Perennial ground covers: A mitigation strategy to nitrous oxide (N_2O) emissions for maize cropping systems

by

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The student author, whose presentation of the scholarship herein was approved by the academic plan committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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NOMENCLATURE

BBG	bulbous bluegrass (Maize+BBG),
KBG	Kentucky bluegrass (Maize+KBG).
WCCs	winter cover crops (WCCs)

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ABSTRACT

Agricultural intensification in the U.S. Midwest has led to widespread of nitrogen (N) losses through nitrate (NO_3^-) leaching and nitrous oxide (N_2O) emissions, two processes that threaten water and atmospheric quality. Traditional winter cover crops (WCCs) have proven to be effective in reducing NO_3^- leaching but remain limited in adoption due to economic and management challenges. Perennial ground cover (PGC) systems integrate non-leguminous perennial grasses in between annual row crops offering a potentially optimized alternative that maintains continuous soil cover without requiring annual replanting. However, their influence on soil nitrous oxide emissions and soil nitrogen and carbon pools remain poorly explored.

In this study we used three PGC treatments: 1) Zea mays L. without PGC (Maize Control), 2) maize with Poa bulbosa, a summer-dormant grass commonly called bulbous bluegrass (Maize+BBG), and 3) maize with Poa pratensis, or Kentucky bluegrass (Maize+KBG) to analyze the impact of PGC on N_2O emissions and soil N and C pools. Both PGC treatments reduced interrow cumulative N_2O emissions by 38–81% relative to the control in both years ($p < 0.01$), although row-position emissions were more variable and influenced by fertilizer placement. Despite these consistent interrow reductions, field-level cumulative N_2O emissions were not significantly different among treatments in either year. Overall, our results indicate that PGC does not affect field-level soil N_2O emissions , while maintaining similar levels of plant-available N, and had no effect on maize yield.

CHAPTER 1. GENERAL INTRODUCTION: CAN PERENNIAL GROUND COVERS REPLACE TRADITIONAL COVER CROPS?

1.1 Abstract

Agricultural intensification in the U.S. Midwest has led to widespread of nitrogen (N) losses through nitrate (NO_3^-) leaching and nitrous oxide (N_2O) emissions, two processes that threaten water and atmospheric quality. Traditional winter cover crops (WCCs) have proven to be effective in reducing NO_3^- leaching but remain limited in adoption due to economic and management challenges. Perennial ground cover (PGC) systems comprising non-leguminous perennial grasses intercropped between annual row crops offer a potentially optimized alternative that maintains continuous soil cover without requiring annual replanting. However, the biogeochemical and agronomic consequences of PGC systems, particularly their influence on soil N transformations and gaseous losses, remain poorly understood. This two-year study investigates the effects of PGC integration on soil nitrous oxide (N_2O) emissions and plant-available nitrogen (NO_3^- and NH_4^+) dynamics in maize-based systems. Continuous gas flux monitoring and seasonal soil N measurements were used to quantify differences between PGC and conventional systems. Understanding these interactions is essential for assessing whether PGC systems can mitigate reactive nitrogen losses while sustaining crop productivity. The outcomes of this research aim to inform sustainable N management strategies and guide the future integration of perennial cover systems into Midwestern agriculture.

1.2 Introduction

As the global population steadily expands, ensuring food security for all remains an ongoing and formidable challenge. With projections estimating a global population of 10.3 billion by 2085, concerns persist about agriculture's capacity to meet rising food demand (UN, 2024). Projections suggest that food production will need to increase by 59% to 98% by mid-

century to maintain human well-being and prevent widespread food shortages, placing immense pressure on agricultural systems to scale up efficiently and sustainably (Sands et al., 2023).

With no expected increase in arable land, the burden of increased food production will fall on existing agricultural land. This scenario will likely intensify the use of key agricultural inputs such as synthetic fertilizers, pesticides, and irrigation water. Although unintended, these agricultural inputs can have consequences that lead to contamination of drinking water sources or air pollution (Gillette et al., 2018; Kaspar & Singer, 2015). As a result, there is a critical need to enhance the efficiency of agroecological management practices to balance high productivity with sustainable land stewardship.

One of the most pressing environmental concerns related to agriculture is its substantial contribution to air and water pollution. In particular, the extensive use of nitrogen-based synthetic fertilizers has led to elevated nitrate concentrations in waterways and made agriculture the primary anthropogenic source of nitrous oxide (N_2O) emissions (Millar et al., 2010; Fixen & West, 2002). N_2O is a potent greenhouse gas with a global warming potential approximately 300 times greater than CO_2 (Zhang et al., 2024).

Soil nitrous oxide emissions result from microbial nitrification and denitrification, processes strongly influenced by mineral nitrogen availability, particularly nitrate (NO_3^-). Elevated nitrate concentrations not only increase N_2O emissions but also drive leaching losses that contribute to water pollution issues such as hypoxic zones in the Gulf of Mexico (Robertson & Groffman, 2024; Millar et al., 2010; Jones et al., 2018). In temperate agroecosystems like Iowa, heavy fertilizer use and limited spring plant uptake leave soils vulnerable to nitrate losses during snowmelt, while simultaneously reinforcing the need for further fertilizer inputs that drive additional N_2O emissions (Ruan & Robertson, 2016). Addressing nitrogen losses while meeting

crop demand remains a major challenge (Greer & Pittelkow, 2018; Mandrini et al., 2022). Several sustainable management practices have been considered to mitigate soil nitrogen losses, with the incorporation of traditional cover crops being the most common in the Midwestern region of the U.S. (Johnson et al., 2024).

1.3 Cover crops as a tool to manage soil nitrogen losses

In the absence of active plant uptake, substantial amounts of previously applied synthetic nitrogen (N) fertilizers are lost through nitrate (NO_3^-) leaching and nitrous oxide (N_2O) emissions (Cassman et al., 2002).

Cover crops are primarily cultivated to sustain plant uptake following the harvest of annual crops and to provide soil cover that reduces erosion and nutrient losses (Getahun & Keefer, 2022). The most used categories of cover crops include grasses, legumes, non-legume broadleaves, and brassicas (Dabney et al., 2010). A suitable cover crop largely depends on management objectives, climatic conditions, and annual crop rotation (Snapp et al., 2005). In the U.S. Midwest, grass winter cover crops, such as winter rye, are most widely adopted due to their winter hardiness and capacity to scavenge residual nutrients, which is why they are often referred to as “catch crops” (Kaspar et al., 2011). In addition, grass cover crops contribute to higher soil carbon-to-nitrogen ratios, enhance soil microbial activity, and substantially reduce nitrate leaching losses (Kaspar et al., 2011; Shu et al., 2021; Thapa et al., 2018).

Extensive research has established cover crops to be an effective management strategy to control soil N losses, particularly nitrate losses through leaching(Kaspar & Singer, 2015; Thapa et al., 2018). Although influenced by planting date and shoot biomass, a meta-analysis by Thapa et al. (2018) concluded that non-leguminous cover crops, also commonly known as winter cover crops (WCC) reduced nitrate leaching by 56% on average across 27 studies. Leguminous cover

crops, due to their rich N composition, can become a source of nitrogen susceptible to leaching after termination, before the full establishment of the cash crop. As a result, some suggest a companion crop to close the gap between cover crop termination and cash crop establishment (usually 2–6 weeks) (Thapa et al., 2018). This implies that, although cover crops can efficiently reduce nitrate leaching, their fate after termination should be well calculated, as for they induce the N losses instead of preventing them. Across multiple studies, non-leguminous cover crops' root systems have been identified as the main factor contributing to their efficiency in reducing nitrate leaching. Their extensive roots can simultaneously provide labile carbon and effectively scavenge residual N, using it for shoot biomass production and thereby increasing soil microbial activity and N immobilization (Dabney et al., 2010; Kaspar et al., 2011; Thapa et al., 2018).

Unlike nitrate, soil gaseous losses occur in smaller amounts, but their impact on the environment remains significant, particularly for soil nitrous oxide. Nitrous oxide is a greenhouse gas (GHG) with a warming potential approximately 300 times greater than carbon dioxide, and in the U.S. alone, agriculture is responsible for roughly 75% of total nitrous oxide emissions (United States Environmental Protection Agency, 2025). Nitrogen fertilizer applications enhance plant growth and significantly increase grain yields. However, although unintentional, the application of nitrogen-based fertilizer is the primary driver of increased nitrous oxide emissions from agricultural systems (Hoben et al., 2010; Millar et al., 2010). The mineral nitrogen remaining in the soil profile during fallow periods provides additional substrate to soil microbes and is potentially lost as nitrous oxide, increasing anthropogenic GHG emissions (Bremner, 1997; Behnke et al., 2022; Morse & Bernhardt, 2013). Due to their efficiency in soil residual nitrogen uptake and enhanced microbial N immobilization, non-leguminous cover crops

are commonly suggested as a solution to mitigate soil nitrous oxide emissions (Johnson et al., 2024).

While the role of cover crops in reducing soil nitrate availability is well established, their influence on soil nitrous oxide emissions is far less consistent, with studies reporting mixed and often contradictory results (Basche et al., 2014). Nitrous oxide emissions from soils are a natural outcome of microbial activity, yet agricultural soils remain the dominant anthropogenic source of this potent greenhouse gas due to increasing N-fertilizer inputs (Liang & Robertson, 2021). Among the microbial processes involved, both nitrification and denitrification contribute to N_2O production, but denitrification is generally considered the primary pathway in managed agricultural systems (Pan et al., 2022).

Soil temperature, bulk density, and texture are well-established regulators of denitrification rates. However, when these physical factors remain relatively constant, nitrous oxide (N_2O) emissions are primarily controlled by the availability of nitrate (NO_3^-), labile carbon substrates, and soil oxygen and water dynamics (Senbayram et al., 2009).

Soil denitrification is a microbial respiratory process in which facultative anaerobic bacteria reduce nitrate or nitrite into gaseous forms of nitrogen, predominantly nitrous oxide (N_2O) and dinitrogen (N_2). This process occurs under oxygen-limited (anaerobic) conditions, and the relative proportions of N_2O versus N_2 produced depend strongly on oxygen availability. Carbon substrates serve as the required electron donors for microbial respiration, making soil mineralizable carbon a key limiting factor in the denitrification pathway (Weier et al., 1993; Richardson et al., 2009). In the absence of oxygen, nitrate functions as the alternative terminal electron acceptor. Complete reduction of nitrate yields N_2, whereas N_2O, an intermediate of this process, accumulates when reduction is incomplete. Thus, N_2O production is jointly controlled

by oxygen availability, nitrate concentrations, and the supply of labile carbon in soils (Firestone & Davidson, 1989; Senbayram et al., 2009).

Labile carbon exerts a dual control on denitrification outcomes. In nitrate-rich, anaerobic soils, elevated labile carbon concentrations enhance microbial activity, thereby increasing total denitrification rates and absolute N_2O production, but also facilitating complete reduction to N_2 (Almaraz et al., 2024). Conversely, under conditions of limited carbon availability, denitrification rates decline overall, but the process is more likely to stall at intermediate steps, leading to a higher proportion of N_2O emissions relative to N_2 (Weier et al., 1993). Under strictly anaerobic conditions, denitrification tends toward completion, resulting predominantly in N_2 production (Senbayram et al., 2009; Almaraz et al., 2024). Collectively, these dynamics underscore that soil denitrification is governed primarily by the interaction of oxygen status, nitrate concentration, and mineralizable carbon availability.

Non-leguminous cover crops mitigate nitrate availability in soils by scavenging residual mineral nitrogen, thereby lowering substrate supply for denitrification and reducing potential N_2O emissions. This nitrate uptake provides a strong mechanistic basis for the role of cover crops in mitigating soil N_2O emissions (Basche et al., 2014; Foltz et al., 2021). However, cover crops also contribute additional labile carbon inputs, which may stimulate microbial activity and increase denitrification, potentially offsetting reductions in N_2O emissions. This tradeoff between nitrate removal and carbon addition helps explain the mixed outcomes reported in the literature. For instance, Foltz et al. (2021) demonstrated that rye cover crops decreased peak N_2O emissions by 39–95% in a year-round field study integrating model, field, and laboratory approaches. Similarly, a meta-analysis of 26 studies and 106 observations found that cover crops had a 40% probability of reducing, but a 60% probability of increasing, N_2O emissions (Basche et al.,

2014). More recently, O'Brien et al. (2022) concluded that cover crops have no consistent effect on soil N_2O emissions, further illustrating the complexity of cover crop–denitrification interactions.

Although widely recognized as one of the most effective agricultural conservation practices to mitigate environmental pollution, adoption of cover crops in the U.S. Midwest remains relatively low due to a range of barriers (Plastina et al., 2018). Many of these barriers are either directly or indirectly financial. The initial establishment and integration of cover crops into existing farming systems require a substantial economic investment that may not be feasible for all producers (Arbuckle & Ferrell, 2012; Singer et al., 2005). Establishment costs include the annual purchase of seed, the labor and machinery expenses associated with planting, and the costs of terminating cover crops prior to planting the main cash crop. Moreover, while cover crops provide important ecosystem services, they rarely generate direct monetary returns for farmers, which limits their perceived value as an investment (Roesch-McNally et al., 2018; Ranjan et al., 2019).

In addition to financial considerations, agronomic challenges also play a role in low adoption rates. For example, the nutrient scavenging efficiency of cover crops has been linked in some cases to yield drag in subsequent cash crops, particularly when cover crop management is not well aligned with local environmental and cropping conditions (Thapa et al., 2018; Wortman et al., 2012). Narrow planting and termination windows further complicate management, particularly in the Midwest where growing seasons are short and weather variability limits flexibility (Singer et al., 2005). Despite these challenges, cover crops are well documented to reduce soil erosion, decrease nitrate leaching, and, in some systems, mitigate nitrous oxide emissions (Basche et al., 2014; Thapa et al., 2018). However, adoption requires that farmers

make repeated annual investments in seed and management, adjust their operations to accommodate cover crop planting and termination, and accept that the practice provides little to no direct financial return.

Given these challenges, achieving mass agricultural production without environmental degradation will require exploring more practical alternatives that retain the environmental benefits of cover crops while reducing the financial burden on producers. Perennial ground covers (PGCs) are similar to cover crops but incorporated into farming systems more strategically.

1.4 Perennial ground covers as an alternative to nitrogen losses

Perennial ground covers (PGCs), also referred to as living mulches, are perennial, primarily non-leguminous grasses established between the rows of annual crops. This intercropping system integrates perennial species within conventional annual cropping systems (Figure.1). Living mulch systems are commonly used in perennial fruit production (e.g., apple orchards) but have also been adapted to annual row crops to influence weed suppression and regulate soil water and nutrient dynamics (Qian et al., 2015; Westbrook et al., 2021). Integrating PGCs into row-cropping systems has been proposed as a strategy to simultaneously enhance productivity and environmental sustainability. Similar to non-leguminous cover crops, PGC systems provide a wide range of ecosystem services. For example, perennial vegetation has been shown to increase water infiltration by 11–19 times and reduce sediment loss by at least 90% (Baker et al., 2021; Helmers et al., 2012). Moreover, PGC systems maintain year-round soil cover, including during early spring fallow periods, thereby reducing soil erosion and nitrate leaching (Moore et al., 2019). Enhanced infiltration also limits anaerobic microsites, reducing

denitrification potential and possibly decreasing soil nitrous oxide (N_2O) emissions (Basche et al., 2014; Schlautman et al., 2021).

Consistent carbon (C) inputs play a pivotal role in sustaining soil health and fertility by promoting soil organic matter (SOM) formation (Gilmullina et al., 2023). Both winter cover crops (WCCs) and PGC systems reduce nutrient and SOM losses through runoff, while senesced or harvested biomass provides significant annual C inputs that enhance SOM accumulation and carbon sequestration (Kaspar et al., 2011; Schlautman et al., 2021). Acting as living mulches, PGC systems also stabilize microbial activity by providing continuous inputs of labile C (Moore et al., 2019). These consistent carbon inputs promote nutrient retention and microbial function but may also introduce tradeoffs through increased competition with the cash crop for nutrients and water.

Nutrient competition remains one of the greatest challenges to PGC adoption. While PGCs effectively scavenge residual nitrate and contribute organic C to the soil, they can also limit nitrogen (N) availability for the primary crop. For example, maize–PGC systems have exhibited chlorosis and stem thinning symptoms indicative of N deficiency (Flynn et al., 2013). Similarly, light competition can trigger a shade avoidance response (SAR) in cash crops, leading to elongated stems, narrower leaves, and imbalanced shoot-to-root ratios (Schlautman et al., 2021; Carlson, 2018). Water competition has also been observed: winter rye regrowth in spring reduced water availability for wheat (Johnson, 2020), and decreased soil moisture was reported in living mulch systems (Sanders et al., 2017). Together, these findings highlight that nutrient, light, and water competition can constrain cash crop productivity, emphasizing the need for optimized management practices that enhance crop compatibility.

Yield drag remains a central concern for both WCC and PGC systems. In WCC systems, yield reductions ranging from 0–84% have been reported (Carlson, 2018). Yield responses under PGC systems, however, are more variable. Some studies report no maize yield reductions over two years (Chen et al., 2022), while others observed only initial penalties with recovery in later years (Bartel et al., 2017). In central Iowa, PGC systems have produced equivalent or higher maize yields than conventional systems by the fourth year (Wiggans et al., 2012). Conversely, modeling studies have predicted yield reductions of up to 19%, primarily due to light competition, though nutrient and water competition also contributed (Bartel et al., 2022). Similarly, Kimmelshue et al. (2022) observed a 50% yield reduction under PGC systems. These contrasting results suggest that the success of PGC integration is highly dependent on system design, crop compatibility, and management precision.

The U.S. Midwest Corn Belt was historically dominated by perennial vegetation such as tallgrass prairie, and the adoption of PGC systems represents a means of reintroducing elements of that ecological legacy while supporting modern agricultural production (Kaul & Wilsey, 2020). Strategically establishing PGC species between maize rows provides year-round cover, enhances soil microbial activity, increases infiltration, and improves nutrient cycling (Schlautman et al., 2021). Unlike WCC systems, PGCs do not require annual termination or replanting, minimizing time sensitivity and reducing labor costs (Moore et al., 2019). Current research is focused on minimizing interspecific competition and yield penalties through breeding and selecting compatible perennial grasses, maize, and soybean varieties (Chen et al., 2022; Bartel et al., 2022; Kimmelshue et al., 2022). An optimized PGC system thus represents a promising and economically viable alternative to WCC, combining the conservation benefits of perennial vegetation with the productivity demands of conventional row-crop agriculture.

1.5 Rationale for this study

Agriculture is under increasing pressure to balance the dual demands of food security and environmental sustainability. In the U.S. Midwest, maize–soybean systems are among the most productive globally, yet they contribute disproportionately to nitrogen losses in the form of nitrate leaching and nitrous oxide emissions. These losses not only degrade water quality, contributing to hypoxic conditions downstream, but also intensify greenhouse gas emissions increasing climate change. Addressing these challenges requires rethinking how agricultural landscapes are managed to retain nutrients while maintaining profitability and yield stability.

Winter cover crops (WCCs) have long been promoted as a key conservation practice for reducing nitrogen losses. They efficiently scavenge residual soil nitrate during fallow periods and improve soil structure, yet their adoption remains low due to the recurring costs of establishment, termination, and labor, coupled with limited economic return. Furthermore, WCC effects on nitrous oxide emissions are inconsistent—while nitrate uptake often lowers substrate availability for denitrification, the additional labile carbon inputs from decomposing cover crops can enhance microbial activity and increase N_2O emissions. These mixed outcomes suggest that while WCCs provide important ecological benefits, they are not a one-size-fits-all solution.

As of perennial ground covers (PGCs) represent a more sustainable and potentially cost-effective evolution of the cover cropping concept. By maintaining a living mulch of perennial grasses between rows of annual crops, PGC systems ensure year-round soil cover and consistent carbon inputs without the need for annual replanting. This continuous vegetative cover may stabilize soil nitrogen cycling by promoting N immobilization and improving infiltration while simultaneously reducing nitrate leaching and erosion. However, the implications of PGC systems

for nitrous oxide production are uncertain. The coexistence of perennial roots and cash crop roots may alter oxygen diffusion, moisture distribution, and carbon availability—factors that regulate the balance between complete and incomplete denitrification.

Despite growing interest, data on the biogeochemical outcomes of PGC systems remain limited, especially regarding N_2O fluxes and soil mineral N dynamics in Midwestern row-crop systems. This study, conducted over two years at Iowa State University, was designed to address this knowledge gap by quantifying how PGC integration affects (1) nitrous oxide emissions and (2) plant-available nitrogen in the form of nitrate (NO_3^-) and ammonium (NH_4^+). Through continuous flux monitoring and soil sampling across seasons, this research seeks to identify whether PGC systems can maintain productive conditions while mitigating environmental losses of reactive nitrogen.

Ultimately, this work contributes to a broader effort to develop regenerative and climate-resilient agricultural systems. By understanding how PGC systems influence soil nitrogen cycling and gaseous emissions, the study provides insights necessary to assess their feasibility as a practical, scalable alternative to traditional cover crops in the U.S. Corn Belt. The findings will help determine whether perennial ground covers can meet the goals of environmental protection, soil health, and high-yield agriculture in intensively managed landscapes.

1.6 References

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1.7 Figures

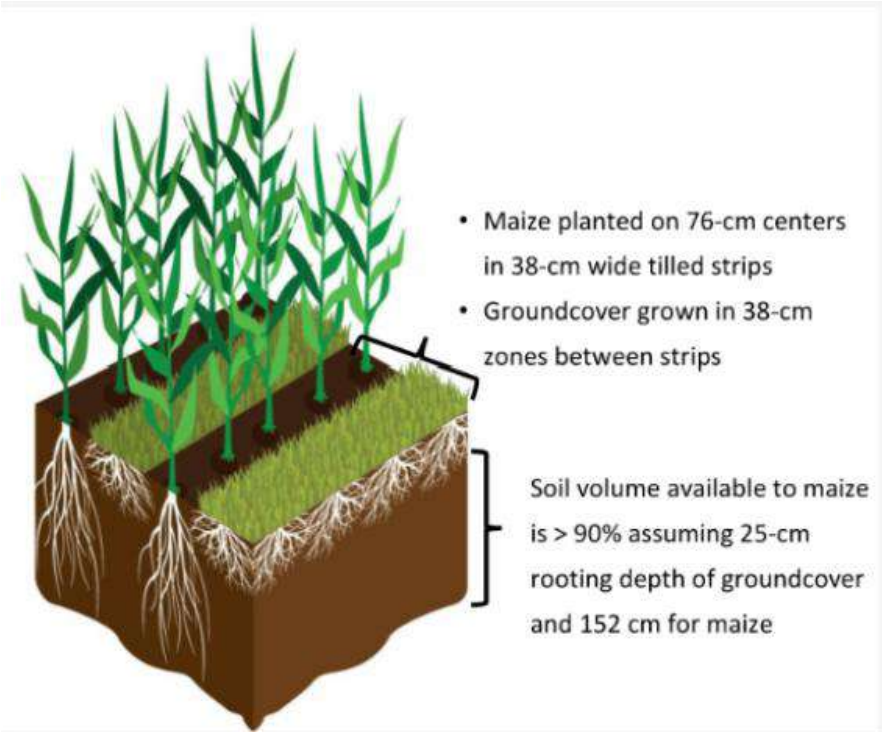

Figure 1.1 One version of the perennial groundcover (PGC)with description and dimensions described. Figure adapted from Moore et al. 2019

CHAPTER 2. PERENNIAL GROUNDCOVER EFFECTS ON N_2O EMISSIONS IN CONTINUOUS MAIZE CROPPING

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2.1. Abstract

Nitrous oxide (N_2O) emissions from agricultural soils remain a major contributor to greenhouse gas emissions and represent a significant challenge for sustainable maize and soybean production in the Midwest US. Perennial ground cover (PGC), or perennial grasses growing between these main cash crops, are capable of maintaining continuous plant cover while sustaining grain crop production. Our objectives were to quantify the impact of three systems ranging in PGC intensity on soil N_2O emissions and dynamic soil carbon (C) and nitrogen (N) pools seasonally (nitrate, ammonium, extractable organic, and microbial biomass). Our gradient of PGC intensity included three treatments: 1) Zea mays L. without PGC (Maize Control), 2) maize with Poa bulbosa, a summer-dormant grass commonly called bulbous bluegrass (Maize+BBG), and 3) maize with Poa pratensis, or Kentucky bluegrass (Maize+KBG).

Both PGC treatments reduced interrow cumulative N_2O emissions by 38–81% relative to the control in both years ($p < 0.01$), although row-position emissions were more variable and influenced by fertilizer placement. Despite these consistent interrow reductions, field-level cumulative N_2O emissions were not significantly different among treatments in either year. Overall, our results indicate that PGC does not affect field-level soil N_2O emissions , while maintaining similar levels of plant-available N, and had no effect on maize yield.

[1] This chapter is a work in progress. The content may differ from the published version.

2.2. Introduction

Agricultural soil nitrous oxide (N_2O) emissions remain a major environmental burden for much of the world, including Midwest US Zea mays L. (maize) production in Midwest US. N_2O is a key contributor to stratospheric ozone depletion and a potent greenhouse gas (Parkin and Kaspar, 2006; Millar et al., 2010; Cavigelli et al., 2012), so mitigating agriculture N_2O emissions is critical to making agriculture sustainable. In agricultural soils, N_2O fluxes primarily driven by nitrification and denitrification which are regulated by soil moisture, temperature, organic carbon (C), and inorganic nitrogen (N) in soils(Weier et al., 1993; Cavigelli et al., 2012; Mitchell et al., 2013; Zhang et al., 2024a). These drivers, and their relative importance, however, fluctuate across time and space; and thus soil N_2O emissions are known for high spatial and temporal variability (McDaniel et al., 2017; Zhang et al., 2024b; Yang et al., 2026). Management practices have a vital role in exacerbating or mitigating soil N2O emissions.

Management practices that reduce excess bioavailable N or modify soil water dynamics have the potential to mitigate N_2O emissions. Traditional winter cover crops, such as cereal rye (Secale cereale), have shown some potential to mitigate soil N2O emissions (Parkin et al., 2006; Jarecki et al., 2009; Basche et al., 2014b). However, the effectiveness of cereal rye depends on complex interactions with management and environmental conditions and can even exacerbate soil N_2O emissions when high nitrogen availability coincides with anaerobic conditions and sufficient carbon inputs to stimulate denitrification (Parkin et al., 2016; Reicks et al., 2021). However, the uncertainty in soil N2O mitigation, combined with the lack of adoption of winter cover crops in the Midwest US (Plastina et al., 2020; Sawadgo and Plastina, 2021) have led researchers and farmers alike to look toward alternatives.

Perennial ground cover (PGC), or non-harvested perennial plants growing in the interrow between maize, offers an appealing alternative for a few reasons. First, PGC is planted once and can overwinter multiple years reducing the need to plant and terminate winter cover crops in autumn and spring, respectively (Snapp et al., 2005; Flynn et al., 2013; Basche et al., 2014a). Second, some PGC species do not even require suppression, such as Poa bulbosa (bulbous bluegrass or BBG), which enters dormancy in mid-June (Galland et al., 2026). Third, PGC roots, although dormant at times, provide greater spatial and temporal coverage than traditional winter cover crops, which can help reduce excess soil moisture and bioavailable N. This may potentially tighten the N cycle and thereby mitigate soil N2O emissions.

PGC systems, also sometimes called “living mulch”, have been found to provide similar benefits to winter cover crops with relatively little trade-offs with crop yield. Using a clover as PGC, researchers found it reduced nitrate-N leaching from 31 to 75% (Ochsner et al., 2010a). This N loss reduction, Ocshner et al. (2010) found, was partially due to decreased soil moisture (and drainage). Other studies using different PGC species have observed similar soil moisture decreases, but find that this reduction is typically short-lived, as PGC tends to go dormant or is chemically suppressed (Banik et al., 2020; Ginakes et al., 2020). Perennial rooting systems PGC could also enhance water dynamics by improving infiltration by modifying soil structure and aggregation, either directly or indirectly via soil microorganisms.

The scant studies on PGC in maize have shown some benefits soil health – particularly increasing soil biomass and/or activity. The increased root mass and extended supply of C via rhizodeposits is thought to be the main mechanisms driving these benefits of PGC. Indeed, PGC has been shown to increase soil microbial biomass and N mineralization (Banik et al., 2020b; Ginakes et al., 2020), and even promote greater microbial diversity (Li et al., 2023; Sui et al.,

2025) However, relatively little is known about how these interacting benefits influence N2O emissions.

There are too few studies on PGC (or living mulch) and soil N2O emissions in general, but particularly with the newer grass species. Only two studies have looked at soil N2O emissions with leguminous clovers as living mulch system with maize (Turner et al., 2016; Peters et al., 2020a); and both of these studies showed that soil N2O emissions were 30 to 240% greater than maize alone. While legumes are thought to supply biologically fixed N, and reduce need for N fertilizer for maize, these studies suggest that the increased organic N and perhaps greater biological activity increase soil N2O in PGC or living mulch systems compared to without. Could more N-demanding PGC species – like BBG or turf-like grasses – perform better than leguminous PGCs and decrease soil N2O emissions?

We designed an experiment to quantify the impact of three systems ranging in PGC intensity – or in other words, a spectrum of greater PGC root biomass and active growth (Figure 2.1) – on soil N_2O emissions and dynamic soil C and N pools. Our gradient of PGC intensity includes three treatments: 1) maize without PGC (Maize Control), 2) maize with bulbous bluegrass (Maize+BBG), and 3) maize with unsuppressed Poa pratensis, or kentucky bluegrass (Maize+KBG). While we would not recommend a farmer to use Maize+KBG without some chemical suppression of the PGC, because it would be too competitive with maize (Galland et al. 2026), it does reflect the maximum PGC intensity and prioritizes all other ecosystem services than grain production and a goal. We sampled soil N2O emissions frequently in the row and interrow zones of the cropping of the PGC system over two years (Figure 2.1); we also sampled soil extractable nitrate, ammonium, organic C, organic N, and microbial biomass in these zones three times a year. We hypothesized that with PGC intensity would reduce residual ammonium

and nitrate but increase salt-extractable organic C and N and microbial biomass, and reduce soil N2O emissions. And that these effects would be strongest in the interrow zone (where the PGC is growing).

2.3 Methods

2.3.1. Site description, management, and experimental Design

This study was carried out at the Iowa State University Agronomy and Agricultural Engineering Research Farms at the Sorensen field located in Boone, IA (42.00944° N, −93.74111° W). The site is characterized by soils developed under Wisconsin glacial till, with dominant soil series consisting of 61% Webster clay loam and 39% Nicollet loam (USDA NRCS, 2023). These soils share similar physical characteristics and high productivity potential when properly managed, although their drainage characteristics differ, with Webster classified as poorly drained and Nicollet as somewhat poorly drained. The 50-year climate record at nearby weather station show a mean annual temperature of 8.3 ± 0.9 °C (mean ± standard deviation) and mean annual precipitation of 945 ± 214 mm (Iowa Environmental Mesonet, 2025). Averaged across all treatments, positions replications, soils at the study site had a mean pH 6.5, a cation exchange capacity of 16.9 meq 100 g-1, and 2.54 % soil organic matter (Table 2.11). Mean soil bulk density was 1.48 g cm^{-3} (Galland et al., 2026; McDaniel et al. in review; Eness et al. in prep).

Established in 2019, the PGC experiment baseline is a continuous maize production system with precision strip tillage, broadcast banded fertilizer applications, targeted pesticide treatments, and other routine plot maintenance. The PGC treatments are arranged in a randomized complete block design (RCBD) consisting of four blocks, each containing 11 treatment plots and one control plot (continuous maize only). All other non-control treatments featured various perennial grasses interseeded between continuous maize rows. The PGC grasses

were seeded in 2019 at different rates; BBG seeded at 684 kg ha-1 and KBG seeded at 98 kg ha-1.

Individual plot dimensions were 6.86 × 6.10 m, accommodating eight maize rows (Figure 2.1). The experiment focused on three treatments: Poa bulbosa (bulbous bluegrass, BBG) interseeded between maize rows, Kentucky bluegrass (KBG) interseeded between maize rows, and a maize-only control treatment (Control). More experimental details can be found in Galland et al., (2026).

Strip tillage was performed annually in all treatments between April and early May on 76.2 cm row centers using a 4-row Unverferth 330 Ripper-Stripper, followed by a Maschio gang tiller equipped with 38.1 cm disks on 76.2 cm row centers (Figure 2.1). Maize was planted at a density of 82,800–83,600 seeds ha^{-1} using Kinze or John Deere planters with Pioneer P1185 or DeKalb DKC57-75 hybrids, typically between 20 April and 10 May.

We fertilized twice early in the growing season using surface banding, with a base pre-plant application followed by a post-plant application shortly after planting. The pre-plant application consisted of a blend of diammonium phosphate (DAP) or monoammonium phosphate (MAP) and potash (KCl), while the post-plant application consisted of sulfur-encapsulated urea (43–0–0–4) applied directly over maize rows. Total annual N application rates ranged from 201 to 221 kg N ha^{-1} (Table 2.1).

2.3.2. Soil nitrous oxide flux measurements

Within each plot, two 20.32 cm diameter PVC rings (12 cm height), hereafter referred to as soil collars, were installed to facilitate gas flux measurements. Each collar was beveled at one end for ease of insertion to soil, and were inserted using a mallet to 2 cm height above soil surface (or 10 cm belowground). One collar was placed within the sixth maize row, and the other positioned between the sixth and seventh maize rows, within the PGC zone of BBG and KBG

treatments (Figure 2.1). To minimize disturbance, N_2O flux measurements began no less than 24 h after collar installation. Collars remained in place throughout the sampling period and were only temporarily removed for essential field operations (tillage, planting, and harvest). They were immediately reinstalled following these events to maintain consistency in sampling locations.

N_2O emissions were measured using a closed static chamber method with a portable cavity-enhanced absorption spectrocopy LI-7820 N_2O/H_2O Trace Gas Analyzer paired with the 8200 Smart Chamber (LI-COR Biosciences; Lincoln, NE). The chamber includes an integrated soil probe that simultaneously records soil temperature and moisture at 5 cm depth. The analyzer and chamber are connected and are operated wirelessly via an internal Wi-Fi hotspot /URL-based application on a smart device (phones, tablets computers), with data stored internally until exported.

All soil N2O measurement events used a standardized 2-min sampling duration excluding a 10-s closure period and a 45-s deadband to ensure pressure headspace stabilization. Flux measurement events began by placing the chamber over the permanently installed soil collars, while the soil probe was inserted 5-7cm distance adjacent to the collar but in the same sampling zone (row or interrow) to record soil temperature and moisture. The SoilFluxPro software, using the analyzer measurements and chamber configuration, provides soil N2O flux in μmol N2O m-2 h-1. We then convert this to g N2O-N ha-1 d-1 to provide daily N flux estimates on a per ha basis. This makes the assumption that the 24-h soil N2O flux is comparable to that measured between 0800 and 1400 hours. While studies have shown daily N2O fluxes can be variable diurnal fluctuations are typically associated with soil temperature changes within the day and not effected by treatments (Parkin et al., 2003; McDaniel et al., 2014; Reeves et al., 2016).

A total of 58 sampling events were carried out between February 2024 and November 2025 with sampling frequency adjusted to capture post-fertilization emission dynamics. Sampling occurred daily during the first week following nitrogen fertilizer application, three times per week during the following two weeks, and twice weekly until fluxes stabilized near baseline levels. Once stabilized, sampling frequency was reduced to weekly, biweekly, and eventually monthly intervals after maize harvest. Sampling was suspended during winter due to low temperatures affecting instrument performance and typically low soil N2O fluxes. Raw data were exported to cloud-based storage and imported into SoilFluxPro for initial visualization and organization, then exported as CSV files for further processing.

2.3.3. Soil sampling and laboratory procedures

Composite soil samples were collected from each plot by extracting eight soil cores (0–15 cm depth) using a 2-cm diameter probe. Cores were taken from two distinct positions or zones within each plot: the maize row and the interrow (where the PGC was established). Sampling was conducted three times (spring, V6, and fall after harvest) in both years of the experiment. In the field, samples were placed on ice and transported to the laboratory, where they were stored at 4 °C until further processing.

In the laboratory, composite samples were sequentially sieved through 8-mm and 2-mm mesh screens. The <2 mm soil fraction was refrigerated and used for fresh analyses. A 15 g subsample of the <2 mm fraction was weighed and oven-dried at 105 °C for 24 h to determine gravimetric water content (GWC). The >2 mm fraction was air-dried at 24 °C until reaching a stable weight (approximately one month) and stored for potential future analysis.

2.3.4. Soil microbial biomass

To quantify microbial biomass carbon (C) and nitrogen (N), twin replicate subsamples were prepared from each composite soil sample and extracted using chloroform-fumigation and the extraction method (Brookes et al., 1985; Vance et al., 1987). For fumigated samples, 5 g of <2 mm soil was weighed into a 50 mL glass beaker and placed inside an airtight glass desiccator containing 25 mL of ethanol-free chloroform. A vacuum was applied for 2–3 min to ensure vapor dispersion, after which samples were left to fumigate at room temperature for 24 h. Simultaneously, a second 5 g subsample was weighed into a 50 mL plastic centrifuge tube and left unfumigated to serve as the control. All samples, fumigated and unfumigated, were stored in the dark during the fumigation period.

Following fumigation, microbial biomass was extracted from both sub-samples using 25 mL of 0.5 M K_2SO_4. Samples were then shaken at 200 rpm for 1 h, then centrifuged at 1200 rpm for 3 min. The supernatants were filtered into 20 mL polyethylene vials using Whatman filter paper and subsequently stored at –20 °C until analysis.

Non-purgeable organic carbon (NPOC) and total nitrogen were quantified using a Shimadzu TOC-L analyzer with total nitrogen capabilities (Shimadzu Corporation, Kyoto, Japan). Final values were corrected using established extraction efficiency coefficients of 0.45 and 0.54 for MBC and MBN respectively (Brookes et al., 1985; Joergensen, 1996).We also use the unfumigated extracts to report salt-extractable organic carbon (SEOC) and salt-extractable organic nitrogen (SEON), respectively. For SEON, we subtracted out inorganic N to get final SEON value.

2.3.5. Inorganic Nitrogen: Nitrate and Ammonium

Using the previously extracted (0.5 M K_2SO_4) unfumigated samples, salt-extractable inorganic nitrogen (SEIN), including ammonium (NH_4^+-N) and nitrate (NO_3^--N), was quantified

using a colorimetric microplate approach. Extractable NO_3^- was determined using the vanadium (III) chloride reagent with absorbance measured at 540 nm (Doane et al., 2003), while NH_4^+ was analyzed using the salicylate–cyanurate reagent with absorbance measured at 595 nm (Sinsabaugh et al., 2000). Each sample was analyzed in duplicate in a 96-well microplate, after which reagents were added and plates were read at the respective wavelengths using a Synergy™ HTX Multi-Mode Microplate Reader (BioTek Instruments, Winooski, VT, USA) with Gen5™ software.

2.3.6 Statistical analyses

All statistical analyses were conducted in Rstudio (version 4.5.1). Prior to statistical modeling, all datasets were examined following standard data exploration procedures described by Zuur et al. (2010). Normality and homogeneity of variance were assessed using the ggResidpanel package (version 0.3.0). When violations of these assumptions were detected, response variables were transformed as necessary prior to analysis. All measured variables were analyzed using linear mixed models (LMMs). The linear mixed model framework is widely used in agroecosystem studies because it accounts for spatial and temporal dependence inherent in repeated measurements while isolating fixed effects and controlling site-specific variability through random effects (Basche et al., 2014; Mitchell et al., 2013; Daly et al., 2022). Linear mixed models were fitted using the lme4 package (version 1.1-37). For soil N_2O flux measurements, perennial ground cover treatment, collar position, and day of year were specified as fixed effects. Plot replication, and its interactions were included as random effects to account for repeated temporal measurements and spatial variability. For soil physicochemical variables measured from seasonal soil samples, perennial ground cover treatment and sampling position were also treated as fixed effects. In these models, plot replication, sampling season, and their

interaction were specified as random effects to account for non-independence from repeated seasonal sampling.

For all fitted models, analysis of variance (ANOVA) was conducted to evaluate the significance of fixed effects and their interactions using Type III sums of squares. Marginal significance was defined at $\alpha < 0.10$, and statistical significance at $\alpha < 0.05$. When significant effects were detected, estimated marginal means were calculated using the emmeans package (version 2.0.0). Pairwise contrasts among estimated marginal means were then used to identify differences between treatment levels.

Additional Paired t-tests were conducted to evaluate treatment effects on raw daily values of soil temperature, volumetric water content (VWC), and nitrous oxide (N_2O) fluxes, as well as cumulative N_2O emissions. For each sampling date, paired differences between PGC treatments and the control were calculated within the same block and position and tested against zero using two-tailed paired t-tests to assess whether mean treatment effects differed from the control.

2.4. Results

2.4.1 Weather and historical climate

According to the historical precipitation in the area (Figure 2.2), 2024 had accumulated 963 mm, which is near the 30-year average (only 0.8% increase from MAP of 955 mm). Whereas 2025 was the 6th wettest year within the same timeframe, with 1156 mm of annual precipitation (21% above the 30-year average). A good portion 2025 precipitation, 29%, occurred between 1 May and 1 July. As far as temperature, 2024 was the 3rd warmest year in growing degree days within the last 30 years and the average GDD was 9% above the 30-year average. Not surprising giving the worldwide increase in global temperatures, but 2025 had the greatest GDD within the last 30 years with a total of 3990 GDD exceeding the 30-year average by 19%.

2.4.2 Soil microclimate (temperature and water content)

Measured soil temperatures ranged from 0.2 to 38.2 °C across both growing seasons (Figure 2.3). During the growing season, mean (± standard deviation) was 25.1 ± 5.4 °C. The PGC treatments had a significant effect on soil temperature (Table 2.2, Figure 2.3). On average, in 2024 the PGCs both increased soil temp by 0.3 and 0.5 °C for BBG and KBG respectively (Figure 2.3). Soils under KBG were also warmer than BBG by 0.3 °C. In 2025, however, there was no difference in soil temperature between the Control and KBG, but both had warmer temperatures relative to BBG by 0.3 and 0.4 °C, respectively. Although these shifts in soil temperature were modest in magnitude, their consistency across sampling dates contributed to statistically detectable differences among treatments.

Measured soil VWC ranged from 0.04 to 0.45 $m^3\ m^{-3}$ (Figure 2.4). In 2024, the drier year, the soil moisture was relatively high between mid-April through 1 June (0.3 to >0.4 m3 m-3). However, soil VWC reached its lowest in June and August, below 0.1 m3 m-3, near the estimated permanent wilting point. In 2025, the wetter year, soil VWC remained relatively high, between 0.2 and >0.3 m3 m-3, nearly throughout most of the growing season with only a dip in August to September.

Across both years PGC had a significant effect on VWC (Table 2.3). In 2024, BBG decreased soil VWC compared to the control by 0.021 $m^3\ m^{-3}$ (1.05 mm), whereas KBG had no effect. In 2025, both PGC treatments reduced VWC compared to the control, with BBG decreasing VWC by 0.024 $m^3\ m^{-3}$ (1.20 mm) and KBG by 0.015 $m^3\ m^{-3}$ (0.75 mm) at a 5 cm depth. Overall, the PGCs had a consistent impact on soil microclimate that varied strongly across dates reflecting seasonal and environmental patterns.

2.4.3 Soil C and N pools

The salt-extractable organic fractions were variable and not all that dynamic within seasons or across the two years (Figure 2.5; Figure 2.6). The SEOC concentrations ranged from 20 to 124 mg C kg^{-1} and averaged approximately 56 mg C kg^{-1} across all sampling events, treatments, and positions (Figure 2.7). In both study years, the PGC did not have main or interactive effect with row position on SEOC concentrations (Table 2.4).

Unlike SEOC, SEON was more dynamic across seasons but similar between years, and did show some PGC treatment effects (Figure 2.6). Across all measurements, SEON ranged from <1 to 33 mg N kg^{-1} and averaged 10.7 mg N kg^{-1}. There was a significant main effect of PGC treatment (Table 2.4). The KBG treatment had 2.7 mg N kg^{-1} greater SEON, a 27% increase, compared to that of Control across both years and positions (Figure 2.6, $P < 0.04$).

Across the two-year study, MBC and MBN varied little and showed little response to PGC treatments (Figures 2.5, 2.6). Soil MBC ranged from 28 to 278 mg C kg-1 , with average of 115 mg C kg-1. In 2025, there was a weak interactive effect between PGC and position whereby BBG in the interrow increased MBC by approximately 30 mg C kg^{-1} (≈31%) compared to the crop row (Figure 2.5). Soil MBN ranged from 4.3 to 35.3 mg N kg-1 across both years, with average of 15.0 mg N kg-1. Although we did not set out to test for this, but soil 2024 MBN was greater compared to 2025 (Figure 2.6). The mean 2024 soil MBN was 3.4 mg N kg-1 greater, on average, than 2025.

Throughout the two years of this study, soil inorganic N concentrations were variable but had only a moderate response to PGC treatments. Across all measurements in both years, NH_4^+–N ranged from 0.3 to 18.7 mg N kg^{-1} and averaged 5.6 mg N kg^{-1}. In 2025 PGC moderately effected soil NH_4^+–N concentration where BBG exhibited approximately 1.5 mg N kg^{-1} greater NH_4^+–N than the Control, representing a ~37% increase across positions. Soil NO_3^-–N

concentrations ranged from 1 to 29 mg N kg^{-1} and averaged 9.8 mg N kg^{-1} across both years and positions. In both years, there was no detectable main or interactive effect on soil NO_3^-–N concentrations (Table 2.4).

2.4.4 Soil nitrous oxide emissions

Daily soil N2O emissions in 2024 were highly variable, with the majority (81.2%) of those larger fluxes, those above 10 g N2O-N ha^{-1} d^{-1}, occurring between April 1 and June 1 (Figure 2.8). The greatest observed flux was 2.5 kg N2O-N d-1 in the BBG row, which was 17.1% of that treatment's mean cumulative flux. In 2024, Raw daily emissions showed that BBG increased emissions relative to the Control in the row by 51.4 g N_2O–N ha^{-1} d^{-1}. However, KBG showed no significant effect on daily emissions across either year or position (Table 2.5). However, on average across all treatments, the cumulative row soil N2O emissions were 17.4% less than the interrow. There was significant PGC treatment × position interaction ($P < 0.001$). For the row, BBG and KBG treatments had 57% and 38% greater cumulative soil N2O emissions than the Control. For the interrow, it was the opposite, BBG and KBG had 39% and 83% less soil N emitted as N2O compared to the Control.

Soil N2O emissions in 2025, a relatively wet year, were markedly different (Figure 2.9). In 2025, about half of the highest fluxes (48.3%) occurred later in the growing season between June and August instead of in April and June , but these fluxes accounted for 79.8% of all high fluxes in this year (Figure 2.9). Contrary to 2024, in 2025 BBG reduced emissions in the interrow by 13.3 g N_2O–N ha^{-1} d^{-1}. Also, a notable difference in 2025 compared to 2024, is the cumulative N2O emissions were much larger and primarily from the maize row. The maize row was 183% greater than the interrow across all treatments. Like in 2024, there was also a significant PGC treatment × position interaction ($P = 0.046$). For the row, there were no

treatment differences. However, for the interrow, BBG reduced cumulative soil N2O fluxes by 58% compared to the Control ($P < 0.001$; Figure 2.8).

2.4.5 Field-level soil N_2O emissions

Despite positional variability, no treatment differences were detected in field-level cumulative N_2O emissions (Table 2.6; Figure.10). In 2024, total emissions averaged across all treatments were approximately 11.8 kg N ha^{-1}, with 5.3 kg N ha^{-1} coming from the row and 6.5 kg N ha^{-1} from the interrow. BBG had the highest emissions (13.6 kg N ha^{-1}), followed by KBG with a total of 11.7 kg N ha^{-1}, and the Control at 10.2 kg N ha^{-1}. In 2025, total emissions averaged across treatments were 11.0 kg N ha^{-1}, with 8.2 kg N ha^{-1} coming from the row and 2.8 kg N ha^{-1} from the interrow. In 2025, BBG had the lowest emissions (8.2 kg N ha^{-1}), KBG had the highest emissions (13.3 kg N ha^{-1}), and the Control was 11.5 kg N ha^{-1}.

When analyzed by position, cumulative emissions varied across treatments and years. In 2024, interrow emissions were fairly stable across treatments, ranging from 6.0 to 7.0 kg N ha^{-1}. However, row emissions showed stronger variation across treatments but with lower magnitudes: BBG = 7.1 kg N ha^{-1}, KBG = 5.7 kg N ha^{-1}, and Control = 3.2 kg N ha^{-1}. Contrarily, in 2025, interrow emissions were significantly lower compared to row emissions, remaining stable across treatments within a range of 2.4 to 3.0 kg N ha^{-1}, whereas row emissions ranged more broadly across treatments (5.8 to 10.7 kg N ha^{-1}). However, these positional trends were averaged at the field level and resulted in PGC treatments having no detectable effect on total cumulative emissions.

2.4.6 Maize grain yield

Grain yield responses varied between the years of this study but little to no treatment effect was observed. In 2024, total grain yields across all treatments averaged approximately 5.1 Mg ha^{-1}, with BBG producing the highest yield at 6.9 Mg ha^{-1}, followed by the Control at 5.1

Mg ha^{-1}, and KBG with 3.2 Mg ha^{-1}. However, in 2025 grain yields were considerably higher across all treatments compared to 2024. KBG had the leading yield at 11.2 Mg ha^{-1}, BBG at 10.6 Mg ha^{-1}, and the Control at 10.5 Mg ha^{-1}.

A treatment difference was observed in 2024 (Figure.11), with BBG yielding approximately 3.7 Mg ha^{-1} greater than KBG. However, 2025 grain yield uniformly increased across all treatments by approximately 5–8 Mg ha^{-1}. The absence of a PGC effect on grain yield in 2025 may suggest that weather-related dependency, given that 2025 was a wetter year compared to 2024.

2.5. Discussion

Soil N2O emissions from agricultural fields remain a central challenge for regional and global land managers. Traditional winter cover crops have low adoption rates, although greater than PGC adoption, and have shown variable effectiveness at mitigating soil N2O emissions (Basche et al. 2014). Furthermore, the few studies on PGC or living mulch maize systems have been on leguminous PGC species, mostly clovers, and most have shown the PGC increases soil N2O emissions (Turner et al., 2016; Peters et al., 2020b). We wanted to not only quantify soil N_2O emissions under an intensity gradient of PGC (Figure.1), but also to measure soil properties that could help explain any treatment differences. Namely, we measured surface soil temperature, moisture, and various C and N pools that may help to explain any differences among treatments. Here we discuss our findings in context of the other few analogous studies, but also draw upon traditional winter cover crop and intercropping literature for further insights.

2.5.1 Perennial groundcover had consistent effects on soil microclimate

Soil temperature and moisture are key features of agroecosystems that drive many soil biological processes, like N2O production, not to mention ultimately regulate plant growth for a

given year (and determine crop yield). We found that in two warmer- and wetter-than-normal years that the PGC had differing effects on soil microclimate that depended on the year. Some of the findings on PGC effects on soil microclimate were more expected than others.

Soil temperatures were slightly warmer, approximately +0.17 °C on average, under PGC compared to the control (Figure 2.3). This unexpected finding is likely attributed to the reduction in VWC observed under PGC treatments, as the Control maintained greater VWC than PGC by approximately 0.016 $m^3\ m^{-3}$ (≈ 0.8 mm of water at 5 cm depth) on average across both years. Soil temperature and moisture are closely coupled in field systems, as VWC directly influences soil evaporation rates and associated soil heat fluxes(Heitman et al., 2008; Jackson, 2015). Decreased soil water content can reduce soil surface evaporation, thereby limiting evaporative cooling and reducing latent heat flux. As a result, less energy is dissipated through vaporization, allowing greater heat accumulation and consequently higher soil temperatures (Heitman et al., 2008; Jackson, 2015). For example,Wilczewski et al., 2023) reported that wetter soils remained cooler due to greater evaporative losses, whereas Marquis (1967) reported lower soil moisture conditions resulted in increased soil temperature. However, PGC's effect on soil moisture was more predictable despite variability over the years.

Soil moisture showed more expected trends. The presence of additional perennial roots in a continuous maize cultivation, extending plant growth periods and providing year-round soil coverage, reduced VWC compared to the maize-only control by approximately 0.017 $m^3\ m^{-3}$ (~0.95 mm) on average across positions and both years. Several studies show that unsuppressed PGC treatments decreased soil moisture compared to controls with no perennial cover growing in between rows(Nielsen and Vigil, 2005; Qi and Helmers, 2010; Ochsner et al., 2010b; Krueger

et al., 2011; Galland et al., 2026). These studies show that soil moisture under perennial groundcover, cover crop, and living mulch systems is consistently reduced relative to the control, with decreases ranging from approximately 24 to 104 mm with an average of 45 mm depending on system type, management intensity, environmental conditions, and sampling depth. Among PGC species, this study found BBG to have a greater affinity to reduce soil moisture compared to KBG, which is contrary to previous studies.

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BBG reduced VWC by approximately 0.022 $m^3\ m^{-3}$ (~1.23 mm) compared to the Control across years, whereas KBG had no effect on VWC in 2024 and only reduced VWC by 0.015 $m^3\ m^{-3}$ (~0.84 mm) in 2025. Additional raw daily averages showed a modest reduction in the row position only in 2024 (Table 2.3). In contrast, a recent study conducted at the same experimental site over three prior years reported that KBG decreased soil water storage by approximately 24 to 38 mm to a depth of 45 cm, while BBG had no effect on soil water over that period (Galland et al., 2026). The authors attributed these differences to species-specific growth patterns, where BBG entered summer dormancy, thereby reducing its water uptake, whereas KBG maintained continuous growth and water use throughout the season. The exact reason why soil moisture reductions were greater under BBG compared to KBG in this study remains unclear; however, this may be related to variation in annual precipitation. The study by Galland et al. was conducted under three drier-than-average years, whereas the present study experienced two wetter-than-average years (Figure 2.2). (Wiggans et al., 2012) found that KBG had no effect on soil moisture in a three-year study in which two years had above-average rainfall. Together, these findings suggest that the responses observed here likely reflect how BBG and KBG behave under wetter conditions, whereas the results from Galland et al. more strongly represent system

responses under drier conditions. Furthermore, our discrepancies may also be attributed to differences in sampling depth, as this study assessed VWC only in the top 5.6 cm of soil, whereas Galland et al. quantified soil water storage across a 0–45 cm profile.

2.5.2 Perennial groundcover had little effect on soil C and N pools

Soil C and N pools are measures of agroecosystem functioning, used as indicators of soil health, and most importantly directly linked to soil N2O emissions. We measured four pools of N (NH4+, NO3-, SEON, and MBN) and two pools of C (SEOC and MBC) at three timepoints over the growing season. We hypothesized that increased root biomass and rhizodeposition from continuous living cover would enhance soil C and N in labile organic pools. Overall, PGC exerted only a modest influence on these pools.

Only SEON showed a relatively consistent response to PGC (Figure 2.6). While both PGC treatments increased SEON, only KBG significantly increased SEON in both the interrow and row positions by ~2.4 to 5.3 mg N kg^{-1} (≈14 to 33%). This response is consistent with previous studies reporting that PGC systems can maintain or increase soil organic N pools when intercropped with maize (Andrews et al., 2018; Sui et al., 2025). For example, Banik et al. conducted a two-year study at the same site and reported an 8.5% increase in potential mineralizable N, indicating an expansion of the soil organic N pool. Outside of SEON, only a modest positional effect was observed within BBG treatments for MBC in 2025, where concentrations were greater in the interrow than in the row. This localized increase in MBC is consistent with findings by (Ginakes et al., 2020) who reported a significant positional effect on MBC in living mulch systems ($p < 0.005$; between rows > within rows).

For inorganic nitrogen pools, we hypothesized that increasing PGC intensity would reduce residual ammonium and nitrate through increased plant uptake and microbial

immobilization. However, only soil ammonium was affected by PGC, and overall concentrations were very low (< 3 mg N kg^{-1}). BBG had no effect in 2024 but increased NH_4^+–N by ~0.4 to 0.9 mg N kg^{-1} compared to the Control in 2025 (≈20 to 55%, Figure 2.7).

A previous study from this site measured plant available nitrogen (PAN = $NH_4^+ + NO_3^-$) and found that KBG had minimal effect on PAN over a three-year period, whereas BBG reduced soil PAN concentrations by 22–45% across both positions in two out of three years (Galland et al., 2026). Another two-year study at this site reported that KBG reduced both NH_4^+ and NO_3^- availability(Banik et al., 2020b). Both studies concluded that these findings were partially driven by short- or long-term drier conditions (low or limited rainfall) compared to this study, and that the reductive mechanisms likely reflected a combination of plant uptake, microbial immobilization, leaching, and/or gaseous losses. Both also acknowledged competition between PGC and maize for available nitrogen. In contrast, this study was carried out during two wetter-than-average years (Figure 2.2), and the increase in NH_4^+ concentrations under BBG was likely driven by enhanced mineralization associated with root C exudates. Although limited research is available, biological nitrification inhibitors (BNI) have been identified in some grasses (Subbarao et al., 2013; Villegas et al., 2020) in the form of root exudates, and if present in BBG, this may partially explain the observed increase in NH_4^+.

Overall, increases in SEON and MBC under PGC marginally support our hypothesis, whereas increases in ammonium contrast with it. These results, combined with PGC's limited effect on MBN, SEOC, and nitrate, suggest a relatively balanced system; however, potential N losses through nitrous oxide emissions must also be considered.

2.5.3 Perennial groundcover affected soil N2O emissions at plot level; but net, field-level results show no difference among treatments

Soil N_2O emissions from our field experiment were within range of those typical for the Midwest US ~2–17 kg N_2O–N ha^{-1} yr^{-1}(Lawrence et al., 2021) However, some of our cumulative values may be notably high (Figure.2.8; Figure.2.9). PGC effects on soil N2O emissions were consistent but complex; and depended on the year and whether measurements were in row or interrow.

Daily N2O emissions were highly variable and PGC had modest effects on them (Table 2.7). In both years, the majority of peak emissions occurred within a relatively narrow time window (April 1 and June 1), coinciding with fertilizer application followed by large rainfall events (Figure 2.8; Figure 2.9). As a result, between 40 -81% of total cumulative N_2O emissions in each year occurred during this window, demonstrating a clear “hot moment” ; an observation recorded across previous studies(Jacinthe ’ and Dick,; Bremner et al., 1981; Wisconsin et al., 1987; Cates and Keeney, 1987; Baggs et al., 2003; Sehy et al., 2003; Parkin and Kaspar, 2006) suggesting that annual cumulative emissions are largely driven by short-lived, rainfall-induced events, and strongly influenced by interannual climatic variability.

PGC affected cumulative N_2O emissions in both years, but responses were strongly dependent on spatial distribution. In 2024, cumulative emissions were consistently greater in the interrow than in the row by 22.6% across all treatments. Both KBG and BBG reduced interrow emissions by 16.1% and 6.6%, respectively, relative to the Control. This reduction was not observed in the row, where cumulative emissions were significantly greater under KBG and BBG (Figure 2.8). Similar spatial patterns have been reported (Goodroad et al., 1984; Baggs et

al., 2003; Parkin and Kaspar, 2006). However, Parkin and Kaspar, (2006) reported higher interrow emissions to fertilizer band misplacement, where chambers captured concentrated N zones; a similar mechanism may have occurred here, although this cannot be confirmed it would explain the higher emissions observed in the interrow including the Control treatment.

Reductions in interrow emissions under PGC align with previous findings that grass species can decrease or have neutral effects on N_2O emissions in maize cropping systems (Parkin and Kaspar, 2006; Jarecki et al., 2009; Behnke and Villamil, 2019; Foltz et al., 2021) mostly through mineral N uptake and enhanced microbial immobilization. In contrast, greater row emissions under PGC may reflect additional labile carbon inputs enhancing localized denitrification (Griffiths and Robinson, 1992; Parkin et al., 2006; Banik et al., 2020b; Seitz et al., 2024).

In 2025, BBG reduced cumulative interrow emissions by 25.6% relative to the Control, but PGC had no effect on row emissions. Some studies using grass cover crops have reported decreases ranging from approximately 39% to as much as fivefold (~80%) depending on biomass production, nitrogen scavenging efficiency, and environmental conditions(Behnke and Villamil, 2019; Foltz et al., 2021). However, cumulative row emissions were substantially greater than interrow emissions (Figure 2.9), reversing the 2024 pattern. Interrow emissions exceeded row emissions by 22.6% in 2024, whereas in 2025 row emissions were 217.7% greater than interrow across treatments. Compared to several studies conducted nearby in drier years (Flater et al., in review; Parkin and Kaspar, 2006; Parkin et al., 2006, 2016; Lawrence et al., 2021), our cumulative emissions were notably higher, suggesting that interannual climatic variability, particularly precipitation, and site-specific conditions likely contributed to elevated emissions (Figure 2.2).

Relative to the applied N fertilizer rate of 221 kg N ha^{-1}, the IPCC Tier I emission factor (1% of applied N) would estimate approximately 2.21 kg N_2O–N ha^{-1} yr^{-1}. In contrast, mean cumulative N_2O emissions in this study reached 11.5 kg N_2O–N ha^{-1} in 2024 and 10.8 kg N_2O–N ha^{-1} in 2025 (Figure 2.10). Although values of this magnitude are not unprecedented, reported means are often lower. For example, Jarecki et al. (2009) reported 7.5 and 5.3 kg N_2O–N ha^{-1} yr^{-1} at 175 kg N ha^{-1}, while Parkin and Kaspar (2006) reported 11.3 and 15.4 kg N_2O–N ha^{-1} yr^{-1} at 215 kg N ha^{-1}, and Smith et al., (2011) reported 3.8 and 5.1 kg N_2O–N ha^{-1} yr^{-1} at 195 kg N ha^{-1}. These comparisons indicate that cumulative N_2O emissions vary substantially across studies and that fertilizer rate alone does not explain the observed variation, suggesting that broader environmental and system-level controls likely influenced the elevated cumulative emissions observed here.

Studies evaluating the effects of living mulch or PGC on N_2O emissions in maize systems are limited. Among those available, a few have examined perennial clover used as a living mulch within maize–soybean rotations(Turner et al., 2016; Peters et al., 2020b). These studies generally concluded that legume living mulches increase N_2O emissions due to additional mineral N inputs from the decomposition of low C:N biomass. This aligns with findings from(Basche et al., 2014b) which indicate that legume cover crops tend to increase N_2O emissions, whereas grass species are more likely to reduce emissions by limiting nitrate availability. This suggest that PGC treatments use in this study have more potential to reduce N_2O emissions than legumes living mulches.

We initially hypothesized that PGC would reduce soil N_2O emissions, with the strongest effects occurring in the interrow zone where the PGC is actively growing. Our findings do not fully support this hypothesis. While PGC reduced interrow N_2O emissions at the plot level, these effects did not translate to the field scale. By combining soil N_2O fluxes across both row and interrow positions, which presumably each represent half of the field, we scaled emissions to estimate net field-level N_2O fluxes for the PGC systems. When aggregated, there were no significant differences among treatments (Figure 2.10), highlighting the importance of measuring both positions to accurately assess field-level emissions. Similar patterns have been reported in previous studies (Parkin and Kaspar, 2006), where grasses reduce N_2O emissions locally but their effects become insufficient when scaled to the field level

Some treatment differences were observed in 2024, but only between BBG and KBG, and overall yields were notably lower compared to 2025 (Figure 2.11). Following increased precipitation, grain yields were substantially higher across all treatments in 2025, which was a wetter year. Although previous studies have reported negative effects of PGC on maize grain yield (Bartel et al., 2017; Banik et al., 2020b; Galland et al., 2026), this was not observed in the present study. PGC treatments had no effect on maize grain yield relative to the Control (Figure 2.11). These results suggest that grain yield in this system may be more sensitive to environmental conditions, particularly precipitation, than to PGC treatment effects.

2.6. Conclusions

PGC affected soil N_2O emissions in both years, but responses were consistent yet complex and strongly dependent on spatial distribution. In both years, PGC reduced interrow N_2O emissions relative to the Control, while row emissions were more variable and influenced by fertilizer placement and possibly labile C inputs. These reductions were likely associated with

improved soil aeration, reduced soil water content, and N immobilization in PGC interrow zones, which limited conditions required for denitrification. Despite these spatial patterns, cumulative N_2O emissions at the field level were not significantly different among treatments.

Overall, PGC had a consistent impact on soil microclimate, particularly by reducing soil water content, while exerting only modest influence on soil C and N pools. These interacting effects may have influenced soil N_2O emissions. While treatment effects were clearly expressed at the plot and positional scale, they did not translate into detectable differences at the field level. Together, these findings indicate that PGC systems do not affect soil N_2O emissions at the field level in maize systems, but show potential, maintain similar levels of plant-available N, and have no negative effect on maize grain yield.

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2.8. Tables and Figures

Table 2.1Agronomic fertilizer management practices for the study period.

Application timing	*Application window*	*Fertilizer source*	*Fertilizer rate (kg ha⁻¹)*
Pre-plant (base)	April 12 – April 20	Diammonium phosphate (DAP) or monoammonium phosphate (MAP) + muriate of potash (KCl)	44–53 (N) – 112–135 (P) – 123–179 (K)
Post-plant	April 25 – May 12	Sulfur-encapsulated urea (43–0–0–4)	157–168 (N)
Total annual	—	—	201–221 (N)

Table 2.2 Three-way ANOVA results from 2024 and 2025 from soil temperature.

Source of Variation	2024 Df	2024 F-statistic	2024 p-value	2025 Df	2025 F-statistic	2025 p-value
Treatment	2	9.77	0.0001	2	4.31	0.014
Position	1	0.36	0.550	1	0.12	0.728
Time	26	893.01	0.000	21	541.00	0.000
Treat.×Pos.	2	0.18	0.839	2	0.12	0.887
Treat.×Time	52	2.53	0.000	42	0.97	0.519
Treat.×Pos.×Time	52	0.16	1.000	42	0.15	1.000

Table 2.3 Three-way ANOVA results from 2024 and 2025 from soil volumetric water content.

Source of Variation	2024 Df	2024 F-statistic	2024 p-value	2025 Df	2025 F-statistic	2025 p-value
Treatment	2	11.42	1.4e-05	2	10.63	3.1e-05
Position	1	0.12	0.733631	1	16.84	4.9e-05
Time	26	108.50	<0.000001	21	39.00	<0.000001
Treat.×Pos.	2	3.97	0.019396	2	0.66	0.519847
Treat.×Time	52	0.84	0.775827	42	1.50	0.027293
Treat.×Pos.×Time	52	0.76	0.886258	42	0.81	0.797894

Table 2.4 Two-way ANOVA on soil carbon and nitrogen pools.

Year	Soil Carbon or Nitrogen Pool	Treatment	Position	Treat.×Pos.
2024	Ammonium	0.2188	0.3716	0.5905
	Nitrate	0.0947	0.3866	0.7545
	Salt-extractable Organic N	0.0445	0.1567	0.2566
	Microbial Biomass N	0.6782	0.8068	0.9478
	Salt-extractable Organic C	0.2640	0.1546	0.6848
	Microbial Biomass C	0.9772	0.1556	0.9583
2025	Ammonium	0.0478	0.0023	0.4963
	Nitrate	0.4531	0.0823	0.7796
	Salt-extractable Organic N	0.0379	0.1137	0.9624
	Microbial Biomass N	0.5272	0.2817	0.8348
	Salt-extractable Organic C	0.8801	0.2078	0.3833
	Microbial Biomass C	0.7531	0.1200	0.0495

Table 2.5 Pairwise on raw, daily soil nitrous oxide emission differences between perennial groundcover treatments – bulbous bluegrass (BBG) and Kentucky bluegrass (KBG) – and Control, maize only (paired with Figure 2.8).

Position	Treatment Comparison	n	2024 Mean Difference (m^3 m^{-3})	p-value	n	2025 Mean Difference (m^3 m^{-3})	p-value
Row	BBG vs. Control	108	51.3641	0.036297	88	-30.8816	0.257891
	KBG vs. Control	108	35.8989	0.067837	88	20.7196	0.496261
Interrow	BBG vs. Control	108	21.4171	0.3642	88	-13.2701	0.038725
	KBG vs. Control	108	2.3099	0.873645	88	-10.2540	0.127461

Table 2.6 Summary of two-way ANOVA for field-level, cumulative soil N_2O flux.

Source of Variation	Df	F-statistic	p-value
Treatment	2	0.12	0.883762
Year	1	0.15	0.700662
Treat.×Pos.	2	0.71	0.506084

Table 2.7 Three-way ANOVA results from 2024 and 2025 from daily soil nitrous oxide emissions (paired with Figure 2.8).

Source of Variation	2024			2025		
	Df	F-statistic	p-value	Df	F-statistic	p-value
Treatment	2	3.63	0.027171	2	2.72	0.067046
Position	1	0.02	0.893529	1	116.21	<0.000001
Time	26	64.91	<0.000001	21	63.09	<0.000001
Treat.×Pos.	2	3.06	0.047748	2	3.74	0.024678
Treat.×Time	52	1.34	0.064432	42	2.35	1.1e-05
Treat.×Pos.×Time	49	0.75	0.891787	42	0.58	0.983967

Table 2.8 Summary of three-way ANOVA for cumulative soil N_2O flux.

Source of Variation	2024			2025		
	Df	F-statistic	p-value	Df	F-statistic	p-value
Treatment	2	4.25	0.014845	2	12.99	3e-06
Position	1	206.88	<0.000001	1	188.69	<0.000001
Time	26	67.61	<0.000001	21	146.42	<0.000001
Treat.×Pos.	2	25.83	<0.000001	2	3.58	0.028693
Treat.×Time	52	0.31	0.999999	42	0.81	0.793615
Treat.×Pos.×Time	52	0.18	1	42	0.28	0.999999

Table 2.9 Pairwise on raw soil temperature difference between perennial groundcover treatments – bulbous bluegrass (BBG) and Kentucky bluegrass (KBG) – and Control, maize only.

Position	Treatment Comparison	n	2024 Mean Difference (m^3 m^{-3})	p-value	n	2025 Mean Difference (m^3 m^{-3})	p-value
Row	BBG vs. Control	108	0.328	0.0692	88	-0.313	0.0920
	KBG vs. Control	108	0.563	0.0001	88	0.164	0.2830
Interrow	BBG vs. Control	108	0.262	0.0668	88	-0.303	0.0214
	KBG vs. Control	108	0.429	0.0028	88	0.045	0.6310

Table 2.10 Pairwise on raw soil volumetric water content differences between perennial groundcover treatments – bulbous bluegrass (BBG) and Kentucky bluegrass (KBG) – and Control, maize only

Position	Treatment Comparison	n	2024 Mean Difference (m^3 m^{-3})	p-value	n	2025 Mean Difference (m^3 m^{-3})	p-value
Row	BBG vs. Control	108	-0.0259	5.3e-05	88	-0.0286	0.000176
	KBG vs. Control	108	-0.0197	0.005348	88	-0.0142	0.053901
Interrow	BBG vs. Control	108	-0.0156	0.011749	88	-0.0195	0.003981
	KBG vs. Control	108	0.0050	0.397485	88	-0.0165	0.078051

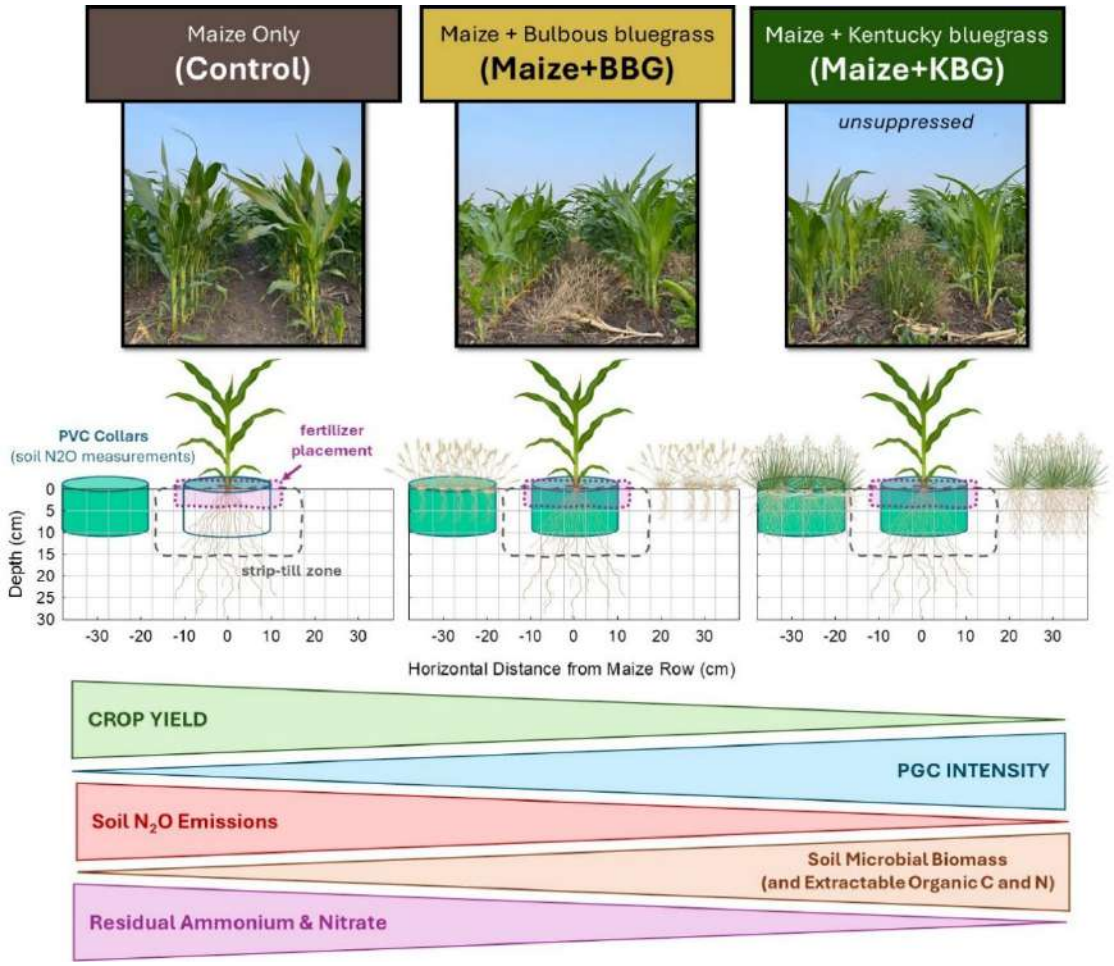

Figure 2.1 Conceptual overview of perennial ground cover (PGC) systems and their expected functional impacts on soil processes and crop performance. Three treatments are illustrated: maize-only (Control), maize intercropped with Poa bulbosa (BBG), and maize intercropped with Kentucky bluegrass (KBG, unsuppressed). Photographs (top) depict canopy structure and ground cover presence across treatments. The schematic (middle) shows the spatial configuration of maize rows, PGC distribution, strip-tillage zone, fertilizer band placement, and PVC collar positions used for N_2O flux measurements (row and interrow). The lower conceptual diagram summarizes anticipated system-level responses along a gradient of increasing PGC intensity, including potential trade-offs and synergies among crop yield, soil N_2O emissions, residual inorganic nitrogen (NH_4^+ and NO_3^-), and soil microbial biomass and extractable organiccarbon and nitrogen.

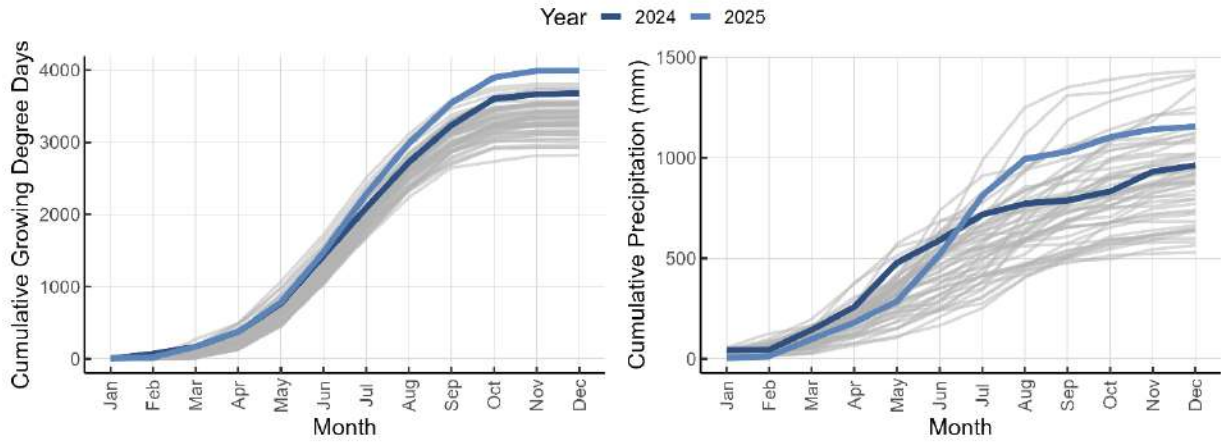

Figure 2.2 Cumulative growing degree days (GDD; *left panel*) and cumulative precipitation (*right panel*) for the 2024 and 2025 growing seasons. Bold colored lines represent seasonal cumulative for 2024 and 2025, while gray lines represent historic 50-year trends. Data are derived from Iowa Environmental Mesonet (IA Mesonet). The GDD was calculated on base 10°C.

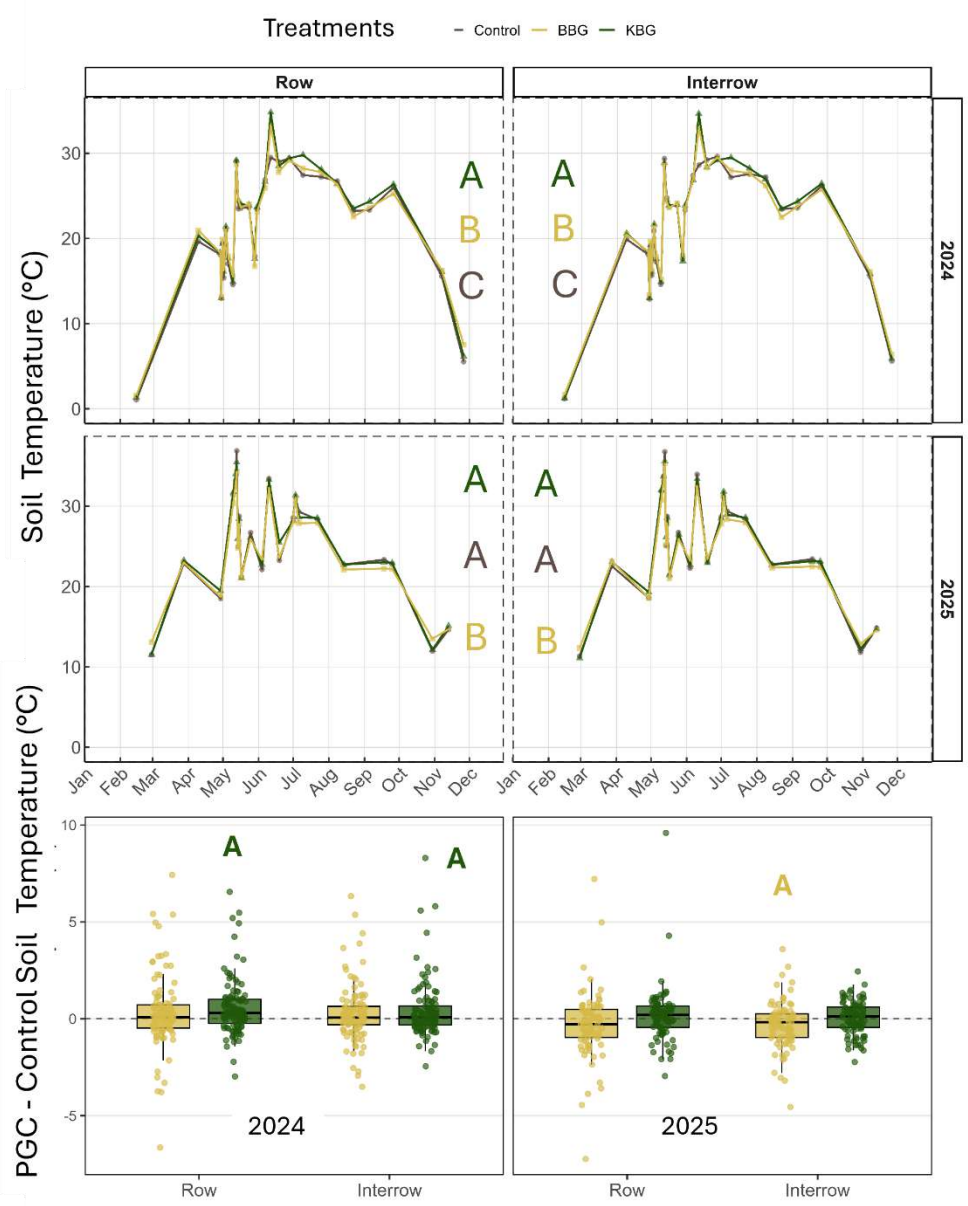

Figure 2.3 Seasonal soil temperature (°C) patterns as influenced by perennial ground cover (PGC) treatments across row and interrow positions in 2024 and 2025. Line plots (top panels) show temporal trends for Control, *Poa bulbosa* (BBG), and Kentucky bluegrass (KBG). Letters within panels indicate significant treatment differences within each position and year ($P \leq 0.05$). Bottom panels present daily raw differences (PGC − Control), illustrating the magnitude and variability of treatment effects relative to the Control.

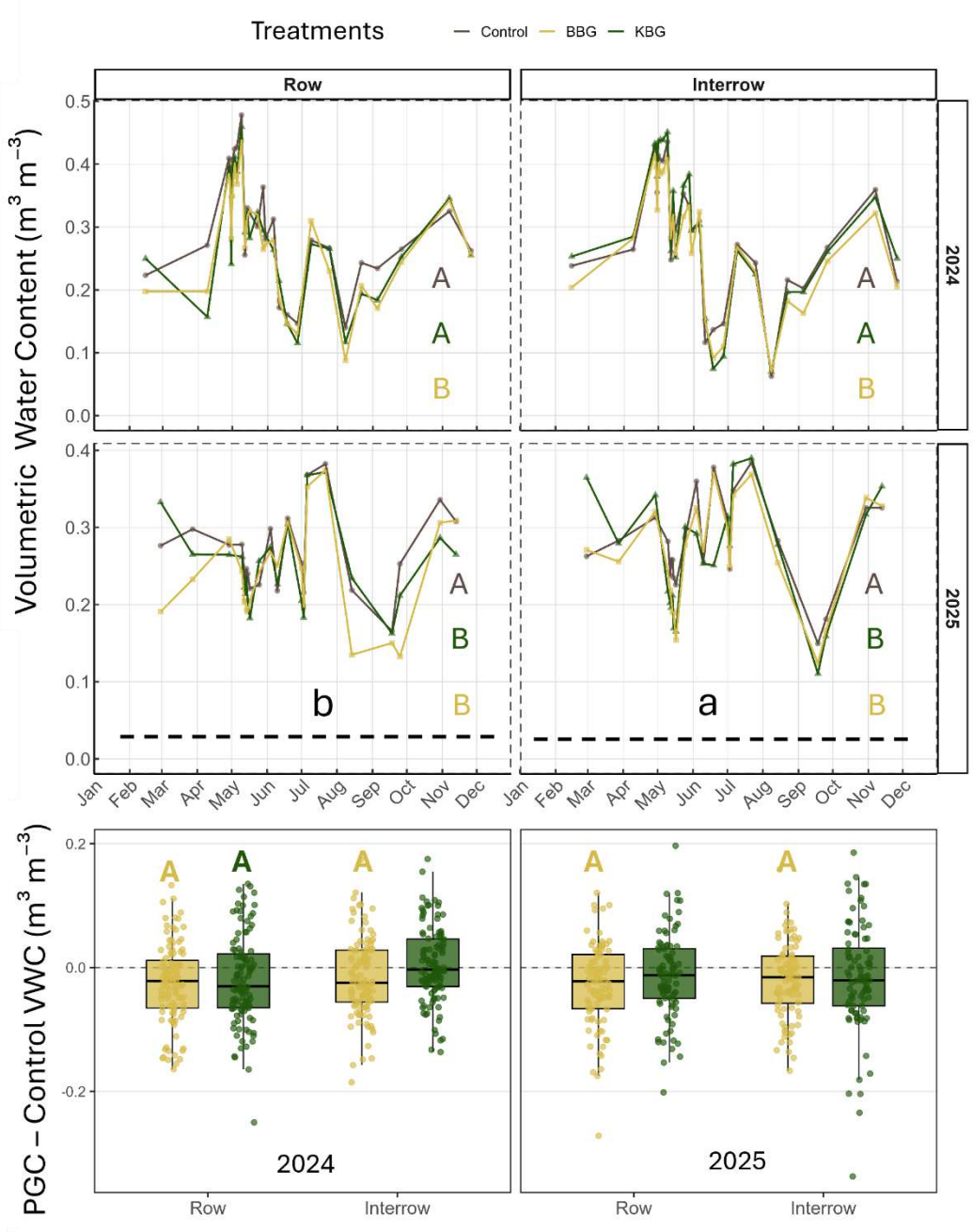

Figure 2.4 Seasonal soil volumetric water content (VWC; $m^3\ m^{-3}$) as influenced by perennial ground cover (PGC) treatments across row and interrow positions in 2024 and 2025. Line plots (top panels) show temporal dynamics for Control, *Poa bulbosa* (BBG), and Kentucky bluegrass (KBG), with letters indicating significant treatment differences within each position and year ($P \leq 0.05$). Bottom panels display daily raw differences (PGC − Control), highlighting treatment-induced shifts in soil moisture.

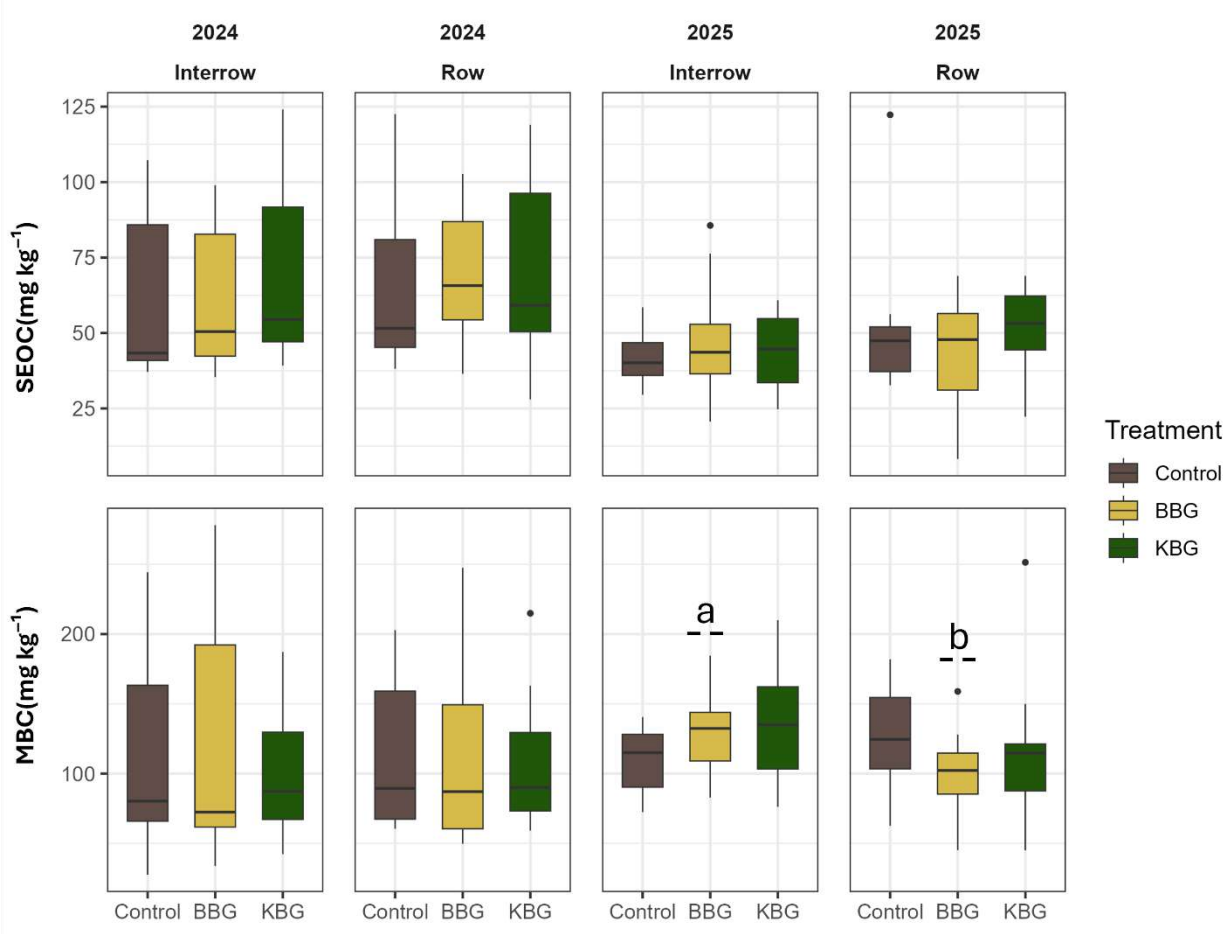

Figure 2.5 Salt extractable organic carbon (SEOC; mg kg^{-1}) and microbial biomass carbon (MBC; mg kg^{-1}) during the 2024 and 2025 growing seasons, shown separately for interrow and row positions. Boxplots represent treatment distributions (Control, BBG, KBG). Uppercase letters (color-coordinated) indicate treatment significance within a given position and year ($P \leq 0.05$). Lowercase letters with the shortened dashed line denote a significant treatment × position interaction ($P \leq 0.05$), specifically indicating differences between row and interrow positions within the BBG treatment only. The reduced width of the dashed line indicates that the positional comparison applies exclusively to BBG rather than all treatments.

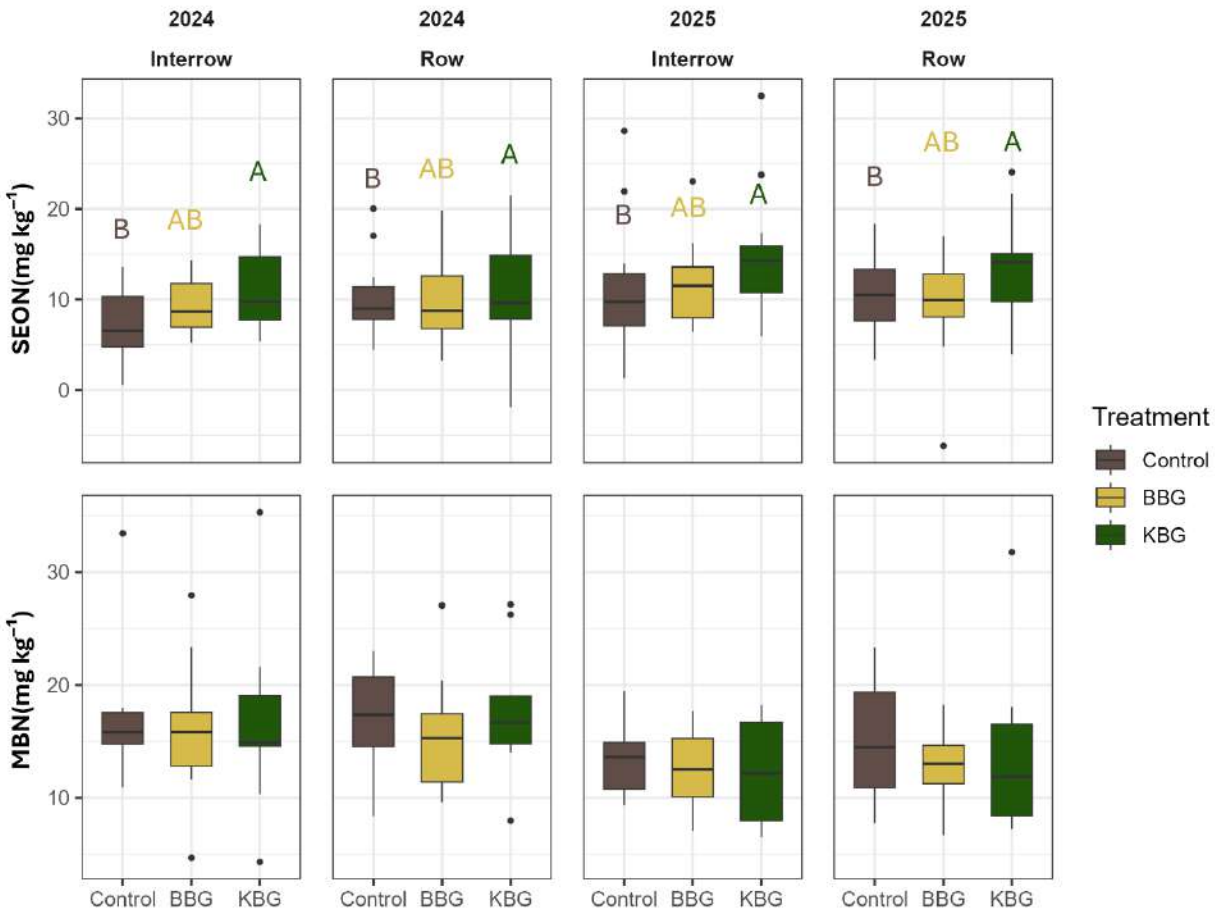

Figure 2.6 Salt extractable organic nitrogen (SEON; mg kg^{-1}) and microbial biomass nitrogen (MBN; mg kg^{-1}) during the 2024 and 2025 interrow and row positions shown separately. Boxplots represent treatment distributions (Control, BBG, KBG). Uppercase letters (color-coordinated) indicate significant treatment differences within a given position and year ($P \leq 0.05$).

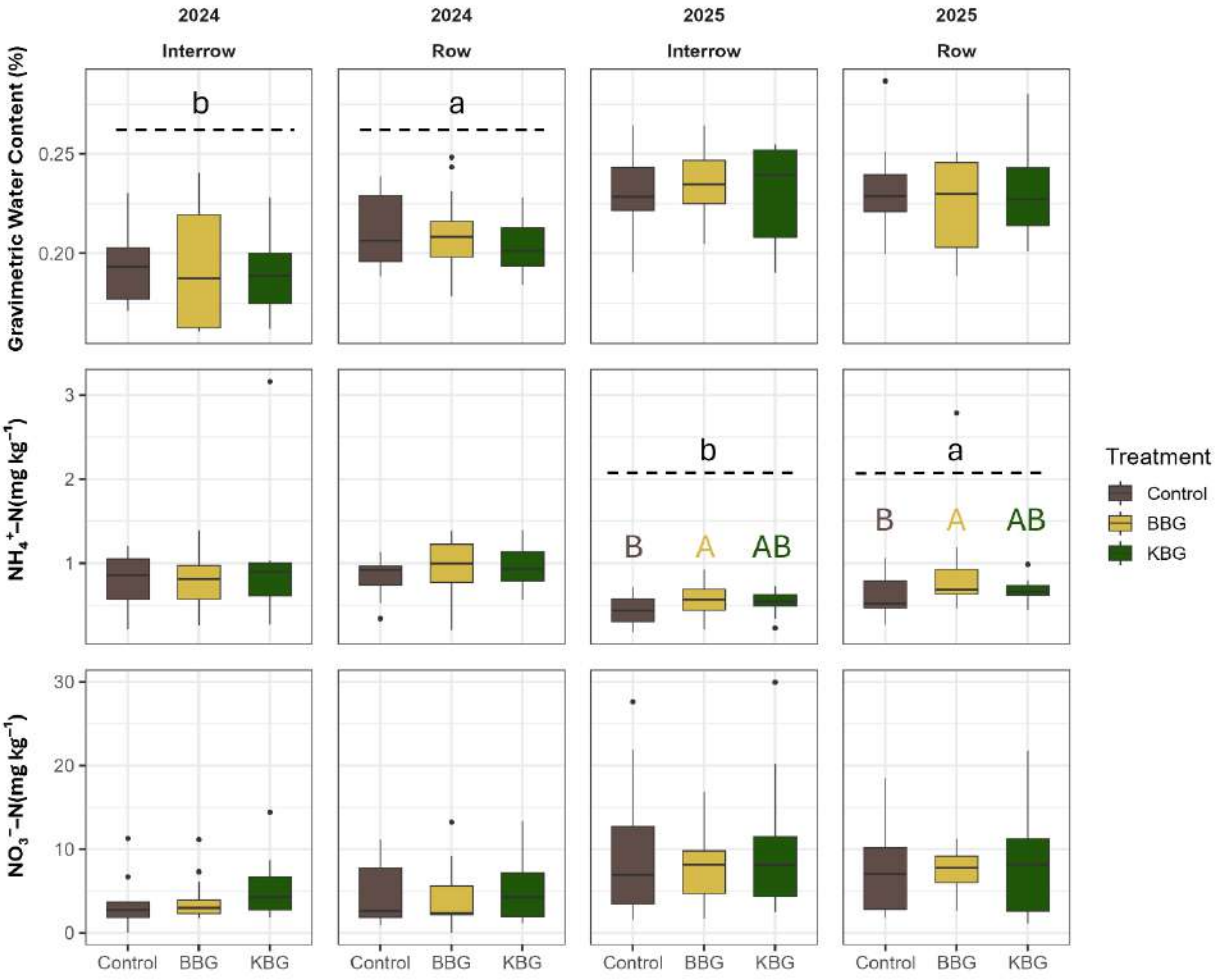

Figure 2.7 Gravimetric soil water content (%), ammonium-N (NH_4^+–N; mg kg^{-1}), and nitrate-N (NO_3^-–N; mg kg^{-1}) measured during the 2024 and 2025 growing seasons, separated by interrow and row positions. Boxplots represent the distribution of observations within each treatment (Control, BBG, and KBG). Capital letters (color-coordinated with treatments) indicate treatment significance within a given position and year ($P \leq 0.05$). Lowercase letters associated with the horizontal dashed line denote statistically significant differences between positions (row vs. interrow) within the same year ($P \leq 0.05$). The dashed line spans the width of the panel to indicate that the positional comparison applies collectively across all treatments within that year.

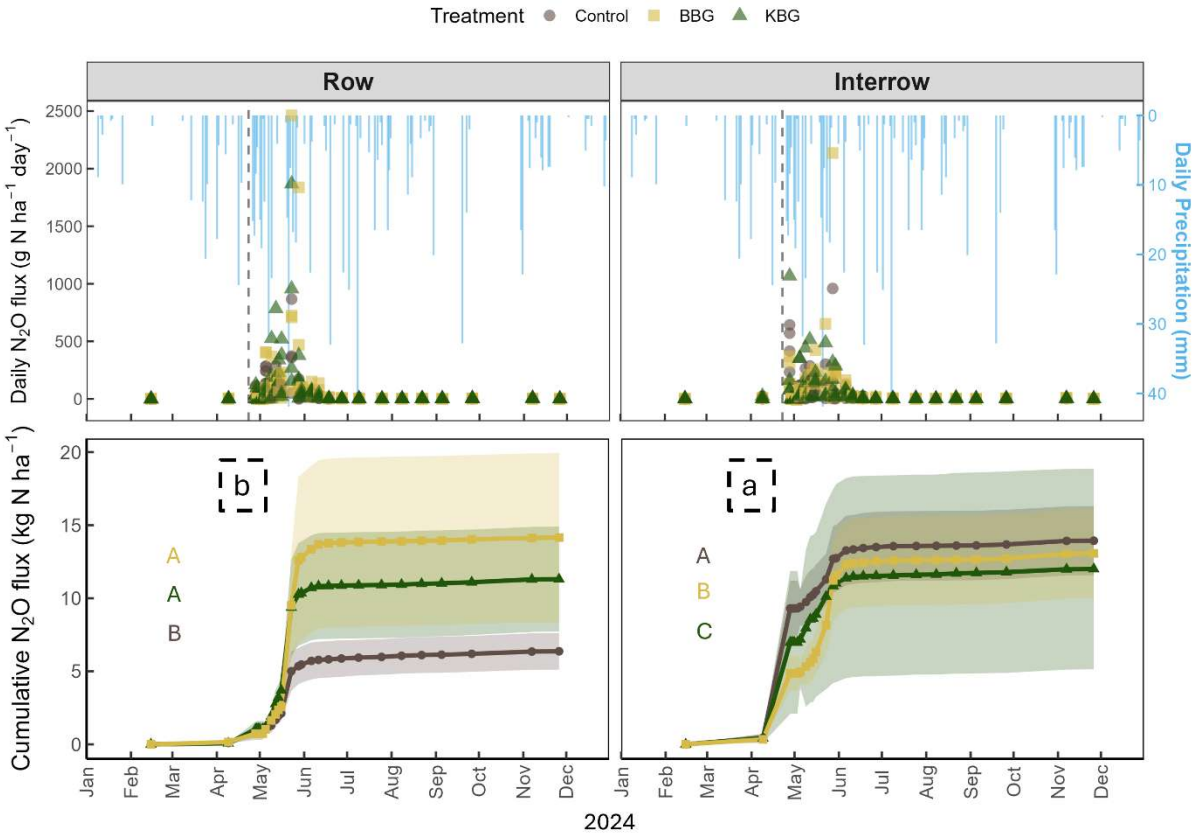

Figure 2.8 Daily nitrous oxide (N_2O) flux (g N ha^{-1} day^{-1}; upper panels) and cumulative N_2O flux (kg N ha^{-1}; lower panels) during the 2024 growing season, shown separately for interrow and row positions. Daily precipitation (mm) is displayed on the secondary y-axis in the upper panels, and the vertical dashed line indicates the fertilizer application date. Shaded regions in the cumulative panels represent ± standard error of the mean. Uppercase letters (color-coordinated with treatments) indicate treatment significance within a given position ($P \leq 0.05$). Lowercase letters enclosed in dashed boxes denote statistically significant differences between positions (row vs. interrow) within the same year ($P \leq 0.05$).

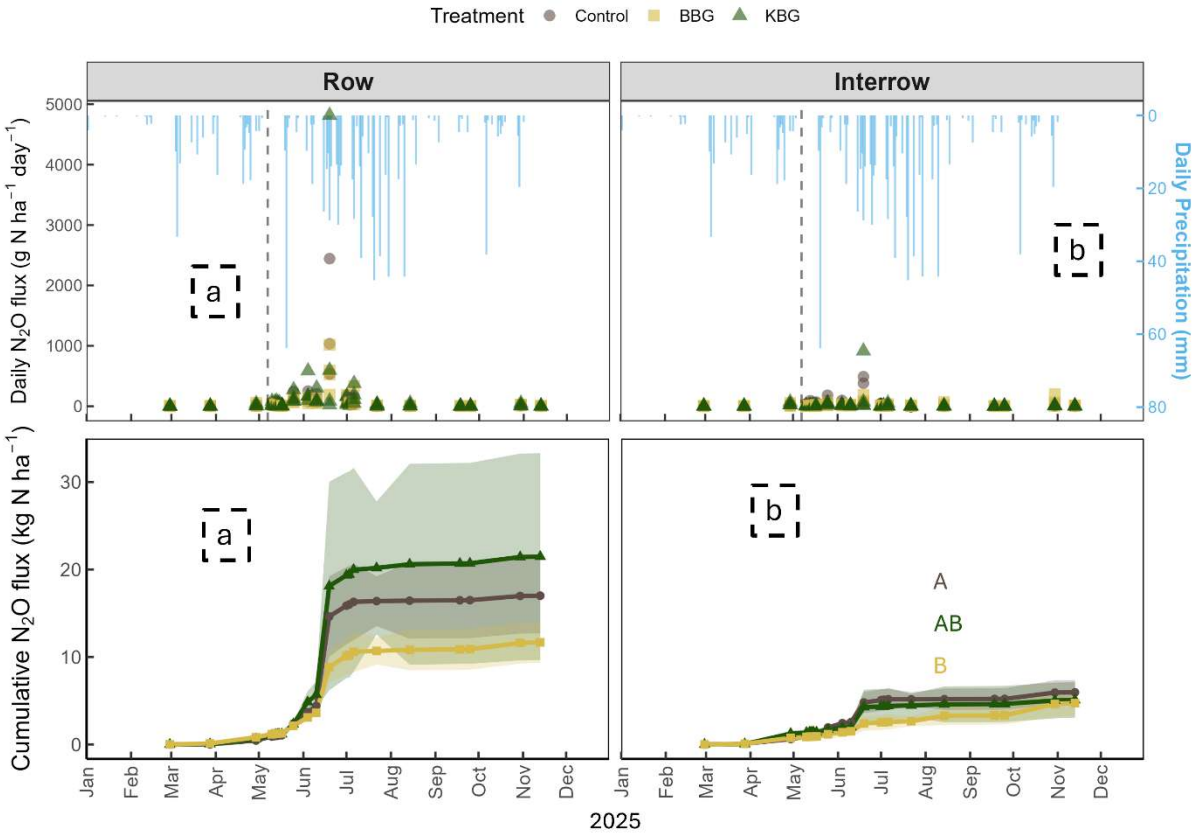

Figure 2.9 Daily nitrous oxide (N_2O) flux (g N ha^{-1} day^{-1}; upper panels) and cumulative N_2O flux (kg N ha^{-1}; lower panels) measured during the 2025 growing, separated by interrow and row positions. Daily precipitation (mm) is shown on the secondary y-axis in the upper panels. The vertical dashed line indicates the date of fertilizer application. Shaded regions in the cumulative panels represent ± standard error of the mean. Uppercase letters (color-coordinated with treatments) denote treatment significance within a given position ($P \leq 0.05$). Lowercase letters enclosed in dashed boxes indicate statistically significant differences between positions for all treatments ($P \leq 0.05$).

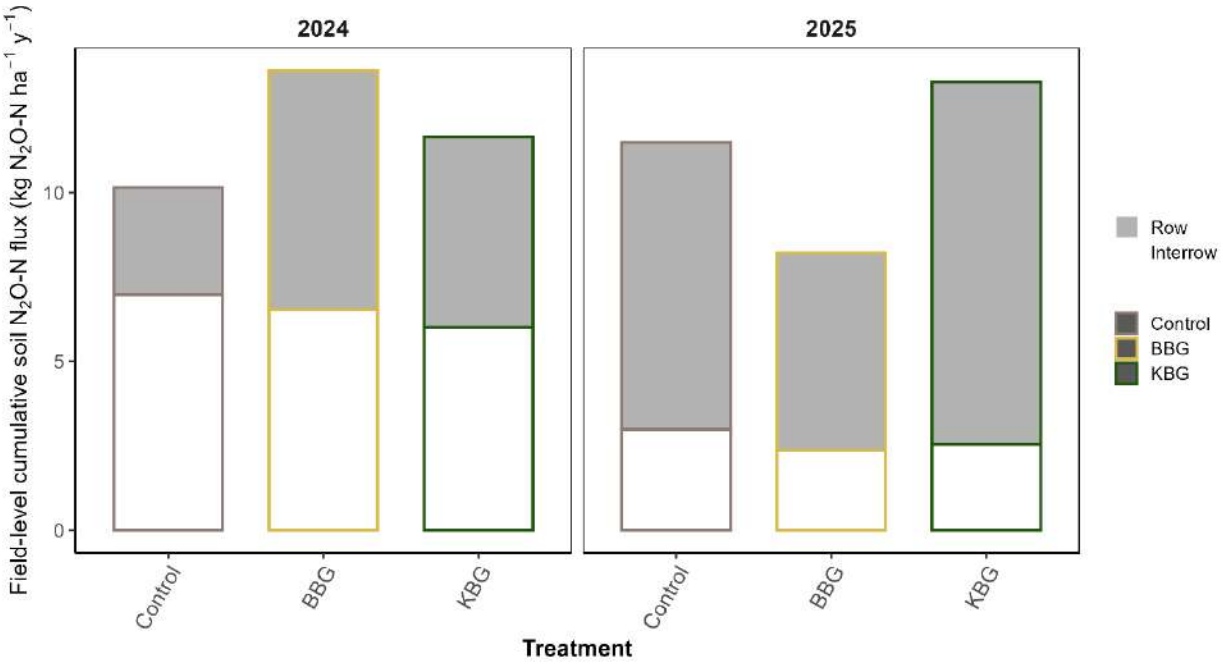

Figure 2.10 Field-level cumulative soil nitrous oxide (N_2O–N) flux (kg N_2O–N ha^{-1} yr^{-1}) as influenced by perennial ground cover treatments in 2024 and 2025. Bars represent treatment means for Control, *Poa bulbosa* (BBG), and Kentucky bluegrass (KBG), partitioned by sampling position (row and interrow), with each position contributing equally (50%) to the field-level estimate.

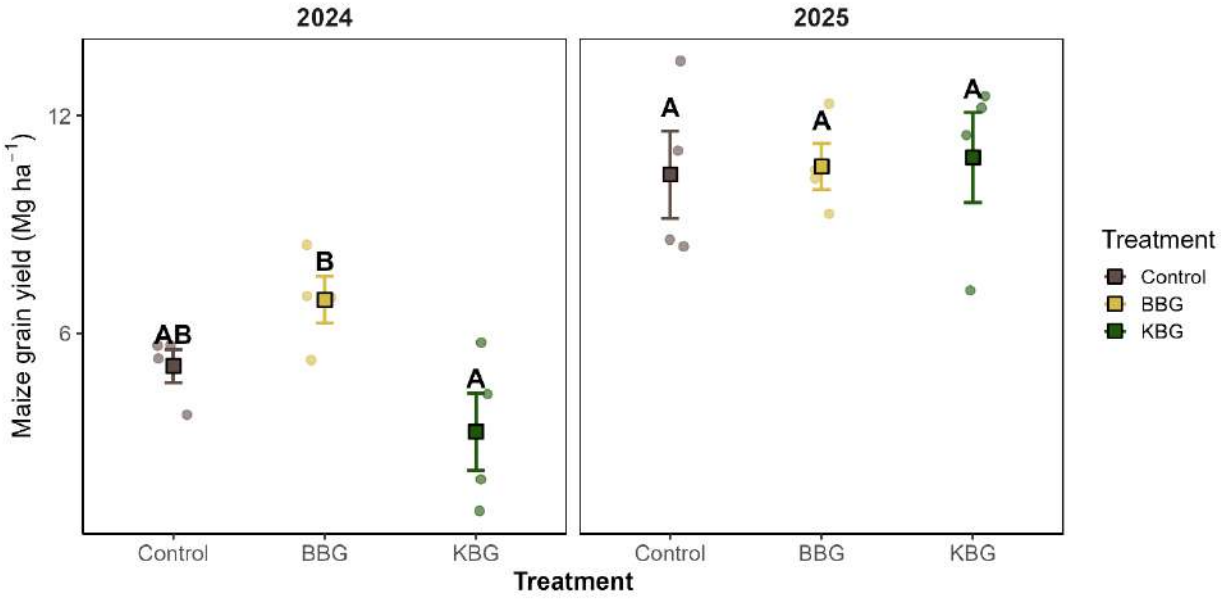

Figure 2.11 Maize grain yield (Mg ha^{-1}) as affected by perennial groundcover (PGC) treatments across 2024 and 2025. Points represent individual plot-level observations, while squares and error bars indicate treatment means ± standard error. Letters denote significant differences among treatments within each year based on pairwise comparisons ($\alpha < 0.05$).

Appendix

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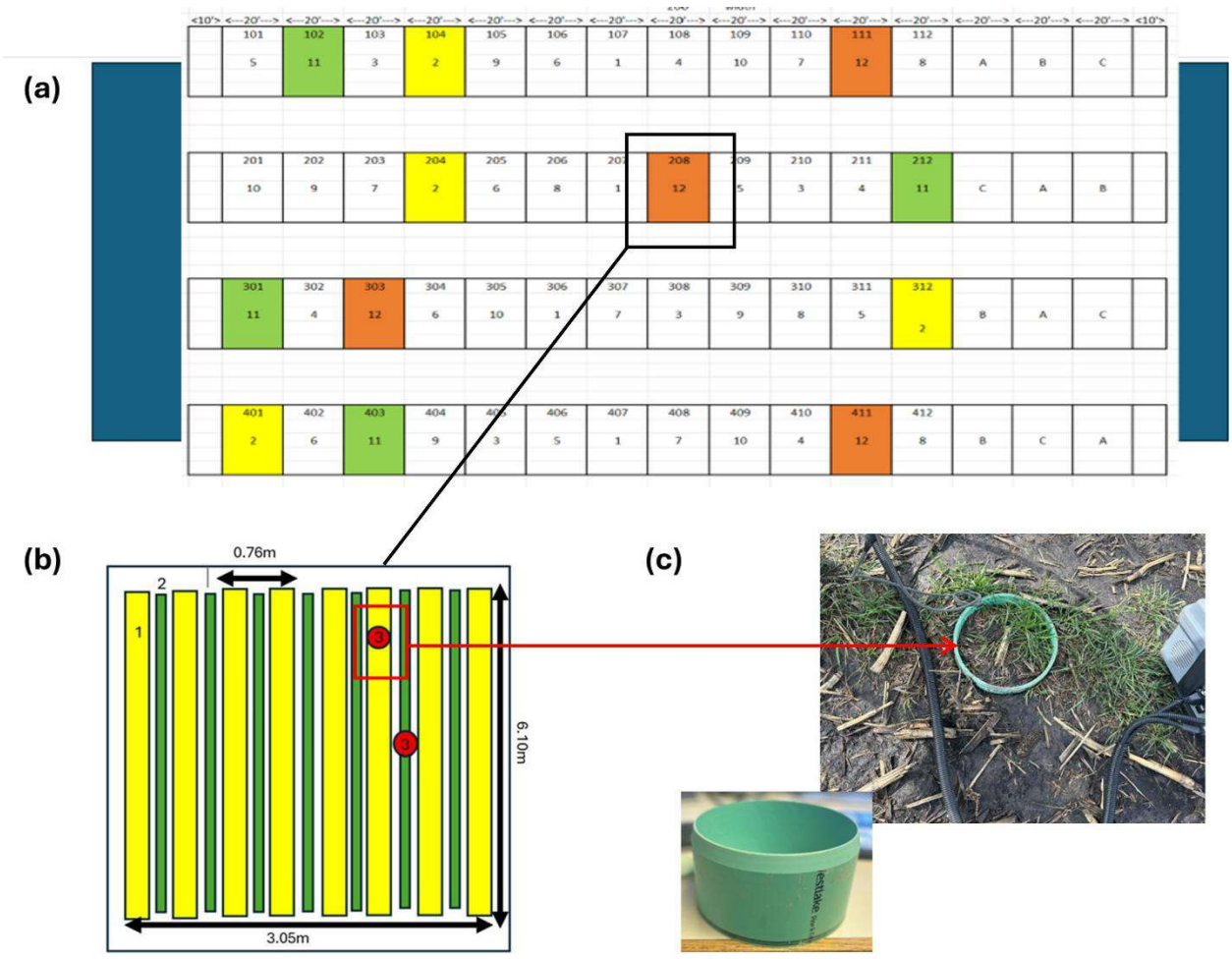

Figure 2.12 Experimental design and gas sampling configuration for the perennial ground cover (PGC) experiment. (a) Field layout showing the randomized complete block design consisting of four blocks, each containing treatment plots representing 2 perennial ground cover treatments and a maize-only control. Colored plots indicate treatment assignment within each block. (b) Schematic representation of the plot-level configuration (3.05 m × 6.10 m) consisting of eight maize rows spaced 0.76 m apart. Perennial ground cover strips were established between maize rows. Two gas flux collars were installed per plot, one positioned within the maize row and one within the interrow groundcovers trip. (c) Example of a PVC soil collar installed in the field and used for closed chamber measurements of N_2O fluxes.

Table 2.11 Site-level mean ± standard deviation of soil physical and chemical properties.

Soil Property	**Mean ± SD**
Bulk Density (g cm^{-3})	1.48 ± 0.20
Gravimetric Water Content (%)	0.16 ± 0.03
pH	6.25 ± 0.26
Sand (%)	35.55 ± 7.46
Clay (%)	27.11 ± 3.44
Silt (%)	37.34 ± 4.52
Cation Exchange Capacity	16.93 ± 1.78
Soil Organic Matter (%)	2.54 ± 0.41

CHAPTER 3. GENERAL CONCLUSIONS

The future of agriculture will likely depend on meeting the dual challenge of ensuring food security for an estimated global population of 10.3 billion while simultaneously minimizing environmental degradation. Achieving this goal will require innovative approaches to agricultural land management, particularly because increases in production will most likely occur on existing arable land. In recent decades, synthetic nitrogen fertilizers have helped sustain increases in crop production, but have also led to greater nitrogen losses through nitrate leaching and nitrous oxide emissions (Fixen & West, 2002; Millar et al., 2010)

Together, nitrate and nitrous oxide losses represent some of agriculture's most significant contributions to environmental degradation due to their impacts on water quality and climate change(United States Environmental Protection Agency, 2025; Jones et al., 2018). Traditional cover crops, also known as winter cover crops in the U.S. Midwest, are among the most effective conservation practices to reduce soil nitrogen losses and improve soil health (Kaspar et al., 2011; Thapa et al., 2018)However, due to constraints such as narrow planting windows, annual replanting, and termination requirements, farmer adoption rates remain low (Plastina et al., 2020a, 2020b; Singer & Kohler, 2005).

Perennial groundcover (PGC) systems represent a developing alternative to winter cover crops, as they provide similar ecological benefits while maintaining year-round soil cover due to their perennial nature. Previous research has established that PGC influences soil water dynamics, nutrient cycling, and microbial activity (Banik et al., 2020; Galland et al., 2026; Moore et al., 2019; Schlautman et al., 2021), , yet their effects on nitrous oxide emissions and inorganic nitrogen pools remain understudied.

Therefore, the primary objective of this study was to assess and quantify the effects of PGC systems on nitrous oxide emissions and inorganic nitrogen availability within continuous

maize production in the U.S. Midwest. Although nitrous oxide responses were not uniform, we demonstrated that integrating PGC systems into continuous maize production can affect and potentially reduce nitrous oxide emissions depending on spatial distribution at the plot level. In each year of the study, at least one PGC treatment reduced interrow nitrous oxide emissions relative to the maize control. However, when positions were aggregated to the field level, PGC treatments had no effect on N_2O emissions across both years, suggesting that PGC effects are primarily limited to the plot scale.

We conclude that these observations were likely associated with a combination of soil water dynamics, nitrogen immobilization, and interannual climate variability, particularly precipitation. Consistent with previous studies, we also observed the "hot moment" phenomenon, where early-season emissions accounted for the majority of cumulative annual nitrous oxide emissions. These results indicate that PGC systems did not override the well-established temporal and spatial variability of nitrous oxide emissions (Parkin & Kaspar, 2006).

Overall, PGC systems had only a modest influence on soil carbon and nitrogen pools and microbial activity dynamics. However, KBG treatments consistently increased SEON concentrations relative to the control, likely reflecting rapid microbial turnover associated with perennial root-derived carbon inputs (Andrews et al., 2018; Graham et al., 2021; McDaniel & Grandy, 2016; Sui et al., 2025). Spatial differences were also observed in BBG treatments, where microbial biomass carbon was greater in soils under the bulbous grass relative to the maize row. In addition, ammonium concentrations were elevated in BBG treatments relative to the control, suggesting a modest increase in soil mineralization.

Despite these localized differences, the overall stability observed in soil carbon and nitrogen pools indicates that nutrient cycling processes remained tightly coupled within the

system. Processes such as mineralization, plant uptake, and microbial immobilization likely occurred simultaneously, resulting in relatively balanced nitrogen dynamics across treatments(Daly et al., 2022)

Beyond the insights generated by this study, several questions remain. While we demonstrated that PGC systems can alter nitrous oxide emissions in continuous maize production, the mechanisms driving the observed spatial inconsistencies require further investigation. PGC responses to interannual variation, particularly precipitation, may help identify the conditions under which PGC systems can mitigate N_2O emissions at the field scale. Previous studies have shown that PGC effects on soil nutrients and processes shift with changes in precipitation (Banik et al., 2020; Galland et al., 2026; Wiggans et al., 2012). As a result, long-term research examining how interannual precipitation variability influences plant-available nutrients and N_2O emissions would provide valuable insight.

in summary, this study highlights that PGC systems have the potential to contribute to more sustainable agricultural systems, although further research is needed to optimize their effectiveness. More broadly, these findings emphasize the importance of designing cropping systems that improve productivity while minimizing environmental impacts. Future research should build on these results to refine PGC systems in ways that maximize ecological benefits and crop productivity, ultimately contributing to more resilient agricultural landscapes. Although, adaptation of sustainable agriculture practices like PGC can be culturally supported or not, researchers should also consider developing financial incentives (markets) to increase chances of farmers adopting PGC systems in the future.

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