soils, especially in presence of cover crop residue mulch, can limit anoxia and reduce denitrification rate; 3) increased macroporosity in pulverized soils after tillage can increase oxygenation to reduce N_2O production; however, it may also allow rapid escape of N_2O to the atmosphere (Dhaliwal et al., 2024a; Elmi et al., 2003); 4) incorporation of PL and cover crop residues accelerates microbial activity, which could lead to early N release before the peak crop N demand (**Fig. 16 and 17**). The increased microbial activity as a result of tillage operation could lead to the formation of microbial respiration-induced anoxia to promote N_2O production from denitrification in the presence of NO_3^- from decomposing residues. This process likely contributed to the high N_2O emissions observed in the corn phase during early summer (Abalos et al., 2022; Chen et al., 2013; Lussich et al., 2024). This result was further supported by random forest model predicting close association between CO_2 and N_2O emissions in these systems (**Fig. 20**). The increased CO_2 concentration due to microbial respiration could deplete the oxygen level in soil pores, resulting in anoxic condition favorable for N_2O emissions. Consistent with our result, Saha et al. (2021) also reported CO_2 fluxes as an important predictor of N_2O emissions from cover crops and dairy manure-based corn-production in the US Mid-Atlantic region. Unlike synthetic fertilizers in conventional systems, these findings further underscore the importance of coupled soil C and N cycling in controlling N_2O emissions from organic systems employing organic fertility inputs that supply N as well as C, two critical ingredients for N_2O production by heterotrophic denitrification (Dhaliwal et al., 2024c). Biological systems (certified organic) receiving N from only clover (similar to our LIS) equally emitted N_2O (4.2 kg N_2O-N ha^{-1}) as other mineral N fertilized systems (Dhaliwal et al., 2024c). This further stresses the greater role of C associated N than the total N input. Therefore, from N_2O mitigation perspective, managing organic N sources is equally important to managing synthetic N sources, as N_2O emissions showed exponential relationship with organic N inputs (Davis et al., 2019) – just like synthetic N fertilizer (Hoben et al., 2011; Shcherbak et al., 2014).

Among the fertilized cropping systems (HTS, MTS, and LTS), N_2O emissions from the corn phase, contributing the highest to the cumulative emissions, was statistically similar as all systems employed tillage to incorporate PL and cover crop residues before corn planting (**Fig. 13; Fig. 24**). In a study, Saha et al. (2021) reported reduced N_2O emissions with the avoidance of tillage in silage corn by NT cover crop (hairy vetch and triticale mixture) termination using roller crimper and applying manure in preceding fall at cover crop planting, suggesting tillage enhances N_2O emissions. In our study, lower N_2O emissions from tilled corn in LIS were driven by the exclusion of N input from PL and low N input from cover crops due to poor cover crops, highlighting that fertility inputs significantly influence N_2O emissions. Delayed application of split PL in the 2023 corn season may have resulted in better synchronization with crop demand, as notable in reduced peaks of mineral N availability (**Fig. 16**), limiting N_2O emissions. However, this data should be interpreted with caution as the effect was confounded by relatively lower precipitation in the 2023 growing season than the other years. Therefore, while tillage is inevitable in organic systems for weed control, cover crop termination, cash crop establishment, and fertility management, reducing tillage in corn phase receiving large C and N inputs from PL and cover crops presents the greatest opportunity to reduce N_2O emissions from organic rotations. However, novel reduced-till agronomic practices ensuring adequate and timely nutrient supply, cover crop termination, and crop establishment is a critical need to provide soil health benefits (Pearsons et al., 2023), and help mitigate N_2O emissions (Mutegi et al., 2010; Petersen et al., 2011).

Reduced tillage effect on N_2O reduction was more prominent and significant in soybean followed by wheat (**Fig. 24**). Tillage effect was magnified when cover crops are present as they supply C and N at varying rate depending on how they are incorporated. For example, the incorporation of flail mowed cover crop residues into the soil by disking in HTS before soybean planting likely accelerated the microbial decomposition of the cover crop residues (Baggs et al., 2000; Baggs et al., 2003; Hu et al., 1997; Jahanzad et al., 2016; Lacey et al., 2020). This rapid N mineralization increased mineral N availability shortly after planting when plant N uptake was low (**Fig. 17**). Consequently, this resulted in elevated N_2O emissions in HTS immediately following cover crop termination and soybean planting. In contrast, lower emissions from MTS, LTS, and LIS in soybean phase were attributed to the reduced soil disturbance under NT and slower decomposition of thick triticale cover crop residue mulch (Mutegi et al., 2010). However, the lack of tillage effect on N_2O emissions from DC-soybean (HTS vs MTS) was likely due to management and weather conditions (**Fig. 15**). Double crop soybean was planted approximately one month after corn and soybean planting (at the end of June or first week of July). The drought period following 2021 DC-soybean planting and relatively less precipitation in 2023 might have offset the tillage effects (HTS vs. MTS) on N_2O emissions from DC-soybean phase.

3.6 Conclusion and potential implications of the study

Our study provides valuable insights into N_2O emissions in three-year soybean-wheat-corn rotations under various organic management regimes and highlights potential N_2O mitigation opportunities by managing tillage, cover crops, and PL—the three pillars of management of organic systems.

Results from three years of soybean-wheat-corn rotation revealed that reducing tillage could be a potential approach to mitigate N_2O emissions. The soybean phase, which received reduced tillage (MTS, LTS, and LIS), showed relatively lower emissions than tilled HTS. In addition to reduced soil disturbance, slow decomposition of cover crop residue under NT limits C and N availability, resulting in a reduction in N_2O emissions. However, tillage is often inevitable in corn to manage weeds, cover crop residues, and fertility inputs. Alternative strategies such as subsurface banding of PL manure could potentially slow N release and better synchronize it with crop uptake during the corn phase of rotation. However, banded PL variably influenced N_2O emissions depending on cover crop treatments (Davis et al., 2019), hence further research is needed.

Our study observed surplus N from three years of organic rotations systems (Chapter II), which likely contributed to N_2O losses due to poor synchronization between N supply and crop demand. While reducing PL application rates can decrease N surplus, ensuring adequate N for crop needs remains challenging. Therefore, the following strategies may synchronize crop N supply and demand, reduce surplus N, and mitigate N_2O emissions: i) Improve timing of cover crop termination and PL application, for example, reducing the number of days between cover crop termination and subsequent cash crop planting, ii) Consider a gap between topdress and sidedress PL application to better synchronize N supply and demand; however, this result from 2023 in our study was confounded by relatively lower precipitation and needed further validation using longer-term data, and iii) Use cover crop mixtures (legume + grass cover crop) instead of pure legume cover crop, which can slow down N release from cover crop biomass decomposition and N_2O emissions (Davis et al., 2019).

Our study also highlights the importance of strategic crop selection during the transition period. Beginning the transitional rotation with crops with lower N and tillage requirements, such as soybean and wheat, could potentially reduce initial N_2O losses to negate higher emissions stemming from initial tillage for land preparation and weed control. This approach may reduce emissions during the early stages of transition to organic farming systems.

Furthermore, except for soybean, there was a general decreasing trend in N_2O emissions from all crop phases with years since transition. Although yearly weather variations-controlled emissions and may have confounded our interpretation, this could be an indication that N_2O emissions may subside with time under organic rotations and underscores the need of long-term management and monitoring of organic systems.

Appendix

Table 3. Mean growing season daily N_2O fluxes (g N_2O-N ha^{-1} day^{-1}).

	Cropping systems	2021	2022	2023	Mean
Corn	HTS	296.73^{a}	115.08^{ab}	41.90^{a}	151.23^{a}
	MTS	162.99^{ab}	143.10^{ab}	31.41^{a}	112.50^{a}
	LTS	150.76^{ab}	207.23^{a}	27.68^{a}	128.55^{a}
	LIS	42.45^{b}	27.73^{b}	9.31^{b}	26.49^{b}
Soybean	HTS	33.75^{a}	20.82^{a}	15.79^{a}	23.45^{a}
	MTS	17.64^{ab}	22.70^{a}	6.60^{ab}	15.64^{ab}
	LTS	9.72^{b}	16.72^{a}	4.47^{ab}	10.30^{bc}
	LIS	15.97^{b}	5.24^{b}	3.10b	8.10^{c}
DC-soybean	HTS	8.69^{a}	10.93^{a}	40.10^{a}	19.90^{a}
	MTS	8.17^{a}	9.39^{a}	11.99^{b}	9.85^{b}
Wheat	HTS		10.38^{a}	6.66^{a}	8.52^{a}
	MTS		5.81^{ab}	6.10^{a}	5.95^{ab}
	LTS		3.29^{bc}	2.33^{a}	2.81^{bc}
	LIS		2.76^{c}	1.78^{a}	2.27^{c}
Mean		74.68^{a}	43.69^{a}	15.78^{b}	

Different letters across cropping systems in each year indicate Tukey's honestly significant difference at $p < 0.05$. HTS: Higher tillage system, MTS: Moderate tillage system, LTS: Least tillage system, and LIS: Low input system. Gas samples were collected from the summer of 2021 through the fall of 2023. Therefore, we were not able to collect gas samples for the wheat phase of rotation in 2021.

Table 4. Yield scaled nitrous oxide (kg N_2O-N Mg^{-1} yield) from a three-year crop rotation.

Treatments	Corn	Soybean	DC-Soybean	Wheat	Mean
HTS	3.89^{a}	6.68^{a}	0.71^{a}	0.52^{a}	2.95^{a}
MTS	5.01^{a}	3.26^{ab}	0.54^{a}	0.79^{a}	2.4^{a}
LTS	5.68^{a}	2.65^{ab}		0.24^{a}	2.85^{a}
LIS	0.99^{b}	2.73^{b}		0.40^{a}	1.37^{b}
Mean	3.89^{a}	3.83^{b}	0.62^{cd}	0.48^{d}	

Different letters across cropping systems in each crop indicate Tukey's honestly significant difference at $p < 0.05$. HTS: Higher tillage system, MTS: Moderate tillage system, LTS: least tillage system, and LIS: Low input system. DC-soybean was not practiced in LTS and LIS cropping systems. Therefore, LTS and LIS systems do not have data.

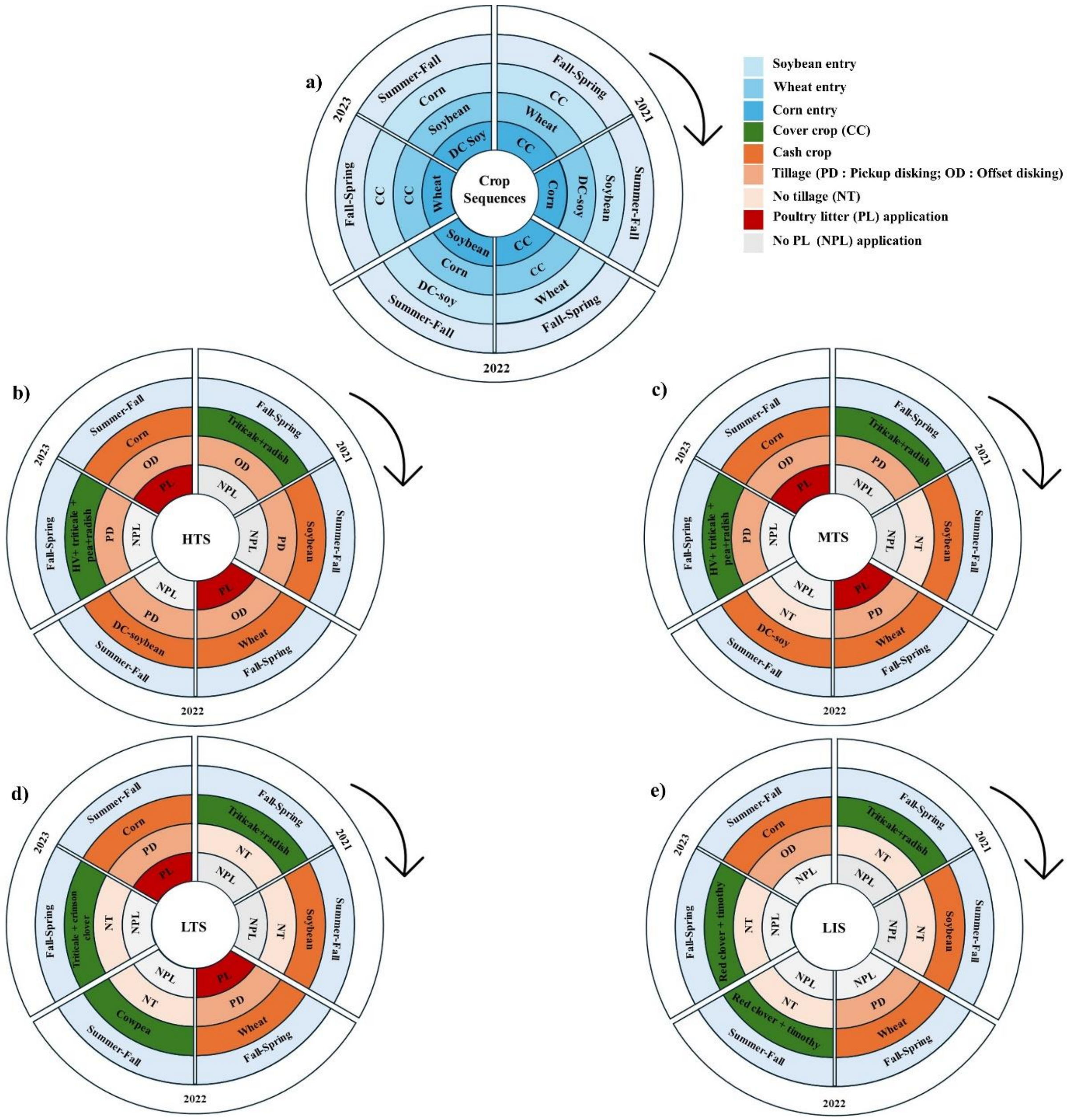

Figure 13. Three-year crop rotation with four different cropping systems. a) Three sequences of crop rotation systems, b) HTS: Higher tillage system, c) MTS: Moderate tillage system), d) LTS: Least tillage system, and e) LIS: Low input system. HV: hairy vetch, DC-soybean: double crop soybean, CC: cover crop, PD: Pickup disking, OD: Offset disking, NT: No tillage, PL: Poultry litter, and NPL: No poultry litter.

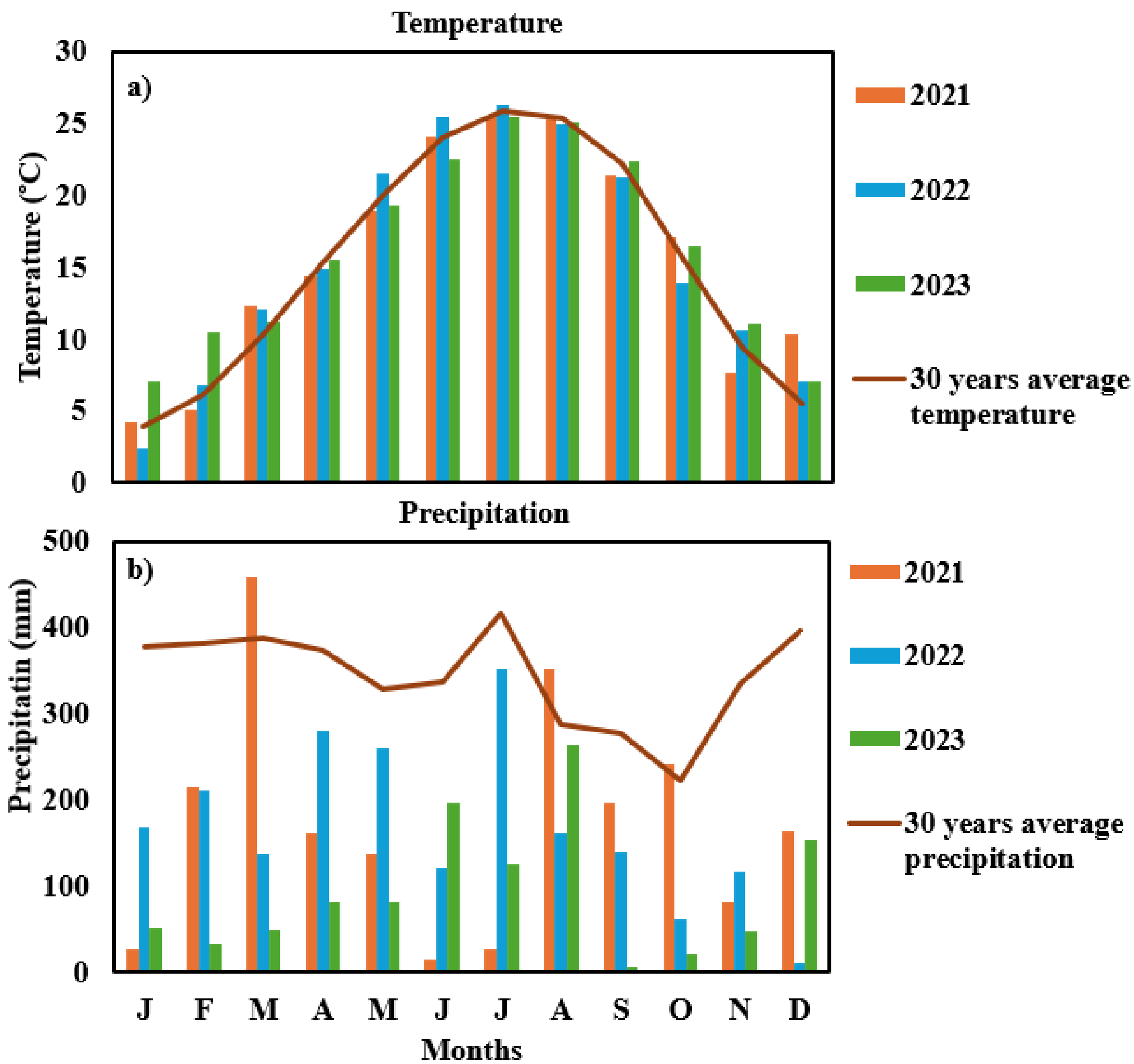

Figure 14. Temperature and precipitation during the research period. a) monthly mean temperature, and b) cumulative monthly precipitation during the research period.

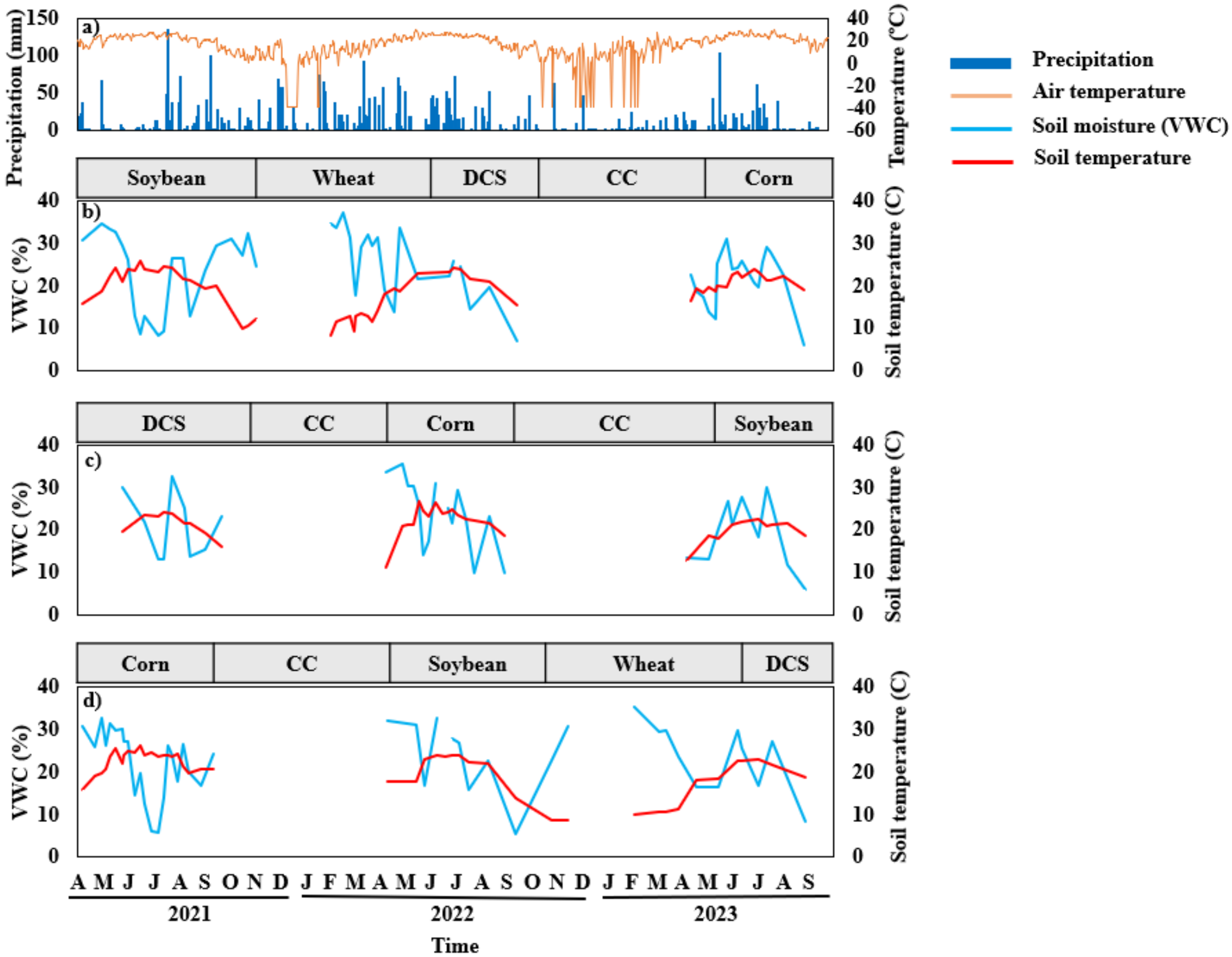

Figure 15. Daily weather, soil temperature, and soil moisture during the study period (2021-2023). a) Daily mean air temperature and daily precipitation during soybean, wheat, double crop soybean, and corn phases of crop rotation, b) soil temperature and soil moisture–soybean start (soybean-wheat-corn), c) Soil temperature and soil moisture–wheat start (wheat-corn-soybean), and d) Soil temperature and soil moisture–corn start (corn-soybean-wheat). DCS: Double crop soybean, VWC: Volumetric water content (%).

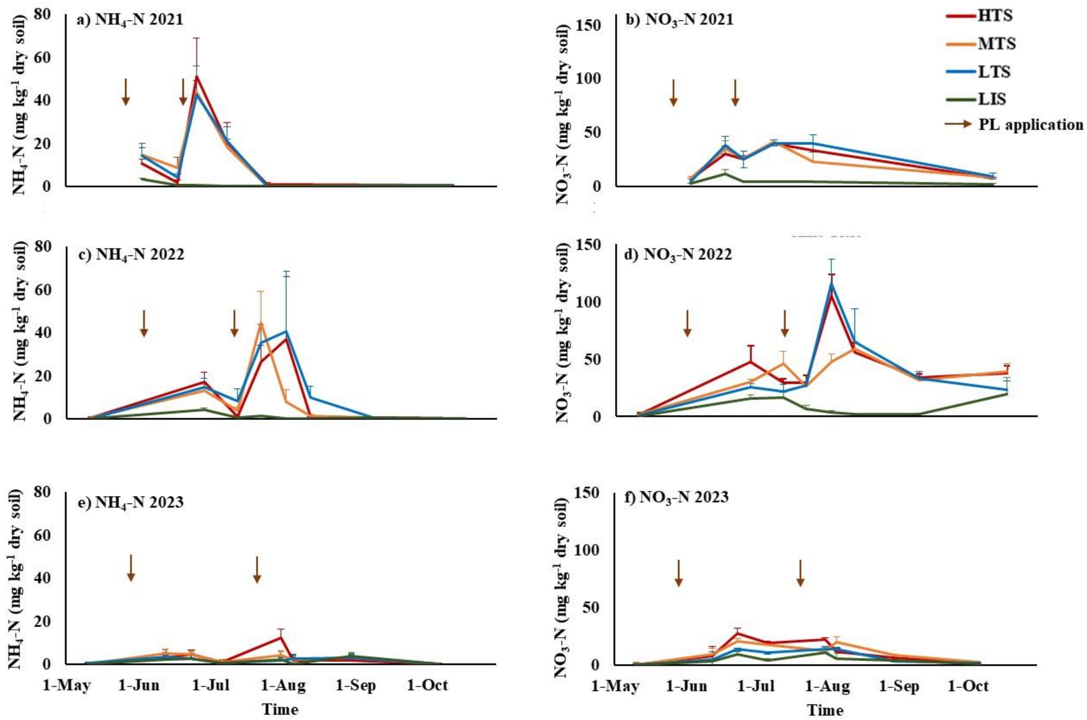

Figure 16. Soil mineral nitrogen dynamics during corn phase of organic grain rotation systems. a) NH_4-N 2021, b) NO_3-N 2021, c) NH_4-N 2022, d) NO_3-N 2022, e) NH_4-N 2023, and f) NO_3-N 2023. The vertical brown arrow represents the time of poultry litter (PL) application. HTS: Higher tillage system, MTS: Moderate tillage system, LTS: Least tillage system, and LIS: Low input system.

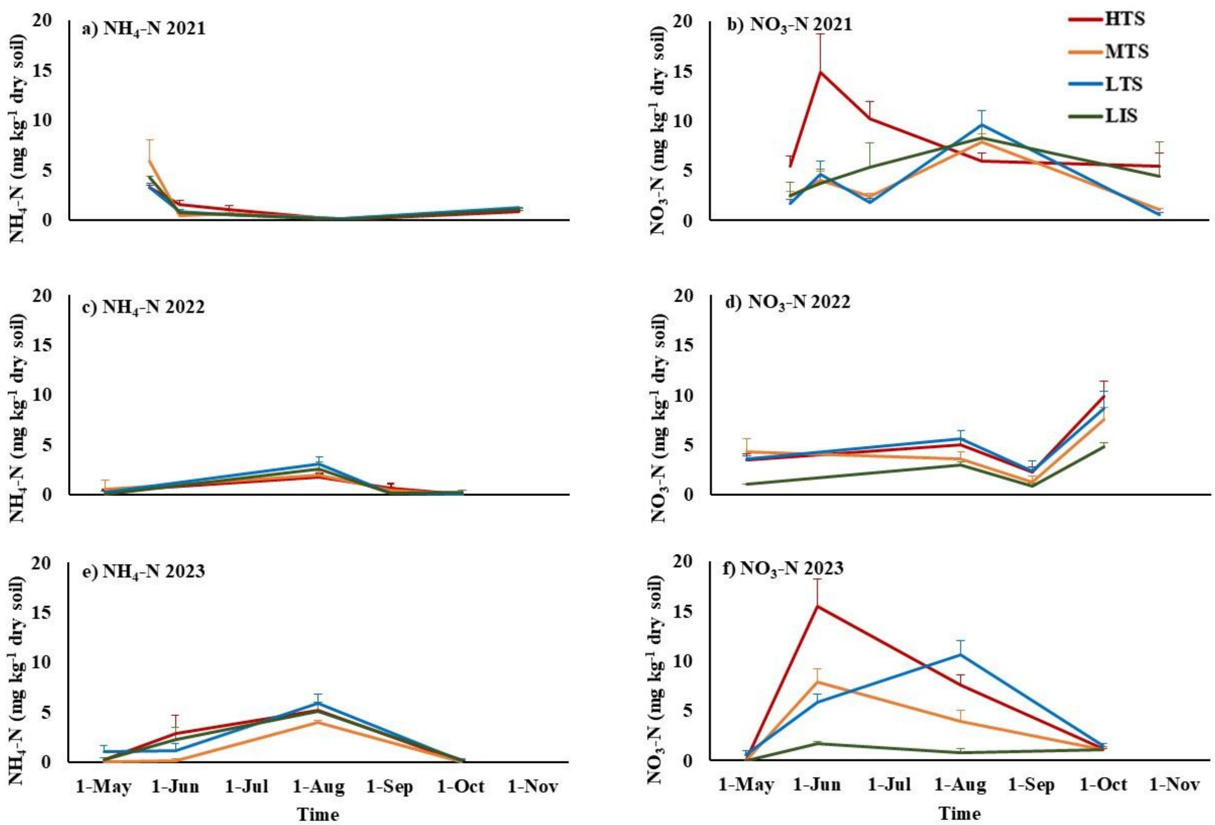

Figure 17. Soil mineral nitrogen dynamics during soybean phase of organic grain rotation systems. a) NH_4-N 2021, b) NO_3-N 2021, c) NH_4-N 2022, d) NO_3-N 2022, e) NH_4-N 2023, and f) NO_3-N 2023. HTS: Higher tillage system, MTS: Moderate tillage system, LTS: Least tillage system, and LIS: Low input system.

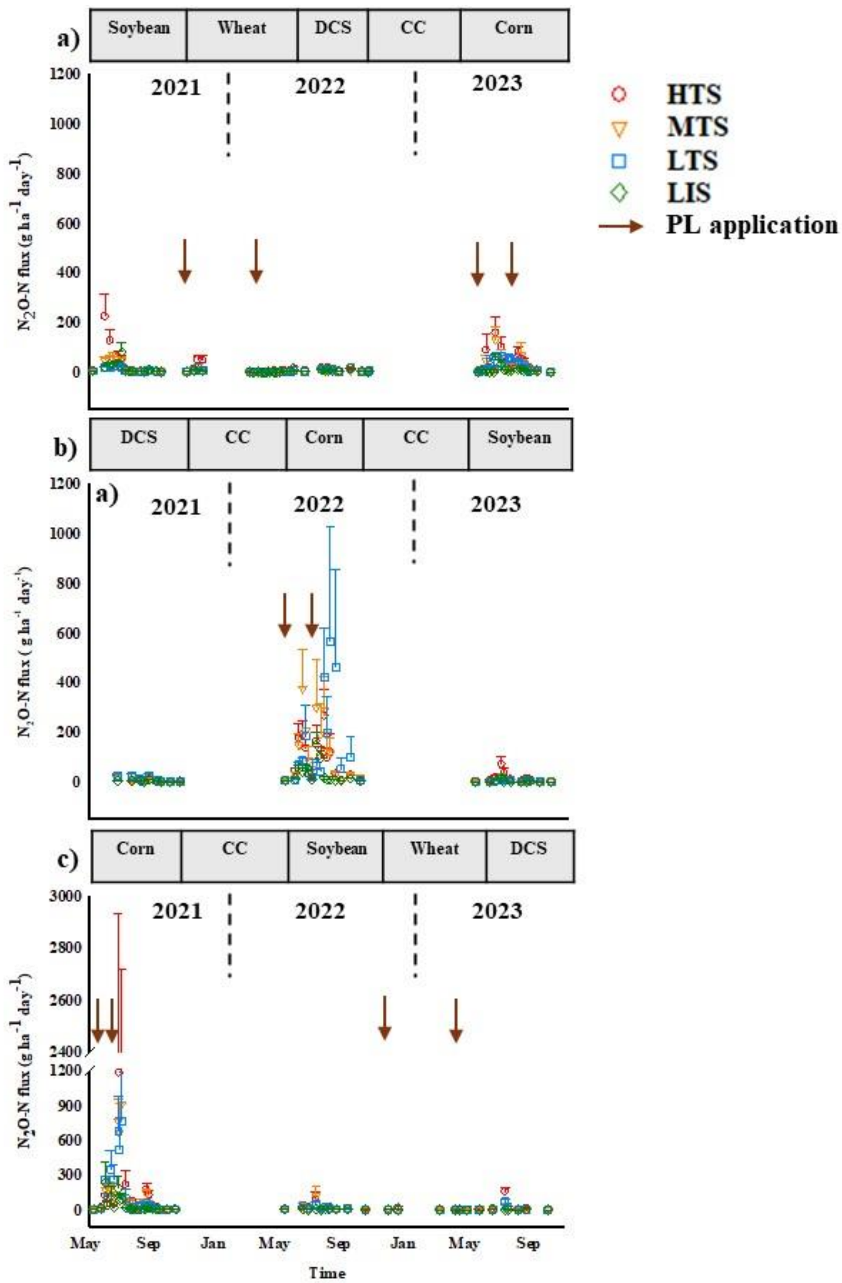

Figure 18. Daily N_2O emissions (± standard error) during soybean, wheat, double crop soybean (DCS), and wheat phases of the cropping systems. Each panel represents the crop start in a three-year rotation: a) soybean start (soybean-wheat-corn), b) wheat start (wheat-corn-soybean), and c) corn start (corn-soybean-wheat). The horizontal bars with crop name at the top of each panel represent cash and cover crop growing periods. The broken vertical lines separate the year, and vertical brown arrows represent the time of PL application. HTS: Higher tillage system, MTS: Moderate tillage system, LTS: Least tillage system, LIS: Low input system, DCS: Double crop soybean, and CC: Cover crop.

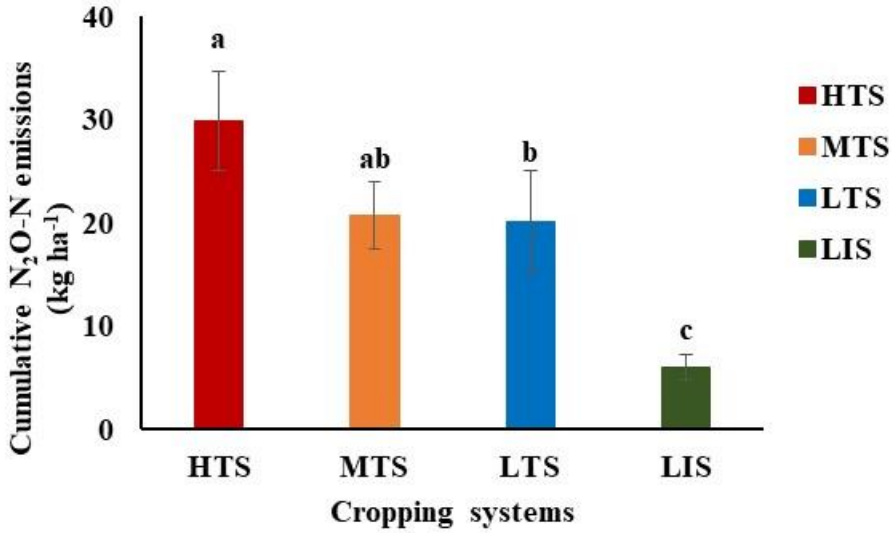

Figure 19. Cumulative N_2O emissions from three-year organic grain rotation systems. HTS, MTS, LTS, and LIS. HTS: Higher tillage system, MTS: Moderate tillage system, LTS: Least tillage system, and LIS: Low input system. Different letters indicate significant differences ($p < 0.05$) in cumulative N_2O emissions between cropping systems.

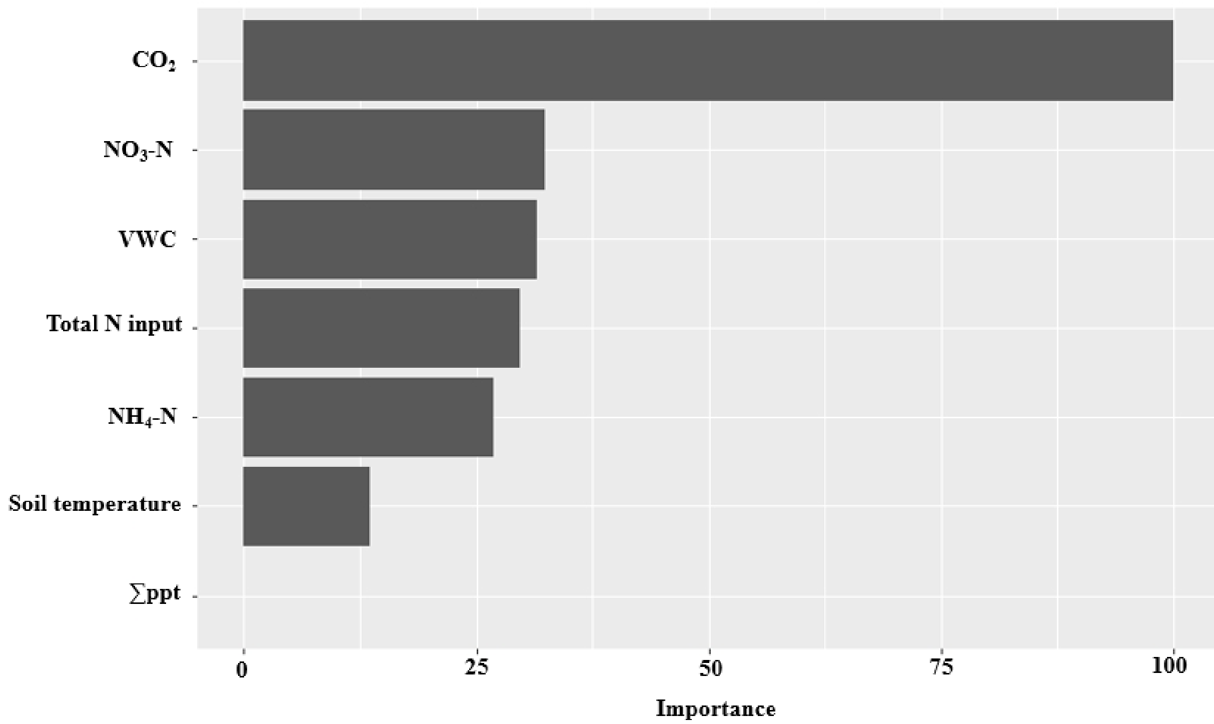

Figure 20. Variable importance plot based on a random forest model. CO_2: Carbon dioxide; Total N input: sum of total N applied from poultry litter (PL) and cover crop; VWC: soil volumetric water content (%); ∑ppt: two days cumulative precipitation prior to gas sampling (mm).

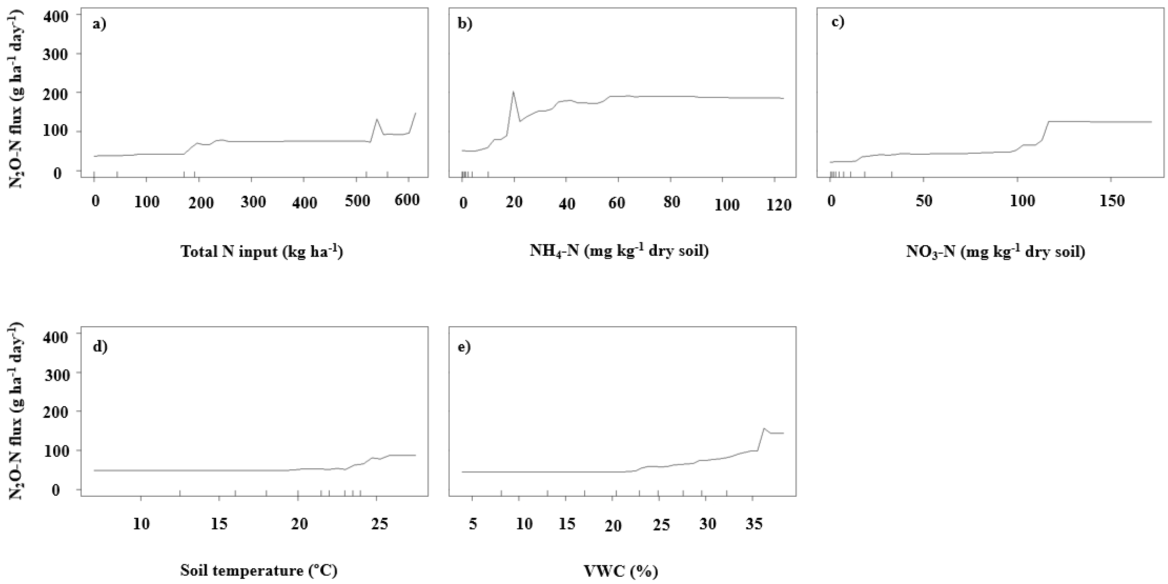

Figure 21. One dimensional partial dependence of predictor variables on N_2O emissions as predicted by random forest. X-axes represent the fractile of key input variable values and indicate the data density. a) Total N input: sum of total N applied from poultry litter (PL) and cover crop; b) NH_4-N: ammonium N; c) NO_3-N: Nitrate N; d) Soil temperature (°C), and e) Soil moisture (volumetric water content %).

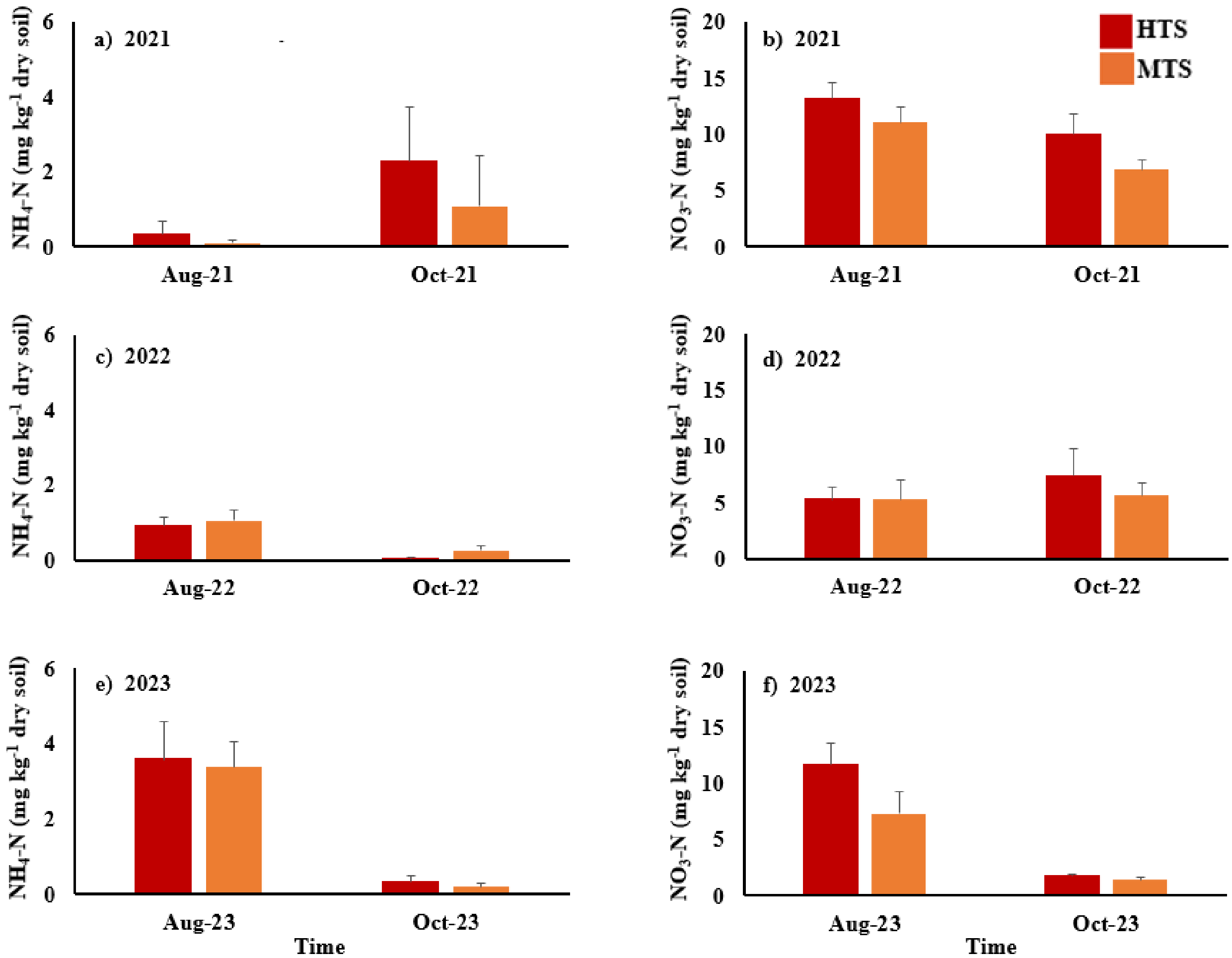

Figure 22. Soil mineral nitrogen dynamics during double crop soybean phase of organic grain rotation systems. a) NH_4-N 2021, b) NO_3-N 2021, c) NH_4-N 2022, d) NO_3-N 2022, e) NH_4-N 2023, and f) NO_3-N 2023. HTS: Higher tillage system and MTS: Moderate tillage system MTS.

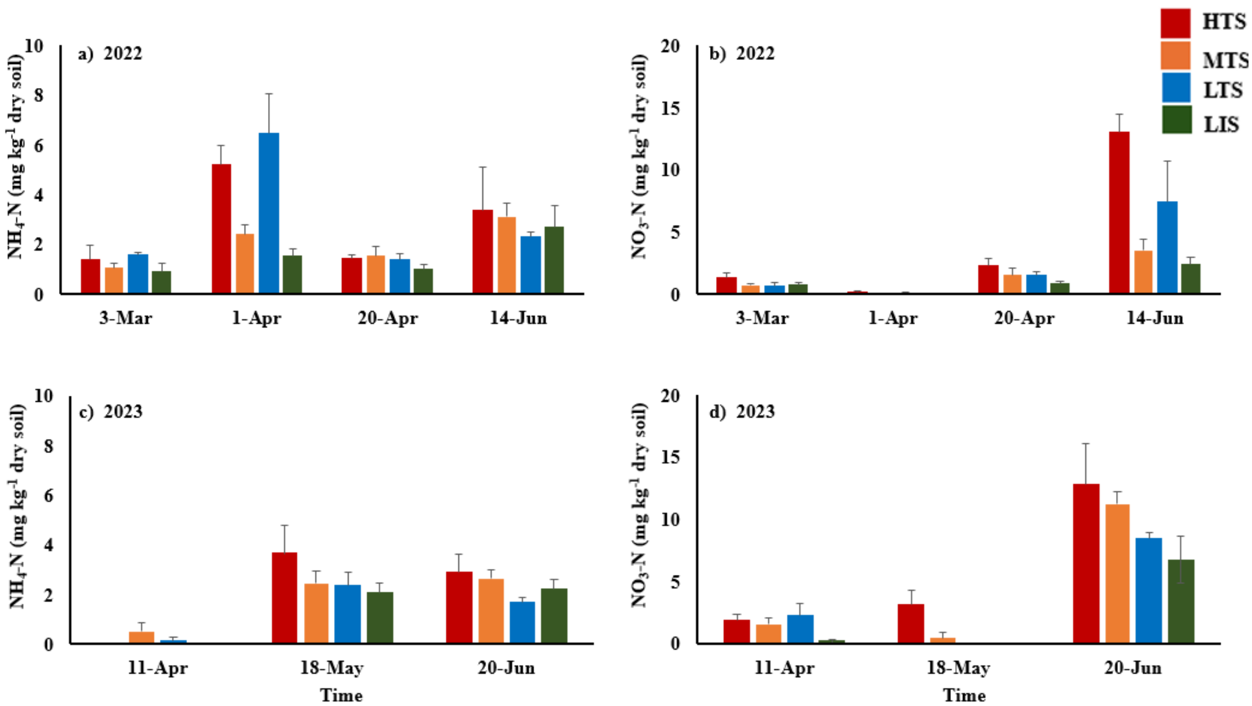

Figure 23. Soil mineral nitrogen dynamics during wheat phase of organic grain rotation systems. a) NH_4-N 2022, b) NO_3-N 2022, c) NH_4-N 2023, and d) NO_3-N 2023. HTS: Higher tillage system, MTS: Moderate tillage system, LTS: Least tillage system, and LIS: Low input system.

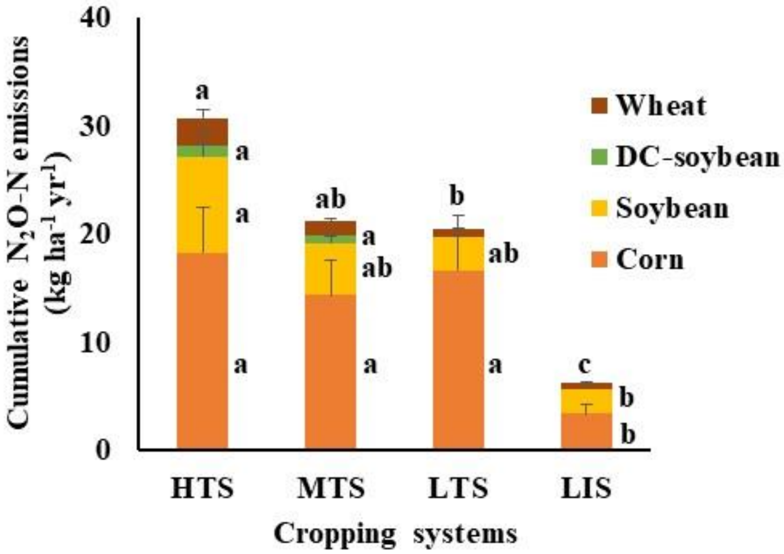

Figure 24. Cumulative N_2O emissions from soybean, wheat, double crop soybean, and corn phases across all cropping systems – Higher tillage system (HTS), Moderate tillage system (MTS), Least tillage system (LTS), and Low input system (LIS). The data for each crop phase in each cropping system represents the mean cumulative emissions over a three-year period (2021–2023). Different letters indicate significant differences ($p < 0.05$) in cumulative N_2O emissions between cropping systems within the crop phase. DC-soybean: Double crop soybean.

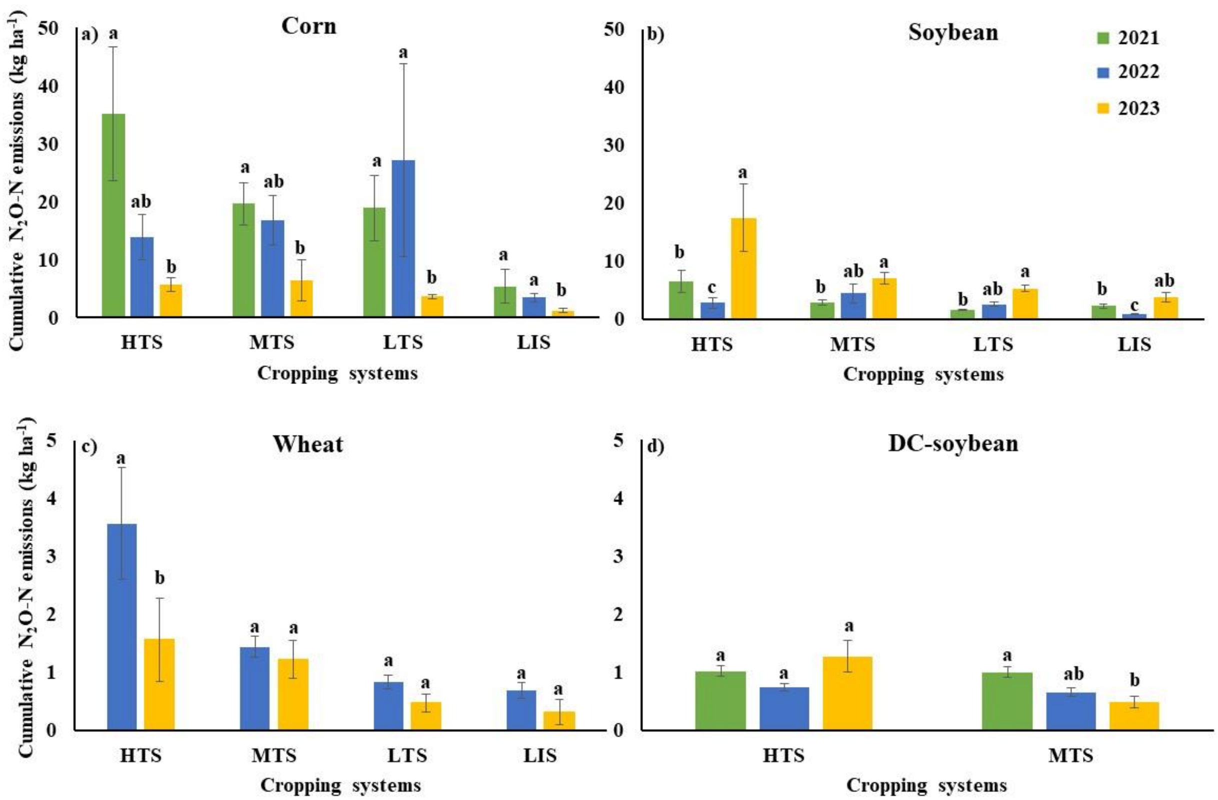

Figure 25. Cumulative N_2O emissions from a) Corn, b) Soybean, c) Wheat, and d) Double crop soybean phases in 2021 (green), 2022 (blue), and 2023 (yellow). HTS: Higher tillage system, MTS: Moderate tillage system, LTS: Least tillage system, and LIS: Low input system. Different letters indicate significant differences ($p < 0.05$) in cumulative N_2O emissions between years within the cropping system. DC-soybean: Double crop soybean.

Chapter 4. Effect of poultry litter on soil nitrous oxide production processes and its dynamic controls during progressive cover crop residue decomposition in organic system

4.1 Abstract

Cover crops provide numerous benefits, such as reducing soil erosion and nitrate (NO_3^-) leaching, enhancing carbon sequestration, and fixing atmospheric nitrogen (N) (by legumes). However, its impact, especially when incorporating along with poultry litter (PL) on N_2O emissions, is unpredictable. The objectives of this study were to observe the effect of PL on cover crop decomposition and subsequent N_2O emissions and determine the microbial processes contributing to N_2O emissions during progressive cover crop decomposition. Nylon mesh litter bags filled with cover crop mixture (triticale [× *Triticosecale* Wittm.], hairy vetch [*Vicia villosa* R.], and winter pea [*Pisum sativum* L.]) were buried in PL applied (CC+PL) and PL excluded plot (CC). Litter bags were retrieved at one to two-week intervals and determined the cover crop residue mass, carbon (C), and N remaining. Gas samples were collected from each treatment for measuring N_2O flux and N_2O isotopomer analysis, and soil samples were collected for mineral N (NH_4-N and NO_3-N), water extractable organic carbon (WEOC), and microbial genomic DNA (*amoA*, *nirK*, and *nosZ*) relevant to N cycling processes. Two years of the study showed no effect of PL on cover crop residue decomposition rate, C remaining, and N remaining. At the end of the corn growing season, 83% (averaged across treatments and year) of cover crop residue was decomposed with a release of 71 %N (averaged across treatments and year). Nitrous oxide emission was significantly greater in CC+PL (11.7 N_2O-N kg ha^{-1} averaged across years) than in CC (2.7 kg N_2O-N ha^{-1} averaged across years) treatment. Higher emissions from CC+PL than CC suggest that N_2O emissions were driven by PL rather than cover crops. Poultry litter addition to the cover crop (CC+PL) showed greater *amoA* and *nirK* gene copy numbers than when PL was excluded from the cover crop. However, the low site preference (SP) value from our study

suggests the greater contribution of denitrification to N_2O emissions during progressive cover crop decomposition.

4.2 Introduction

Organic fertility inputs from manure and biologically nitrogen (N)-fixing legume cover crops could reduce agricultural usage of synthetic N fertilizers, which contribute to the unprecedented increase in nitrous oxide (N_2O) emissions- a potent greenhouse gas (GHG) (IPCC, 2014; Tian et al., 2020). Nitrous oxide is an ozone-depleting, long-lived GHG with 265 times more atmospheric heat trapping capacity than carbon dioxide (IPCC, 2014). While organic N inputs from animal manure and legume cover crops are an integral part of soil fertility and crop nutrition management in organic systems (Carr et al., 2020), previous studies have reported equally high risks of N_2O emissions from organic N sources as mineral N fertilizer (see Chapter III; Davis et al., 2019; Saha et al., 2021). Microbial mineralization of carbon (C) and N during decomposition of manure and legume containing cover crop residues – largely controlled by environmental conditions – can result in substantial N_2O emissions from microbial processes, especially when N release exceeds crop N demand or when in asynchrony with crop N uptake.

Autotrophic nitrification and denitrification (including heterotrophic and nitrifier denitrification) are two major microbial processes responsible for N_2O emissions (Firestone & Davidson, 1989). Nitrification occurs in aerobic conditions when NH_4^+ is available, producing a steadier background rate of N_2O emissions, while denitrification is more episodic, occurring under anaerobic conditions with the availability of C and NO_3^- (Butterbach-Bahl et al., 2013). Soil management practices influence these microbial processes and, consequently, affect N_2O emissions (Skiba & Smith 2000; Thangarajan et al. 2013; Venterea et al., 2012). In particular, the coupling of organic C and N from organic inputs (cover crops and manure) in organic cropping systems likely enhances denitrification, a key microbial pathway for N_2O emissions (Saha et al., 2021; Barton & Schipper, 2001; Rochette et al., 2000). However, the rate of N mineralization or immobilization from organic inputs depends on the quality of these organic inputs and their management.

Given the increasing adoption of organic farming systems in the United States (OTA, 2023; Research Institute of Organic Agriculture, 2020), cover crops are a promising approach to control N dynamics within organic farming systems. Grass cover crops, for example, can scavenge residual soil N, while legume cover crops provide biologically fixed atmospheric N to subsequent cash crops upon termination, potentially reducing the need for external N inputs (Dabney et al., 2001). However, predicting the availability of mineral N and the associated loss as N_2O emissions from cover crops is challenging (Mitchell et al., 2013). Cover crop species and their management practices significantly influence N_2O emissions, with the biochemical composition of the cover crops playing a critical role in determining mineralization rates (O'Connell et al., 2015) and subsequent N_2O emissions (Oliveira et al., 2022). While legume cover crops, characterized by a low C:N ratio, are major contributors to N_2O emissions (Davis et al., 2019; Drost et al., 2020; Saha et al., 2021), grass cover crops with a higher C:N ratio show variable impacts on N_2O emissions (Fiorini et al., 2020; Rosecrance et al., 2000; Gomes et al., 2009). Some studies have even reported minimal or no impact of cover crops on N_2O emissions (Behnke & Vilamil, 2019), suggesting that the variability in emissions may be due to complex interactions between cover crop composition, their management, and external N inputs.

Incorporation of manure along with cover crop residues is a common practice among organic growers in the United States (Carr et al., 2020). For N demanding crops such as corn, it is crucial to supplement N released from cover crop residues with additional N inputs from manure (Isbell et al., 2021; Davis et al., 2019; Wallace et al., 2017; Spargo et al., 2016). Incorporating cover crop residues with manure can accelerate the decomposition of cover crop residue, leading to a rapid release of available N, whereas excluding manure tends to slow the N mineralization rate from cover crop residues (Poffenbarger et al., 2015). Excluding above-ground cover crop residues (mainly legumes) during dairy manure incorporation can lead to a 62% decrease in N_2O emissions from organic corn compared to excluding manure (Saha et al., 2021). The magnitude of N_2O emissions with the exclusion of legume cover crops was similar to the control treatment, where both manure and cover crops were excluded (Saha et al., 2021). The rapid decomposition and N release shortly after cover crop residue incorporation has raised concerns about crop N demand-supply asynchrony during progressive residue decomposition, which could lead to increased N losses, including N_2O emissions (Davis et al., 2019; Saha et al., 2021). Accelerated cover crop residue decomposition and N release rate in presence of additional N inputs from manure could further aggravate the risks of N_2O emissions. This could offset the carbon (C) sequestration benefits of the organic production system.

Addressing this potential trade-off requires a deeper understanding of the complex interactions among various factors, such as cover crop attributes (for example, biomass quantity and composition, especially legume vs. non-legume), manure application, and tillage incorporation of these inputs, as well as their effects on soil biogeochemical processes driving microbial N_2O production. The objectives of this study were to examine the effect of poultry litter (PL) on cover crop mixture residue decomposition, N release from cover residues, and subsequent N_2O emission. Additionally, this study aimed to provide insights into microbial processes that drive N_2O emissions during the progressive decomposition of cover crop residues. The study hypothesizes that: i) The incorporation of both PL and cover crop residues increases residue decomposition rate, thereby resulting in higher N_2O emissions from the treatments when PL and cover crop residues were incorporated than the treatments with only the cover crop residue incorporation and ii) the coupling of both C and N from PL and cover crop enhances denitrification as a major microbial process contributing to N_2O emissions.

4.3 Materials and methods

4.3.1 Site Description and experimental design

The research was conducted from May 2022 to October 2023 on a certified organic field in University of Tennessee – East Tennessee Research and Education Center's Organic Crop Unit (35°52′55.4″, -83°55′32″; 270 m above sea level) in Knoxville, TN. The soil at the research site is classified as Decatur Silt loam (thermic Rhodic Paleudults). The research site has an mean annual temperature of 15.3°C and receives an annual precipitation of 1319 mm (National Oceanic and Atmospheric Administration, NOAA).

The experimental set up in our study included full entry design, where all the main crops (soybean, wheat, and corn) were planted each year. This research was conducted during the corn phase of organic grain (soybean-wheat-corn) rotation systems because preliminary data from 2021 showed that the corn phase of rotation emitted high N_2O emissions in response to the high N inputs from legume cover crops and PL. Within the main corn plot (6 m × 12 m), a poultry litter (PL) exclusion strip (6 m × 5 m) was established to isolate the cover crop effects with and without PL amendments. Therefore, there were two N input treatments: i) cover crop residue without PL (CC), and ii) cover crop residue with PL (CC+PL). Each treatment was replicated four times, resulting in eight experimental units. Two treatments were randomly assigned in each replication block, resulting in a randomized complete block design (RCBD).

4.3.2 Litter bag preparation and installation

The cover crop mixture, consisting of triticale (× *Triticosecale* Wittm.), hairy vetch (*Vicia villosa* R.), and winter pea (*Pisum sativum* L.) was planted in October 2021. Before corn planting in the next spring, cover crops were terminated by flail mowing and then incorporated into the soil using offset disking. Poultry litter in the CC+ PL plots was manually broadcasted and subsequently incorporated along with cover crop residues. Poultry litter was applied in two split doses, topdressing 4.9 Mg ha^{-1} before planting and 9.8 Mg ha^{-1} as sidedressing at the V5-V6 growth stage of the corn.

Before cover crop termination, aboveground cover crop biomass was sampled using 0.5 m × 0.5 m quadrats, followed by oven drying at 65°C for 72 hours to calculate the total cover crop residue biomass and species composition. Prior to termination, a portion of cover crop biomass was allocated for litter bag preparation. Nylon mesh litter bags (0.15 m × 0.20 m dimensions, 1-mm mesh size) were used for the cover crop decomposition study. The cover crop mixture was chopped to approximately 2–4 cm length, resembling the flail–mowed residues, and placed in nylon mesh litter bags in proportion to the dry weight of total cover crop biomass and species composition measured in the field. In 2022, 81 litter bags were prepared (including 2 unburied bags as day 0 samples, and 2 bags retrieved per plot at each of the 6 sampling events; except in from block 4 in which number litter bags installed were less than other blocks), whereas in 2023, 58 litter bags were prepared (2 unburied bags as day 0 samples, 1 bag retrieved per plot at each of the seven sampling events; calculated as: 2 initial bags + 1 bag retrieved at each sampling event × 7 sampling events × 2 treatments × 4 replications = 58 litter bags). After cover crop termination, litter bags were buried at a depth of approximately 10 cm, which is a typical tillage depth. During filed management practices, such as tillage for weed control or PL incorporation, the litter bags were temporarily removed to prevent physical damage to the litter bags and then reburied immediately following management practices.

4.3.3 Sampling and analysis

4.3.3.1 Litter bag extraction and analysis

Litter bags were sampled at one to two weeks intervals depending on weather conditions, agronomical management practices, and observed trends of N_2O flux magnitudes. The retrieved litter bags were cleaned to remove any adhering soil debris and oven-dried at 65°C for 72 hours. The oven-dry mass of the cover crop was recorded as the mass remaining at each sampling time. The dried cover crop residue was then ground and approximately 150 mg of the sample was analyzed for total C and N using the dry combustion method (Elementar vario TOC cube, Hanau, Germany). The soil contamination was accounted for by ashing 1 g of subsample at 500°C for 4 hours (Thermo Scientific Thermolyne Furnance), followed by adjusting the dry biomass to an ash free basis.

4.3.3.2 Nitrous oxide flux measurements

Nitrous oxide emissions were monitored from May (before cover crop termination) to September (corn harvest) in 2022 and 2023 growing seasons following the protocol outlined by Parkin & Venterea (2010). Gas sampling frequency was adjusted based on expected emission levels: biweekly sampling was done to capture background emissions, such as during dry periods, while high frequency sampling (once or twice a week) was done when high N_2O fluxes were anticipated, such as after precipitation events, PL application, or tillage. For N_2O flux measurement, a cylindrical, static, vented gas chamber made up of polyvinyl chloride (PVC) with an overhead space volume of 0.01245 m^3 and a diameter of 0.25 m was installed in each plot. At each sampling event, the chambers were closed for one hour and headspace samples were collected at 0, 20, 40, and 60 minutes of chamber closure. Prior to sampling, 2–3 flushes were performed using a 25 ml syringe to mix the air within the chamber headspace. Gas samples were then collected using a 25 ml syringe inserted into the chamber through a rubber septum fitted in the chamber lid and stored in high pressure (20 ml) in 12 ml pre-evacuated Labco exetainer vials (Labco Limited, United Kingdom) until analysis. Nitrous oxide and CO_2 concentrations in the gas samples were analyzed using a Gas Chromatogrphy (GC) equipped with Electron Capture Detector (ECD) and FID (Flame Ionization Detector) (Shimadzu GC-2014). Additionally, during each gas sampling event, soil moisture and temperature at a 10-cm depth were recorded using a soil moisture sensor (HS2 Handheld Soil Moisture Sensor, Campbell Scientific) and a soil thermometer sensor (Reotemp Thermometer), respectively.

4.3.3.3 Soil biogeochemical measurements

Soil samples were collected from 0–15 cm soil depth at bi-weekly to monthly intervals for soil mineral N (ammonium, NH_4–N and nitrate, NO_3–N) analysis. From each plot, three soil samples were collected using a 1.9 cm probe and then homogenized. After homogenization, 10 g of fresh soil samples were extracted with 40 ml of 2M KCl solution and allowed to shake for 1 hours at 200 rpm. The resulting solution was filtered, and the extracts were stored at –20°C until further analysis. Concentrations of NH_4–N and NO_3-N in the extracts were determined colorimatrically using a Microplate reader (Bio Tek Instruments, Winooski, VT) (Doane & Horwáth, 2003; Verdouw et al., 1978). Additionally, 10 g of soil was oven-dried at 105°C to determine the gravimetric water content.

For water extractable organic carbon (WEOC), soil samples collected from 0–15 cm were air dried, ground, and sieved through a 2-mm mesh size. Four grams of air-dried soil samples were extracted with 40 ml milliQ water. After 5 minutes of shaking at 120 rpm, samples were centrifuged at 1096 g for 1 hour and the resultant solutions were filtered, and extracts were analyzed for WEOC analysis using Elementar vario TOC cube CN analyzer in liquid mode (Elementar, Hanau, Germany).

4.3.3.4 Microbial source of N_2O

4.3.3.4.1 Molecular analysis

Soil samples collected at each litter bag extraction time point aligning with high N_2O fluxes and were analyzed for microbial gene (*amoA*, *nirK*, and *nosZ*) abundance to understand N_2O production processes. Soil samples were collected from 0–15 cm depth using a 1.9 cm diameter probe from three locations within each plot, composited, and then stored at –80°C until further analysis. Soil sampling for microbial genomic DNA abundance analysis was performed only in 2022. A total of 52 samples were collected (with a sampling strategy of 4 initials before splitting the plots + 4 blocks × 2 treatments × 6 sampling events = 52 samples).

Microbial N cycling pathways in the soil samples were characterized and quantified using a qPCR, targeting well-established molecular markers. Soil genomic DNA was extracted from 0.25 g soil using the DNeasy PowerLyzer PowerSoil Kit (Qiagen, Hilden, Germany) following manufacturer's protocol. The concentration of extracted DNA was measured using a NanoDrop One Spectrophotometry (NanoDrop Technnologies, Wilmington, DE), and the extracted DNA was stored in –20°C. For the quantification of functional genes related to N_2O transformation processes, qPCR of *amoA (*AOB*)*, *nirK*, and *nosZ* was done on a CFX96 Optical Real-Time Detection System (Bio-Rad, Laboratories Inc., Hercules, CA, USA). During nitrification, *amoA* gene encodes A subunit of ammonia monooxygenase (AMO) enzyme, which is responsible for oxidation of ammonium to hydroxylamine (Norton et al., 1996). During the denitrification process, the *nirK* gene encodes the copper-containing nitrite reductase (CuNIR) that governs the reduction of nitrite to nitric oxide (Glockner et al., 1993). The reduction of N_2O to dinitrogen gas is catalyzed by nitrous oxide reductase (NOS) enzyme, which is encoded by *nosZ* gene (Chan et al., 1997). Each 20 µl qPCR reaction consisted of 10 µl Maxima SYBER Green qPCR Master Mix (Thermo Scientific, USA), 0.5 µl PCR forward and reverse primer, 2.5 µl DNA template, and 6.5 µl nuclease-free water. The primers and qPCR conditions are detailed in **Table 7** (Hu et al., 2021). Standard curves were obtained from serial dilution ranging from 10^2 to 10^8 of respective gene copy numbers, which were previously obtained through cloning and plasmid DNA extraction as described by Hu et al. (2021). Plasmid DNA concentrations were quantified using a Qubit 2.0 Fluorometer.

4.3.3.4.2 N_2O isotopomer analysis

Nitrous oxide isotopomer analysis was performed for source partitioning of N_2O emissions. Gas samples for N_2O isotopomer analysis were collected from the regular gas sampling setup when high N_2O fluxes were anticipated. The gas samples were collected after 30 minutes of chamber closure and stored in pre-evacuated 100 ml crimp-top glass serum bottles (Sigma-Aldrich, St. Louis, MO). Gas samples for N_2O isotopomer analysis were collected only in 2022. There were six sampling events with a total of 66 gas samples including three ambient gas samples at each sampling event. The collected gas samples were sent to the UC Davis Stable Isotope Facility for site preference (SP-relative abundance of heavy and light N atoms in N_2O molecule) analysis in a GasBench-Precon isotope ratio mass spectrometry.

This isotopomer analysis measures the distribution of ^{15}N within the N_2O molecule (center or alpha vs peripheral or beta position) as outlined in the equation (1), enabling the identification of the dominant microbial pathways responsible for N_2O production (nitrification vs. denitrification) (Sutka et al. 2006). Site preference values of 33 to 37‰ indicate N_2O

emissions from nitrification + fungal denitrification (hereafter referred to as nitrification), while values between –10 and 0‰ suggest emissions from nitrifier denitrification and heterotrophic denitrification (hereafter referred to as denitrification). These SP values were used to determine the microbial processes contributing to N_2O emissions (Ostrom & Ostrom, 2012). Conservative estimates of the proportion of N_2O derived from nitrification and denitrification employ broader SP ranges of 37 and –10‰, respectively, while non-conservative estimates use narrower SP ranges of 33 and 0‰, respectively. To estimate the range of microbial processes contributed to N_2O emissions, we used a two end-member isotope mixing model (Ostrom & Ostrom, 2012).

$SP=\delta^{15}N^{\alpha}- \delta^{15}N^{\beta}$, (i)

Where, $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ are the ^{15}N abundance at the α and β position of N_2O molecule, respectively.

4.3.4 Statistical analysis

A single-pool first-order exponential decay function (ii) was employed to model the proportion of cover crop residue mass, C, and N remaining over time.

$M(t)=M_0\exp^{-kt}+b$, (ii)

Where, $M(t)$ represents the proportion of cover crop residue mass, C or N mass remaining at time t, M_0 represents the proportion of initial cover crop residue mass or C or N mass, k is the decay constant, and b represents the proportion of cover crop residue, or C or N mass resistant to decay. Data were fitted to a model using the minpack.lm package in R software (version 4.3.1). To compare the decomposition rate (decay constant, k) between the two treatments, we performed a linear mixed effect model, with treatment as a fixed effect and block as a random effect, followed by an analysis of variance (ANOVA) to assess statistical significance at $p \leq 0.05$.

Daily N_2O flux data were linearly interpolated to calculate cumulative N_2O emissions during the corn growing season. To assess the effects of treatments on cumulative N_2O emissions, corn yield, and microbial gene abundance, linear mixed effect model was used, with treatment as the fixed effect and block as the random effect, followed by one-way ANOVA to evaluate statistical significance at $p \leq 0.05$.

We conducted a Principal Component Analysis (PCA) using the PCA FactoMiner package in R (version 4.3.1). Before performing PCA analysis, the data were scaled to transform variables to a common scale with a mean zero and unit variance. Principle Component Analysis reduces the dimensionality of the dataset and identify the most important variables contributing to the observed variance. A variable factor map was created to visualize the relationship among the variables in the reduced space of the principal components. The variables included in the PCA were microbial gene abundance (*nosZ, amoA,* and *nirK*), NH_4-N (mg kg^{-1} dry soil), NO_3-N (mg kg^{-1} dry soil), WEOC (mg kg^{-1} dry soil), N_2O-N (g ha^{-1} day^{-1}), CO_2 (kg ha^{-1} day^{-1}), soil temperature (°C), VWC(%), and two days precipitation before sampling (mm).

4.4 Results

4.4.1 Weather conditions during the growing seasons

Daily air temperature was comparable during the two growing seasons (May through September 2022 and 2023); however, 2023 was relatively drier than 2022 (see Chapter II, III; **Fig. 30a and b**). Cumulative precipitation in the 2022 growing season (May through September) was 1033 mm, while during the same period, cumulative precipitation in 2023 was 673 mm.

4.4.2 Residue quality of cover crop mixture in the litter bags

The proportion of cover crops used in the litter bags was in proportion to the measured cover crop species composition in the field. In 2022 and 2023, the proportion of grass cover crops was higher than legume cover crops (**Table 5**). In 2022, triticale accounted for 82%, while hairy vetch and winter pea accounted for 8% and 10%, respectively. However, in 2023, the proportion of legume cover crop was increased to 34% (hairy vetch 19% and winter pea 15%), while reducing the proportion of triticale to 66%. The CN ratio of cover crop mixture in the litter bag at harvest was 40 and 22 in 2021 and 2022, respectively.

4.4.3 Cover crop residue mass remaining, and C and N release during progressive cover crop decomposition

A single-pool first-order exponential decay function was best fitted to the cover crop mass remaining (R^2=0.97). Cover crop decomposition was not significantly influenced by PL in both the 2022 and 2023 growing seasons ($p > 0.05$, **Fig. 26**). Regardless of treatments, approximately 75% of the cover crop mixture was decomposed by 111 days of cover crop litter bag burial in 2022. Whereas approximately 80% of cover crop mixture was decomposed by 83 days of litter bag installation in 2023, suggesting an increase in legume proportion likely increased decomposition rate.

Similar to cover crop decomposition, there was no significant effect of PL on cover crop residue C and N remaining (**Fig. 27**). Approximately 20% of initial C was remaining after 111 days of litter decomposition in 2022, whereas less than 20% of the residue C was left within only 83 days of litter bag installation in 2023. Unlike C, in 2022, N release from cover crop was gradual with N remaining approximately 46% of initial cover crop residue N by 111 days of litter bag installation, while relatively rapid release of N was observed in 2023, with less than 20% N remaining by 83 days of litter bag installation, suggesting increasing legume cover crop proportion possibly accelerated mineralization.

4.4.4 Soil mineral nitrogen availability and water extractable organic carbon

Temporal variability in soil mineral N (NH_4-N and NO_3-N) availability exhibited high variability within and between two corn growing seasons (**Fig. 28**). Averaged across two growing seasons, CC+PL treatment had five times higher NH_4-N (6.5 vs 1.4 mg N kg^{-1} dry soil) and three times higher NO_3-N (22.1 vs 6.5 mg N kg^{-1} dry soil) than the CC treatment, respectively (**Table 6**). Larger peaks of mineral N were observed in CC+PL treatment than in CC treatment in response to management practices and weather conditions (**Fig. 28**). For example, a peak of NH_4-N (44.4 mg N kg^{-1} dry soil) on July 22, 2022, was observed after 10 days of PL application. Likewise, NO_3-N peaks in 2022 corresponded to two PL applications and precipitation events. The largest peak of NO_3-N (46.1 mg N kg^{-1} dry soil) in 2022 was associated with the first PL application, which occurred 32 days following the first PL application. Similarly, the largest peaks of 47.2 and 59.2 NO_3-N mg kg^{-1} dry soil in 2022 were observed 21 and 31 days after the second PL application. Despite higher legume cover crop proportion, 2023 exhibited relatively less mineral N availability than 2022, with diminished treatment differences. Unlike mineral N, WEOC concentrations were comparable between CC+PL and CC treatments ($p > 0.05$), with a mean value of 52.6 mg C kg^{-1} dry soil (CC+PL) and 49.6 mg C kg^{-1} dry soil (CC) across different sampling events in 2022 and 2023 growing seasons, respectively (**Fig. 29; Table 6**).

4.4.5 Nitrous oxide emissions

We observed temporal and inter-annual variation in N_2O emissions, likely driven by management practices and weather conditions, with consistently higher emissions from CC+PL than CC treatment in both growing seasons (**Fig. 30**). Averaged across two growing seasons, mean daily fluxes from CC+PL were four times higher than CC treatment (93 vs 21 g N_2O-N ha^{-}

^{1} day^{-1}, **Table 6**). Temporal N_2O emissions from the CC+PL treatment was characterized by two distinct peaks associated with two PL split applications. In 2022, the first peak of 377 g N_2O-N ha^{-1} day^{-1} was observed five days after the first PL application, and the second peak of 474 g N_2O-N ha^{-1} day^{-1} was observed within two days of the split PL application. Whereas the CC treatment only showed one peak emission of 148 g N_2O-N ha^{-1} day^{-1}, much lower in magnitude than the CC+PL, shortly after residue incorporation (**Fig. 30c**) Contrasting to 2022, the N_2O peaks in 2023 were much smaller in magnitude with the highest peak of 235 g N_2O-N ha^{-1} day^{-1}, which is observed four days following second PL application. The CC treatment exhibited no large peaks during the 2023 growing season.

Cumulative N_2O emissions were significantly higher in CC+PL than CC treatment in 2022 and 2023 (**Fig. 31**); however, the magnitude of treatment differences notably varied between the growing seasons. Cumulative N_2O emissions were higher in 2022, with high variability in CC+PL (16.8 kg N_2O-N ha^{-1} in 2022 vs. 6.5 kg N_2O-N ha^{-1} in 2023), whereas it was relatively less variable in CC treatment (3.7 kg N_2O-N ha^{-1} in 2022 vs. 1.5 kg N_2O-N ha^{-1} 2023).

4.4.6 Effect of PL exclusion on corn yield

Despite high N_2O emissions with large differences between the CC+PL and CC treatments, corn yield was comparable in 2022 ($p > 0.05$; **Fig. 31b**). In contrast, decreased N_2O emissions in 2023 was concomitant with 40% reduction in mean corn yield from 2022, and PL exclusion in CC treatment suffered the greatest yield reduction that was significantly different from CC+PL (**Fig. 31b**).

4.4.7 Microbial processes and drivers of N_2O emissions during cover crop residue decomposition

The CC+PL treatment showed a significantly higher abundance of ammonia-oxidizing bacteria (AOB) *amoA* gene than the CC treatment in four out of the seven sampling dates in 2022, while *nirK* showed a significantly higher abundance in only two sampling events (**Fig. 32a, b**). Across all sampling events, *nosZ* gene abundances did not significantly differ between the two treatments (**Fig. 32c**).

Site preference (SP) values from CC+PL were generally lower (ranged: -10.8‰ to 20.5‰; mean: 8.6‰) than that from CC treatment (ranged: -1.5‰ to 55.7‰; mean: 11.4‰), suggesting that bacterial denitrification was the major contributor of N_2O emissions in 2022 (**Table 8**). In both the treatments, the increase in SP values with the waning of N_2O emissions earlier in August of 2022 was associated with decreasing N_2O contribution from denitrification and increasing contributions from nitrification process (**Fig. 30c, 33; Table 8**).

Principal component analysis (PCA) could explain 45.9% variability, with the first principal component (Dim1) explaining 28.2% variability and the second principal component (Dim2) explaining 17.7% variability (**Fig. 34**). Relatively greater association of daily N_2O fluxes with biogeochemical variables such as VWC, CO_2 fluxes, WEOC, and soil temperature further suggests that denitrification might be major N_2O contributing process (**Fig. 34, Fig. 35**). The *amoA* and *nosZ* abundances were poorly associated with daily N_2O fluxes than *nirK*.

4.5 Discussion

While cover crops have several soil health benefits such as weed control, C sequestration and soil conservation, N release during residue decomposition and subsequent loss of N as N_2O is complex and often difficult to predict. Especially in warm, humid climatic conditions, where a warm climate could accelerate cover crop residue decomposition and rapid mineralization of C and N, resulting in asynchronization in crop N demand and supply could result in environmental N losses, including N_2O emissions. In the context of high N demanding crops such as corn, where manure is inevitable to meet the N demands of organic growing system, manure application further enhances mineralization rate of cover crop residues to aggravate these problems. This study, spanned over two growing seasons, suggested that incorporating grass-legume cover crop mixture (containing 20–35% legume) reduced N_2O emissions by 77% compared to PL incorporated along with cover crop residues (*i.e.,* CC+PL). Poultry litter had a limited impact on cover crop residue decomposition rate and residue N release, consistent across two growing seasons. However, significantly higher soil mineral N availability and subsequent N_2O emissions from the PL inclusion treatments indicate a greater role of PL than cover crop mixture residue on N availability and N_2O losses. Furthermore, our results highlighted that denitrification was the major microbial process contributing to the peak N_2O emissions during progressive cover crop decomposition.

4.5.1 Inter-annual variability in cover crop residue decomposition, residue C and N release, soil mineral N availability, and N_2O emissions

We observed relatively lower cover crop residue decomposition and residue C and N mineralization in 2022 than in 2023 (**Fig. 26 and Fig. 27**), which were influenced by management practices such as cover crop composition and weather conditions (e.g., precipitation). Consistent with our result, Poffenbarger et al. (2015), Ranells & Wagger (1996), and Singh et al. (2020) reported inter-annual variation in the rate of cover crop decomposition largely driven by variation in soil moisture condition across years. While other studies reported weather variables as one of the major factors affecting cover crop decomposition and N release, particularly increased precipitation and resulting increase in soil moisture accelerating cover crop residue decomposition rate (Poffenberger et al., 2015; Ranells & Wagger, 1996; Thapa et al., 2021; Thapa et al., 2022), our result revealed accelerated decomposition in relatively drier year (2023). In our study, 2022 growing period experienced well distributed and relatively higher annual precipitation (1033 mm), while 2023 experienced 35% lower annual precipitation (673 mm) with a much drier early summer period following cover crop termination (**Fig. 5**). Despite this, cover crop residue decomposition and N release was higher at the end of the growing season of 2023 than 2022. This discrepancy was due to the difference in cover crop composition affecting residue decomposition rate between the two growing seasons. In 2022 and 2023, C:N ratio of cover crop residue in litter bags were 40:1 (containing 18% legume species) and 23:1 (containing 34% legume species), respectively. Averaging across treatments, within 111 days of litter bag installation, cover crop residue remaining in 2022 was 25%, while it was only 9% in 2023. Higher proportion of high C:N ratio grass cover crop in 2022 (82%) than 2023 (66%) resulted in higher cover crop residue mass remaining towards the end of 2022 growing season. Cover crops with a high C:N ratio (e.g., grass cover crop) tend to decompose more slowly than those with a lower C:N ratio (e.g., legume cover crop). This slower rate of decomposition in high C:N ratio cover crops is likely due to the reduced availability of mineral N for microbial communities, which limits their ability to break down the high C:N ratio residue (Huang et al., 2004). Consistent with cover crop residue remaining, N remaining towards the end of corn growing season (111 days of cover crop termination) were 46% and 12% in 2022 and 2023, respectively, suggesting only 54% of cover crop N was released during 2022 corn growing season, while it was 88% in 2023 corn growing season. Consistent with our result, several studies have reported the accelerated cover crop decomposition and N release with high proportion of low C:N ratio cover crops (Jahanzad et al., 2016; Kuo & Sainju, 1998; Poffenbarger et al., 2015; Thapa et al., 2022; Zhang et al., 2008). Results from our study suggested that with increasing legume proportion in incorporated cover crop residue, most of the N from cover crop residue was released by the end of the growing season which is also shown in other studies (Starovoytov et al., 2010).

Despite higher N release from cover crop residue in 2023 than in 2022, N_2O emissions and soil mineral N availability were lower in 2023, which were attributed to weather conditions and management practices. Small peaks of N_2O during corn growing period in 2023, conspicuously during the early phase of the corn growing season were attributed to less precipitation, corresponding soil moisture condition, and temperature (**Fig. 30**). In 2023, dry periods prevailed following PL application with mean soil moisture and soil temperature (following PL application to first N_2O peak obtained) was 17% (VWC) and 18.8°C, respectively, while in 2022, following PL application the highest peak in June was associated with high soil moisture of 29% (VWC) and soil temperature (20.8°C). A similar trend of N_2O peaks was observed following split PL application and was attributed to soil moisture, with 29% and 24% VWC in 2022 and 2023, respectively. Consistent with our result D'Amours et al. (2023) also reported inter-annual variation in N_2O emissions mostly driven by soil moisture and temperature when N availability was not a limiting factor. This result is further supported by PCA analysis with a positive correlation between N_2O and VWC and soil temperature (**Fig. 34b**).

In addition to weather variables, inter-annual variation in N_2O emissions from the CC+PL treatment could be due to timing of PL application. In 2023, a split PL application was performed two months after the first application at corn planting, which might have contributed to better synchronization of crop N uptake and N release from both PL and cover crop residue decomposition. However, this interpretation needs to be done cautiously, as low emissions in 2023 were confounded by the weather variables – precipitation, soil moisture, and soil temperature. Our result was further corroborated by relatively higher peaks of mineral N (NH_4-N and NO_3-N), especially NO_3-N, in 2022 than in 2023. Results from our study depicted that inter-annual variation in cover crop decomposition and N_2O emissions were associated with weather variables along with cover crop and PL management.

4.5.2 Effect of PL on cover crop residue decomposition, N release, and N_2O emissions

The PL application did not significantly impact cover crop residue decomposition and residue N release; however, soil mineral N and N_2O emissions were higher when PL was incorporated with cover crop residues (**Fig. 26; Fig. 27c, d, and Fig. 31a**). Similar rate of cover crop decomposition is reflected in lack of significant difference in decay constants for residue mass, C, and N remaining between CC and CC+PL treatments in both growing seasons. We assumed that N sourced from PL could increase the cover crop decomposition in CC+PL treatment, resulting in relatively higher N release in CC+PL than CC treatment; however, in contrast to our hypothesis, we did not observe the effect of PL on the rate of cover crop decomposition. Temporary removal of litter bags during inter-row cultivation to incorporate PL into soil might not allow mixing the litter bag's cover crop with soil, which is one of the limitations of the litter bag study in cover crop decomposition study. Our result was inconsistent with a previous study by Poffenberger et al. (2015), who reported a higher rate of cover crop residue C mineralization and N release when incorporated with PL than cover crops without PL. Averaging across years, 40% of the cover crop residue was decomposed from both treatments (CC and CC+PL) with a release of 38% and 41% N by 30 days of litter bags burial from CC and CC+PL treatments, respectively. This estimate was lower than Poffenberger et al. (2015), reporting >50% of cover crop residue (with varying proportion of hairy vetch species) decomposed by 30 days of litter bag burial. This discrepancy in cover crop decomposition and N release was attributed to the legume cover crop proportion in the cover crop mixture. In their study, the smallest proportion of hairy vetch in cover crop mixture was 25%, which was more than legume proportion in the cover crop mixture in our study in 2022 (18%), which could have resulted relatively less N release in our study than reported by Poffenberger et al. (2015). However, increased legume proportion in cover crop mixture from 18% in 2022 to 34% in 2023 resulted in a comparable amount of N release (~50%) by 30 days of litter bag burial. This result supports several previous studies that cover crop decomposition and N release increases with a higher proportion of legume in the cover crop mixture (Poffenbarger et al., 2015; Ranells & Wagger, 1996; Starovoytov et al., 2010; Schomberg & Endale, 2004; Singh et al., 2020).

The similar rates of cover crop residue decomposition between CC and CC+PL treatments suggest that N availability did not limit microbial decomposition when PL was excluded. This is likely attributed to the C:N ratio of the cover crop mixture and availability of soil mineral N. While it is commonly assumed that residues with a C:N ratio >25:1 results N immobilization, and those <25:1 favor mineralization (Kaye & Hart, 1997; Chapin et al., 2011), O'Connell et al. (2015), reported that C:N ratio >40:1, can still result in net N mineralization. Consistent with this, C:N ratio of 40:1 in 2022 cover crop mixture and 23:1 in 2023 may have facilitated net mineralization, ensuring sufficient N for microbial decomposition throughout both years. Furthermore, well distributed precipitation along with corresponding high soil moisture and perhaps sufficient background soil N availability in 2022 possibly resulted in net mineralization. Small peaks of mineral N (NH_4-N and NO_3-N) in CC (**Fig. 28**) also supports the availability of background mineral N availability for microbial decomposition of cover crops.

However, larger peaks of mineral N from CC+PL than CC, were likely due to the preferential mineralization from PL.

Corresponding to soil mineral N peaks, peaks of N_2O conspicuously higher in CC+PL were observed following PL application, suggesting that N_2O emissions were mainly driven by the PL. Davis et al. (2019) reported a similar result, suggesting that N supplementation through PL can increase N_2O emissions. Such peaks of N_2O driven by management practices and weather events such as precipitation may account for approximately half of the annual N_2O emissions (Parkin & Kaspar, 2006). We have speculated that microbial preferences to easily mineralizable N from PL (low C:N ratio as compared to cover crop mixture) most likely induced faster N mineralization from PL fueling N_2O emissions. Averaging across the growing seasons, cumulative N_2O emissions were 11.6 kg N_2O-N $ha^{-1}yr^{-1}$ and 2.6 kg N_2O-N $ha^{-1}yr^{-1}$ from CC+PL and CC, respectively. Cumulative N_2O emission from CC+PL in our study was similar to the cumulative emissions of 10.7 kg N_2O-N $ha^{-1}yr^{-1}$ reported by Saha et al. (2021), from corn phase of rotation that received liquid dairy manure and legume dominated cover crop mixture as N source. However, in their study, the exclusion of legume dominant cover crop reduced N_2O emissions by 59% (4.4 kg N_2O-N $ha^{-1}yr^{-1}$), suggesting that N_2O emission in their study was driven by legume cover crops. In our study, the legume proportion in the cover crop mixture was low; therefore, excluding low C:N ratio PL (8:1) while retaining only a high C:N ratio cover crop mixture could have reduced the N_2O emissions. However, in contrast to our results, D'Amours et al. (2023) reported less emissions from the corn phase of rotation with PL and green manure as N source, 1.4 kg N_2O-N ha^{-1} mean emissions across the cropping system and years. The difference in emissions might be due to several reasons, including differences in climatic conditions; for example, D'Amours et al. (2023) reported the research region had a cool, humid climate with a mean annual temperature of 5.3°C, while our research site is characterized by warm, humid climate with a mean annual temperature of 15.3°C.

Although N_2O emissions were highest from CC+PL treatment, corn yield was comparable between treatments in wetter 2022, while significantly higher yield from CC+PL than CC treatment in drier 2023. This suggests that while limited mineral N supply in the CC treatment resulted in lower N_2O, it also reduced yield – especially in a drier year (2023). Therefore, PL application along with cover crops is more critical in drier yields to attenuate yield reduction by supplying additional N. While our interpretation lies in the possibility of better synchronization of N demand and supply in 2023, significant yield reduction in 2023 suggests cautious interpretation of the data regarding synchronization of N release and demand, and subsequent loss as N_2O, as both yield and N_2O emissions are affected by several other factors such as growing season weather conditions (precipitation and temperature). Therefore, we have speculated that mineral N availability from organic inputs (cover crops and PL) is difficult to predict as N mineralization from organic inputs depends on microbial processes, which in turn are affected by soil environment (e.g., soil moisture and temperature).

4.5.3 Microbial processes and drivers of N_2O emissions during cover crop decomposition

Among the two primary microbial processes responsible for N_2O, our findings from N_2O isotopomer analysis indicated that denitrification may be the dominant pathway contributing to N_2O emissions (**Fig. 32, Fig. 33, and Fig. 35, Table 8**). We observed the influence of management practices on the abundance of *amoA* (AOB) and *nirK* gene, with a relatively higher gene copy number in the CC+PL treatment than in the CC treatment in some of the sampling dates. Consistent with our results, Hu et al. (2021), Kong et al. (2010), and Maul et al. (2019), Pereira et al., (2015) reported changes in communities of nitrifier and denitrifier bacterial communities. However, higher microbial gene abundance might not be positively correlated to N_2O emissions (Pereira et al., 2015; Maul et al., 2019); therefore, based on the microbial gene abundance, we were not able to conclude the key microbial processes contributing to N_2O emissions. However, N_2O isotopomer analysis in our study supports denitrification as a major microbial process emitting N_2O. Site preference value of -10 to 0% represents bacterial denitrification, while SP value of 33 to 37% represents end member for nitrification and fungal denitrification (Toyoda et al., 2005). The site preference value of 8.4‰ in CC+PL treatment and 11.8‰ in CC treatment (mean value across sampling dates) from our study suggests that N_2O emissions might be due to the denitrification process. Using a two-end-member isotope mixing model (Ostrom & Ostrom, 2012), we estimated that denitrification accounted for 58– 69% of N_2O emissions, while nitrification contributed 31–42% (**Table 8** – calculated mean across treatments and sampling dates). Consistent with our results, Saha et al. (2021) reported denitrification as a dominant pathway in organic cropping systems contributing to N_2O emissions. Similar to our result, D'Amours et al. (2023) speculated denitrification as a major pathway emitting N_2O from organic cropping systems due to coupling of both C and N from organic inputs.

Our result associated with denitrification as a primary microbial process is further corroborated by higher positive correlation between N_2O emissions and *nirk*, compared to correlation between N_2O and *amoA* (**Fig. 34 and Fig. 35**). The availability of labile C and mineral N (NO_3-N), substrates for denitrification process further supports that denitrification might be the major microbial process for N_2O emissions. The continuing availability of water-extractable organic C during the corn growing period (**Fig. 29a, b**), along with the availability of mineral N likely stimulated the microbial respiration, thereby depleting oxygen levels in soil, eventually creating anaerobic conditions conducive for N_2O emissions via denitrification (Thangarajan et al., 2013; Lussich et al., 2024). The positive correlation between N_2O and CO_2 (**Fig. 9b**) in our study further supports that anaerobic condition developed through microbial respiration enhanced denitrification process. A positive correlation between N_2O and VWC indicates that high soil moisture levels promoted anoxic conditions, favoring denitrification. Therefore, our study further corroborates that denitrification as the key microbial process responsible for peak N_2O emissions from organic cropping systems.

4.6. Conclusion

Poultry litter addition did not influence cover crop decomposition but significantly increased N_2O emissions from increased N availability. However, PL buffered corn yield reduction in a direr year, whereas relying only on cover crops for N source in organic systems could significantly reduce corn yield due to N limitation. This outcome suggests that while the inclusion of only cover crops can lower N_2O emissions, there may be concerns about how this approach can sustain crop yields over time. Delaying PL split application in 2023 reduced emissions and maintained higher yield from cover crops, but lower than in 2022. However, drier weather in 2023 than in 2022 complicates interpretation, and longer-term data is needed.

Increasing legume proportion in the cover crop mixture accelerates the cover crop decomposition. Inclusion of cover crop mixture (30–35% legume cover crop) while excluding PL application could reduce N_2O emissions by 77%, revealing the potential of cover crop to mitigate N_2O emissions. Microbial gene abundance and SP analysis from our study revealed that denitrification was the major microbial pathway contributing to N_2O emissions. The presence of both C and N with cover crop and PL could have accelerated the denitrification process. Anoxic conditions developed due to microbial respiration could have further accelerated the denitrification process, contributing to N_2O emissions.

Appendix

Table 5. Total dry mass of triticale, hairy vetch, and winter pea and their proportion placed in litter bags at the initiation of the cover crop decomposition study. Total dry mass is presented on ash-free basis.

	2022		2023	
Cover crop species	Cover crop biomass (Mg ha^{-1})	Cover crop biomass (proportion)	Cover crop biomass (Mg ha^{-1})	Cover crop biomass (proportion)
Triticale	7.46	0.82	4.37	0.66
Hairy vetch	0.68	0.08	1.23	0.19
Winter pea	0.98	0.10	0.96	0.15

Table 6. Mean growing period mineral N (NH_4-N and NO_3-N), water extractable organic carbon (WEOC), and N_2O flux.

Treatment	NH_4-N (mg N kg^{-1} dry soil)			NO_3-N (mg N kg^{-1} dry soil)			WEOC (mg C kg^{-1} dry soil)			N_2O-N (g N ha^{-1} day^{-1})		
	2022	2023	Mean	2022	2023	Mean	2022	2023	Mean	2022	2023	Mean
CC+PL	9.97	3.04	6.5	28.97	15.19	22.08	55.89	49.45	52.67	143.10	42.52	92.81
CC	1.54	1.33	1.43	6.46	6.63	6.54	51.82	47.40	49.61	29.29	12.04	20.66

Table 7. Primers and qPCR conditions for microbial gene abundance analysis.

Gene	Primers	Sequence (5′-3′)	Reaction parameters
amoA (AOB)	*amoA* 1F *amoA* 2R	GGGGTTTCTACTGGTGGT CCCCTCKGSAAAGCCTTCTTC	95 °C for 3 min × 1 cycle; (94 °C for 1 min, 56 °C for 45 s, 72 °C for 1 min) × 40 cycles; 72 °C for 10 min × 1 cycle
nirK	F1aCu R3Cu	ATCATGGTSCTGCCGCG GCCTCGATCAGRTTGTGGTT	94 °C for 2 min × 1 cycle; (94 °C for 30 s, 58 °C for 1 min, 72 °C for 1 min) × 40 cycles; 72 °C for 10 min × 1 cycle
nosZ	*nosZ*-I F *nosZ*-I R	CGCRACGGCAASAAGGTSMSSGT CAKRTGCAKSGCRTGGCAGAA	94 °C for 5 min × 1 cycle; (94 °C for 40 s, 60 °C for 40 s, 72 °C for 1 min) × 40 cycles; 72 °C for 10 min × 1 cycle

Note: AOB: Ammonia oxidizing bacteria

Table 8. Percentage contribution to N_2O emissions from nitrification and denitrification during the cover crop decomposition.

	2022						2023	
Treatment	Jun 10	Jun 16	Jul 6	Jul 14	Jul 22	Aug 2	Jun 12	Jun 23
CC Nitrification	30.5–42.7	37.0–47.3	17.1–33.3	31.0–43.0	34.1–45.2	51.6–52.6	29.0–36.8	42.4–50.9
CC Denitrification	57.2–69.4	52.6–62.9	66.9–82.8	56.9–68.9	54.7–65.8	47.3–48.3	63.1–70.9	49.0–57.7
CC+PL Nitrification	17.4–33.5	23.8–37.6	16.3–27.4	35.8–46.4	29.5–42.0	39.0–48.6	34.6–45.5	29.6–42.1
CC+PL Denitrification	66.4–82.5	62.3–76.1	72.5–83.6	53.5–64.1	57.9–70.4	51.3–61.0	54.4–65.3	57.8–70.3

Note: CC: cover crop, PL: poultry litter.

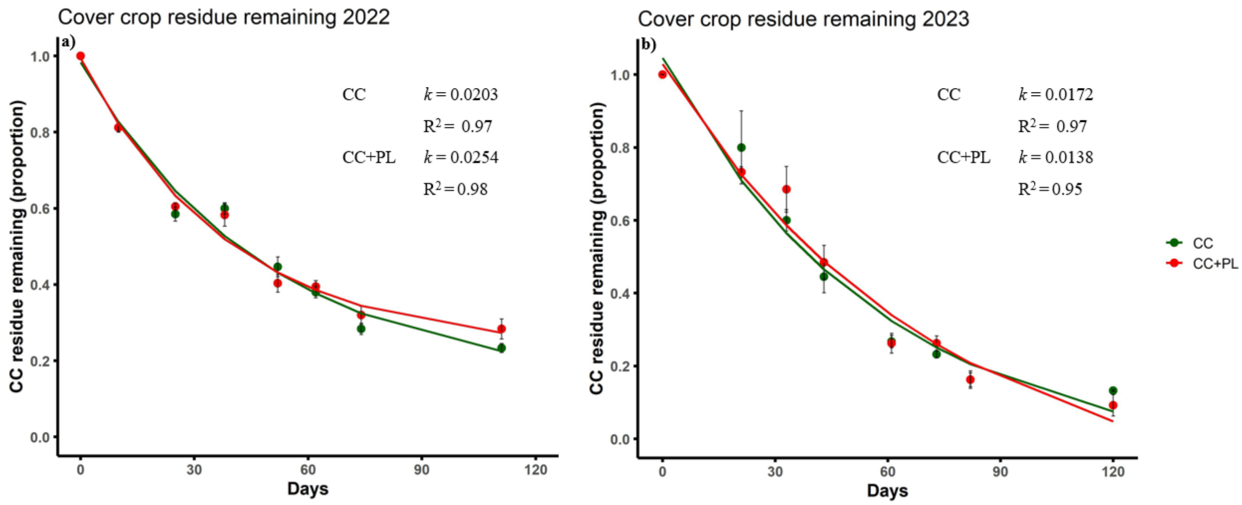

Figure 26. Proportion of cover crop residue remaining in litter bags in a) 2022, and b) 2023. Each point represents the mean of four replicates of a respective treatments at a given time. Curves are single pool first order exponential decay models fit to each cover crop residue proportion within each treatment. Error bars are the standard error. CC: cover crop, PL: poultry litter, and *k*= decay constant.

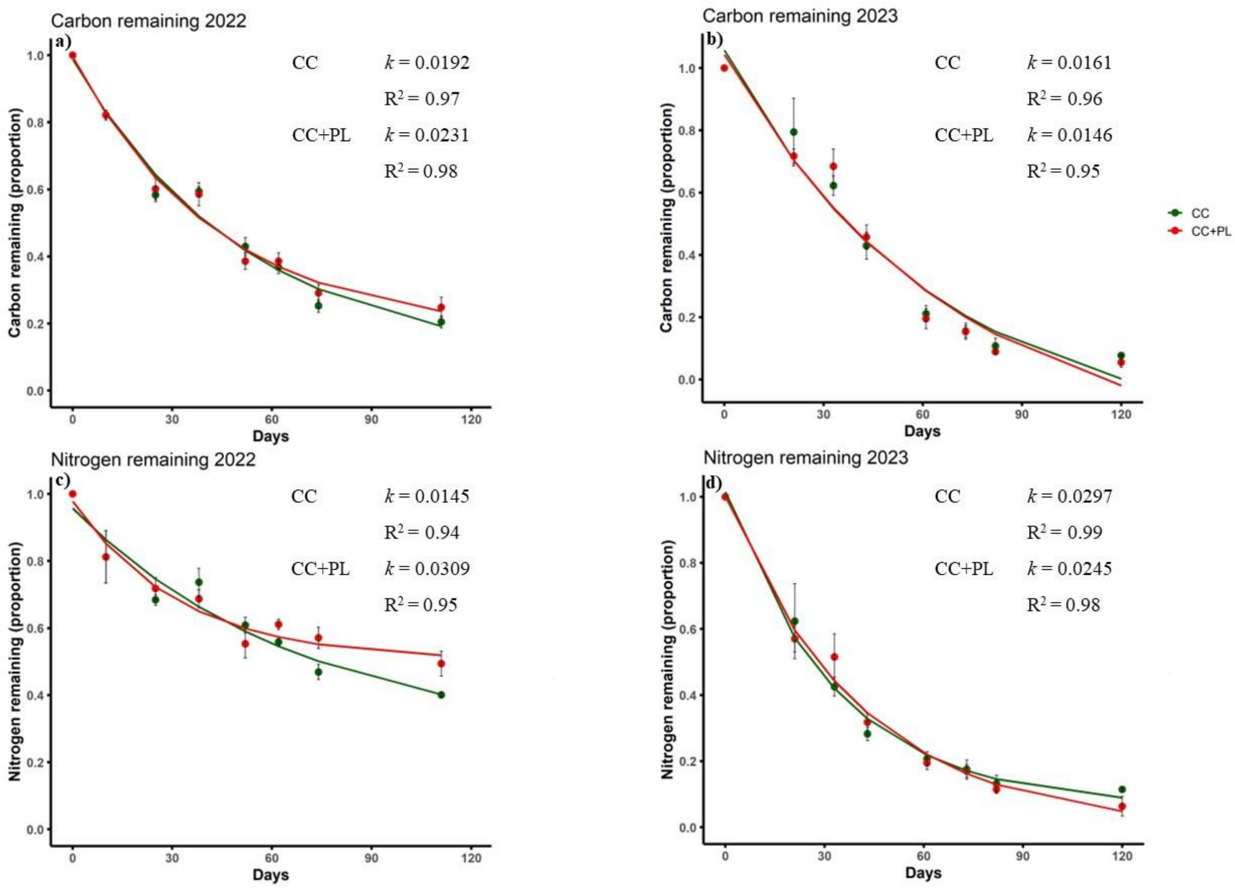

Figure 27. Proportion of carbon and nitrogen remaining in litter bags. a) Carbon remaining in 2022, b) Carbon remaining in 2023, c) Nitrogen remaining in 2022, and d) Nitrogen remaining in 2023. Each point represents the mean of four replicates of a respective treatments at a given time. Curves are single pool first order exponential decay models fit to each carbon and nitrogen remaining within each treatment. Error bars are the standard error. CC: cover crop, PL: poultry litter, and *k*= decay constant.

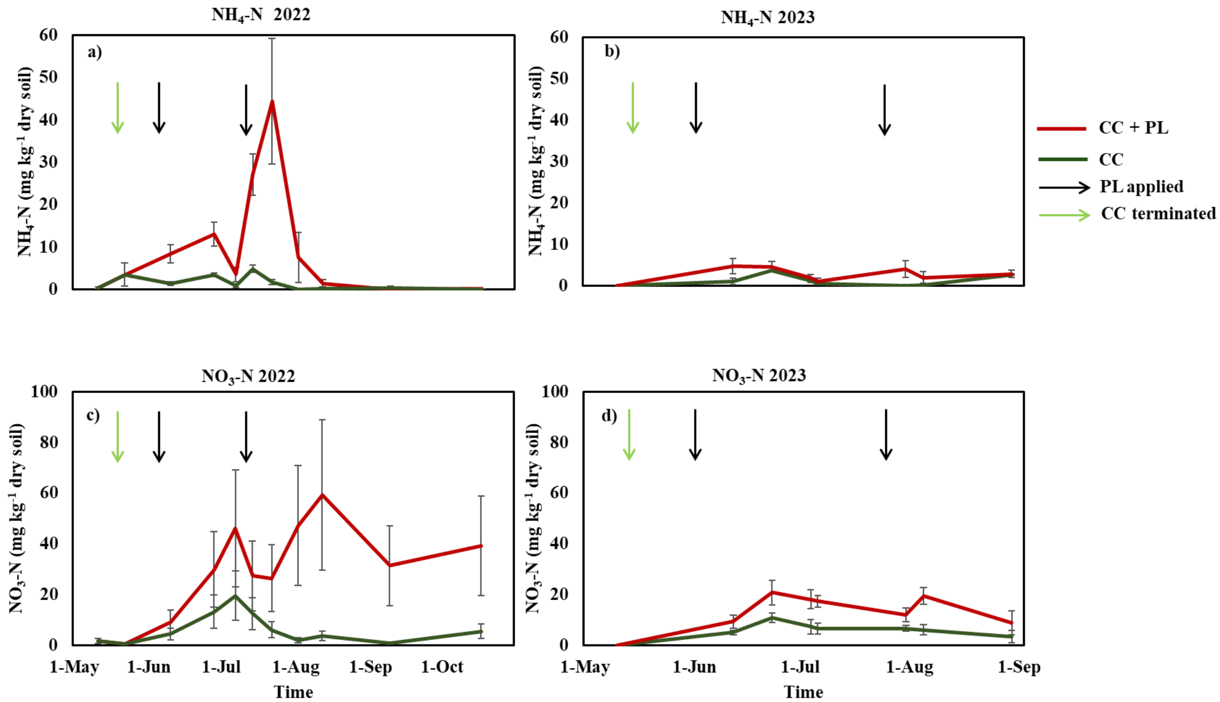

Figure 28. Soil mineral nitrogen availability during the study period (2022–2023). a) NH_4-N 2022, b) NH_4-2023, c) NO_3-N 2022, and d) NO_3-N 2023. The vertical black and green arrows represent the time of PL application and cover crop termination. CC: cover crop, PL: poultry litter.

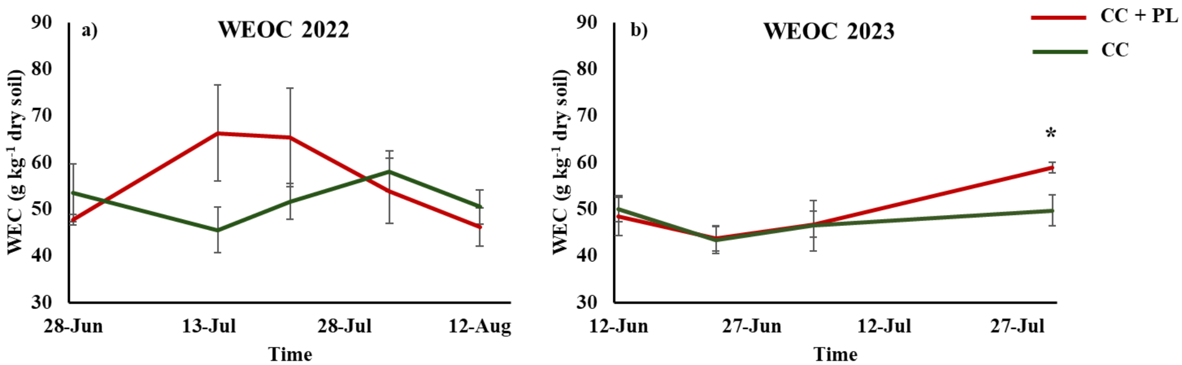

Figure 29. Water extractable organic carbon (WEOC) during the study period (2022–2023). a) 2022, and b) 2023. CC: cover crop, PL: poultry litter.

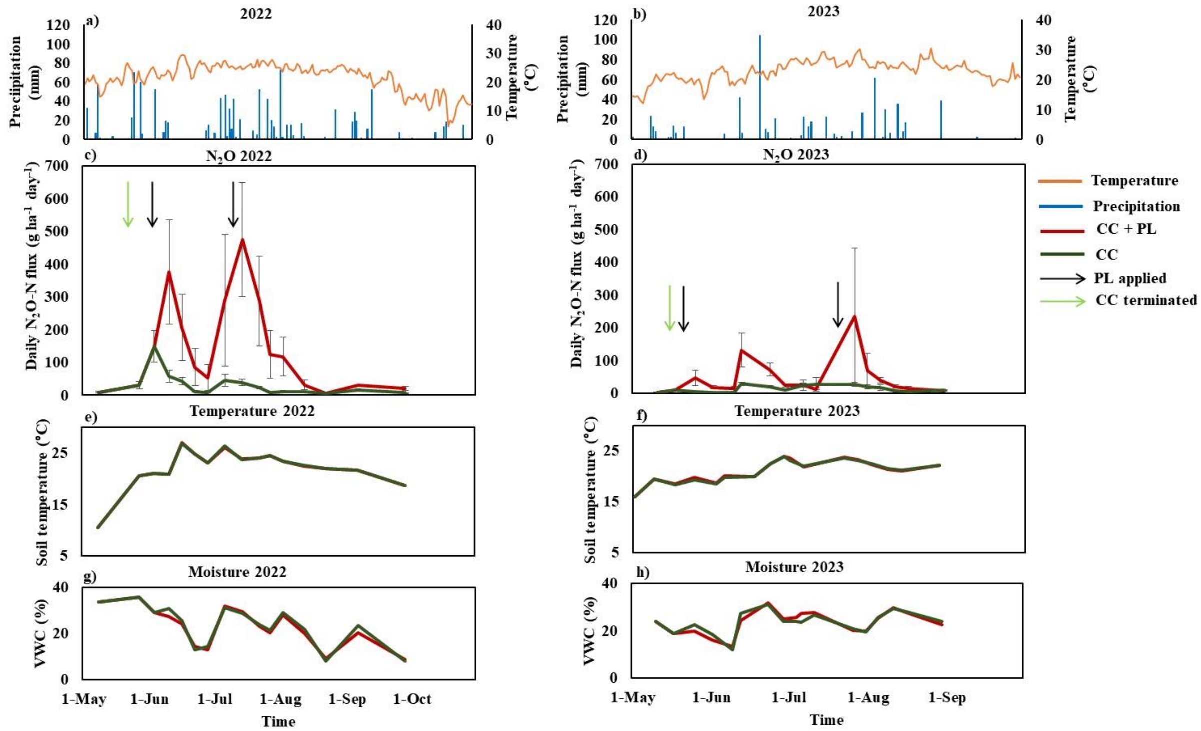

Figure 30. Daily weather, N_2O fluxes, soil temperature, and soil moisture during the study period (2022–2023). a) Daily mean air temperature and daily precipitation in 2022, b) Daily mean air temperature and daily precipitation in 2023, c) Daily N_2O flux in 2022, d) Daily N_2O flux in 2023, e) Soil temperature in 2022, f) Soil temperature in 2023, g) Soil moisture in 2022, and h) Soil moisture in 2023. The vertical black and green arrows represent the time of PL application and cover crop termination. CC: cover crop, PL: poultry litter; VWC: volumetric water content.

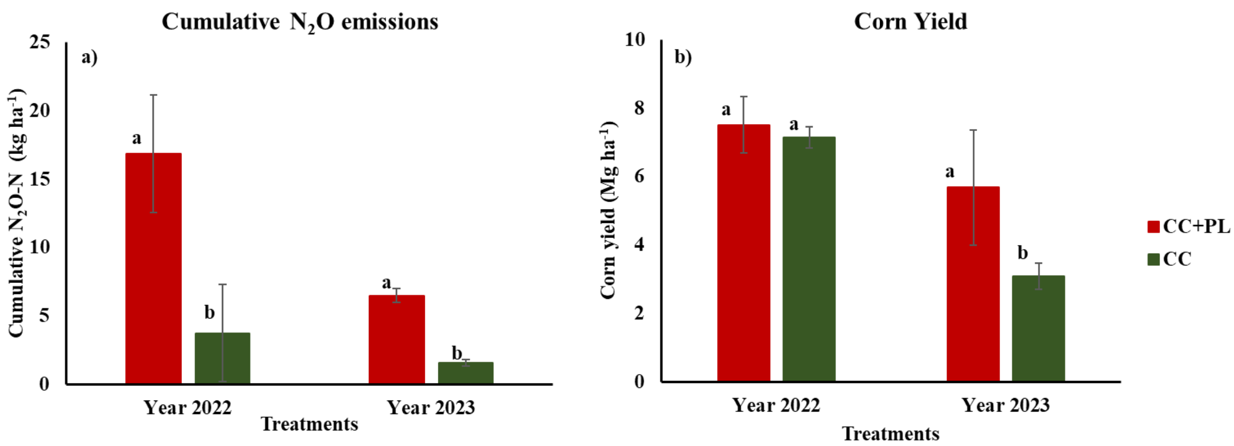

Figure 31. Cumulative N_2O emissions and corn yield during the study years (2022–2023). a) Cumulative N_2O emission, and b) Corn yield. CC: cover crop, PL: poultry litter.

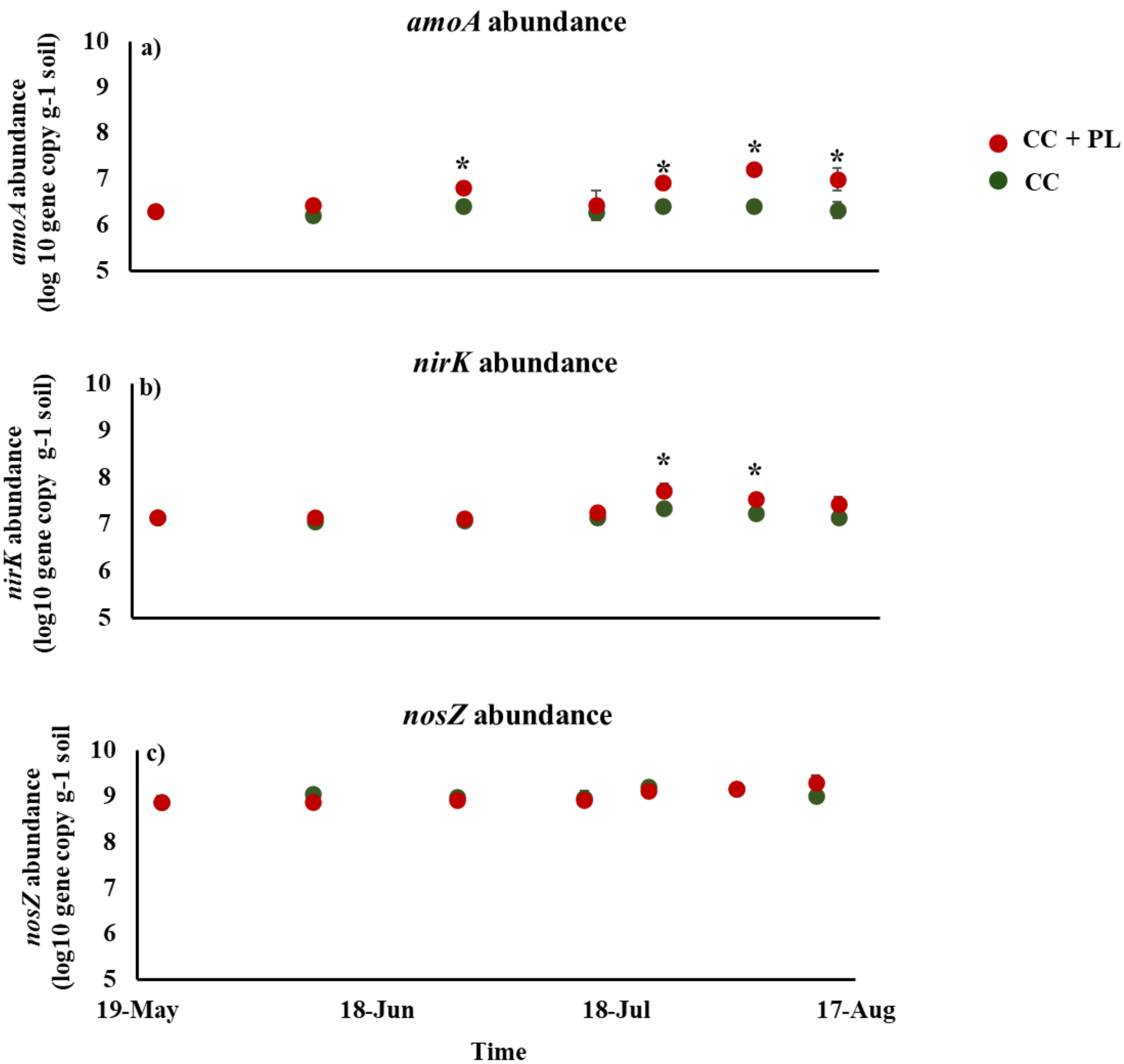

Figure 32. Microbial gene abundance during the cover crop decomposition in 2022. a) Bacterial *amoA* gene copy number, b) Denitrifier *nirK* gene copy number and, c) Denitrifier *nosZ* gene copy number. CC: cover crop, PL: poultry litter.

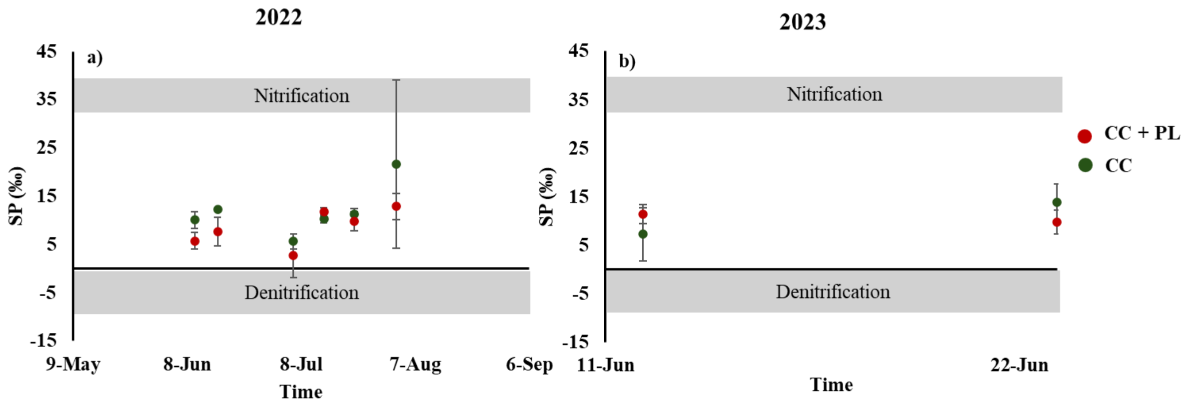

Figure 33. Nitrous oxide isotopomer site preference of $\delta^{15}N$ during the cover crop decomposition study. a) 2022, and b) 2023. CC: cover crop, PL: poultry litter.

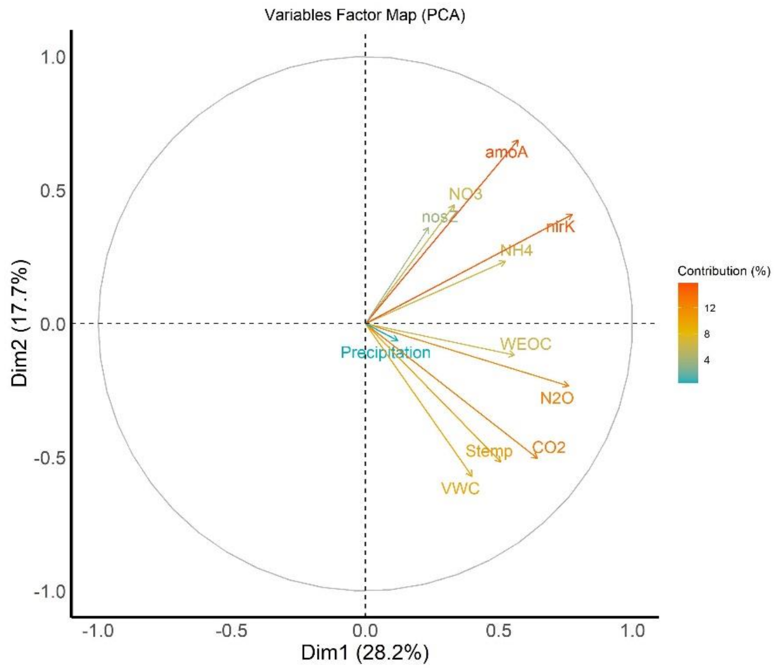

Figure 34. Principal component analysis of variables (PCA). Variables Factor Map–the variables factor map represents the contribution of different variables to the principal components.The direction and length of the arrows indicate the influence of each variable on Dim1 (28.2% of variance) and Dim2 (17.7% of variance). Variables analyzed: *nosZ*, *amoA*, *nirK*, NH_4-N, NO_3-N, water extractable organic carbon (WEOC), N_2O, CO_2, soil temperature, volumetric water content (VWC%), and precipitation. Stemp: soil temperature, CC: cover crop, PL: poultry litter.

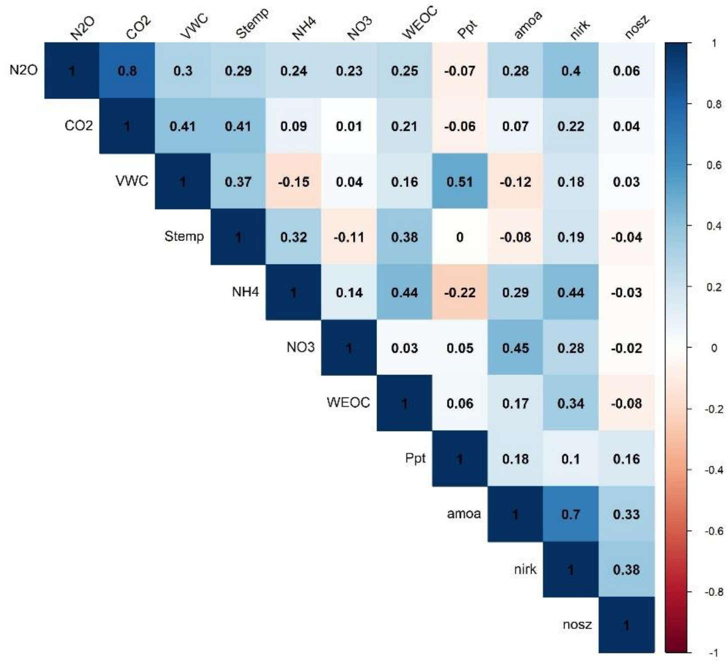

Figure 35. Pearson's correlation matrix among the measured variables. Blue and red colors represent positive and negative correlations, respectively. Variables measured: N_2O, CO_2, soil moisture (VWC%), soil temperature (Stemp), NH_4-N, NO_3-N, water extractable organic carbon (WEOC), 2 days cumulative precipitation prior to sampling (Ppt), nitrifying bacterial gene (*amoA*), denitrifying gene *(nirK*), N_2O reducing gene (*nosZ*).

Chapter 5. Soil greenhouse gas budget in a three-year organic grain rotation system

5.1 Abstract

While organic cropping systems benefit soil health, increased greenhouse gas (GHG) emissions from organic systems may offset the carbon (C) sequestration benefits. This study aimed to determine the effect of three-year corn-soybean-wheat with diverse tillage, cover crops, and poultry litter (PL) management on GHG emissions and net global warming potential (GWP). Soil samples from 0–30 cm were collected in Fall 2020 before cover crop planting and after crop harvest in Fall 2023. Soil organic carbon (SOC) of collected soil samples was analyzed using the dry combustion method. Gas samples were collected from 2021–2023 using a static gas chamber and analyzed in Gas Chromatography (GC). After three years of rotation, SOC stock increased by 30%; however, cropping systems had no significant effect on SOC stock. Across all cropping systems, net global warming potential (GWP) was negative, suggesting organic cropping systems could enhance C sequestration. However, long-term study is essential to understand the C sequestration potential of organic cropping systems.

5.2 Introduction

To meet the increasing demand for organic food and products, increasing land coverage under organic cropping system is expected (Badgley et al., 2007; Reganold & Wachter, 2016; OTA, 2023). The primary benefits associated with organic agriculture include soil health through increased carbon (C) sequestration, improved soil biological activity, and increased soil water-holding capacity, porosity, and aggregate stability (Tully & McAskill, 2020). The possible mechanism of harnessing soil health benefits is associated with incorporation of organic nutrient sources such as cover crops, crop residue, and manure (Lal, 2020). However, while the inclusion of organic inputs in organic systems enhances C sequestration and associated soil health benefits, it can alter coupled soil C and nitrogen (N) dynamics, which eventually can result in the emissions of non-CO_2 greenhouse gases (GHGs) such as nitrous oxide (N_2O) and methane (CH_4).

Nitrous oxide is a potent GHG with a global warming potential (GWP) 273 times greater than CO_2 in a 100-year time period (IPCC, 2023). Nitrous oxide emissions from organic agriculture are associated with soil management (e.g., cover crop or crop residue management, manure application, and tillage practices). The quality and management of cover crop residue could potentially affect N_2O emissions. For example, legume cover crops can increase N_2O emissions (Basche et al., 2014; Guardia et al., 2016; Muhammad et al., 2019) due to their low C:N ratio (Huang et al., 2004), while grass cover crops with high C:N ratio could reduce the emissions (Thomas et al., 2017; Guardia et al., 2016; Muhammad et al., 2019). There are evidence that a mixture of legume and grass cover crops can reduce N_2O emissions (Basche et al., 2014; Rosecrance et al., 2000; Muhammad et al., 2019). However, N_2O emissions from cover crops are further controlled by other management practices, including supplementing N from manure application and tillage management. Previous studies have reported increased N_2O emissions with manure application (Watts et al., 2011; D'Amours et al., 2023; Saha et al., 2021). In addition to organic N inputs, their tillage management practices could influence N_2O emissions. Increased N_2O emissions were reported with increased tillage intensity (Chatskikh & Olsen, 2007; Mutegi et al., 2010; Omonode et al., 2011), which is possibly due to increasing microbial access to organic inputs resulting in microbial decomposition resulting mineral N available for N_2O loss, and increasing microbial respiration, thereby depleting oxygen and creating anoxic conditions favorable for N_2O emissions through denitrification process (Lussich et al., 2024). Organic cropping systems where tillage is inevitable, particularly to suppress the weeds, could potentially offset the C sequestration benefits of organic inputs.

In addition to N_2O, CH_4 is another important GHG, with a GWP of 27–30 times greater than CO_2 in a 100-year time scale (IPCC, 2023), particularly associated with liquid dairy manure (Chadwick et al., 2000; Owen & Silver, 2015; Wattiaux et al., 2019). Methane production from manure generally occurs in anaerobic conditions (Le Mer & Roger, 2001). However, the magnitude of CH_4 emissions depends on the type and management of manure (Petersen et al., 2013). For example, CH_4 emissions from cattle manure could be higher than poultry litter (PL) (Chadwick et al., 2011). Such enhanced non-CO_2 GHGs (N_2O and CH_4) emissions associated with organic inputs and their management practices may offset the carbon sequestration benefits of organic inputs (Grandy et al., 2006; Guenet et al., 2021), therefore, can be an ecosystem service tradeoff for organic cropping systems.

Therefore, mitigating GHG emissions while ensuring improvement in soil health through organic cropping system is challenging. Limited studies have been conducted about the GHG balance encompassing both C sequestration and GHGs emissions from organic cropping systems associated with different organic inputs (e.g., cover crop, manure) and agronomic (e.g., tillage) management practices (Seufert & Ramankutty, 2017). Understanding the impact of organic management practices, whether they sequestered C in soil or offset soil C sequestration benefits through increased soil GHG emissions, is crucial for understanding the net GWP from organic systems. This study aimed to understand the soil GHG balance driven by different organic management practices, including diverse tillage systems with different cover crops and manure management.

5.3 Materials and methods

5.3.1 Research site and experimental design

Research was conducted on organically certified land of the University of Tennessee—East Tennessee Research and Education Center's Organic Crop, Knoxville, TN. The soil at the research site is characterized as Decatur silt loam (thermic Rhodic Paleudults). The climate of the research site is warm and humid, with a mean annual temperature of 15.2 °C and annual precipitation of 1319 mm.

The research was conducted with a three-year soybean (*Glycine max* L.)-winter wheat (*Triticum aestivum* L.)-corn (*Zea mays* L.) rotation in a randomized complete block (RCBD) design. In each block, three main plots (48 m × 6 m) were assigned for each crop start (soybean, wheat, and corn). Each block in the first year was planted with the main crop in the subsequent years; for example, soybean block in the first year transitioned to wheat in the second year and then corn in the third year, thereby resulting in three crop starts: soybean start (soybean-wheat-corn), wheat start (wheat-corn-soybean), and corn start (corn-soybean-wheat). This crop rotation strategy allowed the cultivation of all main crops (soybean, wheat, and corn) over all three years. Each block with each crop start was further divided into four plots for four cropping systems that differed in management practices such as tillage intensity, cover crop management, and PL application.

5.3.2 Description of cropping systems

Four different cropping systems were assigned for each main crop. The cropping systems practiced were: i) Higher tillage system (HTS), ii) Moderate tillage system (MTS), iii) Least tillage system (LTS), and iv) Low input system (LIS). The cropping systems differed regarding tillage intensity, cover crop management, and PL application. Cover crops were grown and terminated between main crops before the subsequent cash crop was planted. Three cropping systems (HTS, MTS, and LIS) of the corn and wheat phase of rotation received PL, while PL was excluded in LIS. A detailed description of the cropping systems is discussed in Chapter II and Chapter III. Brief description of cropping systems is as follows:

Higher tillage system (HTS): In this cropping system, four main crops [soybean, wheat, DC-soybean (double crop soybean), and corn] were grown in three years of rotation. This cropping system received the highest tillage intensity, with three primary tillage [offset disking (OD) to a depth of 25 cm] and three secondary tillage [pickup disking (PD) to a depth of 10 cm].

Moderate tillage system (MTS): Like HTS, four main crops were grown in this cropping system. Primary tillage was performed only to incorporate cover crops before corn planting, while three secondary tillage was practiced for planting cover crops and wheat, and no tillage (NT) was practiced before soybean and DC-soybean planting.

Least tillage system (LIS): Unlike HTS and MTS, in this cropping system, three main crops (soybean, wheat, and corn) were grown in a three-year rotation. This cropping system excluded primary tillage while retaining two secondary tillage and four NT during a three-year period.

Low input system (LIS): Unlike all other cropping systems, this cropping system excluded PL. Likewise, tillage intensity was reduced in this system, with one primary tillage before corn planting and one secondary tillage before wheat planting. Cover crops and soybeans were planted in NT plots.

5.3.3 Measurement of N_2O and CH_4 fluxes

Details on the measurement of N_2O and CH_4 fluxes were described in Chapter III. Briefly, gas fluxes were measured using a static gas chamber, as explained by Parkin & Venterea, (2010). Gas samples were collected from each experimental unit during the main crop growing season from May 2021 through September 2023. During each sampling, the chambers were closed for one hour. Headspace samples were collected at 0, 20, 40, and 60 minutes of chamber closure, and gas samples were stored at high pressure in pre-evacuated Labco exetainer vials (Labco Limited, United Kingdom) until analysis. Nitrous oxide and CH_4 gas concentrations were analyzed using a Gas Chromatography (GC) equipped with an Electron Capture Detector (ECD) and FID (Flame Ionization Detector) (Shimadzu GC-2014, Japan).

5.3.4 Soil sampling and SOC analysis

Two soil cores (0–30 cm) were collected from each experimental unit during fall 2020 (baseline – experiment initiation) and fall 2023 using a hydraulic probe (8 cm diameter). Soil samples from two cores were mixed and homogenized. Following homogenization, soil samples were air-dried, ground, and sieved through a 2-mm mesh. Soil organic carbon was analyzed using a dry combustion method using an Elementar vario Max cube CN analyzer (Hanau, Germany).

5.3.5 Global warming potential (GWP) calculation and statistical analysis

Soil organic carbon stock from each soil depth was calculated using equation (i). After three years of rotation, the change in soil organic carbon was calculated by subtracting the baseline SOC (2020) from the 2023 SOC (ii).

SOC_{stock} = SOC concentration × bulk density × soil depth × area ………………………….(i)

Where, SOC_{stock} is the soil organic C stock.

$\Delta SOC_{stock} = SOC_{stock}(2023) - SOC_{stock}(2020)$………………………………………………...(ii)

Where ΔSOC stock is the change in the SOC stock in megagrams per hectare from 2020 to 2023, covering a complete cycle of a three-year rotation.

Statistical analysis was done using R software (version 4.3.1). Linear interpolation of daily N_2O and CH_4 fluxes was done to calculate the growing season N_2O and CH_4 emissions. Note that the cumulative N_2O and CH_4 emission data only represent the main crop growing season (see Chapter III). While winter emissions of these GHGs during the cover crop growing period are often low (Basche et al., 2014) and were not captured by our measurements, our cumulative GHG estimates may underestimate yearly emissions. To evaluate the effect of cropping systems on N_2O and CH_4 emissions, cumulative N_2O emissions and CH_4 from three years were averaged across three crop starts (*i.e.,* corn-soybean-wheat, soybean-wheat-corn, and wheat-corn-soybean) for each cropping system. Then, net CO_2 equivalent global warming potential (GWP CO_2 eq) was calculated using the equation (iii).

Net GWP (CO_2 eq Mg ha^{-1}) $= -\frac{44}{12} \times \Delta SOC_{stock} + N_2O \times 273 + CH_4 \times 30$ ……………………(iii)

Where, Net GWP is the net global warming potential (GWP) in megagrams per hectare of CO_2 equivalents (CO_2 eq Mg ha^{-1}); ΔSOC_{stock} is the change in SOC in megagrams of C per hectare (from 2020 to 2023); N_2O is cumulative N_2O emissions in megagrams per hectare, and CH_4 is cumulative CH_4 emissions in megagrams per hectare; the ratio $\frac{44}{12}$ used to convert ΔSOC $_{stock}$ to CO_2 eq ΔSOC $_{stock}$; 273 and 30 represent the IPCC factors for the conversion of N_2O and CH_4 to CO_2 eq, respectively (IPCC, 2023). A positive Net GWP value represents net emissions of CO_2 in the atmosphere, while a negative value represents a sink of Ceq from the atmosphere.

Statistical analysis was done separately for ΔSOC $_{stock}$, CO_2 eq ΔSOC $_{stock}$, CO_2 eq N_2O emissions, CO_2 eq CH_4 emissions, and net GWP using a linear mixed effect model (lmer4 package), where the cropping system was a fixed effect and crop start and block were random effects.

5.4. Results and discussion

Our result exhibited no significant differences in ΔSOC $_{stock}$ among cropping systems (**Fig. 36**) ($p < 0.05$) with a value of 8.5 Mg ΔSOC ha^{-1} (range: 7.6 –10.2 Mg ΔSOC ha^{-1}) averaging across cropping systems. The least tillage system exhibited slightly higher ΔSOC $_{stock}$ (10.2 ΔSOC Mg ha^{-1}) among cropping systems, although this difference was not significant at $p < 0.05$. All cropping systems exhibited increased SOC $_{stock}$ from 2020 to 2023, with a 30% increase (averaging across cropping systems) over the baseline SOC $_{stock}$. Consistent with our result, Gryze et al. (2010) and Zani et al. (2023) reported an increase in SOC stock under organic crop rotations. The magnitude of ΔSOC $_{stock}$ reported by Gryze et al. (2010) was 1.3 Mg ΔSOC ha^{-1} yr^{-1} (averaged across research sites), whereas it was 0.31 Mg ΔSOC ha^{-1} yr^{-1} in Zani et al. (2023). These estimates are lower rates of SOC accumulation than that observed in our study (2.84 ± 2 Mg ΔSOC ha^{-1} yr^{-1} mean value across cropping systems). High Δ SOC $_{stock}$ in our study was associated with a large amount of C inputs from residues from cover crops and cash crops, and PL.

Similar to ΔSOC_{stock}, there was no significant difference between cropping systems in CO_2 eq C sequestration (Cseq) (**Fig. 37**) ($p < 0.05$). However, CO_2 eq N_2O emissions were significantly higher from HTS (12.8 Mg ha^{-1}) than LTS (8.6 Mg ha^{-1}) and LIS (2.6 Mg ha^{-1}). High CO_2 eq N_2O emissions from HTS than LTS were attributed to higher tillage intensity to incorporate organic N inputs accelerating C and N mineralization and subsequent N_2O emissions. The HTS rotation received three primary tillage (OD) during a three-year rotation. In contrast, LTS did not receive any primary tillage (OD) but received two secondary tillage (PD) and four no-till phases during a three-year rotation. However, the least CO_2 eq N_2O emissions from LIS were associated with low tillage intensity and N inputs. Unlike other cropping systems, LIS did not receive PL and practiced reduced tillage, which likely contributed to low N_2O emissions from LIS. Regardless of cropping systems, high CO_2 eq N_2O emissions were likely attributed to the coupling of C and N inputs from organic inputs (cover crop residue and PL), which enhance denitrification processes contributing to N_2O emissions (Butterbach-Bahl et al., 2013). Additionally, tillage increases microbial access to the substrate, thereby increasing mineralization and subsequent emissions. The temporary development of macropores due to tillage further enhances the escaping of N_2O from the soil layer without allowing further reduction (Dhaliwal et al., 2024a). Consistent with our result, Cavigelli et al. (2009) reported tillage effect on CO_2 eq N_2O emissions with relatively higher emissions from chisel tillage than NT (0.4 vs. 0.3 Mg ha^{-1} yr^{-1}). However, the magnitude of CO_2 eq N_2O emissions from our study was greater than reported by Cavigelli et al., 2009 (2.74 vs 0.73 Mg ha^{-1} yr^{-1}). These results suggest that N_2O emissions from organic input systems can be large and largely offset the C sequestration benefits associated with organic inputs (Grandy et al., 2006; Guenet et al., 2020).

Carbon dioxide equivalent CH_4 emissions were negative for all cropping systems with a mean value of -0.015 Mg ha^{-1}, exhibiting minor sink and did not significantly differ between the cropping systems (**Fig. 37**). Methane emissions from agriculture systems, in general, are associated with animal enteric fermentation and manure management (Dunkley & Dunkley, 2013). Across all cropping systems, we observed negative CO_2 eq CH_4 emissions, suggesting that CH_4 emissions did not offset C sequestration benefits associated with organic systems. Consistent with the result reported by a previous study (D'Amours et al., 2023), our findings suggest that an organic crop rotation system with PL as a supplement N source reduces CH_4 emissions. The negative values for CO_2 eq CH_4 further corroborate that agricultural soil, especially well-aerated and drained soil, may act as the sink for CH_4 (Saunois et al., 2020).

Three years of organic crop rotation showed negative net GWP across all cropping systems, which indicates net sink for atmospheric CO_2 eq GHG into the soil; however, cropping systems did not significantly differ in net GWP (**Fig. 37**). Our findings suggest that organic crop rotation has the potential to sequester atmospheric C as shown in Cavigelli et al. (2009). Averaging across cropping systems, net GWP from three years of rotation was -23 Mg CO_2 eq ha^{-1} (**Fig. 37**). The negative net GWP was most likely driven by soil C sequestration due to large amount of organic C inputs from cover crop and PL application. Supporting our results, Cavigelli et al. (2009) also reported a reduction in GWP from organic cropping systems compared to conventional cropping systems. Cavigelli et al. (2009) reported ~ - 0.87 Mg CO_2 eq Mg ha^{-1} yr^{-1} net GWP, a magnitude that is much lower than our study (-7.67 Mg CO_2 eq ha^{-1} yr^{-1} (averaged across cropping systems). This might be due to the inclusion of emissions associated with energy consumption during farm operations. However, in our study, net GWP was calculated based on changes in SOC $_{stock}$, CH_4, and N_2O while excluding emissions associated with farm operation.

Our study further indicates that C sink potential of organic systems can further be enhanced by mitigating soil N_2O emissions. Averaged across the cropping systems, N_2O alone could offset 32% of the CO_2 eq SOC sequestration benefits, more so intensively tilled HTS (54%) due to higher N_2O emissions but similar SOC sequestration as compared to other systems (**Fig. 38**). This underscores the importance of N_2O in holistic assessment of soils CO_2 eq emissions/sequestration potential under different management practices. For example, CO_2 eq N_2O emission was two times higher than CO_2 eq sink by SOC gain in the US corn belt, hence offsetting 100% of SOC sequestration benefits (Lawrence et al., 2021). In our study, if we could reduce N_2O emissions from HTS by 50%, C sink potential could increase by 37%, suggesting that reduction in N_2O emissions could potentially enhance C sequestration benefits.

5.5 Conclusion

The increase in SOC_{stock} from 2020 to 2023 across cropping systems suggests organic cropping systems enhanced soil C sequestration. Carbon dioxide equivalent sequestration in organic rotation systems with cover crop residue and PL incorporation can be enhanced with N_2O mitigation. Results from our study further depict that agricultural soil with cover crop and PL incorporation may be the source of N_2O but may not contribute to CH_4 emissions. In this study, although we observed an increase in SOC after three years of rotation, knowledge of the changes in different soil C fractions is crucial to understand the long-term stability of the sequestered C. Therefore, a long-term study of organic rotation systems is essential to understand the effect of organic inputs on C sequestration and the reduction of GHG emissions.

Appendix

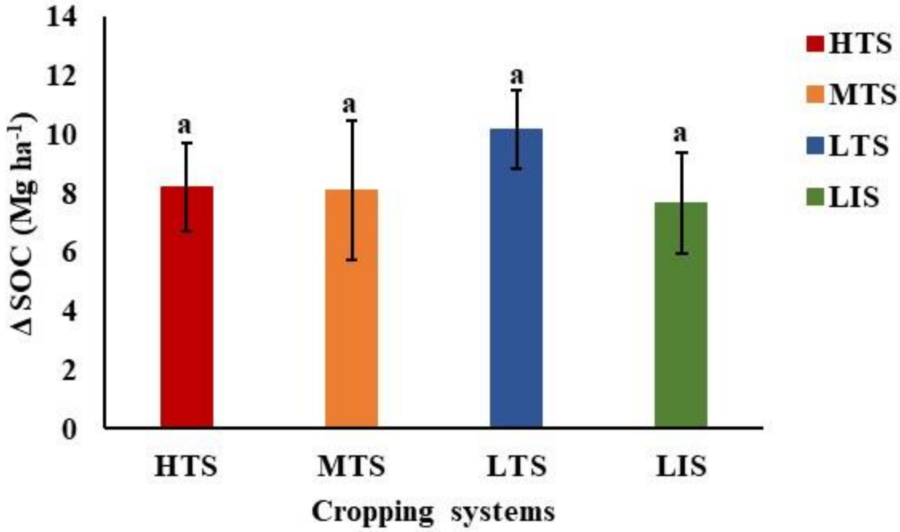

Figure 36. Soil organic carbon from 0-15 cm soil depth after three years of soybean-wheat-corn rotation. HTS: Higher tillage system, MTS: Moderate tillage system, LTS: Least tillage system, LIS: Low input system, and SOC: Soil organic carbon. "ΔSOC" represents a change in SOC from 2020 to 2023 at 30 cm soil depths. Different letters represent significant differences between cropping systems at $p < 0.05$.

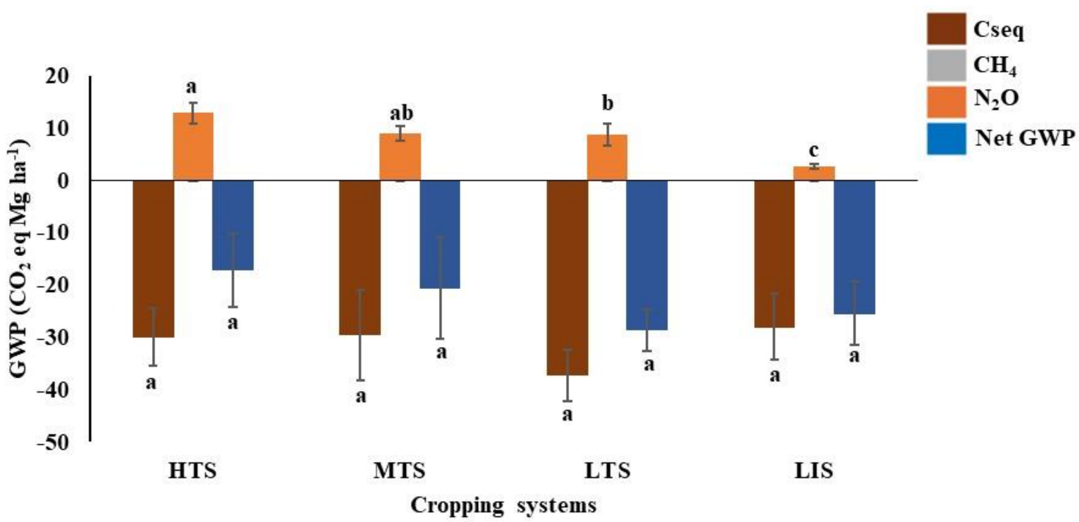

Figure 37. Global warming potential (GWP) from a three-year organic soybean-wheat-corn rotation. Cseq, CH_4, N_2O, and Net GWP represent CO_2 equivalent carbon sequestration, CO_2 equivalent CH_4 emissions, CO_2 equivalent N_2O emissions, and net global warming potential, respectively. HTS: Higher tillage system, MTS: Moderate tillage system, LTS: Least tillage system, LIS: Low input system, and SOC: Soil organic carbon. Different letters represent significant differences between cropping systems at $p < 0.05$. Note: due to a very small value, GWP for CH_4 and respective standard error bars are not visible.

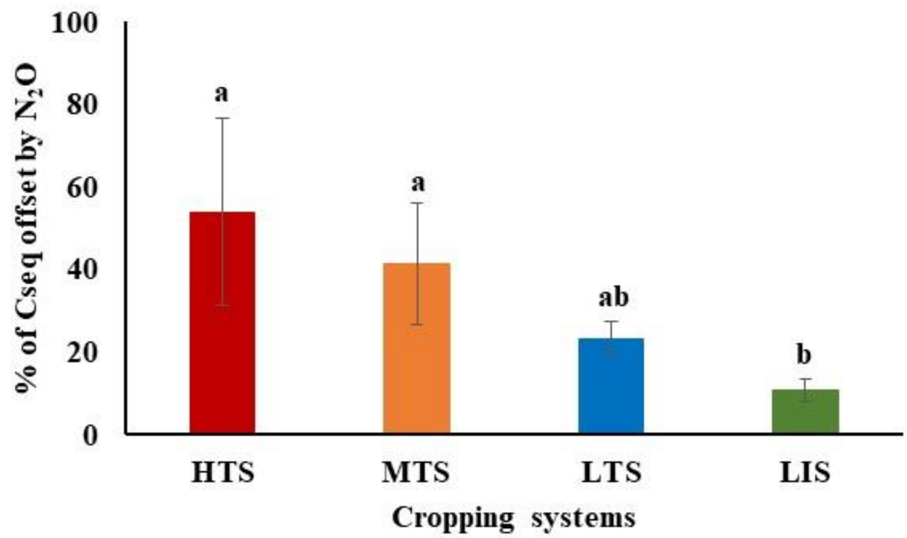

Figure 38. Percentage of carbon sequestration (Cseq) offset by nitrous oxide (N_2O) emissions from three years of organic grain rotation systems. HTS: Higher tillage system, MTS: Moderate tillage system, LTS: Least tillage system, LIS: Low input system. Different letters represent significant differences between cropping systems at $p < 0.05$.

Chapter 6. General conclusion, implications, and future research directions

6.1 General conclusion

Nitrous oxide emissions from organic cropping systems are unpredictable as it rely on microbial processes, which depend on the soil environment (e.g., soil moisture and temperature) and substrate availability. Rapid release of mineral nitrogen (N) from the decomposition of organic inputs, especially in the warm, humid climate of the southeastern US, may not synchronize with N uptake by crops and subsequently may contribute to N_2O emissions, which possibly offset the C sequestration benefits of organic inputs. Although a few studies have been conducted in temperate humid climates, limited research in warm, humid climates may underestimate N_2O emissions from organic cropping systems. The warm, humid climates may accelerate the decomposition rate of organic inputs, resulting in surplus N in soil susceptible to environmental loss, including N_2O emissions. This study provides insight into the N_2O mitigation and C sequestration potential of organic cropping systems in warm, humid climate of east Tennessee.

The results from Chapter 2 revealed a surplus N, which is partially explained by the presence of soil inorganic N (SIN) and total N concentration across the soil profile. The availability of SIN during crop phases was highly attributed to the legume cover crops and poultry litter (PL). Further, high pulses of SIN following organic inputs may result in asynchronization in N availability and crop uptake, suggesting risk to environmental losses.

In Chapter 3, we found that the corn phase of rotation was the major contributor to N_2O emissions in three years of soybean-wheat-corn rotation, which is attributed to tillage intensity and N input from legume cover crops and PL. Despite a similar amount of N input in three cropping systems (higher tillage system-HTS, moderate tillage system-MTS, and least tillage system-LTS), the highest N_2O emissions from HTS suggest that the highest tillage intensity elevates N_2O emissions. Inter-annual variability in N_2O emissions across all crop phases was attributed to weather variables.

The results from Chapter 4 revealed no significant effect of PL on the rate of cover crop decomposition; however, higher N_2O emissions from PL-applied treatments suggest that N_2O emissions were attributed to PL. Inter-annual variations in cover crop residue decomposition rate were likely attributed to cover crop composition, where the decomposition rate was higher in the cover crop mixture with a higher proportion of legume cover crops than in the mixture with a lower proportion of legume cover crops. Microbial gene abundance and N_2O isotopomer analysis depicted that denitrification was the major microbial process contributing to peak N_2O emissions.

In Chapter 5, GHG balance was measured by accounting for changes in SOC stock, N_2O, and CH_4 data from three years of crop rotation. We observed a change in SOC stock that was more significant than CO_2 equivalent emissions from N_2O and CH_4, suggesting that organic grain rotation systems are likely to enhance C sequestration.

To summarize, this research indicates that N_2O emissions from organic cropping systems might be comparable with conventional cropping systems, especially under high N input from organic sources such as PL, along with high tillage intensity. The warm, humid climate, like in the southeastern region of the US, could potentially accelerate the mineralization of organic inputs, subsequently releasing N to meet the N demand for crops. However, surplus N, as observed in Chapter II, suggests the possibility of asynchronization of N release and N uptake by the cash crops in organic cropping systems, with a potential risk of environmental loss. Our study observed that this excess N was partly lost as N_2O across all organic cropping systems.

However, emissions were lower in systems with reduced tillage, suggesting reduced tillage as a possible strategy to mitigate N_2O emissions in organic systems.

While organic cropping systems may act as N_2O source, they also promote C sequestration through higher organic inputs like cover crops and manure, as demonstrated in Chapter V. However, to maximize C sequestration benefits, N_2O emissions–which counterbalance SOC gains–should be minimized. This can be enhanced by better synchronization of N release from organic inputs and crop uptake through comprehensive management practices (tillage, cover crop, and manure management), as discussed in the implications of the study below.

6.2 Implications for mitigating N_2O emissions from transitioning organic cropping systems

Results from three years of soybean-wheat-corn rotation highlight opportunities to mitigate N_2O emissions. Reducing tillage could be a win-win for improving soil health, C sequestration, and mitigating N_2O emissions. Significantly less N_2O emission from the cropping systems with the least tillage system than the highest tillage system revealed that tillage reduction could reduce emissions. The best strategy to reduce emissions is synchronizing N release and N uptake by crops, which, from our result, might be possible through i) improving the timing of cover crop termination and subsequent cash crop planting, ii) considering a gap between top-dress and side-dress PL application, iii) use cover crop mixtures (legume + grass cover crop), which could reduce the N release through slow rate of cover crop residue decomposition.

6.3 Future research direction

Chapter 2 of my study revealed surplus N associated with organic crop rotation, particularly N input from legume cover crops and PL. Surplus N in this study was calculated based on N input from legume cover crops, and PL and N were removed from yield without accounting for environmental loss. Although we observed reduced SIN in the deep soil profile, suggesting limited leaching loss of N, our estimation of leaching loss from two sampling events may not be sufficient to interpret the results. Therefore, including runoff and leaching N loss in the N balance study, particularly in such a complex system, could provide N dynamics within organic cropping systems.

This research generally observed inter-annual variation in N_2O emissions with a decreasing trend over three years, suggesting that N_2O emissions from organic cropping may decrease over time. However, this result is from only three years, which might be confounded by several other variables, for example, weather. Therefore, long-term studies of N_2O emissions from organic cropping systems could provide insight into the possibility of mitigating N_2O emissions from organic systems.

Three years of organic crop rotation revealed the C sequestration benefits of the organic cropping system. The increase in SOC was attributed to a large amount of C added through cover crops and PL. However, in this study, we did not account for the different fractions of C accumulated in the systems, part of which might be susceptible to loss, which could potentially risk the C sequestration benefits. The long-term studies, including studies on different fractions of soil C and CO_2 equivalent loss as methane and N_2O in organic cropping systems, could help to understand the C sequestration benefits of the organic systems.

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