A Social-Ecological Analysis of Ecological Nutrient Management using Cover Crops in the U.S. Midwest

by

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Dedication

To my husband and my parents for their unconditional love and support.

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Abstract

Nitrogen (N) fertilizer inputs to agricultural soils are a leading cause of nitrous oxide (N_2O) emissions, contributing significantly to global climate change. As an alternative to conventional fertilizers, N_2 fixing legumes can provide an organic source of N that recouples N and carbon (C) cycles. Legumes can be added to crop rotations as cover crops, which are non-harvested crops that provide critical ecosystem functions including N supply and retention. Further, mixtures of cover crop species with complementary functional traits can increase multiple ecosystem functions at once. Despite the potential benefits of widespread use of cover crops, adoption is low on farms in the U.S. Midwest. Top-down constraints including policies (e.g., the Farm Bill), dominant knowledge systems and infrastructure, and concentrated grain markets create large barriers to cover crop adoption. This dissertation applies and extends a social-ecological systems framework to link cover cropping as an ecological nutrient management (ENM) practice with the social variables that influence farmer perceptions and adoption of cover crops. Chapter 1 introduces key principles of ENM and the social-ecological systems framework that guided this interdisciplinary research.

To advance ecological knowledge of cover crops, in Chapter 2, I conducted a field experiment at two sites with contrasting soil fertility properties. I tested the hypothesis that a legume-grass cover crop can decrease N_2O emissions compared to a sole legume during the two-week window following tillage because grass litter can decrease N mineralization rates. I found partial support for my hypothesis: the functionally-diverse legume-grass mixture led to a small reduction in N_2O emissions at one site but led to a slight increase at the other. The different

treatment patterns between sites suggest that interactions between cover crop functional types and background soil fertility impact N_2O emissions as cover crops decompose.

In Chapter 3, I tested the hypothesis that a legume-grass mixture would provide similar N inputs as a sole legume to support corn yield, while reducing N_2O losses over the whole corn growing season. The mixture supplied similar N inputs; however, I did not find significant differences in cumulative N_2O emissions between treatments. A six-year N mass balance indicated N inputs and exports are approximately balanced in this agroecosystem. Historical data show that the long-term history of ENM has continued to build soil organic matter stocks, which is likely a more important driver of N cycling processes than the short-term addition of the mixture, explaining similarities between treatments.

Chapter 4 is a case study of the 2017 Cover Crop Champions peer education program. Using qualitative interviews with 24 participants, I demonstrate that bottom-up actions helped farmers overcome structural constraints to cover cropping by training farmers to use language that normalized cover crop adoption, and by leveraging farmer networks to facilitate peer education and mentorship. Despite this progress, decades of research and financial incentives have largely supported large-scale commodity production, reducing access to resources needed for farmers to transition to ENM practices.

Chapter 5 integrates findings across chapters, discusses the broader social-ecological systems impacts of ecological management with cover crops, and proposes further research needs. Overall, this interdisciplinary dissertation addresses gaps in our understanding of how functionally diverse cover crops influence N cycling under different soil conditions, and how social factors including farmer networks influence attitudes towards and adoption of cover crops.

Chapter 1 Introduction

The convergence of global climate change with food, energy, and water crises has called new attention to the sustainability of agricultural management. In the U.S. Midwest, excess nitrogen (N) fertilizer inputs to grain fields have resulted in decades of N losses including nitrous oxide (N_2O) which contributes significantly to global greenhouse gas emissions (Robertson and Vitousek 2009, USEPA 2021). Amid calls for a reduction in greenhouse gas emissions to mitigate the catastrophic effects of a warming planet, it is critical that farmers shift from industrial to ecological management practices that optimize soil fertility management and protect the surrounding environment by reducing nutrient pollution (Drinkwater et al. 2008, IPCC 2019).

1.1 Ecological Nutrient Management

Agroecology applies ecological principles to agroecosystem management, linking concepts from ecology, agronomy, and the social sciences with farmer knowledge and practice (Gliessman 2007, Tomich et al. 2011). Within the broad field of agroecology, my dissertation focuses on ecological nutrient management as a conceptual framework and practice for tightening N cycling on Midwestern grain farms. Ecological nutrient management applies principles of ecosystem ecology to manage soil fertility to support both crop production and environmental sustainability (Drinkwater et al. 2008). Ecological practices provide an alternative to industrial management, for instance, by replacing synthetic fertilizer N inputs with legume N_2 fixation within crop rotations. Legumes can be added to crop rotations as cover crops, which are unharvested crops that are typically planted in the fall and terminated in the spring in temperate agroecosystems. Cover crops increase functional diversity to provide a broad suite of ecosystem services in grain agroecosystems with minimal disruption of typical crop rotations (e.g., corn-soy-wheat) (Snapp et al. 2005, Davis et al. 2012, King and Blesh 2018). In particular, legume cover crops have the potential to reduce or replace conventional fertilizer inputs through biological N_2 fixation (BNF) carried out by symbiotic bacteria. Compared to synthetic N fertilizer inputs, legume N sources have been shown to better balance N inputs to fields with N exported in harvested crops, reducing N losses (Blesh and Drinkwater 2013).

High intensity monoculture agriculture often only supports one ecosystem function – yield – relying on external inputs, such as fertilizer and pesticides, to maintain yield in the absence of species diversity. This reductionist way of managing agriculture has led to serious environmental degradation down-stream and down-wind of agricultural fields (Galloway et al. 2003, Drinkwater et al. 2008). For example, applying inorganic N fertilizer to fields in large pulses results in low N use efficiency. More than 50% of synthetic N fertilizers are lost, adding excess reactive N to the surrounding environment through leaching and gaseous losses (Galloway et al. 2008, Robertson and Groffman 2015).

Legume cover crops can improve agroecosystem N retention compared to soluble N fertilizers because they couple carbon (C) and N cycles, adding both C and N inputs to the soil, and reducing the size of inorganic N pools that are most vulnerable to loss. By increasing the length of time during which photosynthesis is occurring during a crop rotation, cover crops increase C and N assimilation into organic matter, increasing soil organic matter (SOM) stocks while reducing pools of soil inorganic N (Drinkwater and Snapp 2007, McDaniel et al. 2014, King and Blesh 2018, Blesh 2019). Drinkwater et al. (1998) found that differences in the biochemical quality of N inputs (e.g., organic vs. inorganic) affects N cycling processes that improve long-term soil N retention on farms. That is, more legume-derived N than fertilizer-derived N is immobilized in microbial biomass and stored in SOM pools. The increased agroecosystem functional diversity with overwintering legume cover crops can therefore maintain soil fertility and restore the biological linkage between C and N cycling. For example, a study at Kellogg Biological Station's Main Cropping System Experiment found that the microbial community in an organic cropping system managed with legume cover crops had 50% higher C use efficiency and 56% higher microbial growth rates compared to the community in a conventional grain cropping system managed with N fertilizer (Kallenbach et al. 2015). The study suggests that changes in microbial physiology that transform new C from photosynthesis into microbial biomass are key mechanisms for soil organic carbon (SOC) accumulation. Even with lower net C inputs overall, and frequent soil disturbance through mechanical tillage, organic cropping systems managed with legume cover crops can accumulate more SOC than continuous grain rotations (Kallenbach et al. 2015).

SOC can be broken down into particulate organic matter (POM) and mineral-associated organic matter (MAOM) fractions (Cotrufo et al. 2019). POM fractions are influenced directly by plant litter inputs to soil, changing on shorter time-scales than total SOM, and serving as an indicator of soil nutrient supply from decomposition (Wander 2004). MAOM has greater potential to store soil organic N (SON) but has a longer turnover time. However, given its large size and low C:N, this fraction may still be a significant source of N through mineralization (Jilling et al. 2018). Cover crops, which can increase the biochemical diversity of inputs to soils through aboveground biomass and root exudates – and which extend the overall time during which roots are active in the soil – have the potential to build both POM and MAOM pools. These pools turnover at different rates, increasing the capacity of a soil to assimilate, recycle, and store N in SOM (Li et al. 2018, Blesh 2019). In summary, there are multiple mechanisms through which cover crops can stimulate N release from SOM, driving internal N cycling and storage in multiple SOM fractions, resulting in lower N losses (leaching and denitrification) than from synthetic fertilizers (Drinkwater et al. 1998, Syswerda et al. 2012, Kallenbach et al. 2015).

Despite the potential importance of legume cover crops for reducing use of synthetic fertilizers, sole planted rye is still the most common cover crop in Michigan. There is growing interest, however, in planting mixtures of rye with cover crops in the legume family to simultaneously enhance multiple ecosystem functions (Snapp et al. 2005, Hayden et al. 2014, Poffenbarger et al. 2015, Wood et al. 2015, Blesh 2017). In diverse agroecosystems, small increases in functional diversity (e.g., 2-3 species cover crop mixtures with complementary traits) can impact ecosystem function (e.g., SOC, N cycling, microbial biomass, weed suppression) (Drinkwater et al. 1998, McDaniel et al. 2014, Tiemann et al. 2015, Blesh 2017). This is evidenced by Storkey et al.'s (2015) analysis of different combinations of cover crop mixtures (drawing from a pool of 10 legumes and 4 grasses), finding that low to intermediate levels of species richness, particularly when species exhibited contrasting functional traits in terms of both growth habit and phenology, provided an optimal balance between ecosystem services (Storkey et al. 2015). Subsequent studies have also found that selection of crops with complementary functional traits can enhance the multifunctionality of agroecosystems by simultaneously providing services such as BNF, nutrient retention, enhanced soil quality, and weed suppression (Blesh 2017, Finney and Kaye 2016).

This suggests that increasing diversity in crop mixtures should be done intentionally. For example, farmers can strategically choose species based on known functional traits and complementary relationships to maximize ecosystem functions to improve soil quality and tighten nutrient cycles (Blesh 2017). My dissertation research focuses on how legume-grass cover crop mixtures can increase nutrient use efficiency through niche differentiation and complementary N acquisition strategies. Legumes and grasses occupy different niche spaces above and below ground, due to their different rooting depths and growth patterns. Annual grasses tend to have shallow roots that spread out radially from the plant in the top few inches of the soil while legumes have deeper tap roots. This allows the two species to exploit spatially different pools for nutrients and water (Gliessman 2007, Nyfeler et al. 2011). Niche differentiation can increase soil C accumulation through increased root biomass (Fornara and Tilman 2008, Steinbeiss et al. 2008). Complementary aboveground traits of tall grasses and short bushy legumes, allows mixtures to occupy more vertical space to maximize light interception, increasing photosynthesis (Liebman and Dyck 1993).

Legume-grass mixtures also have complementary N acquisition strategies. Legumes can supply newly fixed N_2 through BNF, while grasses enhance N retention by scavenging soil N, along with providing other functions such as weed suppression at higher levels than legumes (Blesh 2017). Additionally, the functions provided by legumes can be enhanced in mixtures with grasses compared to in monoculture stands. Legumes allocate up to 30% of their photosynthate to rhizobia in root nodules in return for fixed N (Minchin and Pate 1973, Warembourg and Roumet 1989). Legumes generally allocate less photosynthate to BNF if sufficient plant-available N is already present in soil. Through competition for soil N with grasses, legumes respond by increasing the energy-intensive processes needed to fix N, increasing BNF rates per plant (Jensen 1996, Hogh-Jensen and Schjoerring 1997). In conclusion, as functional diversity, not simply biodiversity, increases on farms from monocultures to more complex crop rotations, the need to apply external inputs declines, indicating that diversified agroecosystems provide more ecosystem functions than monoculture systems adapted to maximize only yield (e.g., Tiemann et al. 2015, Blesh 2017, Beillouin et al. 2021).

1.2 Overcoming constraints to cover crop adoption

In addition to the complex environmental drivers that moderate cover crop outcomes on working farms, a suite of social conditions also influences cover crop management (Stuart et al. 2015). In the U.S. Midwest, farmers' adoption of conservation practices, including use of cover crops, is voluntary, so the onus is on the individual to manage private land sustainably. However, individual farmers are often constrained from voluntarily adopting cover crops by a range of interacting economic, social, and political barriers (Hendrickson and James 2005, Kremen et al. 2012, Blesh and Wolf 2014). As a result, cover crops are present on only 2-3% of annually harvested cropland in the U.S. (Hamilton et al. 2017). Barriers to adoption include national Farm Bill policy that overwhelmingly supports industrial over ecological management approaches, a supply chain with tight margins that promotes cheap food grown on large-scale monoculture farms, and the erosion of locally-adapted farmer knowledge about conservation practices (Iles and Marsh 2012).

1.3 Conceptual framework and summary of chapters

To understand farmer adaptation strategies for overcoming structural constraints to cover crop adoption in the Midwest, this dissertation evaluates both ecological and social variables that impact farmer decision making. Specifically, I draw on coupled human and natural systems and social-ecological systems frameworks to integrate my dissertation chapters (Figure 1-1). Liu et al.'s (2007) model for coupled human and natural systems considers not only ecological and human variables, but also the complex interactions and feedback loops that connect them. Stuart et al. (2015) applied this framework to analyze farmer decision making about synthetic N fertilizer management, and identified key social, economic, and biophysical factors that drive N losses from industrial agroecosystems. Wittman et al. (2017) also considered interacting processes across temporal and spatial scales in agriculture to develop a social-ecological systems model for assessing the intersection of biodiversity conservation and food security. For instance, constraints to increasing diversity on farms span multiple scales, from declining soil fertility (local) to increasing demand for meat (global) (Wittman et al. 2017). This conceptual framework (Figure 1-1) highlights interactions and feedbacks between multi-scale social (e.g., knowledge, farmer networks, policies, and markets) and biophysical (e.g., climate, soil conditions, and environmental outcomes) factors that mediate farmer decision-making and management of cover crops and resulting impacts on the N cycle. Chapters 2 and 3 address ecological influences on, and outcomes of cover crop management including how soil organic C and N, particulate organic matter, and potentially mineralizable N impact cover crop performance, soil N_2O emissions, and crop production. Chapter 4 addresses social influences of cover crop adoption across multiple scales, focusing on a case study that evaluates how micro- and meso-scale factors can help farmers overcome macro-scale limitations to cover crop adoption.

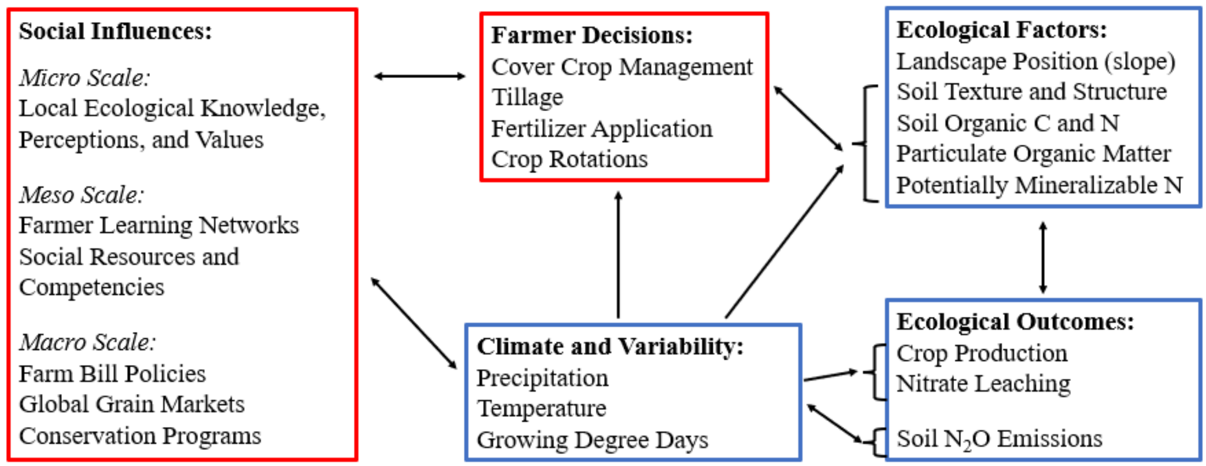

Figure 1-1. A conceptual framework highlighting interactions between social and ecological systems that influence farmer decisions about nutrient management and resulting impacts on the nitrogen cycle. Modified from Stuart et al. 2015.

1.3.1 Summary of dissertation chapters

To evaluate ecological factors that influence farmer decision making, I interviewed 24 grain farmers in Michigan in 2017 prior to developing my research questions. I worked with conservation district staff in southern Michigan to identify grain farmers who were using cover crops or had used cover crops previously. I asked farmers a series of questions about their management practices (i.e., crop rotation, tillage methods, cover crop species, and fertilizer practices) and inquired about why they started cover cropping and how they made the transition. We also discussed how cover crops affected their fertilizer management practices. Generally, interviews revealed that farmers had not reduced N fertilizer application rates after adopting cover crops. Many farmers had only been using grass (e.g., cereal rye, annual ryegrass) cover crops for a few years. Given that rye does not fix N, and that a few years of cover cropping may not be sufficient to increase soil organic matter and internal nutrient cycling capacity, it is reasonable that farmers had not yet adapted their N fertilizer rates. Generally, I found that farmers wanted to know more about N cycling dynamics following use of legume cover crops. Particularly, they wanted to know how much N they could attribute to a legume when making decisions about fertilizer reductions. This, combined with growing concern about agriculture's contribution to greenhouse gas emissions, including N_2O, led me to conduct two field experiments measuring soil N cycling following legume and non-legume overwintering cover crops. The first study was conducted at the University of Michigan Campus Farm (*CF*) during the 2017-2018 overwintering cover crop season. The second study was conducted at the Kellogg Biological Station (*KBS*) Long-Term Ecological Research (LTER) site during the 2019-2020 overwintering cover crop season. At both sites, I planted cereal rye (*Secale cereal L.*), crimson clover (*Trifolium incarnatum L.*), a mixture of these two species, and a weedy fallow control. Cover crops overwintered and were tilled into the soil the following spring, and fields were planted to organic corn.

Chapter 2: Episodic N_2O emissions following tillage of a legume-grass cover crop mixture

Chapter 2 reports results from the first experiment at *CF*, which focused on the first few weeks following tillage when N_2O emissions are expected to be highest following a large soil disturbance (tillage) and pulse input of organic residues, and from the first two weeks of the second experiment at *KBS*. I hypothesized that N_2O flux following tillage of the cover crops would be lower in the mixture and rye treatments compared to the clover treatment, because biochemical properties of rye litter can decrease initial N mineralization rates. I found partial support for my hypothesis with higher N_2O emissions following both treatments with legumes compared to treatments without legumes, with particularly distinct differences in emissions when controlling for differences in soil fertility parameters across sites. The cover crop mixture tended to have a lower N_2O flux at *CF* compared to the legume, but did not at *KBS*, suggesting that interactions between cover crop functional types (legume vs. non-legume) and background soil fertility levels influence N_2O emissions, warranting future studies to evaluate how cover crop composition and performance influences soil N cycling across a wide range of soil conditions.

Chapter 3: Nitrogen cycling dynamics following a legume-grass cover crop mixture in an organic agroecosystem

In Chapter 3, I expanded on work in Chapter 2, measuring N_2O emissions throughout the whole corn growing season (91 days), and measuring corn chemistry and yield outcomes, in an organic agroecosystem at *KBS* that had been managed for 30 years with BNF by a legume cover crop as the sole external fertility input. I hypothesized that the legume-grass cover crop mixture would provide similar BNF inputs while increasing the chemical diversity of the cover crop residue. I expected that the mixture would improve synchrony between cover crop N release and corn N assimilation, reducing N_2O emissions compared to the legume while increasing corn yield compared to the grass. My hypothesis that BNF inputs would be similar between clover and mixture was supported, however, the mixture did not change litter chemistry, reduce N_2O emissions compared to clover, or increase yield compared to rye grown alone. An N mass balance over two full crop rotations (2014-2019) prior to our study period in 2020, and an analysis of historical SOC and SON data showing increases in SOM stock over time, indicate that this organic agroecosystem managed with cover crops is tightening N cycling, increasing soil N retention over time.

Chapter 4: Cover Crop Champions: Linking strategic communication approaches with farmer networks to support cover crop adoption

To evaluate social barriers and opportunities to cover crop adoption, Chapter 4 evaluates the National Wildlife Federation’s 2017 Cover Crop Champions cohort using qualitative methods based on semi-structured interviews with 24 farmers and outreach professionals. Research objectives were to: 1) evaluate the effectiveness of the methods and resources that Champions used to communicate with other farmers about cover crops, 2) identify factors that lead to sustained outreach efforts after participating in the program, and 3) examine if and how farmer perceptions about, and willingness to adopt cover crops changed after engaging with Champions. Findings showed that the program leveraged bottom-up mechanisms including farmer networks and novel communication methods to help farmers overcome constraints to cover crop adoption. Other programs could adopt a similar model of hiring trained facilitators who are experts in social theory to develop localized, strategic communication approaches to mobilize farmer networks. Findings suggested that Cover Crop Champions helped change farmer perceptions about cover crops to overcome structural constraints. Farmers will still face many top-down barriers to diversifying their management systems, but sustained and widespread efforts to increase cover crop adoption across the U.S. can create pressure for institutional changes, which will be necessary for widespread adoption of practices like cover crops.

Chapter 5: Conclusions and next steps

In Chapter 5, I summarize the overarching goal of my dissertation research and discuss the results from each chapter, integrating findings from my mixed-methods approach within a social-ecological systems framework. Cover crops have the potential to serve as a critical ecological nutrient management practice to restore fertility in highly degraded soils, increase agroecosystem resilience to extreme weather events and changing weed and pest pressure in the context of a changing climate, and provide ecosystem services that can replace conventional, fossil fuel intensive inputs like fertilizers and pesticides. Finally, I propose that future ecological studies take key social factors, including farmers' perceptions and attitudes towards cover crops, into consideration by co-designing on-farm experiments with farmers. Such experiments could span years to decades, as well as a wide range of natural, economic, and social conditions to better understand how cover crops respond to real-world variability to impact ecosystem functions on farms and at larger spatial scales.

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Chapter 2 Episodic N_2O Emissions Following Tillage of a Legume-Grass Cover Crop Mixture[1]

Abstract

Nitrogen (N) fertilizer inputs to agricultural soils are a leading cause of nitrous oxide (N_2O) emissions. Legume cover crops are an alternative N source that can reduce agricultural N_2O emissions compared to fertilizer N. However, our understanding of episodic N_2O flux following cover crop incorporation by tillage is limited and has focused on single species cover crops. Our study explores whether increasing cover crop functional diversity with a legume-grass mixture can reduce pulse emissions of N_2O following tillage. In a field experiment, we established crimson clover (*Trifolium incarnatum L.*), cereal rye (*Secale cereal L.*), a clover-rye mixture, and a no-cover control at two field sites with contrasting soil fertility properties in Michigan. We hypothesized that N_2O flux following tillage of the cover crops would be lower in the mixture and rye compared to the clover treatment, because rye litter can decrease N mineralization rates. We measured N_2O for approximately two weeks following tillage to capture the first peak of N_2O emissions in each site. Across cover crop treatments, the higher fertility site, *CF*, had greater cover crop biomass, twofold higher aboveground biomass N, and higher cumulative N_2O emissions than the lower fertility site, *KBS* (413 ± 67.5 g N_2O-N ha^{-1} vs. 230 ± 42.5 g N_2O-N ha^{-1}; $P = 0.0037$). There was a significant treatment effect on daily emissions at both sites. At *CF*, N_2O fluxes were higher following clover than the control 6 days after tillage.

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At *KBS*, fluxes from the mixture were higher than rye 8 and 11 days after tillage. When controlling for soil fertility properties across sites, clover and mixture led to approximately twofold higher N_2O emissions compared to rye and fallow treatments. We found partial support for our hypothesis that N_2O would be lower following incorporation of the mixture than clover. However, treatment patterns differed by site, suggesting that interactions between cover crop functional types and background soil fertility influence N_2O emissions during cover crop decomposition.

2.1 Introduction

Nitrogen (N) losses from grain agroecosystems contribute to climate change through nitrous oxide (N_2O) emissions (Robertson and Vitousek 2009). In the U.S., approximately 75% of N_2O emissions come from agricultural soils (USEPA 2021), and the amount of N added to soil from synthetic fertilizers is the primary driver of these high emissions (Millar et al. 2010; Han et al. 2017; Eagle et al. 2020). Highly mobile forms of inorganic N that are not incorporated into crop biomass, particularly nitrate (NO_3^-), are easily lost from agricultural systems by leaching or through gaseous losses. Denitrification, and to a lesser extent nitrification, convert NO_3^- into N_2O and N_2 gases that are emitted from farm fields.

Diversified grain rotations with legume N sources have lower potential for N losses compared to fields with synthetic fertilizer inputs (Drinkwater et al. 1998; Blesh and Drinkwater 2013; Robertson et al. 2014). Legumes can be added to rotations as cover crops, which are unharvested crops typically planted in the fall and terminated in the spring in temperate regions. As an organic N source, legume litter supplies organic substrates to support microbial processes that can increase soil organic matter (SOM) pools and N retention in SOM (Drinkwater et al. 1998; Syswerda et al. 2012; Blesh and Drinkwater 2013). Further, diversified rotations with legume N sources could reduce or replace the use of synthetic N fertilizers, thereby reducing greenhouse gas emissions associated with fertilizer production and application (Norskov and Chen 2016).

Two key agricultural management factors that affect N_2O emissions are soil disturbance through tillage and crop functional traits (Gelfand et al. 2016). The timing and rate of N release from different cover crop functional types (*i.e.*, C4 vs C3 grasses, N fixing legumes) during decomposition affects the potential for N losses (Millar et al. 2004; White et al. 2017), through effects on soil inorganic N availability. Interactions between the biochemical composition of fresh litter inputs and background soil properties, including the microbial community, are key drivers of microbial decomposition dynamics and N mineralization rates (Kallenbach et al. 2019). Consequently, legume cover crops, which have a high N concentration, may result in higher production of N_2O after disturbances like tillage compared to cover crops that include non-legume species (Alluvione et al. 2010; Huang et al. 2004; Millar et al. 2004). The effects of litter C:N on N mineralization and N_2O flux may be particularly evident when comparing sole legumes with lower C:N ratios (e.g., < 15) to grass cover crops with higher ratios (e.g., > 30) (Baggs et al. 2003). Prior research on legume-grass mixtures – which can have residues of intermediate C:N (e.g., 15-25) – revealed that they reduced N leaching compared to sole legumes, while enhancing N supply compared to sole grasses, providing multiple ecosystem functions (Kaye et al. 2019). However, there is limited data on N_2O losses following cover crops in organically managed agroecosystems, and the effects of mixtures of complementary functional types on N_2O emissions are poorly understood.

Understanding the timing of N_2O emissions is also key to reducing N losses from crop rotations (Wagner-Riddle et al. 2020). Millar et al. (2004) found that N_2O fluxes are episodic in corn rotations with legumes as the sole source of new N. Specifically, 65-90% of N_2O emissions occurred during the first 28 days following tillage of legume cover crops, over an 84-day measurement period. Similarly, Gelfand et al. (2016) observed high temporal variability in N_2O fluxes measured for 20 years in different annual cropping systems and suggested that emissions following tillage were a primary driver of this variation in the two agroecosystems with cover crops. Therefore, there is a need to measure N_2O in the weeks following cover crop termination to understand pulse N_2O fluxes, particularly when legumes are the sole, or primary, source of N additions. Further, to our knowledge no studies have tested whether legume-grass mixtures reduce pulse N_2O during this critical period compared to sole legume cover crops.

Variability in soil conditions also plays an important role in soil N_2O flux. Edaphic characteristics, such as soil texture (Gaillard et al. 2016), SOC (Bouwman et al. 2002; Dhadli et al. 2016), and interannual rainfall patterns can often explain more variation in N_2O emissions than treatment differences (Basche et al. 2014; Ruser et al. 2017). One study with synthetic N fertilizer additions on clayey Oxisols in Brazil found higher N_2O losses from more intensively managed fields with lower labile SOM fractions and total C content (de Figueiredo et al. 2017). In fields with organic N sources, SOM fractions with relatively short turnover times (i.e., years to decades) likely influence N mineralization following cover crop incorporation and resulting N_2O emissions. Free particulate organic matter (fPOM) and occluded particulate organic matter (oPOM), which is physically-protected in soil aggregates, are both indicators of nutrient cycling capacity in soil (Marriott and Wander 2006). Prior studies have found that POM N concentrations are positively correlated with potential N mineralization rates (Blesh 2019), and that this relationship varies with soil texture and management history (Luce 2016). It is therefore critical to assess N_2O emissions in different soil conditions, such as SOM, POM, and nutrient stocks, which reflect land management histories.

In this field experiment, we assessed the effects of a legume-grass cover crop mixture on agroecosystem N cycling processes compared to either species grown alone during the first flux of N_2O following tillage. The experiment was conducted at two sites in Michigan with contrasting soil fertility properties. Our specific objectives were to: (1) quantify cover crop functional traits, including C:N and legume N inputs from biological N_2 fixation (BNF) and (2) test the effects of cover crop treatment on pulse N_2O fluxes following spring tillage, when emissions are expected to be greatest in agroecosystems that rely on legume N sources. Our hypothesis was that the legume-grass mixture would supply the same new N inputs from BNF as the sole-planted legume, while lowering pulse N_2O fluxes due to functional traits of grasses (e.g., high C:N) that would increase diversity of litter inputs and decrease N mineralization rates during the weeks immediately following tillage. Materials and Methods

2.1.1 Site description and experimental design

The study was conducted on two sites in two regions of Michigan, USA. The first site (*CF*) was located at the University of Michigan’s Campus Farm (Lat/Long: N 42° 17' 47", W 83° 39' 19" Elevation: 259.08 m), was previously in a grass fallow with periodic mowing for over 45 years. The experiment at *CF* was conducted in the 2017-2018 overwintering cover crop season. The site resides on a glacial till plain with well drained sandy loam soils in the Fox series which are mixed, superactive, mesic Typic Hapludalfs. The soil had 2.5% organic matter, 21.5% clay, and a pH of 6.35. The site received 1030 mm of rainfall during the experiment (August 2017 – September 2018) with an average temperature of 10.2 °C. The second site (*KBS*) was located in the biologically-based cropping system in the Main Cropping System Experiment (MCSE) of the

Kellogg Biological Station (KBS) Long-Term Ecological Research (LTER) site (Lat/Long: N 42° 14’ 24”, W 85° 14’ 24” Elevation: 288 m). The field has been in a corn-soy-winter wheat rotation managed using organic practices for over 30 years. The experiment at *KBS* was conducted in the 2019-2020 overwintering cover crop season. *KBS*’s soil resides on a glacial outwash plain with well drained loam, sandy loam, and sandy clay loam soils in the Kalamazoo and Oshtemo series which are mixed, mesic Typic Hapludalfs (Crum and Collins, 1995). The soil had 1.74% organic matter, 19.4% clay, and a pH of 6.59. The site receives an average of 933 mm yr^{-1} with an average temperature of 9.2 °C. Neither field received any fertilizer or manure applications before or during the experiment.

In a randomized complete block design, we established four cover crop treatments in 4.5 x 6 m plots in *CF:* (1) cereal rye (seeding rate: 168 kg ha^{-1}), (2) crimson clover (seeding rate: 34 kg ha^{-1}), (3) clover-rye mixture (seeding rate: 67 kg ha^{-1} rye, 17 kg ha^{-1} clover) (4) and a weedy fallow control, in four blocks by broadcasting seed on 16 August 2017. We established four treatments into 3.1 x 12.2 m plots in *KBS*: (1) cereal rye (seeding rate: 100.9 kg ha^{-1}), (2) crimson clover (seeding rate: 16.8 kg ha^{-1}), (3) clover-rye mixture (seeding rate: 50.4 kg ha^{-1} rye, 9.0 kg ha^{-1} clover) (4) and a weedy fallow control, in four blocks with a grain drill on 31 July 2019. Seeding rates were determined based on recommendations from Michigan State University Extension. Seeding rates were reduced for the site planted with a grain drill due to higher likelihood of germination. The cover crops overwintered and were rototilled into the soil on 24 May 2018 (*CF*) and on 26 May 2020 (*KBS*) followed by corn planting on 14 June 2018 (*CF*) and on 1 June 2020 (*KBS*). Cover crops had 4,501 growing degree days at *KBS* and 3,898 at *CF*.

2.1.2 Baseline Soil Sampling

Prior to planting, we collected a composite, baseline soil sample for each replicate block at *CF*, and for each treatment plot within each replicate block at *KBS*, to determine initial soil conditions and characterize soil fertility status at both experimental sites. In each plot, we estimated bulk density from the fresh mass of 10 composited soil cores (2 x 20 cm) and adjusted for soil moisture, determined gravimetrically. Subsamples of ~ 50 g were also analyzed for soil texture using the hydrometer method. Air-dried soil was mixed and soaked with 100 mL of sodium hexametaphosphate and blended for 5 min. The mixture was transferred to a glass sedimentation cylinder and filled to the 1L mark with tap water. The slurry was mixed with a metal plunger and hydrometer readings were taken 40 seconds and 2 hours after the plunger was removed. Percent sand was calculated from the 40 second reading and percent clay from the 2-hour reading.

At sampling, we sieved a subsample of fresh soil to 2 mm and measured extractable and potentially mineralizable N in triplicate for each soil sample. We immediately extracted inorganic N (NO_3^- + NH_4^+) in 2 mol L^{-1} KCl. The amount of NO_3^- + NH_4^+ in each sample was analyzed colorimetrically on a discrete analyzer (AQ2; Seal Analytical, Mequon, WI). We also performed a 7-day anaerobic N incubation and then extracted NH_4^+ in 2 mol L^{-1} KCl. Soil weights for extractions and incubations were adjusted for soil moisture. Potentially mineralizable N (PMN) was calculated by subtracting the initial amount of NH_4^+ in the soil from the NH_4^+ released during the 7-day incubation (Drinkwater et al. 1996).

Particulate organic matter (POM) (> 53 μm) was separated from triplicate 40-g subsamples of unsieved, air-dried soil based on size and density (Marriott and Wander, 2006; Blesh, 2019). To isolate the light fraction POM (also called free POM or fPOM), the subsamples were gently shaken for 1 hour in sodium polytungstate (1.7 g cm^{-3}), allowed to settle for 16 hours, and free POM floating on top of the solution was removed by aspiration. To separate the physically protected, or occluded, POM fraction (oPOM), the remaining soil sample was shaken with 10% sodium hexametaphosphate to disperse soil aggregates and then rinsed through a 53-μm filter (Marriott and Wander 2006). Protected POM was then separated from sand by decanting. The C and N of both POM fractions (fPOM and oPOM) were measured on an ECS 4010 CHNSO Analyzer (Costech Analytical Technologies, Valencia, California, USA). Total soil C and N (to 20 cm) were measured by dry combustion on a Leco TruMac CN Analyzer (Leco Corporation, St. Joseph, Michigan, USA) (Blesh 2019).

2.1.3 Aboveground biomass sampling and analysis

We sampled aboveground biomass from all treatments on 22 May 2018 (*CF*) and on 26 May 2020 (*KBS*), from one 0.25 m^2 quadrat randomly placed in each plot, avoiding edges. Shoot biomass was cut at the soil surface, separated by species (with weeds grouped together), dried at 60 °C for 48 hours, weighed, and coarsely ground (< 2 mm) in a Wiley mill. We analyzed the biomass for total C and N by dry combustion on a Leco TruMac CN Analyzer (Leco Corporation, St. Joseph, MI).

2.1.4 Legume N_2 fixation by natural abundance

We estimated BNF by crimson clover using the natural abundance method (Shearer and Kohl, 1986). Biomass from the clover in monoculture and mixture and rye in monoculture (the non-N_2 fixing reference plant), were collected in the field, dried, weighed, and finely ground (<0.5 mm). Samples were analyzed for total N and $\delta^{15}N$ enrichment using a continuous flow Isotope Ratio Mass Spectrometer at the UC Davis Stable Isotope Facility. The percent N derived from the atmosphere (i.e., %Ndfa) was calculated using the following mixing model:

$$\%Ndfa = 100 \times ((\delta^{15}N_{ref} - \delta^{15}N_{legume}) / (\delta^{15}N_{ref} - B))$$

where $\delta^{15}N_{ref}$ is the $\delta^{15}N$ signature of the reference plant (rye), $\delta^{15}N_{legume}$ is the $\delta^{15}N$ signature of the clover and B is defined as the $\delta^{15}N$ signature of a legume when dependent solely on atmospheric N_2. B values were determined by growing crimson clover species in the greenhouse in a N-free medium following methods in Blesh (2017). After conducting two B-value experiments with crimson clover (one per site), we found an average B-value of -1.57, which we used in our calculation of %Ndfa. We estimated BNF (kg N ha^{-1}) by multiplying field values for aboveground biomass by shoot %N, and then by %Ndfa. The natural abundance method is generally considered reliable when the $\delta^{15}N$ signature of the legume and reference plants are separated by 2 ‰ (Unkovich et al. 2008). At the *KBS* site, this criterion was met; however, we did not find adequate separation between the legume and reference species at *CF*. We therefore estimated BNF at *CF* using the mean %Ndfa values from *KBS* for clover in mixture and monoculture. Given this, we also conducted a sensitivity analysis to test how variation in %Ndfa at *CF* would affect model outcomes.

2.1.5 N_2O flux following soil disturbance

We used the static chamber method (Kahmark et al. 2018) to measure the first pulse of N_2O emissions in each field following tillage of all experimental plots. All measurements occurred between 9 am and noon. In *CF*, we measured N_2O once before and five times after cover crop incorporation over 18 days. In *KBS*, we measured N_2O seven times after cover crop incorporation over 15 days. These periods captured the main episode of N_2O flux following tillage and initial decomposition of cover crop residues. During the N_2O measurement period, each site received the same amount of precipitation (15 mm) and had the same average temperature (20.6 °C).

Static chambers at *KBS* were made from stainless steel cylinders (diameter: 28.5 cm) and chambers at *CF* were made from Letica 3.5-gallon pails with the bottom removed to create a cylinder (diameter at top: 28.5 cm, diameter at bottom: 26 cm). Chamber lids were fitted with O-ring seals to create an airtight container during sampling. Each lid was equipped with a rubber septum port for extraction of gas samples. Before each sampling date, static chambers were installed in the ground and allowed to rest for at least 24 hours to reduce the impact of soil disturbance on measured emissions. The morning before each sampling event, the depth from the lip of the chamber to the ground was measured at three locations inside the chamber to calculate the internal volume. Lids were then placed securely on the chamber and 10 mL samples were extracted using a syringe every 20 minutes over a period of 60 minutes. Each 10 mL sample was stored, overpressurized, in a 5.9 mL, graduated glass vial with an airtight rubber septum (Labco Limited, Lampeter, UK). We analyzed samples for N_2O using a gas chromatograph equipped with an electron capture detector (Agilent, Santa Clara, CA). N_2O flux was calculated as the change in headspace N_2O concentration over the 60-minute time-period. Each set of 4 data points (0, 20, 40, and 60 minutes) were analyzed using linear regression and screened for non-linearity.

2.1.6 Data analysis

For all variables, we calculated descriptive statistics (mean, standard error, and IQRs) and checked all variables and models for normality of residuals and homoscedasticity. We transformed data using a log function for all variables. Within each site, we used repeated measures ANOVA models to test for differences in N_2O flux (g N_2O N ha^{-1} day^{-1}) across treatments for all time points. Models included day as the repeated measure, cover crop treatment as the fixed effect, and block as the random effect. We estimated mean cumulative N_2O

emissions (g N_2O N ha^{-1}) for all treatments by calculating the area under the curve (Gelfand et al. 2016) using this equation:

$$\text{Cumulative } N_2O \text{ Emissions} = \sum_{t_0}^{t_{final}} [(x_t + x_{t+1})/2] * [(t+1) - t]$$

Where t_0 is the initial sampling date, t_{final} is the final sampling date, x_t is N_2O flux at time t, and x_{t+1} is N_2O flux at the following sampling date.

Within each site, we determined the effects of cover crop treatments on cumulative N_2O, total biomass (kg ha^{-1}), total biomass N (kg N ha^{-1}), the C:N ratio, clover N (kg N ha^{-1}), and BNF (kg N ha^{-1}) using separate ANOVA models for a randomized complete block design, with cover crop treatment as the fixed effect and block as the random effect. To understand the effects of cover crop treatments on all response variables across both sites, we used two-way ANOVA models with site and treatment as fixed effects, along with their interaction, and block nested in site as a random effect. For all ANOVAs, post-hoc comparison of least square means was performed using Tukey's HSD, and results were reported as statistically significant at either α = 0.05 or 0.1, for models including N_2O flux, following previous work identifying high variability from unidentified sources in ecological field experiments measuring N_2O emissions (Gelfand et al. 2016; Han et al. 2017). All statistical analyses were performed in JMP Pro 15 software (SAS Institute, Cary NC). Excel and JMP Pro 15 were used to make figures.

2.2 Results

2.2.1 Baseline Soil Fertility

The *CF* site had higher soil fertility compared to the *KBS* site (Table 2-1). Total organic C was 34% higher at *CF* (P = 0.0003). Similarly, we found that *CF* had significantly larger POM pools than *KBS.* The concentration of free particulate organic matter (fPOM) was 44% higher (*P* = 0.0109) and occluded particulate organic matter (oPOM) was 29% higher at *CF* (*P* = 0.0062). The fPOM N concentration was 30% higher at *CF* than *KBS* (*P* = 0.0413) and PMN was 46% higher at *CF* than at *KBS* (*P* = 0.0039). However, oPOM N was not significantly different between *CF* and *KBS* (*P* = 0.2949).

Table 2-1. Soil fertility indicators at each site. P-values are indicated as: * <0.05, ** <0.001 for differences between sites.

	CF		*KBS*	
Soil Series	Fox		Kalamazoo & Oshtemo	
	Mean	Std. Error	Mean	Std. Error
**Bulk Density*	1.48	0.02	1.58	0.02
***% Sand*	65.00	1.29	41.30	2.06
% Clay	21.50	0.96	19.40	1.33
***% Silt*	13.50	0.50	39.30	2.40
pH	6.35	0.20	6.59	0.07
***Total Organic Carbon (Mg ha^{-1})*	44.39	1.81	29.44	1.01
***Total Organic Nitrogen (Mg ha^{-1})*	3.83	0.10	2.81	0.06
**Phosphorus (mg P kg^{-1})*	16.00	1.91	9.31	1.85
Potassium (mg K kg^{-1})	62.25	5.31	60.19	3.18
**oPOM (mg kg^{-1})*	3.89	0.05	2.75	0.14
oPOM N (mg N kg^{-1})	63.20	1.05	56.93	2.95
**fPOM (mg kg^{-1})*	5.26	0.36	2.92	0.13
**fPOM N (mg N kg^{-1})*	62.31	3.69	43.54	2.11
**PMN (kg NH_4^+ N ha^{-1} $week^{-1}$)*	24.62	1.01	13.34	0.90

2.2.2 Cover crop biomass and traits (C:N and BNF)

There was a significant effect of site (*P* = 0.0005), treatment (*P* < 0.0001) and an interaction effect between site and treatment (*P* = 0.0084) on total shoot biomass, which included both cover crops and weed species. Across all cover crop treatments, mean biomass was 40%

higher at *CF* (5430.45 ± 499.26 kg ha^{-1}) than at *KBS* (3259.96 ± 289.65 kg ha^{-1}), with nearly three times more rye biomass and 1.5 times more mixture biomass at *CF* than *KBS.* At *CF*, rye biomass (7709 ± 387 kg ha^{-1}) was 37% higher than biomass in the clover treatment (4846 ± 477 kg ha^{-1}), and almost threefold higher than in the fallow (2775 ± 245 kg ha^{-1}) ($P < 0.0001$). Rye and mixture (6392 ± 206 kg ha^{-1}) were not significantly different from each other, nor were the mixture and clover treatments. At *KBS*, clover (3972 ± 580 kg ha^{-1}) and mixture (4219 ± 297 kg ha^{-1}) treatments had approximately twofold more biomass than the fallow (2006 ± 388 kg ha^{-1}) ($P = 0.0068$). However, mixture and clover biomass did not differ significantly from rye (2842 ± 212 kg ha^{-1}), and rye was not significantly different from fallow (Figure 2-1). At both sites, clover performed well in the mixture, representing 54% of the total mixture biomass at *KBS* and 53% of total mixture biomass at *CF* (Table A 1).

We also found a significant effect of site ($P = 0.0005$), treatment ($P < 0.0001$), and a significant site by treatment interaction ($P = 0.0484$) on total shoot N (biomass N; including both cover crop and weed biomass). Across sites, there was two-fold higher biomass N at *CF* (102.6 ± 8.7 kg N ha^{-1}) than at *KBS* (53.0 ± 7.2 kg N ha^{-1}), with 68% higher biomass N in rye, 44% higher in mixture, and 56% higher in fallow at *CF* compared to *KBS*. At *CF*, there was a significant difference in biomass N between treatments, in which clover (121.2 ± 14.4 kg N ha^{-1}) accumulated twofold more N than the weeds in the fallow (59.0 ± 14.4 kg N ha^{-1}) ($P = 0.0055$); however, clover, mixture (131.28 ± 14.3 kg N ha^{-1}), and rye (98.64 ± 4.6 kg N ha^{-1}) treatments did not significantly differ from each other. At *KBS*, we found significantly higher aboveground N in the clover (80.8 ± 13.5 kg N ha^{-1}) and mixture (73.4 ± 5.8 kg N ha^{-1}) treatments compared to the rye (31.9 ± 1.4 kg N ha^{-1}) and weedy fallow (26.0 ± 6.6 kg N ha^{-1}) ($P < 0.0004$) (Figure 2-1).

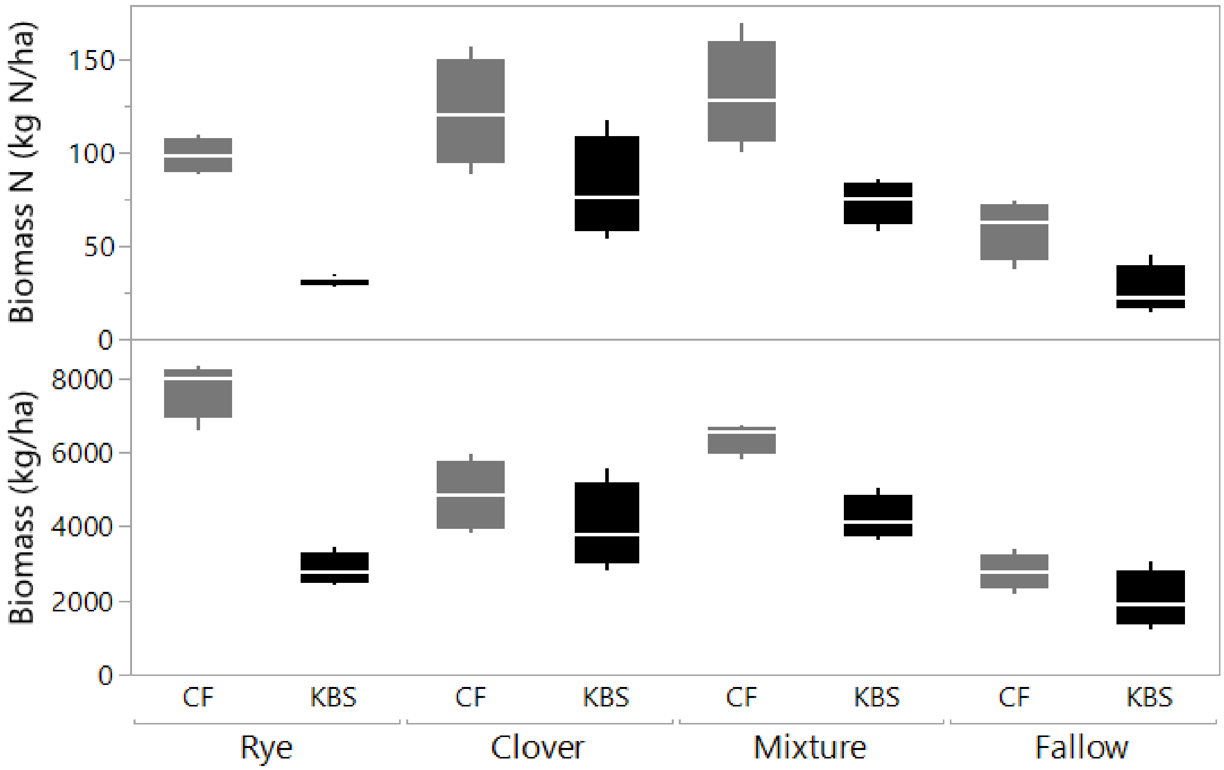


Figure 2-1. Biomass (kg ha^{-1}) and biomass N (kg N ha^{-1}) by treatment (including cover crops and weeds), at two sites (*CF* and *KBS*).

There was also a significant effect of site ($P = 0.0014$), treatment ($P < 0.0001$), and a significant interaction between site and treatment ($P = 0.0052$) on cover crop C:N. Across sites for all treatments combined, C:N was 26% higher at *KBS* (30.7 ± 2.0) than *CF* (23.7 ± 1.8). At *CF*, the C:N of rye biomass was 34.7 ± 1.6, while the mixture had a significantly lower C:N (21.7 ± 1.8). The mixture C:N did not differ from that in clover (17.2 ± 0.67) or weeds in the fallow (21.1 ± 1.6; $P < 0.0001$). At *KBS*, we also found a lower C:N in treatments with legumes (40.3 ± 1.3 in rye and 34.8 ± 1.9 in fallow vs. 25.6 ± 1.1 in the mixture and 21.8 ± 0.3 in clover; $P < 0.0001$). At *KBS*, the difference between clover and mixture was not significant.

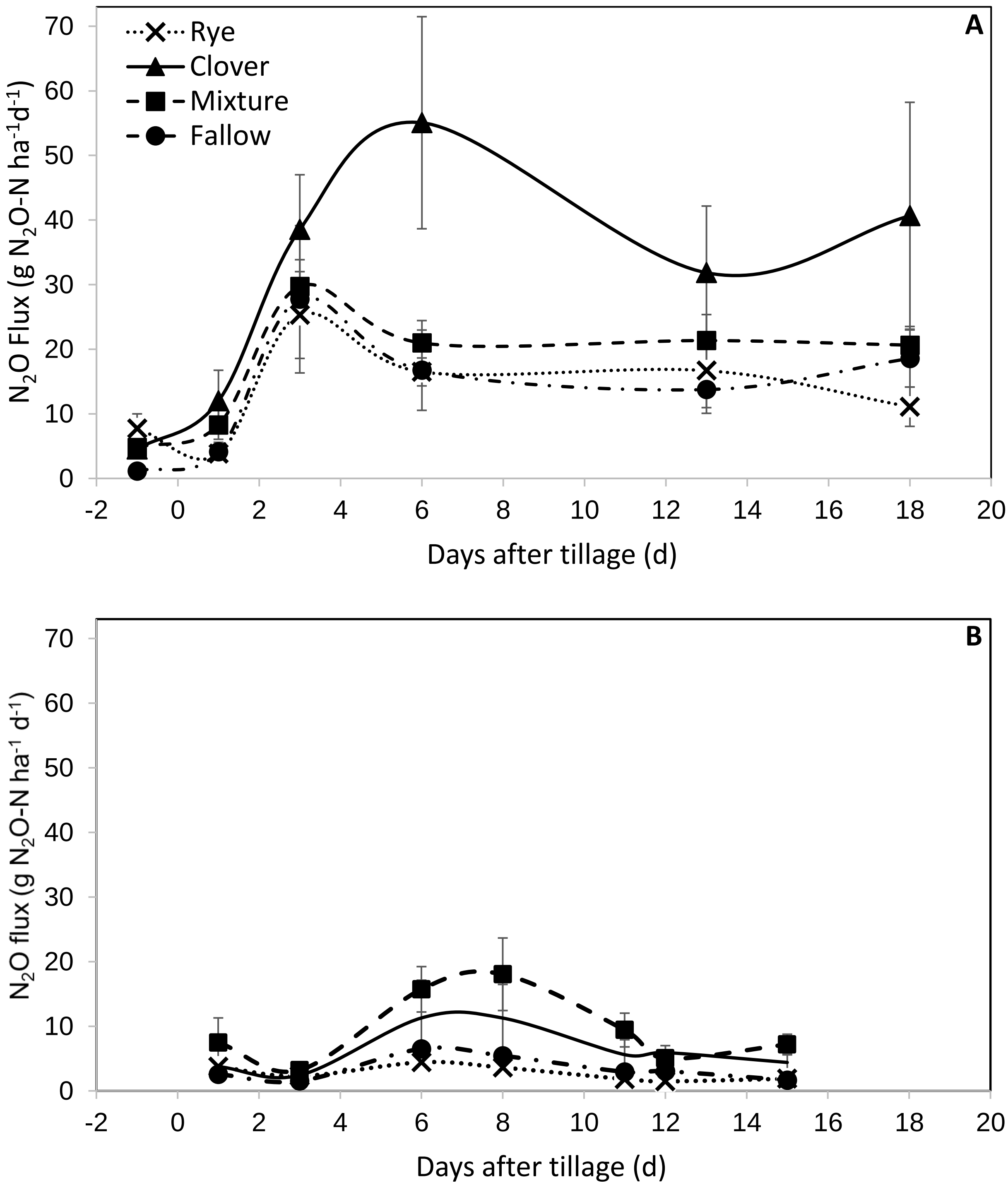
Using stable isotope methods at KBS, we estimated that the clover shoot N derived from fixation was 43.3% when grown alone and 63.3% when grown in mixture with rye, which we applied to estimates of N supply from BNF at both sites. There was a weakly significant effect of site (P = 0.0526) on N supplied by BNF in clover, but no significant effect of treatment (P = 0.7043) and no significant interaction (P = 0.9360). Between sites, with mixture and clover treatments combined, aboveground N from BNF was 38% higher at *CF* (49.5 ± 7.3 kg N ha^{-1}) than at *KBS* (30.6 ± 3.5 kg N ha^{-1}) (P = 0.0526). At *KBS*, BNF in clover (29.2 ± 6.0 kg N ha^{-1}) and mixture (32.1 ± 4.4 kg N ha^{-1}) were not significantly different (P = 0.6772). Similarly, at *CF*, clover (46.2 ± 8.3 kg N ha^{-1}) and mixture (52.7 ± 13.1 kg N ha^{-1}) supplied similar BNF inputs (P = 0.8653). In a sensitivity analysis for BNF at *CF* spanning 40-70 %Ndfa, N from fixation ranged from 42.7 to 74.7 kg N ha^{-1} for the sole clover treatment and from 33.3 to 58.3 kg N ha^{-1} for the clover in the mixture treatment (Table A 2).

2.2.3 Effects of cover crop functional diversity on daily N_2O emissions

In the repeated measures model for daily N_2O flux at *CF*, we found a significant effect of cover crop treatment (P = 0.07), day (P < 0.0001), and a significant interaction between day and treatment (P = 0.005). At *KBS*, there was a significant effect of cover crop treatment (P = 0.0155) and day (P < 0.0001). Individual ANOVA models for each sampling date at *CF* showed that N_2O emissions were higher in the clover (4.5 ± 0.5 g N_2O N ha^{-1}), mixture (4.8 ± 1.3 g N_2O N ha^{-1}), and rye (7.7 ± 2.2 g N_2O N ha^{-1}) treatments than in the fallow (1.2 ± 0.3 g N_2O N ha^{-1}) at the baseline sampling point prior to tillage (P = 0.0017). Six days after incorporating the cover crops by tillage, N_2O emissions in the clover treatment peaked at 55.1 ± 16.4 g N_2O N ha^{-1}, whereas fluxes in the other treatments had started to decline (Figure 2-2 A). On day six, emissions in the clover treatment were significantly higher than in the fallow (16.8 ± 6.2 g N_2O N ha^{-1}) (*P* = 0.032), whereas the mixture (21.0 ± 3.5 g N_2O N ha^{-1}) and rye (16.5 ± 2.2 g N_2O N ha^{-1}) treatments were not different from fallow. Emissions in the clover treatment remained elevated for the rest of the measurement period, however, the difference in emissions between clover, mixture, and rye treatments was not statistically significant on the last sampling date, 18 days after tillage (*P* = 0.15) (Figure 2-2 A).

At *KBS*, N_2O emissions were five times higher in the mixture (18.0 ± 5.6 g N_2O N ha^{-1}) than in rye (3.6 ± 1.0 g N_2O N ha^{-1}) at the peak flux eight days after tillage (*P* = 0.0487) and were also five times higher in mixture (9.4 ± 2.6 g N_2O N ha^{-1}) than the rye (1.8 ± 0.4 g N_2O N ha^{-1}) eleven days after tillage (*P* = 0.0178). Twelve days after tillage, emissions were four times higher in clover (5.9 ± 1.1 g N_2O N ha^{-1}) than rye (1.5 ± 0.6 g N_2O N ha^{-1}) (*P* = 0.018). By the fifteenth and last day, clover (4.4 ± 1.3 g N_2O N ha^{-1}) and mixture (7.2 ± 1.6 g N_2O N ha^{-1}) were higher than rye (1.9 ± 0.4 g N_2O N ha^{-1}) and fallow (1.7 ± 0.3 g N_2O N ha^{-1}) (*P* = 0.0073) (Figure 2-2 B).

Figure 2-2. A: Mean net nitrous oxide (N_2O) flux from the soil (with standard error) over 18 days at *CF*, measured once the day before (d = -1) tillage on 23 May 2018 (d = 0), and then five times following tillage and incorporation of cover crop biomass. **B:** Mean net nitrous oxide (N_2O) flux from the soil (with standard error) over 15 days at *KBS*, measured seven times following tillage on 26 May 2020 (d = 0).



2.2.4 Cumulative N_2O emissions

Both cover crop treatment (P = 0.0016) and site (P = 0.0037) had a significant effect on cumulative N_2O emissions, with no significant interaction (P = 0.1377). The mean N_2O flux following tillage was 1.8 times higher at *CF* (413 ± 67.5 g N_2O-N ha^{-1} vs. 230 ± 42.5 g N_2O-N ha^{-1}; P = 0.0037), which had both higher rates of potentially mineralizable N and larger free and occluded POM fractions (Figure 2-3). On average across both sites, the clover (488.5 ± 129.4 g N_2O-N ha^{-1}) and mixture (388 ± 46.2 g N_2O-N ha^{-1}) treatments led to significantly higher emissions than the rye (193.0 ± 43.4 g N_2O-N ha^{-1}) and fallow (218.0 ± 52.5 g N_2O-N ha^{-1}), with clover producing more than 2.5 times and mixture 2 times higher emissions than rye (P = 0.0016). Emissions from clover and mixture were statistically similar, and emissions from rye and fallow also did not differ significantly.

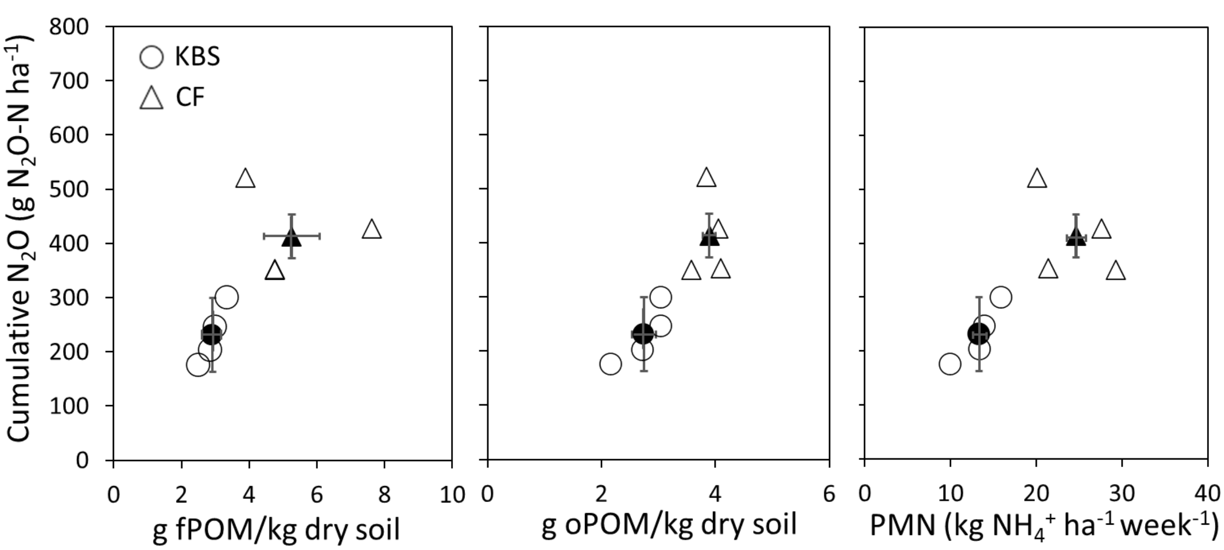


Figure 2-3. Cumulative N_2O plotted against fPOM (g kg^{-1}), oPOM (g kg^{-1}), and PMN (kg NH_4^+ N ha^{-1} $week^{-1}$) at both sites (*KBS* and *CF*). Open symbols are values by replicate block and closed symbols are overall site means. Error bars represent standard error of the means for each site.

When evaluating treatment effects within each site, at *CF*, cumulative N_2O flux tended to be lower in the fallow (291.5 ± 92.0 g N_2O-N ha^{-1}), rye (288.9 ± 48.1 g N_2O-N ha^{-1}), and clover-rye mixture (380.2 ± 44.4 g N_2O-N ha^{-1}) treatments compared to clover grown alone (692.9 ± 204.7 g N_2O-N ha^{-1}), although these differences were not statistically significant (P = 0.112). At *KBS*, cumulative N_2O fluxes were lower in the fallow (144.5 ± 28.2 g N_2O-N ha^{-1}) and rye (97.1 ± 18.3 g N_2O-N ha^{-1}) treatments compared to the clover-rye mixture (397.7 ± 89.1 g N_2O-N ha^{-1}) and clover grown alone (284.1 ± 91.5 g N_2O-N ha^{-1}) (P = 0.008). At this site, the mixture produced four times, and clover three times, higher emissions than rye (Figure 2-4).

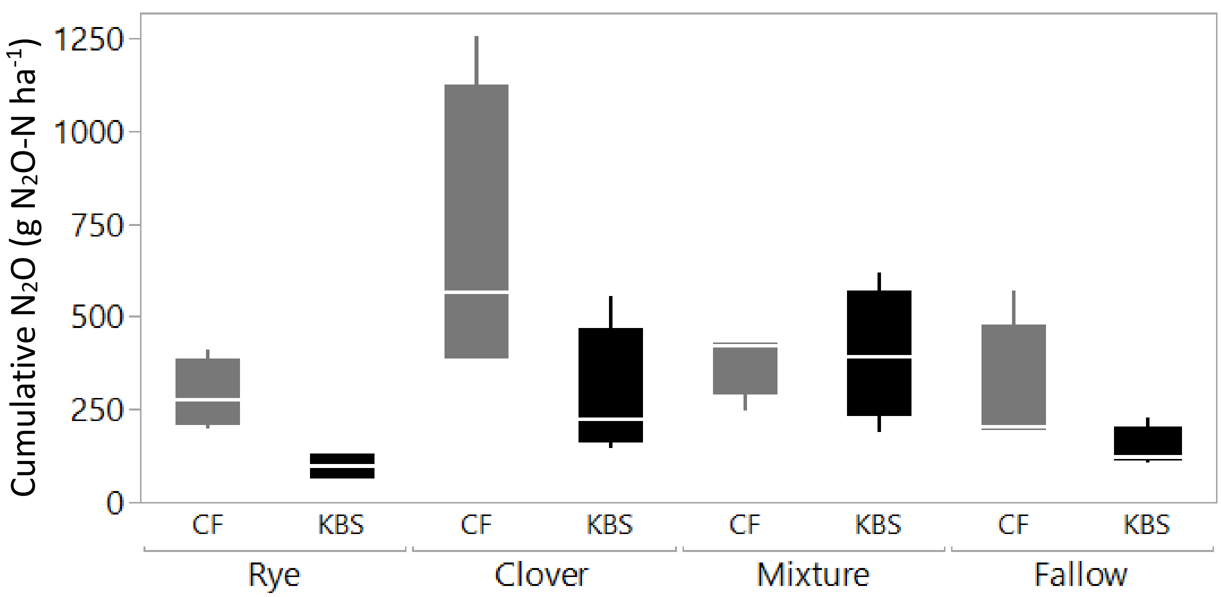


Figure 2-4. Cumulative N_2O flux by treatment, compared between sites.

2.2.5 N_2O fluxes normalized by soil fertility indicators or cover crop biomass

Given the contrasting soil fertility properties at the two experimental sites, we normalized N_2O emissions by POM levels and PMN rates (i.e., cumulative N_2O to POM, or PMN, ratios). When controlling for differences in soil fertility, all ratios had significant treatment effects, with clover resulting in the highest N_2O emissions at *CF* and mixture producing the highest emissions at *KBS* (Table 2-2). There was no significant effect of site on cumulative N_2O when expressed per unit fPOM or PMN. However, when normalizing for differences in oPOM, oPOM N, and fPOM N across sites, there was a significant site effect. Specifically, compared to *KBS*, mean N_2O emissions at *CF* were 22% higher when normalizing for oPOM ($P = 0.0112$), 43% higher for oPOM N ($P = 0.0013$), and 26% higher for fPOM N ($P = 0.0268$). When normalized by POM fractions or PMN, the cumulative N_2O emissions across sites were 1.9 – 2.8 times higher in clover and mixture than in fallow or rye (Table 2-3).

When N_2O was normalized by cover crop biomass, site was not significant ($P = 0.1795$), but we found a significant treatment effect ($P = 0.0031$) with lower emissions following rye than the other treatments. There was no effect of either treatment ($P = 0.1712$) or site ($P = 0.4696$) when expressing N_2O emissions as a ratio of cover crop biomass N (Table 2-4).

Table 2-2. Mean ± standard error for ratios of g N_2O/g POM and g N_2O/ kg PMN by treatment and site. P-values are indicated as: * <0.05, ** <0.001 for differences between treatments, and ^ <0.05 for differences between sites.

Site	Treatment	N_2O/ fPOM*	N_2O/ oPOM*^	N_2O/ fPOM N*^	N_2O/ oPOM N*^	N_2O/ PMN**
CF	Rye	0.19 ± 0.03	0.25 ± 0.04	16.12 ± 3.08	15.36 ± 2.35	12.09 ± 2.48
	Clover	0.51 ± 0.19	0.60 ± 0.18	41.44 ± 14.96	37.82 ± 11.77	29.95 ± 11.04
	Clover/Rye	0.26 ± 0.04	0.33 ± 0.03	21.38 ± 3.31	20.27 ± 2.15	16.17 ± 2.84
	Fallow	0.17 ± 0.03	0.25 ± 0.08	14.94 ± 2.53	15.26 ± 4.29	11.67 ± 3.06
KBS	Rye	0.10 ± 0.02	0.13 ± 0.02	6.65 ± 1.38	5.82 ± 0.82	7.43 ± 1.14
	Clover	0.30 ± 0.09	0.34 ± 0.10	19.81 ± 6.54	15.80 ± 4.66	23.61 ± 6.49
	Clover/Rye	0.50 ± 0.12	0.47 ± 0.11	32.64 ± 8.50	22.00 ± 5.44	33.41 ± 7.85
	Fallow	0.16 ± 0.03	0.15 ± 0.03	10.50 ± 1.97	7.00 ± 1.39	9.33 ± 1.55

Table 2-3. Mean ± standard error for ratios of g N_2O/g POM and g N_2O/ kg PMN averaged across both sites by treatment. Significant treatment differences are indicated by different letters.

Treatment	N_2O/ fPOM	N_2O/ oPOM	N_2O/ fPOM N	N_2O/ oPOM N	N_2O/ PMN
Rye	0.15 ± 0.03*b*	0.19 ± 0.03*b*	11.39 ± 2.37*b*	10.59 ± 2.14*b*	9.76 ± 1.54*b*
Clover	0.40 ± 0.11*a*	0.47 ± 0.11*a*	30.63 ± 8.59*a*	26.81 ± 7.19*a*	26.78 ± 6.05*a*
Clover/Rye	0.38 ± 0.08*a*	0.40 ± 0.06*a*	27.01 ± 4.73*a*	21.13 ± 2.73*a*	24.79 ± 5.05*a*
Fallow	0.17 ± 0.02*b*	0.20 ± 0.04*b*	12.72 ± 1.71*ab*	11.13 ± 2.61*b*	10.50 ± 1.65*b*

Table 2-4. Mean ± standard error for ratios of g N_2O to kg cover crop biomass and g N_2O to kg cover crop biomass N averaged across both sites by treatment. Significant treatment differences are indicated by different letters.

Treatment	N_2O/biomass	N_2O/biomass N
Rye	0.036 ± 0.0049*b*	2.98 ± 0.34*a*
Clover	0.12 ± 0.034*a*	5.12 ± 1.48*a*
Clover/Rye	0.076 ± 0.011*a*	4.17 ± 0.70*a*
Fallow	0.087 ± 0.012*a*	5.37 ± 0.60*a*

2.3 Discussion

Reducing greenhouse gas emissions from agriculture is necessary to meet global targets for limiting climate change (IPCC 2019). Generally, greenhouse gas emissions are greater from grain agroecosystems with fertilizer additions compared to legume N sources (Robertson et al. 2014; Han et al. 2017; Westphal et al. 2018) and are higher in rotations with only annual crops compared to those with perennial crops (Gelfand et al. 2016). Overwintering cover crops can help "perennialize" annual agroecosystems by providing continuous plant cover, building soil organic C (King and Blesh 2018), and supporting related functions such as soil nutrient supply and storage. In diversified rotations with cover crops, however, N_2O emissions can peak during the weeks following tillage when cover crop biomass is incorporated into the soil, increasing N mineralization rates (Han et al. 2017). Our experiment tested whether increasing cover crop functional diversity with a legume-grass mixture would reduce pulse N_2O emissions following cover crop incorporation by tillage at two field sites. Understanding these critical moments of N_2O flux can inform how to adapt management of diversified cropping systems to reduce N losses, and further reap their environmental benefits compared to fertilizer-based management practices.

2.3.1 Effects of cover crop functional diversity on N_2O flux

The sampling period (15-18 days) of this experiment captured the first peak of N_2O emissions following tillage of cover crop biomass at both sites. Our analysis of cover crop treatment effects on cumulative N_2O emissions in this period shows the strong influence of biomass N inputs, particularly for the legume species, which supplied an external N source through BNF. When normalized for differences in soil fertility across sites, the clover and mixture treatments led to significantly higher pulse losses of N_2O than rye or fallow (Table 2-3), providing strong evidence that BNF inputs from the treatments that included clover were a driving factor of N_2O losses. By adding new N, legumes increase inorganic N in the soil compared to non-legumes (i.e., grasses), increasing denitrification potential, particularly in soils with readily available organic carbon (i.e., POM) (Robertson and Groffman 2015, Bernhardt and Schlesinger 2013).

While our study tested the role of legume N inputs, past meta-analyses have been dominated by studies with N inputs from synthetic fertilizer and manure sources (Han et al. 2017; Eagle et al. 2017; Basche et al. 2014). The only studies included in these meta-analyses that had legumes as the sole N source were Robertson et al. (2000) and Alluvione et al. (2010). Gelfand et al. (2016) extended the data reported in the Robertson et al. (2000) study by another decade and found that legume N sources did not significantly reduce N_2O fluxes from soil compared to fertilizer N sources. Our findings contribute evidence that legume cover crops release more N_2O compared to treatments without legumes, within the context of agroecosystems that have only received legume N inputs for several decades.

Despite clear differences between treatments with clover and those without, we did not find strong support for our hypothesis that the legume-grass mixture would reduce pulse N_2O flux. This may be explained by the lack of difference in total BNF inputs between clover grown alone and in mixture within each site, as well as the similar C:N ratios of litter biomass in both treatments. Litter chemistry for clover and mixture both fell into the intermediate C:N range (17.2-25.6) expected to lead to net N mineralization, potentially increasing the soluble inorganic N pool and driving N_2O fluxes following tillage, compared to the much higher C:N range in rye (31.5-44.1) across sites, which likely led to net N immobilization, especially during the two-week window when we measured N_2O following tillage (Robertson and Groffman 2015; Kramberger et al. 2009; Rosecrance et al. 2000; Wagger et al. 1998).

When N_2O fluxes were normalized by aboveground biomass N inputs to soil, emissions were the same for all treatments regardless of the source of N (soil or external inputs of atmospheric N_2). Furthermore, we found that three times higher rye biomass N at *CF* corresponded with 1.6-2.6 times higher N_2O emissions when normalized to control for differences in multiple soil fertility parameters across the two sites. In the clover treatment, 1.5 times higher BNF inputs at *CF* corresponded with 1.2-2.3 times higher N_2O emissions when normalized for differences in soil fertility. The magnitude of new N inputs from BNF was higher at *CF*, due to greater clover biomass in both treatments with clover, which corresponded with significantly higher emissions at that site. However, when N_2O fluxes were normalized by aboveground biomass across sites, emissions were significantly lower following rye than the other treatments, including weeds in the fallow, indicating that the biochemical composition and other traits of rye residue influence N_2O emissions. For example, higher C:N in the rye compared to the treatments with clover may have reduced N_2O emissions per unit biomass input. These results reflect the importance of cover crop functional type, and the impact of legume N inputs on episodic N_2O emissions, which is supported by prior studies showing that higher total N inputs lead to higher N mineralization rates and higher N_2O fluxes (e.g., Han et al. 2017) and that legume cover crops can lead to pulse N_2O fluxes following incorporation by tillage (Baggs et al. 2003; Millar et al. 2004; Basche et al. 2014).

Within each site, the specific treatment effects differed. At *CF*, the clover treatment produced the highest pulse of N_2O, while at *KBS*, the mixture produced the highest flux, with the magnitude of the treatment effect being much more pronounced. N_2O fluxes were four times higher following mixture than rye at *KBS*, compared to just over two times higher in clover than rye at *CF*, suggesting that the new N input from BNF was a stronger driver of treatment differences at *KBS*. At *CF*, the mixture did slightly reduce cumulative N_2O emissions compared to clover (380.2 v. 692.9 g N_2O-N ha^{-1}), a difference which was likely ecologically meaningful even though it was not statistically significant. In contrast, the mixture slightly increased mean N_2O at *KBS*, from 284.1 g N_2O-N ha^{-1} in clover to 397.7 g N_2O-N ha^{-1} in mixture; however, at this site both treatments with clover produced significantly higher N_2O emissions than the non-legume treatments. At both sites, the clover was competitive in mixture, representing just over half of the total stand biomass in this treatment. Given that mixture composition likely drives the quality of cover crop residue inputs to soil (Finney, White, and Kaye 2016), there is a need for future studies to assess the effects of legume-grass mixtures across a wide range of contexts, with larger variation in mixture establishment and evenness. For example, it is possible that if rye had produced more biomass in the mixture in our experiment, we would have observed lower N_2O emissions in the mixture compared to the clover treatment.

2.3.2 Differences in N_2O flux between sites

The different treatment patterns for daily emissions between sites, and the larger pulse emissions overall at *CF*, both provide insights into mechanisms governing N_2O fluxes following cover crop incorporation. There is substantial evidence indicating that new N inputs to agroecosystems, and soil N mineralization rates, are primary drivers of soil N_2O emissions (e.g., Han et al. 2017; Robertson and Groffman 2015). However, in our study, mean BNF inputs did not significantly differ between clover and mixture treatments; thus, the different baseline soil fertility levels, and plant-soil interactions that drive N mineralization, likely played a key role in the contrasting effects of the mixture across sites. For instance, prior studies have found positive correlations between total SOC and N_2O flux (Bouwman et al. 2002; Dhadli et al. 2016). In a meta-analysis of 26 studies, Basche et al. (2014) found that SOC and cover crop biomass had a significant effect on denitrification potential and N_2O emissions. These studies highlight that ecosystem state factors that influence fertility, such as soil parent material and organic C content, drive N_2O emissions.

Here, we found approximately twofold higher cumulative N_2O fluxes at the site with larger soil POM fractions and higher POM N concentrations (*CF*) (Figure 2-3), suggesting that POM fractions may influence cover crop growth and N_2O fluxes. POM fractions are robust indicators of soil fertility that respond to changes in management over shorter time scales than total SOM and play an important functional role in soil N cycling and N availability to crops (Wander 2004; Luce et al. 2016). For instance, the *CF* site also had approximately twofold higher rates of N mineralization (PMN) compared to *KBS*. The total amount of soil N assimilated by cover crops (in the absence of external N inputs) is also an integrated indicator of soil inorganic N availability over the cover crop season. Rye aboveground biomass N was threefold higher at *CF*, while N in weed biomass in the fallow control was 2.3 times higher at *CF* than at *KBS*. In diversified agroecosystems, plant-mediated N acquisition from SOM pools can couple the release of inorganic N with plant N uptake in the rhizosphere, making organic N inputs, such as those from legume residues, less susceptible to loss than inorganic fertilizer inputs (Drinkwater and Snapp 2007). When cover crops grow in higher fertility soils, they are thus likely to have higher net primary productivity, and to release more root C into the soil, which increases microbial growth and turnover rates, and mineralizes more soil N. The roots, in turn, take up more N and produce more biomass (Hodge et al. 2000; Paterson et al. 2006). This positive feedback loop may have led to the significantly higher cover crop biomass production at

CF, which was especially pronounced in the rye treatment (7709 kg ha^{-1} at *CF* compared to 2842 kg ha^{-1} in at *KBS*).

Mechanistically, interactions between background soil fertility and cover crop functional types likely drive soil inorganic N availability and N_2O emissions. For instance, the highest N_2O emissions measured in our study were from the clover treatment at *CF*, which had both the highest new N inputs to soil from BNF and the largest POM pools. This site also showed a small reduction in emissions with the legume-grass mixture. After clover incorporation, the large, relatively labile C and N input to soil, in combination with larger background POM pools, may have primed greater overall N mineralization in *CF* compared to *KBS*, with some of this N lost as N_2O. Since corn had not yet established during this two-week time frame after tillage, there were no active roots to couple N release with N uptake, leaving a window of opportunity for N losses.

Even when controlling for fertility differences across sites (i.e., the analysis of N_2O to POM or PMN ratios), we found that cumulative N_2O emissions per unit oPOM, oPOM N, and fPOM N were significantly higher at *CF*. This site difference was highest for the oPOM N stock, with about 43% more emissions per oPOM N at *CF*. Prior studies have shown that oPOM N is a strong indicator of SOM quality, N fertility, and soil inorganic N availability from microbial turnover of SOM (Wander 2004; Marriott and Wander 2006; Blesh, 2019). Our contrasting findings across experimental sites indicate a need for future studies that assess the effects of cover crops on N_2O emissions across soils with a wide range of POM pool sizes.

2.3.3 Episodic N_2O emissions following tillage of cover crops

To understand the relative importance of N_2O fluxes following cover crop incorporation, it is important to interpret the magnitude of these episodic emissions within the context of N_2O fluxes for a complete crop rotation. In a 20-year study in the biologically-based cropping system in the MCSE at KBS (the *KBS* site in our experiment), Gelfand et al. (2016) reported mean annual N_2O emissions of approximately 1.08 kg N ha^{-1} yr^{-1} during a corn year, which was defined as the 380-day window between corn planting and soybean planting the following year. They also calculated an average of 2.2 kg N ha^{-1} yr^{-1} over the course of the three-year corn-soy-wheat crop rotation at this site (Gelfand et al. 2016). These values are likely a slight underestimate because their sampling did not include emissions during winter thaws, and occurred every 2-4 weeks, potentially missing periods of high emissions. In a meta-analysis, Han et al. (2017) reported average annual N_2O fluxes of 2.3-3.1 kg N ha^{-1} yr^{-1} and Gelfand et al. (2016) reported 2.4 kg N ha^{-1} yr^{-1} for annual cropping systems with inorganic fertilizer additions. Therefore, prior studies indicate that average annual N_2O emissions from soil do not differ significantly between grain cropping systems with synthetic vs. organic N sources.

Specifically, within the context of a corn year, we contextualized the emissions during the two weeks following tillage using Gelfand et al.'s estimate of 1.08 kg N ha^{-1} yr^{-1}. The two-week cumulative flux we measured post-tillage of clover would represent 64% of crop year emissions at *CF* and 26% at *KBS*, while the flux following tillage of the mixture biomass would represent 35% of the crop year estimate at *CF* and 37% at *KBS*. Using the estimate of 2.2 kg N ha^{-1} yr^{-1} for the complete crop rotation, the two-week cumulative flux we measured post-tillage of clover would represent 31% of annual emissions at *CF* and 13% at *KBS*, while the flux following tillage of the mixture biomass is 17% of that annual estimate at *CF* and 18% at *KBS*. After incorporating sole clover biomass, the average daily flux was 36 g N ha^{-1} d^{-1} at *CF* and 19 g N ha^{-1} d^{-1} at *KBS*, and after mixture biomass, was 20 g N ha^{-1} d^{-1} at *CF* and 26 g N ha^{-1} d^{-1} at *KBS* which are approximately three- to twelve-fold greater than the annual average daily flux reported for the organic cropping system at *KBS* (Gelfand et al. 2016), highlighting the relative importance of these peak events. Given the large spatial and temporal variability in N_2O emissions, sampling frequently during the days and weeks following tillage of cover crops is therefore important for advancing knowledge of episodic emissions.

2.4 Conclusion

We tested the impacts of cover crop functional type on short-term N cycling dynamics following tillage in the context of diversified agroecosystems that rely on legume N. Given that gaseous N fluxes are episodic, it is critical to understand how they are influenced by management practices during periods of high susceptibility for N losses. Overall, N_2O flux was higher in the clover and mixture treatments than in rye and fallow when emissions were normalized by soil fertility properties. We found that the functionally-diverse legume-grass cover crop led to a small reduction in N_2O losses at *CF* but not at *KBS*. In contrast to our hypothesis, at *KBS*, the mixture led to higher N_2O emissions than the clover treatment at peak flux following tillage. We also found a more pronounced treatment effect at *KBS*, indicating that new N inputs from both treatments with legumes were a larger driver of N_2O emissions at the site with lower soil fertility. Overall, the clover treatment at *CF* led to the highest emissions across sites, suggesting a synergistic effect of BNF inputs and soil fertility on N_2O. These contrasting findings across sites shed light on the drivers of N_2O losses following cover crop incorporation. Our results show that higher aboveground cover crop biomass can lead to higher N_2O emissions during cover crop decomposition, particularly for cover crops that include legumes.

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Chapter 3 Nitrogen Cycling Dynamics Following a Legume-grass Cover Crop Mixture in an Organic Agroecosystem

Abstract

Legume cover crops are central to an ecological nutrient management approach that can reduce nitrogen (N) losses from agricultural fields. When used to replace synthetic N fertilizers, legumes can reduce emissions of N_2O, a potent greenhouse gas. Increasing the functional diversity of cover crops, by planting legumes in mixtures with non-legumes, has the potential to further tighten the N cycle in agroecosystems. We hypothesized that a legume-grass cover crop mixture would provide inputs of biologically-fixed N_2 similar to a sole legume cover crop, while reducing N_2O losses during decomposition following tillage, by increasing the diversity of cover crop residues. We tested this hypothesis by establishing four cover crop treatments (crimson clover (*Trifolium incarnatum L.*), cereal rye (*Secale cereal L.*), clover-rye mix, and fallow control) in an organic grain agroecosystem that had been managed for 30 years with legume cover crops as the only external source of N. We measured biological N_2 fixation (BNF) in clover grown alone and in mixture with rye, aboveground biomass C:N, soil inorganic N concentrations throughout the growing season, N_2O fluxes for three months following cover crop incorporation by tillage, and corn yield and N assimilation. We found similar litter chemistry (total N and C:N) and BNF inputs in clover and mixture treatments, and no differences in cumulative N_2O emissions (across the 91-day measurement period) between treatments. During the first peak of N_2O emissions in the two weeks following tillage, significantly higher emissions occurred in clover and mixture treatments relative to rye and fallow, with no differences between

clover and mixture. During the second N_2O peak after the first major rain event, we found no differences between treatments. The mixture reduced corn yield compared to other treatments, with the highest yields in the fallow and clover treatments. Therefore, our hypothesis that BNF inputs would be similar between clover and mixture was supported, however, the mixture did not change litter chemistry, reduce N_2O emissions compared to clover, or increase yield compared to rye grown alone. We contextualized our findings for the 2020 corn growing season by calculating a 6-year partial N mass balance (2014 – 2019) for this agroecosystem, representing two full crop rotation cycles. We found a positive partial N mass balance of 13.48 ± 1.88 kg N ha^{-1} yr^{-1}, which became slightly negative (-6.8 ± 0.8 kg N ha^{-1} yr^{-1}) when accounting for historical mean annual losses of N through N_2O emissions and nitrate leaching. However, annual gains in soil organic C and N reported for this cropping system over several decades suggest that nitrate leaching losses may be lower than the historical mean, and that N mineralization from an aggrading SOM pool is likely a missing N source in our balance. While the functionally diverse mixture did not reduce N_2O emissions compared to a sole clover cover crop after one year, the N balance suggests that long-term use of ecological nutrient management has promoted efficient N cycling in this agroecosystem.

3.1 Introduction

The convergence of global climate change with food, energy, and water crises has called new attention to the sustainability of agricultural management. In the U.S. Midwest, excess nitrogen (N) inputs (e.g., synthetic inorganic N fertilizer and manure) contribute to climate change by increasing nitrous oxide (N_2O) emissions (Eagle et al. 2020). In the United States, approximately 75% of N_2O emissions come from agricultural soils (USEPA 2021, Robertson and Vitousek 2009). As the international scientific community calls for significant reductions in global greenhouse gas emissions (IPCC 2019), and as soil quality continues to degrade under industrial management, it is critical that farmers employ ecological management practices that tighten the N cycle, improve soil quality, and reduce N losses (Drinkwater et al. 2008).

Ecological nutrient management applies principles from ecosystem ecology to optimize soil fertility management for both crop production and sustainability (Drinkwater et al. 2008). Ecological practices provide an alternative to industrial management, for instance, by replacing synthetic fertilizer N with legume N_2 fixation within crop rotations (Blesh and Drinkwater 2013). Legumes can be added to crop rotations as cover crops, which are unharvested crops that are typically planted in the fall and terminated in the spring in temperate agroecosystems. Cover crops increase functional diversity to provide a broad suite of ecosystem services in grain agroecosystems with minimal disruption of typical crop rotations (e.g., corn-soy-wheat) (Snapp et al. 2005, Davis et al. 2012, King and Blesh 2018).

Cover crops can reduce N losses from agroecosystems because they recouple carbon (C) and N cycles. For example, cover crops increase the length of time during which photosynthesis is occurring, increasing C and N entering soil organic matter, thereby increasing soil organic matter (SOM) stocks (Drinkwater and Snapp 2007, King and Blesh, 2018, Blesh 2019). Furthermore, legume cover crops can reduce or replace synthetic N fertilizer inputs through biological N_2 fixation (BNF) carried out by symbiotic bacteria. Compared to synthetic N fertilizer inputs, legume N sources can better balance N inputs to fields with N exported in harvested crops, reducing N surpluses and potential for loss (Blesh and Drinkwater 2013). Legume biomass inputs, which have a low C:N ratio, also stimulate higher rates of microbial activity, driving both internal N cycling and storage, which can result in lower N losses (leaching and denitrification) compared to synthetic N fertilizer inputs (Drinkwater et al. 1998, Syswerda et al. 2012, Kallenbach et al. 2015).

Despite these benefits compared to Haber Bosch N, when compared to other functional types of cover crops, sole stands of legumes can result in greater N losses to the environment (Huang et al. 2004, Millar et al. 2004, Alluvione et al. 2010, White et al. 2017). As a result, there is growing interest in planting mixtures of grasses, such as cereal rye, with cover crops in the legume family to simultaneously enhance multiple ecosystem functions (Snapp et al. 2005, Hayden et al. 2014, Poffenbarger et al. 2015, Wood et al. 2015, Blesh 2017). In a legume-grass cover crop mixture, the legume provides a N source through BNF, while the grass enhances N retention and other functions such as weed suppression. Rather than maximizing one function at the cost of others, then, legume-grass mixtures can supply multiple functions at thresholds that meet sustainable management goals (Blesh 2017, Kaye et al. 2019). Additionally, the functions provided by legumes can be enhanced in mixtures with grasses compared to in monoculture stands. Legumes allocate up to 30% of their photosynthate to rhizobia in root nodules in return for fixed N (Minchin and Pate 1973, Warembourg and Roumet 1989). Legumes generally allocate less photosynthate to BNF if sufficient plant-available N is already present in soil. Through competition for soil N with grasses, legumes respond by increasing the energy-intensive processes needed to fix N_2, increasing BNF rates per plant (Jensen 1996, Hogh-Jensen and Schjoerring 1997, Li et al. 2016). This could result in similar N inputs from BNF from legumes planted at half-rate in a mixture compared to sole legume cover crops while also providing ecosystem functions from grasses.

Past experiments have found differences in N_2O emissions between different cover crop functional types, suggesting that legumes produce higher emissions than grasses. For example, in their meta-analysis, Basche et al. (2014) found that out of 106 observations from 26 publications, 40% of fields with cover crops had lower N_2O emissions than fields with no cover crop, while 60% had increased emissions. Generally, higher N inputs to soil lead to higher N mineralization rates during decomposition, and thus higher N_2O losses (e.g., Han et al. 2017). In the context of cover crop N inputs, Basche et al. (2014) found that legumes, which supply new fixed N to agroecosystems, can result in higher N_2O emissions than non-legume cover crops that assimilate and recycle greater quantities of soil N. However, this meta-analysis only included six observations for agroecosystems in which legume cover crops were the only N source (Basche et al. 2014), limiting our ability to generalize these results. Given this limited data and the high variability of N_2O emissions within treatments and over time, there is a need for further study in organically managed agroecosystems where legumes provide the sole source of N. In addition, even fewer studies have quantified N_2O emissions from legume-grass mixtures. By increasing the C:N and molecular diversity of organic compounds entering soil from litter (Finney et al. 2016, Kallenbach et al. 2019), legume-grass mixtures have the potential to slow down N mineralization early in the growing season to improve synchrony between mineralization and crop N assimilation, reducing N losses while still providing substantial N to future crops.

To better understand the dynamics of N cycling with legume-grass cover crop mixtures, we planted an experiment testing a crimson clover-cereal rye mixture compared to clover and rye grown alone within a grain agroecosystem at the Kellogg Biological Station (KBS) Long-Term Ecological Research (LTER) site. The mixture treatment represents an increase in both species diversity and spatial diversity of cover crops in this cropping system, which had been managed for 30 years with winter cover crops of red clover frost seeded into winter wheat and annual rye following corn. In this agroecosystem, red clover BNF is the sole external N source. Our hypothesis was that the crimson clover-cereal rye mixture would produce the highest biomass overall due to complementary traits and interactions between rye and clover. We also predicted similar total new N inputs between the mixture and clover treatment due to higher rates of BNF when clover was competing with rye for soil N, compared to clover grown alone. However, we also expected the mixture to increase the C:N of litter inputs than the clover treatment, which could slow litter decomposition, better synchronizing soil inorganic N availability with corn N assimilation, reducing overall N_2O emissions throughout the three-month growing season. Although better synchrony may reduce N losses, we expected that corn yields would decline as C:N ratios increased from clover to mixture to rye, reducing potential N mineralization. Finally, we expected the six-year, partial N mass balance for the site to indicate that N inputs from BNF approximately balance N removal in harvested crops given its long history of ecological nutrient management.

3.2 Methods

3.2.1 Site description and experimental design

We conducted our experiment from 2019 to 2020 in sub-plots of the biologically-based cropping system in the Main Cropping System Experiment (MCSE) (replicates 1-4) of the KBS LTER site (Lat/Long: N 42° 14’ 24”, W 85° 14’ 24” Elevation: 288 m) (Figure A 1). The site receives an average of 933 mm yr^{-1} with an average temperature of 9.2 °C. This site has been managed with a corn, soy, wheat rotation since 1989. The site resides on a glacial outwash plain with well-drained loam, sandy loam, and sandy clay loam soils in the Kalamazoo and Oshtemo series which are mixed, mesic Typic Hapludalfs (Crum and Collins, 1995). Methods for baseline soil sampling and detailed soil fertility data for this experiment can be found in Bressler and Blesh (In Review) (Table 2-1). The only N inputs at this site since 1989 have been from legume

N_2 fixation by medium red clover (*Trifolium pratense L.*), which is frost-seeded into winter wheat every three years, and soybeans (*Glycine max L.*), which are planted every three years. The rotation also includes a cereal rye (*Secale cereale M.Bieb*) overwintering cover crop planted after corn and incorporated before soybean.

In a randomized complete block design, we established four treatments in 3.1 x 12.2 m plots: (1) cereal rye (seeding rate: 100.9 kg ha^{-1}), (2) crimson clover (seeding rate: 16.8 kg ha^{-1}), (3) rye/clover mixture (seeding rate: 50.4 kg ha^{-1} rye, 9.0 kg ha^{-1} clover) (4) and a weedy fallow control, in four blocks with a grain drill on 31 July 2019. Seeding rates were determined based on recommendations from Michigan State University Extension. The cover crops overwintered, and all four treatments were rototilled into the soil on 26 May 2020. Viking Organic Seed Corn Brand (O.84-95UP) Variety (A1025726) was planted on 1 June 2020 at a rate of 12,950 seeds ha^{-1}.

3.2.2 Cover crop and corn biomass and N assimilation

We sampled aboveground biomass from all treatments in fall 2019 and spring 2020 in one 0.25 m^2 quadrat placed randomly in each replicate plot avoiding edges. Shoot biomass was cut at the soil surface, separated by species, dried at 60 °C for 48 hours, weighed, and coarsely ground (< 2 mm) in a Wiley mill. We analyzed the biomass for total C and N content by dry combustion on a Leco TruMac CN Analyzer (Leco Corporation, St. Joseph, MI). Spring aboveground biomass (cover crops and weeds) was sampled in all plots prior to tillage on 26 May 2020. We harvested corn on 28 October 2020. To reduce edge effects, we sampled corn from the middle 8.5 m of the plots. Using a Kincaid 8XP Plot Combine (Kincaid Manufacturing, Haven, KS), we measured grain yield and moisture using the on-board Mirus Harvest Master computer software (Juniper Systems, Logan, UT) from the middle two rows (1.5m) of each treatment. From the combine bin, we collected a grain subsample for chemical analysis. Corn grain was dried for at least 48 hours at 60° C, weighed, ground to the consistency of flour using a coffee grinder, and analyzed for total C and N by dry combustion on a Leco TruMac CN Analyzer (Leco Corporation, St. Joseph, MI).

3.2.3 N_2O flux from incorporation of cover crops to corn maturity

We used the static chamber method (Kahmark et al. 2018) to measure N_2O for three months following tillage of all treatments (cover crops and fallow). All measurements occurred between 10 am and noon. We measured N_2O every few days after cover crop incorporation and then every two weeks over 91 days for a total of 13 sampling events. This period captured the main episodes of N_2O flux from tillage and subsequent decomposition of organic matter. We analyzed samples for N_2O using a gas chromatograph equipped with an electron capture detector (Agilent, Santa Clara, CA). Static chambers were made from stainless steel cylinders (diameter: 28.5 cm). Chamber lids were fitted with O-ring seals to create an airtight container during sampling. Each lid was equipped with a rubber septum port for extraction of gas samples. Before each sampling date, static chambers were installed in the ground and allowed to rest for at least 24 hours to reduce the impact of soil disturbance on emissions values. The morning before each sampling event, the depth of the lip of the chamber to the ground was measured at three locations around the inside edge of the chamber to calculate the internal volume. Lids were then placed securely on the chamber and 10 mL samples were extracted using a syringe every 20 minutes for 60 minutes. Each 10 mL sample was stored, over pressurized, in a 5.9 mL, graduated glass vial with an airtight rubber septum (Labco Limited, Lampeter, UK). We analyzed samples for N_2O using a gas chromatograph equipped with an electron capture detector (Agilent, Santa Clara, CA). N_2O flux was calculated as the change in headspace N_2O concentration over the 60-minute time-period. Each set of 4 data points (0, 20, 40, and 60 minutes) were analyzed using linear regression and screened for non-linearity.

3.2.4 Soil inorganic nitrogen sampling

Beginning the day after tillage on 27 May 2020, we measured soil inorganic N (NH_4^+ + NO_3^-) near the static chambers every two weeks for the duration of the 91-day N_2O sampling period. We collected four to six, 2 cm diameter soil cores to 10 cm depth, within 1 m of each static chamber. Samples were immediately homogenized, sieved (2 mm), extracted with 2 M KCl, and analyzed for soil moisture using the gravimetric method. Extractions were stored at -20 ºC and later thawed and analyzed for NO_3^- and NH_4^+ colorimetrically on a discrete analyzer (AQ2; Seal Analytical, Mequon, WI).

3.2.5 Legume N_2 fixation by natural abundance

We estimated BNF by crimson clover using the natural abundance method (Shearer and Kohl, 1986). Aboveground biomass from the clover in monoculture and mixture and rye in monoculture (the non-N_2O fixing reference plant), were collected in the field, dried and weighed, and finely ground (<0.5 mm). Samples were analyzed for total N and $\delta^{15}N$ enrichment using a continuous flow Isotope Ratio Mass Spectrometer at the UC Davis Stable Isotope Facility. The percent N derived from the atmosphere (%Ndfa) was calculated using the following mixing model:

$$\%Ndfa = 100 \times ((\delta^{15}N_{ref} - \delta^{15}N_{legume}) / (\delta^{15}N_{ref} - B))$$

where $\delta^{15}N_{ref}$ is the $\delta^{15}N$ signature of the reference plant (rye), $\delta^{15}N_{legume}$ is the $\delta^{15}N$ signature of the clover and B is defined as the $\delta^{15}N$ signature of a legume when dependent solely on atmospheric N_2. B values were determined by growing crimson clover species in the greenhouse in a N-free medium following methods in Blesh (2017). We found a mean B-value of -1.57, which we used in our calculation of %Ndfa. We estimated BNF (kg N ha^{-1}) by multiplying field values for aboveground biomass by shoot %N, and then by %Ndfa.

3.2.6 Nitrogen Balance

To help interpret the single season N cycling dynamics measured in our experiment, we used long-term data from the KBS MCSE to calculate a partial N mass balance (Robertson and Vitousek 2009) for six years (2014 – 2019) in the biologically-based cropping system, following the approach in Blesh and Drinkwater (2013). This period spanned two full crop rotation cycles. The only external N inputs to this agroecosystem are BNF from the red clover cover crop, which is planted every three years, and inorganic N from atmospheric deposition. Based on values collected from the southwest Michigan station by the National Atmospheric Deposition Program, we estimated N inputs from atmospheric deposition to be 10.5 kg N ha^{-1} $year^{-1}$. To estimate N inputs from BNF, we first calculated total aboveground biomass N of the red clover using the historical shoot biomass data (in kg ha^{-1}) from the KBS LTER MCSE data repository (Robertson and Snapp 2020), which we multiplied by a mean N concentration of 3.4% to calculate total shoot N (kg N ha^{-1}). We then multiplied total aboveground N by %Ndfa, which we estimated to be 70% based on Wilke's (2010) study conducted in the biologically-based cropping system at KBS. Finally, belowground N inputs from red clover were assumed to be 40% of aboveground N (Hammelehle et al. 2018).

Because BNF rates can change over time, particularly with changes in soil fertility, we conducted a sensitivity analysis to understand how a range of red clover BNF rates would affect the partial N balance. Past studies have measured red clover %Ndfa ranging from 35% (Heichel et al. 1995) to 90% (Rochester and Peoples 2005). A more recent study by Schipanski and

Drinkwater (2010), also conducted in temperate grain agroecosystems, reported that when grown with grains, red clover had an average %Ndfa of 72% across 15 farms. Based on their dataset, we took the mean of the five lowest and five highest observations and used a range of 50-80 %Ndfa to conduct a sensitivity analysis for our experimental site.

The primary N exported from the agroecosystem is in the harvested corn, soybeans, and wheat crops. We calculated grain N export using historical yield and grain %N data stored on the KBS LTER data repository (Robertson and Snapp 2019, and Robertson 2020a). Specifically, we used a mean grain N concentration of 6.4% in soybeans, 1.2% in corn, and 1.7% in wheat. We multiplied the grain %N by grain dry matter yield (kg ha^{-1}) to calculate N removed in harvest (kg N ha^{-1}). Since soybeans are legumes, we accounted for BNF when calculating soil N exported in the beans. To calculate total biomass N, we assumed that soybeans had an 80% N harvest index (David and Gentry 2000). We then used an estimate of 80 %Ndfa (Gelfand and Robertson 2015) to calculate the non-fixed N (i.e., soil N) exported in the beans by multiplying the total biomass N in soybeans (kg N ha^{-1}) by 20%. The partial N balance was then calculated using the following equation:

$$N_{balance} = (N_{fixed} + N_{deposited}) - (NH_{corn} + NH_{wheat} + NH_{soybean})$$

Where N_{fixed} is the estimated inputs from red clover BNF, $N_{deposited}$ is estimated atmospheric deposition, and NH is the net N removed during harvest for each crop over the six-year period (2014 – 2019). $N_{balance}$ was divided by six to estimate the average annual N balance. We then used historical data from the site to estimate annual N_2O losses and NO_3^- leaching to supplement the partial N mass balance. Finally, we used data from the site assessing changes in SOC and SON from deep soil cores (1 meter) collected in 1989 and 2013, and from the topsoil (20 cm) data we collected in 2019, to interpret the N mass balance results.

3.2.7 Data Analysis

For all variables, we calculated descriptive statistics (mean, standard error, and IQRs) and checked all variables for normality of residuals and homoscedasticity. We transformed daily and cumulative N_2O emissions using the natural log function. We used repeated measures ANOVA models to test for differences in N_2O flux (g N_2O N ha^{-1} day^{-1}) across treatments for all time points. Models included day as the repeated measure, cover crop treatment as the fixed effect, and replicate as the random effect. We estimated mean cumulative N_2O emissions (g N_2O N ha^{-1}) for all treatments by calculating the area under the curve (Gelfand et al. 2016) using this equation:

$$\text{Cumulative } N_2O \text{ Emissions} = \sum_{t_0}^{t_{final}} [(x_t + x_{t+1})/2] * [(t+1) - t]$$

Where t_o is the initial sampling date, t_{final} is the final sampling date, x_t is N_2O flux at time t, and x_{t+1} is N_2O flux at the following sampling date.

We determined the effects of cover crop treatments on cumulative N_2O, total cover crop biomass (kg ha^{-1}), total biomass N (kg N ha^{-1}), biomass C:N, clover N (kg N ha^{-1}), and BNF (kg N ha^{-1}) using separate ANOVA models with cover crop treatment as the fixed effect and replicate as the random effect. When ANOVA models were significant, post-hoc comparisons of least square means were performed using Tukey's HSD, and results were reported as statistically significant at either $\alpha = 0.05$ or 0.1 (for models including N_2O flux), following previous work identifying high variability from unidentified sources in ecological field experiments measuring N_2O emissions (Gelfand et al. 2016, Han et al. 2017). All statistical analyses were performed in JMP Pro 15 software (SAS Institute, Cary NC).

3.3 Results

3.3.1 Cover crop biomass and traits (C:N and BNF)

On average, the clover and mixture treatments had two-fold higher total aboveground biomass (cover crop and weed species) compared to the fallow ($P = 0.0068$). However, biomass in the mixture, clover, and rye treatments did not differ significantly, and the rye treatment was not significantly different from fallow (Table 3-1). Aboveground biomass N assimilation across plots ranged from a low of 14.2 kg N ha^{-1} in one fallow plot to a high of 117.7 kg N ha^{-1} in one clover plot, with two to three-fold higher aboveground biomass N (cover crop and weed species) in the clover and mixture treatments compared to the rye and weedy fallow ($P < 0.0004$). Across treatments, rye biomass was highly correlated with rye biomass N (r = 0.95), as were clover biomass and clover biomass N (r = 0.99).

We found a significantly lower C:N in treatments with clover, with C:N decreasing from 40.3 ± 1.3 in rye and 34.8 ± 1.9 in fallow to 25.6 ± 1.1 in the mixture and 21.8 ± 0.3 in clover ($P < 0.0001$). The difference between clover and mixture C:N was not significant. In the clover-rye mixture, clover produced more biomass compared to rye and weeds in three of the four replicates making up an average of 54% of the total mixture biomass (Table A 3). Using stable isotope methods, we estimated that the mean clover shoot N derived from fixation was 43.4% when grown alone and 63.3% when grown in mixture with rye. Total new aboveground N inputs from BNF between the clover (min: 17.8; max: 45.9 kg N ha^{-1}) and mixture (min: 19.8; max: 40.4 kg N ha^{-1}) treatments did not significantly differ ($P = 0.6772$) (Table 3-1, Figure 3-1).

Table 3-1. Means (standard error) for aboveground biomass, aboveground biomass nitrogen (N), and biological N_2 fixation (BNF) by species across treatments.

	Total		Clover			Rye		Weeds	
	Biomass (kg ha^{-1})	Biomass N (kg N ha^{-1})	Biomass (kg ha^{-1})	Biomass N (kg N ha^{-1})	BNF (kg N ha^{-1})	Biomass (kg ha^{-1})	Biomass N (kg N ha^{-1})	Biomass (kg ha^{-1})	Biomass N (kg N ha^{-1})
Rye	2842.8 (212.2)	31.9 (1.4)				2367.7 (161.8)	25.4 (0.5)	475.2 (89.9)	6.5 (1.1)
Clover	3972.1 (579.7)	80.8 (13.5)	2963.9 (654.8)	67.5 (14.0)	29.2 (6.0)			1008.2 (90.4)	13.3 (1.2)
Mix	4219.1 (297.2)	73.4 (5.8)	2310.0 (380.7)	50.6 (7.0)	32.1 (4.4)	1148.9 (300.9)	13.1 (3.6)	760.3 (43.3)	9.6 (0.6)
Fallow	2005.8 (387.9)	26.0 (6.6)						2005.8 (387.9)	26.0 (6.6)

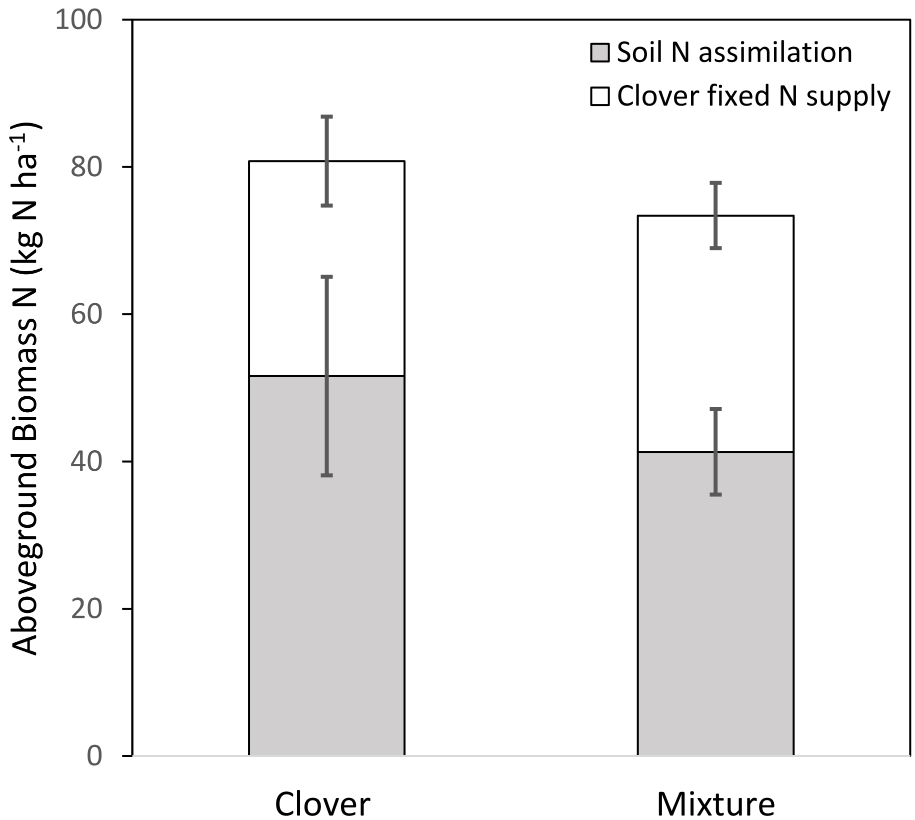

Figure 3-1. Mean aboveground biomass N (kg N ha^{-1}) (with standard error) in the clover and mixture treatments separated between N assimilated from the soil (including clover and weeds in the clover treatment and clover, rye, and weeds in the mixture treatment – gray bars) and N derived from clover BNF (white bars).

3.3.2 Daily N_2O emissions following tillage

In the repeated measures model for daily N_2O flux, we found a significant effect of sampling day ($P < 0.0001$) and cover crop treatment ($P < 0.001$), with no interaction between day and treatment ($P = 0.3460$). During the first peak following tillage, N_2O emissions were five times higher in the mixture (18.0 ± 5.6 g N_2O N ha^{-1}) than in rye (3.6 ± 1.0 g N_2O N ha^{-1}) eight days after tillage ($P = 0.0487$) and five times higher in mixture (9.4 ± 2.6 g N_2O N ha^{-1}) than the rye (1.8 ± 0.4 g N_2O N ha^{-1}) eleven days after tillage ($P = 0.0178$). Twelve days after tillage, emissions were four times higher in clover (5.9 ± 1.1 g N_2O N ha^{-1}) than rye (1.5 ± 0.6 g N_2O N ha^{-1}) ($P = 0.018$). By the fifteenth day, both clover (4.4 ± 1.3 g N_2O N ha^{-1}) and mixture (7.2 ± 1.6 g N_2O N ha^{-1}) were higher than rye (1.9 ± 0.4 g N_2O N ha^{-1}) and fallow (1.7 ± 0.3 g N_2O N ha^{-1}) ($P = 0.0073$). We did not find any other significant differences between treatments for the remainder of the 91-day N_2O sampling period (Figure 3-2B).

Across treatments, there was a significant correlation between soil inorganic N (NO_3^- + NH_4^+) and daily N_2O flux six days after tillage (r = 0.53), but not at any other sampling date. At the second peak, which occurred 27 days after tillage, there were no significant differences in soil inorganic N or N_2O flux between cover crop treatments. However, the mean N_2O flux from the rye treatment (60.0 ± 7.3 g N_2O N ha^{-1}) was about 25% higher than from the clover (45.1 ± 15.1 g N_2O N ha^{-1}) and mixture (47.3 ± 17.1 g N_2O N ha^{-1}) treatments, and 40% higher than fallow (36.6 ± 9.5 g N_2O N ha^{-1}) ($P = 0.758$). At this peak, we also found the highest variability in N_2O flux between replicates for all treatments compared to any other sampling date. For the remainder of the sampling period, background N_2O flux was low, with minimal variability within treatments and no differences between treatments (Figure 3-2B). It rained 26 mm on days 25-26 post tillage, right before sampling day 27. It then rained another 50 mm on days 29-30 after tillage, ahead of the sampling point on day 34, which did not have pulse emissions. It rained significantly again 66 (49 mm) and 67 (8 mm) days post-tillage, ahead of the sampling event on day 76 (Figure 3-2A).

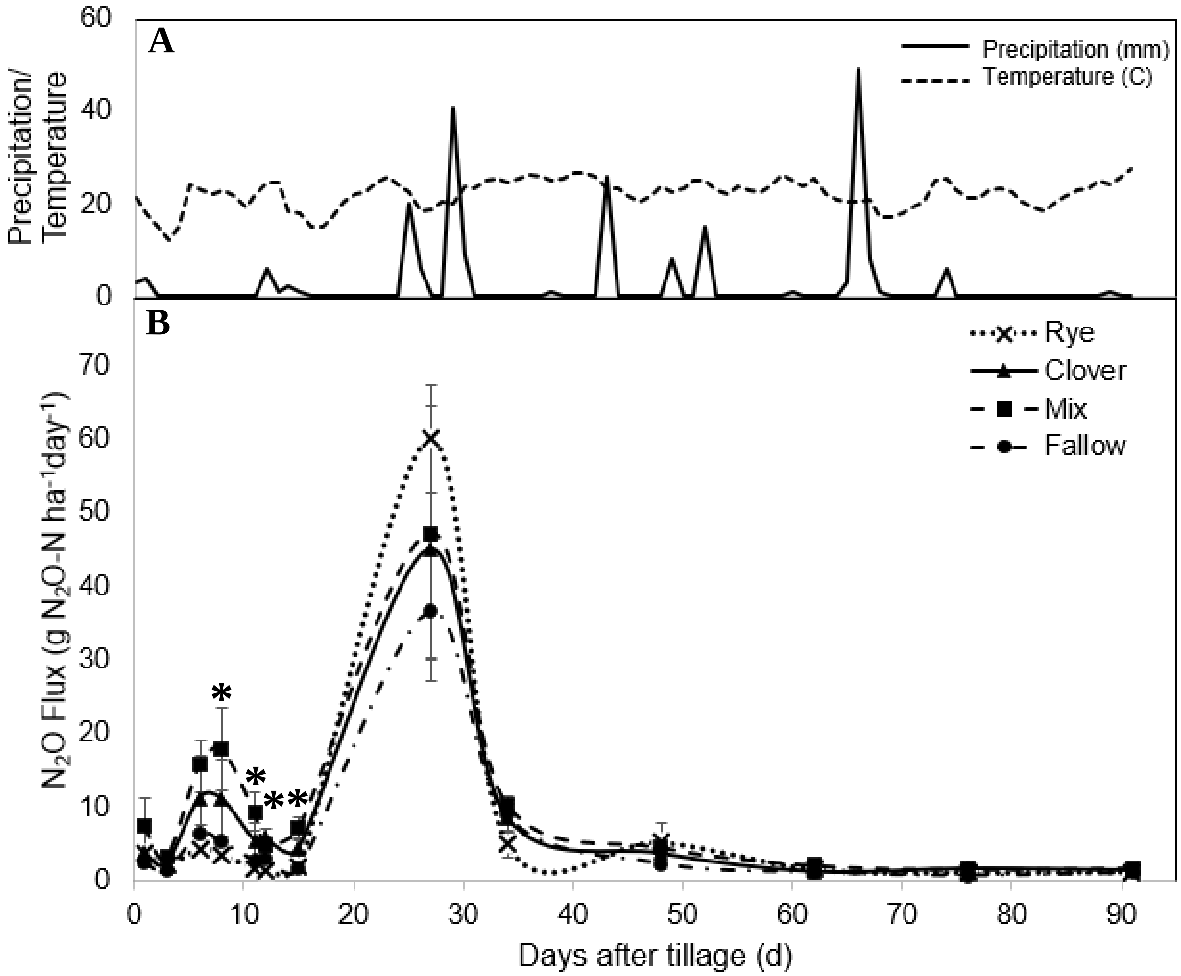

Figure 3-2. A: Precipitation and mean air temperature over the course of the study period. **B:** Mean net nitrous oxide (N_2O) flux from the soil (with standard error) over 91 days following tillage on 28 May 2020 (d = 0). [*] indicates days when we found significant differences between cover crop treatments.

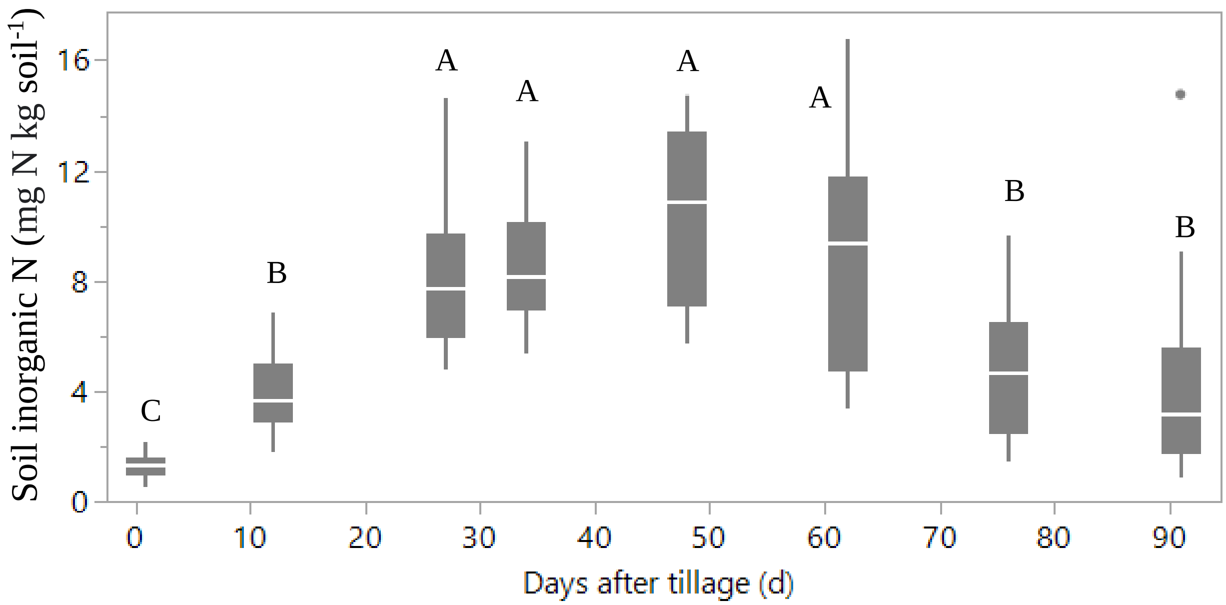

Figure 3-3. Soil inorganic N (NH_4^+ + NO_3^-) concentration throughout the study period (mg N kg soil^{-1}). Letters indicate dates that are significantly different from each other based on Tukey's least square means.

Across soil inorganic N measurements, taken every one to two weeks for the 91-day sampling period, we found a significant effect of sample date ($P < 0.0001$) and a significant effect of treatment ($P < 0.0001$), but no interaction effect ($P = 0.6353$). Across all sampling points, mean soil inorganic N concentrations were higher in the clover (8.29 ± 0.72 mg N kg soil^{-1}) and mixture (7.22 ± 0.75 mg N kg soil^{-1}) treatments compared to the rye (5.06 ± 0.57 mg N kg soil^{-1}) and fallow (4.58 ± 0.55 mg N kg soil^{-1}) treatments (Figure 3-3).

3.3.3 Cumulative N_2O emissions

We found no significant differences in the mean cumulative N_2O emissions (across the 91-day measurement period) among treatments ($P = 0.6875$) (Table 3-2). There was a wider range of cumulative emissions for the clover (min: 1088; max: 3057 g N_2O N ha^{-1}) and mixture (min: 932; max: 3257 g N_2O N ha^{-1}) treatments than for rye (min: 1688; max: 2198 g N_2O N ha^{-}

^{1}) and fallow (min: 1143; max: 1925 g N_2O N ha^{-1}) treatments. Yield-scaled emissions estimates (g N_2O ha^{-1}/g ha^{-1}) also showed no significant differences between treatments (P = 0.1129), but the mean of the mixture (904.8) was more than two times higher than the rye (492.7), clover (438.3), or fallow (303.6).

Table 3-2. Cumulative N_2O by treatment (mean and coefficient of variation) (g N_2O-N ha^{-1} yr^{-1}).

Treatment	Mean N_2O (g N_2O-N ha^{-1} yr^{-1})	CV
Rye	1962.9	10.52
Clover	1926.4	44.08
Mixture	2252.9	43.69
Fallow	1481.1	22.01

3.3.4 N_2O emissions for the full crop rotation

Given that N_2O fluxes vary throughout the three-year crop rotation, it is important to compare the magnitude of the emissions during the corn growing season to N_2O fluxes for a complete crop rotation. Based on the field scale data in the biologically-based cropping system in the MCSE at KBS where the cover crop experiment was conducted, N_2O fluxes were highest during soybean years, followed by corn years, with wheat producing very low levels of N_2O (Robertson 2020b; Table 3-3). The mean cumulative annual N_2O flux from 2015-2020 was 1.98 ± 0.38 kg N_2O-N ha^{-1} yr^{-1}

Table 3-3: Annual cumulative N_2O emissions (kg N_2O-N ha^{-1} yr^{-1}) over two full crop rotations at the study site in the biologically-based cropping system (Robertson 2020b).

Year	Crop	Cumulative N_2O Emissions (kg N_2O-N ha^{-1} yr^{-1})
2015	Soy	2.11
2016	Wheat	1.60
2017	Corn	1.22
2018	Soy	3.45
2019	Wheat	0.63
2020	Corn	2.86
Mean (std. error)		**1.98 (0.38)**

3.3.5 Corn yield and quality

Corn yield differed by cover crop treatment with significantly higher yields from the fallow (5026.4 ± 492 kg ha^{-1}) and clover (4304.2 ± 222 kg ha^{-1}) treatments than the mixture treatment (2914.0 ± 490 kg ha^{-1}), while rye (4057.4 ± 274 kg ha^{-1}) was the same as the other treatments ($P = 0.0097$). Similarly, corn grain N was higher in the fallow (54.6 ± 4.8 kg N ha^{-1}) and clover (56.4 ± 2.7 kg N ha^{-1}) treatments than in the mixture (36.7 ± 6.5 kg N ha^{-1}), while rye (45.4 ± 3.0 kg N ha^{-1}) was the same as the other treatments ($P = 0.033$). The C:N ratio of the corn grain was significantly higher in fallow (40.5 ± 2.2) than in clover (33.8 ± 0.4), while the mixture (35.2 ± 1.0) and rye (39.2 ± 1.0) did not differ from the other treatments ($P = 0.0164$). We found more variable C:N values across replicates in the fallow than in the other treatments. Corn grain %N was significantly higher following clover (1.31 ± 0.02%) than rye (1.12 ± 0.03%) or fallow (1.09 ± 0.06%) ($P = 0.0091$). We found similar corn %N between the clover and mixture (1.25 ± 0.03%); however, the mixture treatment did not differ significantly from rye and fallow treatments (Figure 3-4).

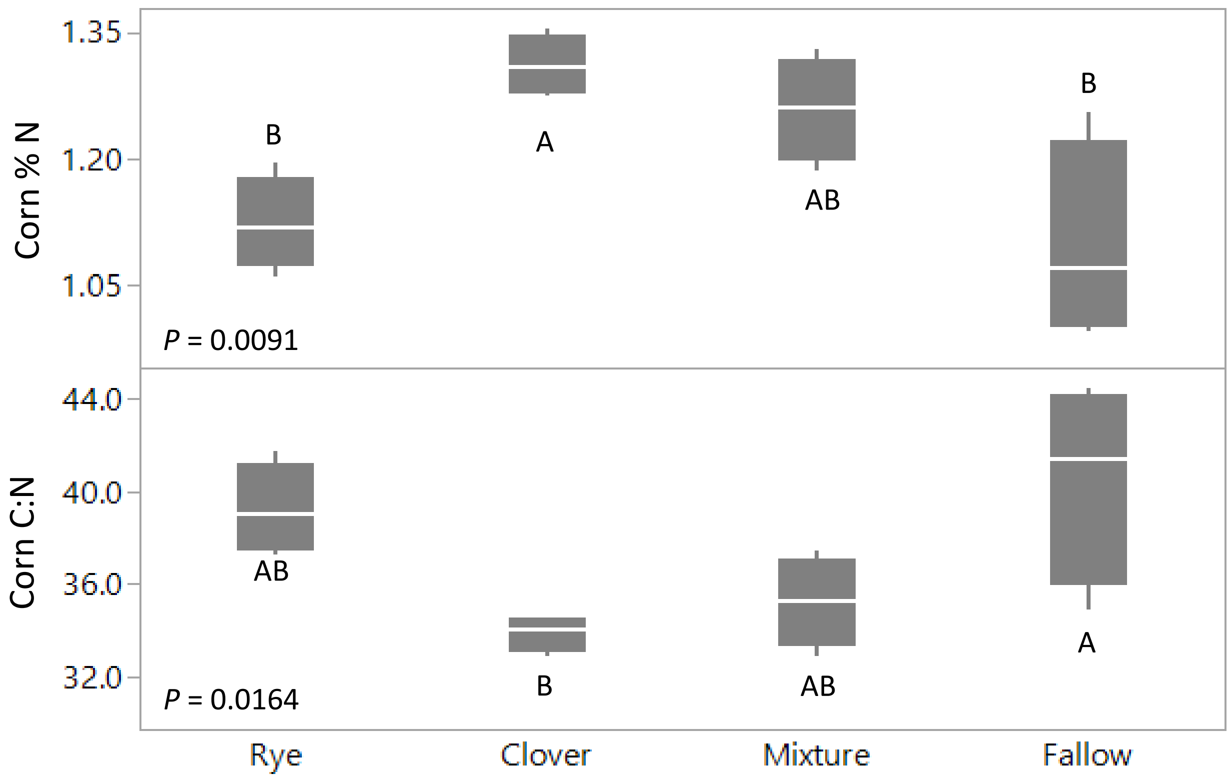

Figure 3-4. Corn grain %N and C:N. Letters indicate categories that are significantly different from each other based on Tukey's least square means.

3.3.6 Agroecosystem N Balance

We constructed a partial N mass balance (i.e., focused on the largest N flows driven by management) for the previous six years to capture two complete cycles of the corn-soy-winter wheat rotation. From 2014-2019, we found a total N import from BNF and atmospheric deposition of 313.41 ± 9.05 kg N ha^{-1} and an N export from harvested crops of 232.55 ± 11.51 kg N ha^{-1} with a net balance of 80.86 ± 11.29 kg N ha^{-1}. The mean annual net N balance was therefore 13.48 ± 1.88 kg N ha^{-1}. To account for potential error in our assumption for red clover %Ndfa, we conducted a sensitivity analysis ranging between 50 and 80 %Ndfa. The low-end estimate of 50% Ndfa changed the total net N import to 260.34 ± 6.98 kg N ha^{-1} over 6 years, resulting in a mean balance of 4.63 ± 1.67 kg N ha^{-1} yr^{-1}. When BNF was estimated at the high end of the range (80 %Ndfa) the mean balance was 17.90 ± 2.00 kg N ha^{-1} yr^{-1} (Table 3-4).

Table 3-4. Partial N balance (kg N ha^{-1} yr^{-1}) estimated for two rotation cycles of corn-soy-wheat (2014-2019) in the biologically-based cropping system at Kellogg Biological Station. Means (std. error) for a sensitivity analysis for red clover BNF with a low-end estimate of 50 %Ndfa, our estimate from the literature of 70 %Ndfa, and a high-end estimate of 80 %Ndfa**.**

%Ndfa	*N import (kg N ha^{-1})*	*N export (kg N ha^{-1})*	*Net N Balance (kg N ha^{-1})*	*Annual Balance (kg N ha^{-1} yr^{-1})*
50	260.34 (6.98)	232.55 (11.51)	27.78 (10.02)	4.63 (1.67)
70	**313.41 (9.05)**	**232.55 (11.51)**	**80.86 (11.29)**	**13.48 (1.88)**
80	339.94 (10.11)	232.55 (11.51)	107.39 (12.02)	17.90 (2.00)

Historically at this site, across all three crops in rotation (corn-soy-wheat) an average of 2.2 kg N $ha^{-1}yr^{-1}$ is lost to N_2O every year (Gelfand et al. 2016) and 19.0 ± 0.8 kg N $ha^{-1}yr^{-1}$ is lost to leaching (Syswerda et al. 2012). When accounting for these potential losses (21.2 kg N $ha^{-1}yr^{-1}$), the N balance across replicate plots of this cropping system ranged from negative (-12.8 kg N $ha^{-1}yr^{-1}$) to neutral (0.7 kg N $ha^{-1}yr^{-1}$). The mean annual N balance was therefore slightly negative (-6.8 kg N $ha^{-1}yr^{-1}$) when accounting for historical mean N_2O emissions and NO_3^- leaching measured from this cropping system.

3.4 Discussion

Increasing agroecosystem functional diversity with legume cover crops can improve the sustainability of soil nutrient management by building labile fractions of SOM and reducing N surplus (Drinkwater et al. 2008, Blesh and Drinkwater 2013, Blesh 2019). To advance understanding of N cycling dynamics in legume-based cropping systems, we tested the hypothesis that planting a more functionally diverse legume-grass cover crop mixture would provide BNF inputs similar to the sole legume cover crop, while reducing N_2O emissions during the following growing season, and increase corn yield compared to the sole grass cover crop. New N inputs from BNF did not differ between crimson clover and clover-rye mixture treatments, supporting our hypothesis that interspecific interactions in mixture could lead to a similar N supply while also increasing cover crop functional diversity. However, our findings did not support the hypothesis that the cover crop mixture would reduce N_2O emissions compared to the clover treatment, or increase yield compared to the rye treatment. The N balance we calculated showed that red clover BNF inputs approximately balanced harvested N exports for the two most recent crop rotation cycles. When interpreting findings from this experiment within the context of historical data from KBS, continued increases in SOM pools over decades in this cropping system further suggest that managing agroecosystems with legume N sources improves the long-term sustainability of N management.

3.4.1 Managing legume N sources in an organic agroecosystem

Compared to soluble N fertilizer inputs, managing agroecosystems with N_2 fixing legumes can reduce N losses by improving synchrony between microbial mineralization of organic N – both from SOM pools and decomposing cover crop litter – and crop N uptake (Drinkwater and Snapp 2007). However, the timing and rate of N mineralization from organic nutrient sources can be difficult to predict, creating uncertainty for farmers. Following cover crop incorporation into soil, the enzymatic depolymerization of SOM to simpler organic monomers is the rate limiting step in soil N mineralization (Schimel and Bennett 2004). The biochemistry of litter inputs to soil affects decomposition by influencing microbial biomass and microbial traits such as C use efficiency (CUE) and N use efficiency (NUE) (Castellano et al. 2015, Kallenbach et al. 2019). On the one hand, increasing the complexity of litter inputs with a cover crop mixture might increase the molecular diversity of organic compounds entering soil. This greater spatial and biochemical heterogeneity could slow initial decomposition rates by increasing the cost of metabolism, while increasing CUE of the microbial community (and SOM persistence) by promoting a microbial community with more diverse traits (Kallenbach et al. 2019, Lehmann et al. 2020). However, in our study the functionally-diverse cover crop mixture had a similar aboveground biomass C:N and total N as the clover grown alone, and these treatments also had similar soil inorganic N concentrations and N_2O emissions throughout the growing season. While the mixture treatment had a significantly lower corn yield than clover, they both resulted in the same corn quality (%N and C:N). These findings suggest that the clover-rye mixture did not alter cover crop residue composition in ways that would affect agroecosystem N cycling dynamics following its incorporation by tillage. Our experiment had strong clover establishment and growth, representing an average of 54% of the total mixture biomass, and rye was terminated in its vegetative stage with a relatively low C:N. It is therefore possible that in other contexts mixtures might have a stronger effect on these N cycling processes (e.g., based on mixture composition and cover crop traits at incorporation).

In addition, prior research focused on rates of N release from plant litter has neglected important plant-microbe-soil interactions that regulate overall N availability. A legume-grass cover crop mixture can occupy more niche space belowground through complementary rooting patterns, which can support a more diverse microbial community compared to single species cover crops (Jilling et al. 2018, Kallenbach et al. 2019). Given that different functional groups of microbes will have different cellular stoichiometry requirements (C:N), and facilitate different

CUE and NUE rates, a more diverse microbial community has the potential to increase the efficiency of nutrient cycling overall (Mooshamer et al. 2014, Jilling et al. 2018). Furthermore, cover crops also increase the presence of living roots in the soil compared to fallows, which provide more continuous labile C inputs that could expand both microbial and plant access to N from turnover of multiple SOM pools through rhizosphere priming (Jilling et al. 2018, Kallenbach et al. 2019). Both the clover and mixture treatments increased soil inorganic N and N_2O emissions during the first peak after tillage compared to rye and fallow, suggesting that the two treatments with legumes supported higher N mineralization and availability initially. However, given that the second peak in N_2O emissions was the same across all treatments 27 days after tillage, including for rye and fallow, it is likely that the long-term history of cover crop use in this cropping system was a more important driver of N mineralization rates throughout the growing season rather than the short-term addition of the mixture.

Our study also provides evidence that crimson clover in mixture with rye upregulated BNF, supporting our hypothesis that the mixture would supply BNF inputs comparable to clover grown alone, which may explain why we found higher N_2O emissions in both the clover and mixture treatments compared to rye and fallow during the first peak immediately following tillage. We also found that the mixture had higher mean biomass (3458 kg ha^{-1}) than the clover (2964 kg ha^{-1}) for the two cover crop species we planted (clover and rye). Taken together, greater cover crop biomass production in mixture, the high proportion of clover biomass resulting in similar residue chemistry, and higher %Ndfa of clover in mixture (63.3% v. 43.4%), likely explain the similarities between the clover and mixture treatments in this experiment. Additionally, baseline soil samples we analyzed for a companion study across all of the annual cropping systems at KBS showed that multiple SOM pools in the organic system were significantly higher than in the conventional management system, including particulate organic matter fractions that increase soil N supplying capacity as well as rates of potentially mineralizable N (Plumhoff et al. In Review). This long-term management context, with relatively high N availability from decomposition of SOM, may in part explain why we found a low BNF rate in the sole crimson clover in our experiment, considering that clover usually has a %Ndfa closer to 70 (Schipanski and Drinkwater 2010, Blesh et al. 2019).

When managing cover crops to reduce N losses, another important consideration is potential tradeoffs between N losses and crop yield. Studies investigating the impact of cover crops on corn yield generally find that legumes have positive or neutral impacts, while sole grasses are more likely to have negative impacts (Tonitto et al. 2006, Kramberger et al. 2009, and Finney et al. 2016). However, in our experiment, none of the cover crop treatments improved corn yield compared to the no-cover control, and our hypothesis that the mixture would improve yield compared to sole rye was not supported. Corn yields in our experimental sub-plots were lower than the mean whole plot yield measured in 2020 in the biologically-based cropping system (7180 ± 439 kg ha^{-1}) (Robertson and Snapp 2019). Lower-than-expected yields in our sub-plots may be due to their location on the edge of the larger experimental plots and thus do not reflect typical yields in this cropping system. In this study, then, yield may not be the best indicator of corn performance. However, we found that corn quality (%N) was significantly higher following the clover treatment than the rye and fallow, suggesting that legume cover crops can improve grain N assimilation compared to grass cover crops, which recycle soil N but do not supply an external N source. The strong performance of clover in mixture likely resulted in sufficient new N inputs to maintain corn quality comparable to sole clover. Further, although we found a tradeoff in this experiment between higher mixture biomass and reduced corn yield, the mixture may hold promise for enhancing a broader suite of ecosystem functions (Blesh 2017, Finney and Kaye 2016) in the long-term due to greater quantity and diversity of plant residue inputs to the soil (Lehmann et al. 2020).

3.4.2 N_2O emissions during the growing season following cover crop incorporation

Across all cover crop treatments, we found temporal trends in N_2O emissions during the corn growing season that indicate asynchrony between N mineralization and crop N assimilation during the first month of the experiment, followed by tighter synchrony for the remaining two months of the experiment. The majority of emissions occurred during the first month after tillage when crop N demand was low, but N was being mineralized during cover crop decomposition increasing the size of the soil inorganic N pool and thus NO_3^- available for denitrification. The first N_2O peak occurring during a two-week period after the cover crop biomass was tilled into the soil and the second peak occurred four weeks after tillage, following the first significant rainfall. During the first peak, corn had just been planted, and soil inorganic N was significantly lower than it was during the second N_2O peak, which produced 2-3 times higher emissions than the first peak. At 27 days post-tillage, the second N_2O peak occurred following a rewetting event (26 mm; 25-26 days post tillage). Even though the soil inorganic N concentration remained high for another 35 days, we did not see additional peaks in N_2O emissions following subsequent rewetting events 29- and 66-days post-tillage. We hypothesize that lower N_2O emissions during this period were in part due to improved synchrony between N supply and crop N assimilation, reducing N availability for denitrification following rain events.

In temperate agroecosystems, the rate of incomplete denitrification generally has the greatest effect on N_2O emissions, with nitrification contributing a small amount (Aronson and Allison 2012, Bernhardt et al. 2017). Denitrification is widespread in terrestrial ecosystems but generally occurs in saturated soils or in the center of aggregates in unsaturated soils where locally anoxic conditions persist. Denitrification also tends to be higher in soils with readily available organic carbon and NO_3^- (Robertson and Groffman 2015, Bernhardt and Schlesinger 2013). Therefore, dentification rates generally increase with increasing soil inorganic N pools and following precipitation events (Bergsma et al. 2002).

In particular, the first rainfall after a lengthy dry period may lead to high rates of denitrification if nitrous oxide reductase (*nosZ*) enzyme activity is low (Robertson and Groffman 2015). Zaady et al.'s (2013) field measurements showed that under arid conditions denitrification during wetting events is an increasingly important component of microbial respiration. In dry areas with few rain events (which is expanding into temperate regions as drought becomes more common), labile C and organic and inorganic N build up in the soil profile between rain events (Zaady et al. 2013). In combination, larger soil C and N pools, and low *nosZ* enzyme activity, can result in high rates of incomplete denitrification, resulting in high emissions of N_2O following rain events. The extended dry period and likely accumulation of labile C and N pools in our study are factors that may have contributed to the first peak of N_2O emissions following cover crop incorporation.

To put this in context of historical N_2O emissions at our study site, Gelfand et al. (2016) reported mean N_2O emissions of 2.2 kg of N ha^{-1} yr^{-1} in a study at KBS from 1991-2011. In a meta-analysis, Han et al. (2017) reported an average annual N_2O flux of 2.3 – 3.1 kg N ha^{-1} yr^{-1} for annual cropping systems with inorganic fertilizer additions. Relative to the mean annual N_2O flux estimate of 1.98 kg N ha^{-1} yr^{-1} from 2015-2020, encompassing two full crop rotations, the cumulative flux we measured post-tillage following the mixture was 14% higher (2.25 kg N ha^{-1} yr^{-1}) while clover and rye produced 97-99% of the emissions and fallow produced 75% of expected annual emissions at this site. Given that the emissions we measured were close to or slightly less than the annual average at this site, we believe we captured the majority of the N_2O flux by conducting measurements over this three-month period during the corn growing season.

3.4.3 Interpreting the partial N balance with historical data

One benefit of conducting this experiment at a long-term cropping systems site is the ability to interpret the N cycling dynamics we measured from wheat harvest in July 2019, through corn harvest in October 2020, using data spanning a longer period. We found a positive mean partial N mass balance of 13.5 kg N ha^{-1} yr^{-1} (Table 3-4) when accounting for the primary N fluxes that are influenced by farm management practices (N inputs from BNF and N exports through harvested grains). Assuming that SOM stocks are close to steady state, partial N balances are a robust indicator of N that is vulnerable to environmental losses based on data that are relatively easy to collect (Robertson and Vitousek 2009, McLellan et al. 2018, Zimnicki et al. 2020). The small N surplus we found here is slightly higher than the mean N balance reported in a previous study using this approach across multiple farms in the Midwest with legume N sources (i.e., 3.7 kg N ha^{-1} yr^{-1}; Blesh and Drinkwater, 2013). This suggests that this agroecosystem has some potential for N losses, which is supported by the historical measurements of N_2O emissions and NO_3^- leaching reported for this site. These losses were lower than the fertilizer-based cropping systems, but higher than treatments in successional communities (Gelfand et al. 2016, Syswerda et al. 2012). However, when including these past measurements of N_2O flux and NO_3^- leaching (21.2 kg N $ha^{-1}yr^{-1}$ total) in our balance, it became negative (-6.8 kg N ha^{-1} yr^{-1}). Furthermore, these estimates may still be missing important fluxes.

First, the loss pathway of total denitrification (N_2O + N_2) is important to consider but difficult to quantify. While denitrification has not been measured in the biologically-based cropping system at the MCSE where we conducted our experiment, Cavigelli and Robertson (2000) and Bergsma et al. (2002) measured denitrification in the conventionally managed treatment, manipulating conditions including pH, NO_3^-, oxygen, and soil moisture. Cavigelli and Robertson (2000) found a range in the relative rate of N_2O production ($\Delta N_2O/\Delta[N_2O + N_2]$) from approximately 0.2 at a lower pH and oxygen level to around 0.85 at a higher pH and oxygen level. Bergsma et al. (2002) found that the N_2O mole fraction ($N_2O/[N_2O + N_2]$) ranged from 0.36 in soils that received water for 48 hours before incubation to 0.9 in soils that received water immediately before incubation. The range in values reported for this site indicate that soil conditions significantly impact the N_2O mole fraction through denitrification pathways in agricultural contexts.

There are several possible N sources that could account for the missing N in our balance, including N mineralization if the steady state assumption for SOC and SON does not hold. When the MCSE experiment started in 1989, the land had been primarily in a corn and soybean rotation since 1954 (Tomecek and Robertson 1996). During the first decade (1991-1999) of the MCSE's establishment, gains of 80-110 kg C ha^{-1} yr^{-1} in the topsoil (7cm) were measured in the organic cropping system, which was attributed to cover crop biomass inputs (Robertson et al. 2000). A more recent analysis of deep soil cores (0-100 cm) collected in 2013 demonstrated further gains of 460 ± 80 kg C ha^{-1} yr^{-1} to the SOC stock and gains of 40 ± 10 kg N ha^{-1} yr^{-1} to the SON stock (Cordova et al. In Prep). These findings show that two decades of organic management, which increased functional diversity with legume N sources, cover crops, and winter wheat, increased both SOC and SON stocks in both surface and deep soils. In addition, comparing the topsoil measurements we took for this experiment to data from 2013, the mean SOC stock of 29 Mg C ha^{-1} we measured to 20cm depth in 2019 suggests a continued increase over time when accounting for different soil depths (i.e., 1.32 Mg C cm^{-1} in 2013 and 1.45 Mg C cm^{-1} in 2019). Given that SOM appears to still be increasing, at least in the top layer of soil, N supplied from mineralization could compensate for the deficit in our N balance. Along with the possibility that SOM is still accruing in this agroecosystem, it is also possible that NO_3^- leaching losses from this cropping system have declined over time. Since Syswerda et al.'s (2012) leaching study ended in 2006, continued organic management of the site using cover crops may have improved soil quality enough to increase soil N retention (Plumhoff et al. In Review).

It is also possible that we underestimated BNF inputs. Red clover BNF rates may have been higher than our estimates, accounting for some of the N deficit. When accounting for potential N_2O and leaching losses and applying the high-end BNF rate to red clover in our sensitivity analysis (80 %Ndfa), we found that the N balance across replicate plots ranged from slightly positive (4.3 kg N $ha^{-1}yr^{-1}$) to negative (-9.84 kg N $ha^{-1}yr^{-1}$), with a mean of -3.3 kg N $ha^{-1}yr^{-1}$, which is approximately in balance. It is also possible that there is associative N_2 fixation that is unaccounted for as an N source (Smercina et al. 2019), or that we underestimated belowground biomass N inputs from the clover cover crop. Alternatively, low soil phosphorus (P) levels (i.e., a mean Bray-1 P concentration of 9.31 ± 1.85 mg P kg^{-1}; Table 2-1), may limit BNF rates in this cropping system (Vitousek et al. 2013, Sulieman and Tran, 2017), which could also help explain the low %Ndfa we measured for sole crimson clover in our experiment and suggests that it is more likely that our partial N mass balance overestimated red clover N fixation. There is a need for more frequent measures of red clover BNF in this agroecosystem to understand whether rates are changing with changes in soil fertility over time.

3.4.4 Ecological Implications

Although legume cover crops can reduce N losses compared to inorganic N fertilizers, they can still produce higher N_2O emissions than other cover crop functional types due to BNF inputs and N-rich residues that can decompose quickly. We hypothesized that a legume-grass cover crop would better couple C and N cycling processes to further tighten N cycling and reduce N_2O emissions. However, short-term emissions following cover crop incorporation were higher in both treatments with legumes – which had similar aboveground biomass N, BNF, and soil inorganic N concentrations – compared to the rye and fallow treatments. This suggests that further gains in N cycling efficiency could come from reducing soil disturbance through tillage, particularly following the legume cover crop. Overall, we did not find significant differences in cumulative N_2O emissions over the corn growing season following any of the treatments. After a long history of ecological nutrient management at this site, a six-year, partial N mass balance indicated that N inputs from BNF approximately balance N removal in harvested crops, with a growing SOM pool over time. The restoration of SOM pools over 30 years in this organic agroecosystem was likely a more important driver of N cycling dynamics compared to increasing the diversity of the overwintering cover crop in a one-year study. However, with longer-term use, cover crop mixtures of complementary functional types have the potential to enhance multiple agroecosystem benefits such as reducing weed and pest pressure, increasing pollinator habitat, reducing nutrient leaching, and maintaining and building SOM.

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Chapter 4 Cover Crop Champions: Linking Strategic Communication Approaches with Farmer Networks to Support Cover Crop Adoption[2]

Abstract

We conducted a case study of the 2017 Cover Crop Champions cohort to understand how the program changes farmers' perceptions of cover crops and helps them overcome structural constraints to their adoption. Based on semi-structured interviews and document review, we found that the program changed the attitudes and behavior of farmers through two key mechanisms. First, Champions were trained in new communication methods, including use of simple language intended to normalize cover cropping, sharing personal success stories, facilitating hands-on field demonstrations, and focusing on tangible benefits. Second, the program facilitated new farmer networks, while strengthening and connecting farmers with existing networks. Champions who were involved in existing networks were more likely to continue outreach after the program ended. This case study improves our understanding of how farmer networks and mentoring relationships, built on effective communication strategies, can help overcome constraints to crop diversification on grain farms in the U.S. Midwest.

4.1 Introduction

The expansion of industrial agriculture has led to widespread habitat destruction and loss of biodiversity, created imbalances in global nutrient cycles, contributed significantly to

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greenhouse gas emissions, polluted freshwater, and left behind degraded soils that threaten farmer livelihoods (Campbell et al. 2017; Garibaldi, et al. 2017). A paradigm shift towards sustainable management practices is therefore needed. Diversified farming systems support ecological interactions and processes that maintain crop production together with other ecosystem functions (Kremen, Iles, and Bacon 2012; Tamburini et al. 2020). In addition to ecological benefits, cropping system diversification can provide social and economic benefits including decreased pesticide exposure (Bacon et al. 2012; Kremen, Iles, and Bacon 2012) and resilience to shocks such as extreme weather events and supply chain disruptions (e.g., Holt-Gimenez 2002, Prokopy et al. 2020, Bowles et al. 2020).

Farmers can diversify their crop rotations through practices such as cover cropping, use of legume nitrogen sources, planting perennial forages, and recycling animal waste, with the goal of restoring soil quality and reducing the use of external inputs (Drinkwater and Snapp 2007; Kremen, Iles, and Bacon 2012). Here, we focus on the use of cover crops in the Midwestern U.S., a region where simplified rotations of corn and soybeans increasingly cover most agricultural landscapes (Lark, Salmon, and Gibbs, 2015). Cover crops are non-harvested crops that provide living plant cover when soils would otherwise be left bare. They represent a key opportunity for conventional grain farmers to increase crop rotation diversity, because they are planted in the off-season between harvested crops. Different cover crop species (e.g., clover, vetch, ryegrass, radish, and rapeseed) can provide functions such as nutrient supply and retention, soil organic carbon storage, erosion and pest control, and improved water storage (King and Blesh 2018; Snapp et al. 2005). However, only 2-3% of annually planted cropland in the Midwestern U.S. was cover cropped in 2012 (Hamilton, Mortensen, and Allen 2017), due to a suite of social and ecological factors that drive specialization of crop and livestock production in this region. Through a case study of the National Wildlife Federation's (NWF) Cover Crop Champions (CCC) program, we sought to understand pathways that can increase the presence of cover crops in the Midwestern U.S.

4.1.1 Conceptual Framework

A growing body of literature discusses structural constraints to farm diversification, including commodity subsidies in the U.S. Farm Bill, existing knowledge systems and infrastructure, consolidation of seed and chemical companies, and global markets for commodity crops (Hendrickson and James 2005; Iles and Marsh 2012; Schewe and Stuart 2017; Roesch-McNally, Arbuckle, and Tyndall 2018; Houser and Stuart 2020). In the context of agriculture, constrained choice theory identifies how these structural barriers limit farmers' choices (Stuart and Gillon 2013; Guerra et al. 2017), restricting their capacity to diversify their farms. Additionally, in the current U.S. policy context, cover crop adoption is voluntary. Given this context, only a select group of farmers who tend to have a conservation-oriented mindset have adopted cover crops (Ma et al. 2012). Increased adoption of diversification practices can occur, however, when changes to structural factors such as federal policies or markets intersect with bottom-up processes, such as changes in farmer values, or interactions with peers or farmer organizations that increase access to knowledge and other resources (Blesh and Wolf 2014; Iles and Marsh 2012). Addressing these multiscale interactions is necessary to expand the use of conservation practices.

Given that structural barriers are typically slow to change, bottom-up action can play a critical role in reducing constraints by fostering local and regional innovations (Geels 2019) such as niches or clusters of farmers who use cover crops (Figure 4-1). One of the most well-studied, bottom-up frameworks for understanding adoption of new practices is "diffusion of innovations"

(Rogers 2003). In this model, uncommon practices such as cover cropping can spread when innovators and early adopters communicate with and influence the majority (Rogers 2003). Based on the current low adoption rates and large structural barriers, cover cropping is an ecological management innovation that is likely restricted primarily to innovators and early adopters. CCC classifies farmers into two categories that encompass Rogers' (2003) four types of adopters: *innovator farmers* (innovators and early adopters) and *target farmers* (majority and laggards).

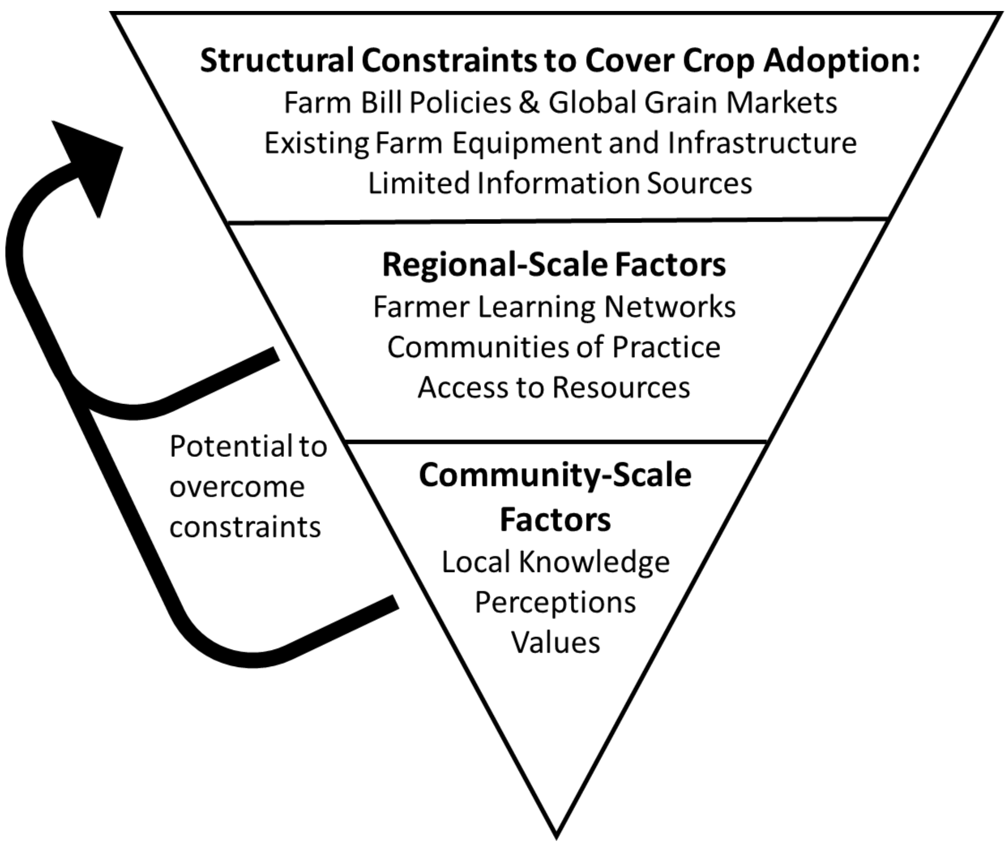

Figure 4-1. Our conceptual framework extends and builds on ideas from Hendrickson and James 2005, Stuart and Gillon 2013, and Guerra et al. 2017. Community and regional-scale factors influence the ability of farmers to mobilize resources and institutions to overcome structural constraints to cover crop adoption.

Farmer networks are critical for overcoming constraints to adoption and helping to extend practices such as cover cropping beyond early adopters. In a meta-analysis of 46 studies about adoption of conservation practices, Baumgart-Getz, Prokopy, and Floress (2012) identified networking and access to and quality of information as strong predictors of adoption. They found that famers' connections to agency personnel, and networks with neighboring farms and grassroots organizations, were particularly influential. Prior studies have identified that established farmer organizations and grassroots networks around the world, such as Brazil's landless workers movement and *La Vía Campesina*, play a critical role in expanding diversified farming systems (Altieri and Toledo 2011; Bell 2004; Blesh and Wittman 2015; Warner 2007). In the U.S. Midwest, Practical Farmers of Iowa (PFI) is a model farmer network that formed in 1985. The organization continues to expand to facilitate knowledge exchange among farmers, and NWF's CCC program collaborates with PFI and similar networks. In these networks, early adopters share their knowledge with others through interactive field demonstrations and mentorship. When local, site-specific ecological knowledge is coupled with peer-peer learning in communities of practice, a larger number of farmers can overcome structural barriers and adopt conservation practices (Laforge and McLachlan 2018; Morgan 2011; Stone 2007).

4.1.2 Research Objectives

Our case study of the CCC addressed three research objectives. First, we evaluated the effectiveness of the methods and resources Farmer Champions used to communicate with *target farmers* about cover crops. Second, we sought to identify factors that lead to sustained outreach efforts by some Farmer Champions after their CCC program cycle ended. Finally, we examined if (and how) *target farmer* perceptions about, and willingness to adopt cover crops changed as a result of participating in CCC outreach activities and interacting with Farmer Champions.

4.2 Methods

Conducting a case study of the CCC program allowed for in-depth study of a cover crop adoption intervention within its real-world context (Yin 2018). To represent the geographic locations covered by the program, our research design focused on multiple cases of subunits (focused on the 8 geographically dispersed teams of Champions who were part of the 2017 cohort – the 4th program cohort) embedded within a broader case (the CCC organization). We conducted a total of 24 interviews: 19 (out of 26) members of the 2017 CCC cohort, the director of the program, and four *target farmers* who had adopted cover crops after interacting with CCCs (See Appendix 1, A1.1, A1.2, and A1.3 for interview guides). To triangulate the interview data, we also analyzed participant applications and orientation materials used to develop messaging strategies.

We focused on the CCC class of 2017 for several reasons. First, 2017 was recent enough that recall was likely to be high when interviews were conducted during winter, 2019. Enough time had also elapsed for participants to reflect on their tenure as Champions and to identify if they continued to promote cover crop adoption in their communities even after their official role with the program had ended. Since the program started in 2014, it had evolved through several iterations before the class of 2017, allowing us to conduct an outcome-oriented evaluation after the program had time to work through initial kinks inherent in any new program (Berk and Rossi 1999). Finally, working with one class ensured that each Farmer Champion had received the same training opportunities through this program.

Given the small number of Champions in the program, we invited all 2017 cohort members to be interviewed. The 2017 cohort was made up of four teams of Champions from Minnesota, two from Wisconsin, one from Missouri, and one that included farmers in both

Illinois and Indiana. The Outreach Champions were all employees of their local Soil and Water Conservation Districts (SWCD); four were conservation technicians, two were soil scientists, and two held SWCD director positions. The Farmer Champions fell along a spectrum of crop diversification in their farm management systems. At the less diverse end of the spectrum, six Farmer Champions grew corn and soy in rotation with cover crop mixtures of 2-8 species. In the middle, five had integrated livestock operations with crop rotations including corn, soy, wheat, and alfalfa. These Farmer Champions planted more diverse cover crop mixes with 8-30 species. Finally, the most diversified Farmer Champion was the youngest interviewee, and was producing high value crops such as sunflowers in a 5-year rotation with cover crops planted on 100% of his acres. All had transitioned to reduced tillage either before or during the transition to cover cropping. They each planted between 50-100% of their acres to cover crops. Seven farmers planted 100% of their cropped acres to cover crops, while the rest aspired to achieve 100% coverage during periods of the year that would otherwise be fallow.

After several rounds of attempted contact through email and phone calls, we were able to interview one or two Farmer Champions from each of the 8 teams and at least one Outreach Champion from all but one team. In total, we interviewed 12 of the 16 Farmer Champions and 7 of the 10 Outreach Champions. Although we were unable to interview all Champions, through our 19 interviews we reached saturation – where we began to hear the same perspectives from Champions we interviewed, or “the point in coding when you find no new codes occur in data” (Urquhart 2013, 194).

Nonresponse is a potential source of bias in our sample of Champions, particularly if only Champions who were active and had success with the program agreed to be interviewed. However, the interviews we conducted, and program records, indicated that the four Farmer

Champions and three Outreach Champions who did not respond were just as active in program trainings and outreach efforts as those who participated in the interviews. Applications indicated that the Farmer Champions who were not interviewed also fell along a similar spectrum of crop diversification and farm management systems as their cohort.

To identify *target farmers*, we asked each Farmer and Outreach Champion to recommend farmers who they had interacted with during their time as CCCs, either directly through a sponsored event or by talking as friends or neighbors. We were only able to interview four *target farmers*. Overall, Farmer Champions were hesitant to share names with us because they did not want to violate trust by sharing contact information. This small sample of *target farmers* is a limitation of the study, because Champions may have recommended farmers most receptive to adopting cover crops. *Target farmer* perceptions, however, were reinforced by observations made by Farmer and Outreach Champions.

Interview questions explored how the intended CCC Program strategies and expected outcomes played out in practice based on different stakeholder experiences, assumptions, and contextual factors. All interviews were conducted by phone and lasted between 30-60 minutes. Interviews were recorded, transcribed, annotated, and analyzed qualitatively in NVivo. See Appendix 3, Table A 4, and A 5 for the Code Books (IRB: HUM00145979). The first two authors conducted the interviews together and coded the data in NVivo. Both authors first coded the same two interviews and then edited the Code Book to incorporate new themes that had been identified and discussed the final Code Book to ensure a shared understanding of the meaning of each code. Remaining coding was split between these two authors, reading through each other's coded interviews to check for consistency. Two CCC program directors also reviewed drafts of the manuscript and agreed that the authors' interpretations appeared valid given their own experience and longer-term perspective of the program.

4.3 Results

Overall, our interviews indicate that the CCC Program supports cover crop adoption. Many Farmer Champions learned more effective communication strategies, expanded their networks, and continued to promote cover crops even after their formal involvement with the CCC program ended. *Target farmers* we interviewed also reinforced the positive and lasting effects that Champions believe they had on cover crop adoption.

4.3.1 Learning effective communication and outreach methods

Although each Farmer Champion came to the CCC Program with previous knowledge and experience with cover crops, they started with different levels of outreach and public speaking skills. According to observations made by their partnering Outreach Champions, one subgroup of Farmer Champions was initially hesitant to speak in public and tended to use early adopter language that was not accessible to a broader spectrum of farmers. Outreach Champions observed that the NWF training around social messaging, together with logistical support from their Outreach Champions, transformed these Farmer Champions into effective, confident speakers who could better communicate with *target farmers*. The second group of Farmer Champions were active speakers who already had experience using language that was accessible to *target farmers*. These Farmer Champions told us that the funding, and logistical and networking support provided by their Outreach Champions allowed them to extend their outreach to more people.

Farmer Champions described how they learned messaging methods through the CCC orientation, webinars, and interactions with Outreach Champions. Specifically, Farmer Champions reported learning how to couple effective peer-peer learning methods with their existing knowledge to frame cover crops as solutions to common problems, as a form of risk reduction, and as an economically advantageous, ecological alternative to expensive inputs such as livestock feed, herbicides, and fertilizers.

One goal of the program was to train Farmer Champions to talk about cover crops as solutions to common on-farm problems. As one Champion noted: "I try to relate it to that person with something they might struggle with. If they have a problem with one thing, I can try to relate how cover crops can help with that problem." [Interviewee 19] Outreach Champions and the director of the program also described how farm demonstration days are often framed around solving problems – such as managing herbicide resistant weeds – rather than narrowly focused on cover crops, to attract a broader group of *target farmers.* Another Farmer Champion described how he would explain: "Look at my field. It's pretty much weed free, and I don't use any herbicides. I'm relying on my cover crops. You could do this on your own farm." [Interviewee 8] Further, he would explain to *target farmers* that because cover crops serve as a weed management tool, they reduce the need to apply herbicides, which can save time and money.

Field demonstration days aimed at *target farmers* were also organized around topics of managing livestock, soil health, and the economics of cover crops. For example, interviewees reported promoting cover crops to improve soil health and reduce soil compaction, so farmers can reduce the use of tillage, which saves time, money, and machine wear and tear. They additionally discussed how cover crops not only build organic matter, improving soil quality over time, but how legume cover crops also add nitrogen to soil, reducing fertilizer application and associated costs. One Farmer Champion would tell *target farmers* how his cover crops allowed him to skip the pre-emergence (applied at planting) fertilizer application, saving him $15 per acre. Most Farmer Champions used similar, simple economic arguments to talk about difficult problems, such as soil health and erosion, disease and weed pressure, and livestock management.

Other interviewees, however, noted that it can be difficult to convince *target farmers* that cover crops have economic benefits, because the financial savings do not show up until on-farm benefits become noticeable. One Outreach Champion worked with farmers to think longer-term about no-till, cover crops, and soil health. He said:

> "You have to get them looking out 4-5 years...once you change the mindset, there's no such thing as failure anymore. It's a learning experience. So, when they can see five years down the road and truly get into the economics, then they can start seeing savings in chemicals and savings in fertilizers." [Interviewee 17]

Champions explained that experienced cover croppers should show *target farmers* how their costs have dropped over time as their farms become less input intensive. Evidence of tangible savings can help *target farmers* view cover crops through a positive economic lens, which may change their perspective on the value of cover cropping.

The last method Farmer Champions used to change *target farmer* perceptions was to replace language about cover crops as economically risky with language about buffering against risks associated with extreme weather. For example, one Outreach Champion explained that farmers who grew cover crops experienced less risk during spring flooding: "it's actually riskier to not do these practices because we are going to continue to have extreme weather events in the future." [Interviewee 19] Another Champion noted that this risk can be visualized by showing target farmers a field that has been washed out by a storm, resulting in soil loss that is difficult to replenish. One farmer noted, “People are tired of watching [washouts] happen and having to go in and fix it the next year. Some have seen [cover crops] working for me and will try it.” [Interviewee 12] In general, interviewees described how, as floods and droughts become more common, cover crops can reduce associated on-farm risks such as soil erosion, waterlogged soils that prevent planting in the spring, and drought-stricken crops in the summer.

Champions observed that these new messaging methods were best shared through experiential learning approaches that framed cover crops as a “normal” solution to common on-farm problems. However, both Farmer and Outreach Champions found that the quality and nature of this peer-peer learning varied depending on how outreach events were designed. Two main types of events were reported: field demonstration days, and small, open-format meetings. Champions reported that both formats required audience engagement through discussions and hands on activities. Champions perceived field demonstration days to be beneficial because they exposed a larger number of people (i.e., 50-100) to the idea of cover cropping and facilitated informal networking as *target farmers* milled around the farm discussing test plots and learning together. They found that older farmers responded better to field demonstration days and farm shows, while younger farmers preferred smaller open-format settings, such as coffee shop talks. They also reported that open-format meetings with fewer than 20 people encouraged farmers to actively participate. These meetings allowed for in-depth conversations about the “nuts and bolts of cover cropping” that sent farmers home with tangible tools to try on their farms while also building new networks and strong relationships.

4.3.2 Harnessing the potential of farmer networks

The CCC program developed networks of Champions, connecting new and former Champions with each other, while also strengthening connections within their existing networks. For instance, Farmer Champions highlighted the important role CCC played in connecting them to other *innovator farmers*, reducing their feelings of isolation. One Farmer Champion said, “Cover Crop Champions brought lots of groups together. They let you know [that] you’re not alone, and [they] connected you with people.” [Interviewee 10] These new networks enabled Farmer Champions to exchange knowledge about cover crops with other *innovator farmers* and Outreach Champions. This nexus of knowledgeable farmers also became an asset for Outreach Champions to connect *target farmers* with specific information sources. Several Champions mentioned developing spreadsheets with names, phone numbers, and cover crop experience of early adopters in their networks who could serve as mentors or resources to both *innovator* and *target farmers* interested in growing cover crops. Outreach Champions were often the bridge linking experienced cover croppers with those looking to learn; thus, access to an expanded network of early adopters allowed them to connect more *innovator farmers* with *target farmers*.

In addition to building networks of *innovator farmers*, the CCC program harnessed the outreach potential of Champion’s existing farmer networks. Outreach Champions observed that the exchange of information that occurred between *innovator farmers* and *target farmers* through these established (and often extensive) networks was especially effective. One Outreach Champion commented, “Let [the farmers] come up with the ideas. I mean, we’re facilitating; we help set up the field days and we do a lot of the behind-the-scenes work, but being that its farmer led, it leads to better acceptance by other farmers.” [Interviewee 18] Farmer-farmer interactions through field demonstrations and social events hosted by Champions within their existing networks, helped farmers feel “more in their element” and made the learning process more comfortable. Outreach Champions observed that the comfortable educational environment fostered through farmer networks allows farmers to form new, supportive relationships, which has a positive feedback effect that expands networks while sharing information about cover crops. The need to adapt cover crop management to different farm contexts, over time, requires that *target farmers* maintain relationships with *innovator farmers* who can mentor them through uncharted territory. Networks fostered these long-term relationships.

4.3.3 Sustained success of the CCC program

The distinct feature that separated the Farmer Champions who continued outreach from those who did not was involvement in another farmer network. Champions who were members of other farmer networks that also provided funding, logistical support, and encouragement to support outreach activities were more likely to continue outreach than those who depended solely on the CCC's funding and resources. Farmer Champions who did not continue formal outreach activities after the program ended said that funding from the CCC program was a large factor in their ability to orchestrate events, and without it, they could only informally engage in outreach, such as answering questions from neighbors or chatting casually with other farmers about cover crops. Some continued to host smaller, informal events to "sit down in small groups with farmers who are interested and walk them through [cover cropping]." [Interviewee 6] Except for one Champion who did not report any continued outreach, all other Farmer Champions engaged in some level of outreach after the program, including giving up to five public presentations per year, serving in an active leadership role in another farmer network, or maintaining casual conversations with neighbors about cover cropping.

4.3.4 Target farmer perceptions and willingness to adopt cover crops

Both *target farmers* and Champions suggested that the CCC program was largely effective in changing attitudes about cover crops, though they also acknowledged the role of other facilitative factors. Generally, Champions reported that when *target farmers* observed the Champion's cover crops, they asked questions about how they could try the practice on their farms, indicating a change in attitude. Champions also noticed increased adoption of conservation practices such as no-till and cover cropping in the year after their time as Champions. For example, one Farmer Champion noticed that a year after he hosted outreach events on conservation practices, he started seeing those practices on nearby farms. Even if *target farmers* did not attend any official CCC outreach events, their informal interactions with Farmer Champions started to change their perceptions and increase adoption of cover crops.

One Farmer Champion said that he influenced others to change their perceptions about cover cropping and no-till practices just by showing them the soil health benefits those practices provided on his farm. He said that his neighbors "saw my no-till soybeans; they are now starting to use a roller crimper and started to no-till soybeans into cereal rye [cover crops]." [Interviewee 8] This farmer attributed his success to his ability to physically show *target farmers* the clear benefits on his farm, which helped convince them that this method was worth trying. Supporting this, one Outreach Champion believed that: "in-person, in-the-field, seeing some change in cover crops, the biggest being soil health, but actually…seeing firsthand what [cover crops] can do," [Interviewee 18] is a critical step to changing perceptions about cover crops.

Beyond facilitating field demonstrations, another Farmer Champion said that the small-group, peer relationships the CCC program facilitated helped *target farmers* establish lasting connections for ongoing technical support as they started cover cropping. All four *target farmers* we interviewed were still in communication with the Farmer Champions and sought advice from them as new challenges arose. For example, when asked where he turns with questions about cover crops, one *target farmer* replied: “I might call [Farmer Champion X]. I bounced an idea off him last week and he had some pretty good insight [about soil health].” [Interviewee 21] These long-term mentorships were important for farmers who had recently adopted cover crops to help make decisions as conditions change from year to year.

Target farmers observed that their interactions with Champions may have started them down the path of planting cover crops, but other factors such as availability of print and online resources about cover crops were also necessary. Further, access to conservation funding to buy cover crop seeds, and for some, off-farm income, were what allowed them to take financial risks. One Farmer Champion followed up with farmers with whom he had directly communicated about cover crops and estimated that they added about 6,000 acres of cover crops. However, he was unwilling to attribute more than half of that increase to his own actions. He said that “a lot of that probably happened with or without the Cover Crops Champion program. Who knows the other influences?” [Interviewee 12] Even though the *target farmers* who adopted cover crops were also influenced by other factors, it is still likely that the Farmer Champion played a role. One *target farmer* exemplified this by saying “We went to the NRCS asking questions and they gave us [Farmer Champion X’s] number and we went from there…the most important part of doing cover crops was meeting [him]. 90% of the contacts for cover crops have been through [him].” [Interviewee 20] After learning how to plant cover crops from a Farmer Champion, this *target farmer* received generous state conservation payments for cover crops, allowing him to plant cover crops on 100% of his acres, something he would not have been able to accomplish without the funding. All four *target farmers* planned to continue planting cover crops and had, in turn, become mentors to neighboring farmers who had questions about cover crops.

4.4 Discussion

Cover cropping is a diversification practice that can be integrated into many types of farms, including simplified grain farms that contribute to greenhouse gas emissions, water pollution, and other environmental consequences. Overall, our analysis suggests that the greatest potential to overcome the large constraints to cover crop adoption in the U.S. Midwest was realized when the CCC program's communication and outreach strategies (i.e., individual action) were paired with established farmer networks (i.e., collective action). The CCC program also developed new, long-term mentoring relationships, which are essential for supporting farmers as management challenges arise, and for sustaining and expanding the use of cover crops in an unfavorable structural context.

4.4.1 New communication methods coupled with farmer networks reduce the structural barriers to cover crop adoption

We found that *target farmer*-oriented messaging methods were powerful tools for promoting cover cropping when Champions leveraged their personal connections along with the new networks that the CCC program facilitated. Farmer Champions who were previously connected with more formal networks, such as farmer organizations, were able to reach a larger number of farmers. By combining the strategic messaging approaches that they learned through the CCC program with their long-term connections with farmer organizations, they could extend their influence as Champions and continue outreach after the CCC program had ended. For example, Farmer Champions who were also members of the Lower Fox Demonstration Farms network in Wisconsin and the IDEA Farm network in Illinois sustained higher levels of outreach after the program had ended. Building on this idea, NWF could foster more formal collaborations between their Farmer Champions and other farmer networks to change the narrative around cover cropping with larger groups of *target farmers*.

As other research demonstrates, the peer pressure and social and cultural norms that farmers are exposed to in networks can influence their values and beliefs, and ultimately their decisions (Rogers 2003; Carlisle 2016). When individuals are exposed to new ideas and innovations by peers, the perceived risk is diminished, increasing adoption of an innovation (Rogers 2003; Valente 1996). Within the dominant cultural and knowledge system, farmers' decision-making about on-farm management is often influenced by pressure from peers, extension agents, and agronomists who encourage the adoption of technologies that support the industrialization of agriculture (Hendrickson and James 2005; Stuart and Gillon 2013). On the other hand, peer networks are also central to the adoption of ecological innovations (Shaijumon 2018; Wood et al. 2014), helping farmers overcome constraints by increasing access to information (Baumgart-Getz, Prokopy, and Floress 2012), and creating positive narratives about diversification practices, such as the CCC using language tailored to normalize the widespread use of cover crops.

One novel aspect of this case was that the CCC program directly tackled the communication barrier between *innovator* and *target* farmers. Since cover crops are complex to manage and perceived as risky within the industrial farming model, interviewees noted that bridging this communication barrier is a critical bottom-up mechanism that can expand their adoption. For instance, improved communication among *innovator* and *target farmers* increased *target farmers'* access to ecological knowledge. Within the context of simplified commodity production systems, dependence on external inputs such as fertilizers and pesticides has grown since the 1950s, and conventional grain farmers have lost essential skills, knowledge, and equipment needed to manage diversified farming systems that include cover crops. Overcoming this process of "deskilling" (Stone 2007) and building alternative knowledge systems requires both social and environmental learning. Other than conservation-oriented farmer networks, there are few resources available to help Midwestern grain farmers transition to more diversified farming practices, increasing the importance of including more *target farmers* in these learning communities.

Demonstration plots and social events that Champions described in this case study facilitated these social and environmental learning experiences for farmers. On-farm demonstrations provide a social support network to begin to overcome barriers to adoption. The barriers most commonly cited by Farmer Champions in interviews included extreme weather events, costs, and access to seeds and appropriate equipment. Unfavorable policies and markets, and cultural and socio-technical systems that predominately support the production of commodity crops at scale, influence these practical barriers and make overcoming them difficult (Vanloqueren and Baret 2009). Access to farmer-led technical and social support to grow cover crops can begin to help motivated farmers overcome these structural challenges.

The CCC program demonstrates social learning principles that Knowles (1980) describes in his adult learning model. We found that Champions facilitated learning using hands-on, experiential approaches, and by addressing topics that were relevant to farmers' everyday problems. Learning was self-directed by farmers through a bottom-up approach, which was critical to the success of the program. Past farmer-oriented studies also support our observations, showing that farmers value knowledge and information from their peers over other sources (Houser et al. 2019), and learn best using empirical techniques including on-farm tactile activities (e.g., Carlisle 2016; Cooreman et al. 2018; Laforge and McLachlan 2018; Wood et al.

2014). Our case study adds the insight that hands-on, accessible learning activities presented in the context of solving common farm problems can encourage *target farmers* to attend outreach events and improve the quality of peer interactions within farmer networks.

4.4.2 *Complementary Knowledge Between Champions and Farmer-Farmer Mentorships*

Another effective element of the CCC program appeared to be the blending of complementary knowledge types in each Champion team: Farmer Champions contributed place-based knowledge about managing crop diversity while the Outreach Champions facilitated effective networking based on formal training in farmer education and outreach. Together, these teams shared knowledge about cover crops in socially accessible ways with *target farmers*. All Outreach Champions leveraged existing relationships with *target farmers* to help Farmer Champions expand the reach of their messages. Farmer Champions also benefitted from new relationships with previous classes of Farmer Champions who had more experience with cover crop outreach and served as mentors.

The important role of mentorship also carried over to *target farmers* who participated in CCC outreach activities. Generally, we found that when the CCC program connected *target farmers* with networks of experienced cover croppers, lasting relationships formed between farmers with more and less experience. Cover crop management and outcomes are highly variable year to year and from farm to farm; it is therefore important, even for farmers with experience planting cover crops (including Farmer Champions), to have others to consult as they transition to diversified farming systems.

Recent studies have shown that mentorship plays a key role in sustaining small-scale farms in Central New York (Strube 2019) and in organic farming communities in Canada (Laforge and McLachlan 2018). Within a dominant socio-technical system that favors simplified production systems, this research and our case study suggests that it is even more critical for farmers transitioning to diversified agroecosystems to have collaborative relationships and mentors who provide an alternative knowledge system and source of social support.

4.4.3 Future Research Needs

Our interviews with Champions and with a small group of *target farmers* provide initial evidence that *target farmers*' perceptions about cover crops changed because of their interactions with the Champions. To fully understand the effects of CCC on cover crop adoption, longer-term research is needed with more cohorts of Champions to monitor practices on farms within Champions' spheres of influence, including pre- and post-surveys and follow-up interviews with a large, and ideally random, selection of *target farmers* – both those who attend CCC sponsored outreach events and those who were contacted via outreach but chose not to participate. Other data such as total area planted to cover crops in a Farmer Champion's county before and after they serve as Champions, could also shed light on the effectiveness of the program. Such comprehensive program evaluations are uncommon because they require foresight to collect data before the program has started, are logistically difficult, and require significant funding and human resources (Patton 2008). Despite these challenges, it would be valuable to assess innovative programs like CCC over longer periods, combining quantitative and qualitative methods to fully understand their potential impacts on agricultural management systems and associated outcomes.

4.4.4 Policy Implications

Based on our findings suggesting that the CCC strategies help change farmer perceptions of cover crops, we would recommend that other programs with similar aims adopt the CCC model, but also caution that the conditions under which such a program can successfully scale up cover crop adoption may be limited. First, the CCC program developed its strategic communication approaches over several years, based on the context and culture of farmers it was working with in the U.S. Midwest. Directors who trained Champions were also experienced with social theory. Other programs looking to adopt the CCC model would likely need similarly trained facilitators and would have to adapt the CCC communication strategies to their particular contexts. Other efforts to change food systems practices have also found that learning how to avoid "semantic traps" – food systems language and framing that can create opposition for proposed actions (Ilieva 2020, 400) – must be customized to the local political context (Berneche et al. 2017).

Second, while we found that Champions were able to change perceptions, *target farmers* reported that they still needed more knowledge, funding, and proper equipment to manage cover crops on their farms. For example, vast investment in research and development for large-scale commodity production has reduced access to key resources for ecological management practices (Vanloqueren and Baret 2009), such as grain drills for planting cover crops. We also found that sustained outreach by Farmer Champions – after the program ended – depended upon continued funding through existing farmer networks or other farmer outreach programs. Extending more public and private resources to programs like CCC and tying Champions into more farmer networks would therefore provide experienced cover croppers with long-term access to funding for outreach and other resources to support more *target farmers* for a longer period.

While policy and market conditions and the erosion of knowledge constrain the presence of diversified farming systems (Iles and Marsh 2012), expanding programs like the CCC through more sustained outreach and growing farmer networks could ultimately spur the growth of innovation niches (Geels 2019). Eventually, these bottom-up forces might then expand to create pressure for significant institutional change, which is ultimately needed to scale the adoption of cover crops and other diversification practices (Figure 4-1, Blesh and Wolf 2014; Mier y Terán Giménez Cacho et al. 2018).

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Chapter 5 Conclusions and Suggestions for Future Directions

This dissertation applies and extends a social-ecological systems framework to understand the link between the social variables that influence how farmers adopt cover crops, and how cover crops can be used as an ecological nutrient management practice to replace synthetic fertilizers and reduce nitrogen (N) losses from grain farms in the U.S. Midwest. This mixed methods approach addresses gaps in our understanding of how functionally diverse cover crops influence N cycling under different soil conditions within the context of organic grain agroecosystems, and how social factors including farmer social networks influence attitudes towards and adoption of cover crops.

Industrial agriculture has created widespread imbalances in global nutrient cycles (Galloway et al. 2008). Although cover crops hold great potential to restore balance by recoupling C and N cycling, adoption is very low. Due to a range of social, economic, and ecological factors that constrain cover crop adoption, only 2-3% of annually planted cropland in the Midwest was cover cropped in 2012 (Hamilton et al. 2017). Through a case study of the Cover Crop Champions (CCC) program and analysis of multiple N cycling processes following cover crop incorporation at two field sites in Michigan, this dissertation illuminates pathways that can increase the presence and effectiveness of cover crops in the Midwestern U.S.

5.1 Chapter 2: Episodic N_2O emissions following tillage of a legume-grass cover crop mixture

Nitrogen (N) fixing legume cover crops are an alternative to synthetic N fertilizers that can build soil organic matter pools and reduce N surpluses over time, improving sustainability of soil nutrient management (Drinkwater et al. 1998, Syswerda et al. 2012; Blesh and Drinkwater 2013). Legume N sources can reduce nitrous oxide (N_2O) emissions compared to the emissions from fertilizer production and application (Norskov and Chen 2016). Diversifying rotations with legume-grass cover crop mixtures can further tighten C and N cycles with the potential to further reduce N_2O emissions compared to sole legume cover crops. In this chapter, I conducted an experiment at two fields sites in Michigan with contrasting levels of soil fertility to test the impact of a legume-grass cover crop mixture on short-term N_2O emissions compared to either species grown alone.

I focused on a short window immediately following cover crop tillage to capture the first peak in N_2O emissions, when emissions are generally highest in rotations with legume N sources (Millar et al. 2004). I found that when compared to the sole legume treatment, the mixture led to a small reduction in N_2O emissions at one site but not the other. When controlling for soil fertility, the sole legume and mixture treatments resulted in higher N_2O emissions than the sole grass and fallow treatments. Overall, I found that synergistic effects between new biological N fixation (BNF) inputs and soil fertility drove N_2O losses during a short, but critical, window of N_2O emissions following tillage. For example, higher soil fertility, defined by particulate organic matter pools and potentially mineralizable N, can support greater cover crop biomass production. The combination of higher soil fertility and biomass inputs, especially higher N from legumes, can increase N_2O emissions. This chapter highlights the importance of sampling N_2O frequently after soil disturbances such as tillage to advance our knowledge of episodic emissions. Further, this chapter suggests that future studies should measure the effects of cover crop mixture establishment and evenness on N_2O emissions across a soil fertility gradient defined by properties that are responsive to management practices, such as the particulate organic matter fraction.

5.2 Chapter 3: Nitrogen cycling dynamics following a legume-grass cover crop mixture in an organic agroecosystem

Legume cover crops provide a N source in organic cropping systems through BNF. Functionally diverse mixtures of legume and grass cover crops can increase BNF rates to supply a similar amount of N as a sole legume, while potentially increasing total cover crop biomass production and enhancing multiple functions at once. Through interspecific competition for soil N with grasses, legumes respond by allocating more of their photosynthate to the energy-intensive processes required to fix N_2, increasing rates of BNF (Jensen 1996, Hogh-Jensen and Schjoerring 1997, Li et al. 2016). This chapter expands on Chapter 2, by evaluating N cycling dynamics throughout the whole growing season of corn in sub-plots of the biologically-based (i.e., organic) cropping system in the Main Cropping System Experiment (MCSE) at KBS. Long-term datasets collected since this experiment's establishment in 1989 allowed me to calculate a 6-year N mass balance over two complete crop rotations and evaluate decadal changes in soil organic matter (SOM) stocks.

After one year of increasing the functional diversity of a cover crop at a site managed for 30 years with single species cover crops at different points in rotation, I found no significant differences in cumulative N_2O emissions between treatments. This result was contrary to my hypothesis that the mixture would reduce N_2O compared to the sole legume by further tightening

C and N cycling (via slower decomposition rates) and reducing N surpluses. However, I did find support for my hypothesis that BNF rates would be higher in clover in the mixture, due to interspecific interactions with the grass, resulting in a similar N supply with half the seeding rate and additional ecosystem functions from the grass. Although the mixture did not significantly reduce N_2O or improve yield after one year, the mixture supplied the same quantity of new N as a sole legume. Given that this study site has built multiple SOM pools with red clover as a cover crop – increasing the overall quantity and quality of SOM compared to the conventional cropping system (Plumhoff et al. In Review) – it may not need a sole legume cover crop. Rather, over time, more complex cover crop residues from a mixture may help enhance the efficiency of N cycling to further increase SOM accrual (Drinkwater and Snapp 2007, Blesh and Drinkwater 2013, Kallenbach et al. 2019). Further, mixtures provide many other ecosystem functions in addition to long-term SOM storage and N retention, including weed suppression, erosion control, and pest control, which we did not measure. This experiment was limited by its short-term nature and should be expanded over a longer period to evaluate how N surpluses (e.g., N_2O emissions and N leaching) and legume BNF rates – when grown alone and when in mixture – change as soil fertility improves over time.

5.3 Chapter 4: Cover Crop Champions: Linking strategic communication approaches with farmer networks to support cover crop adoption

In this chapter, I interviewed 24 participants from the 2017 Cover Crop Champions cohort to evaluate the effectiveness of the program. I found that the program trained Farmer Champions to use communication methods that normalized cover crops by using accessible language, sharing personal success stories, facilitating hands-on field demonstrations, and focusing on tangible benefits, including buffering against extreme weather events. These communication methods were paired with efforts to build communities of practice by strengthening existing networks and building new networks. The resulting relationships and mentorships between farmers with more and less experience growing cover crops helped farmers overcome barriers to diversification by increasing cover crop adoption and other conservation practices such as no-till. Lessons learned from the program can be applied to programs that work to increase adoption of all conservation-oriented practices. In fact, Cover Crop Champions has renamed themselves “Conservation Champions” since this study was conducted, formally expanding their focus beyond cover cropping. Farmer networks and grassroots organizations such as Practical Farmers of Iowa (in the U.S. Midwest) and *La Vía Campesina* (a global social movement) are critical players in expanding the presence of diversified farming systems (Altieri and Toledo 2011, Bell 2004). The personal relationships and mentorships that form through farmer networks are critical bottom-up forces that can help farmers overcome top-down constraints to cover crop adoption (Strube 2019, Laforge and McLachlan 2018).

A key factor that made the Cover Crop Champions program so effective was that the program’s facilitators were trained in the social theory needed to develop localized, strategic farmer-farmer communication approaches to mobilizing farmer networks. Other programs with similar goals should hire experts with training in social theory and facilitation to spend significant time up front customizing this program’s model to their local political context (Berneche et al. 2017). Sustained outreach efforts to grow farmer networks and expand programs like Cover Crop Champions can create innovation niches to help farmers overcome structural constraints and foster transitions to cover crop adoption on a larger scale (Geels 2019, Iles and Marsh 2012, Blesh and Wolf 2014, Mier y Terán Giménez Cacho et al. 2018).

5.4 Future Directions

5.4.1 Extend focus to ecosystem functions beyond yield

High intensity monoculture agriculture has historically been managed primarily for one ecosystem function: yield, relying on external inputs, such as fertilizers and pesticides, to provide ecosystem functions that other species in rotation used to provide. This reductionist approach to managing agriculture has led to declining biodiversity across entire regions (e.g., Midwest, Mississippi River Basin), reducing ecosystem services and resulting in widespread social and environmental costs, such as eutrophication, polluted drinking water, and increased greenhouse gas emissions. The most well-known example of this in the U.S. is the expanding "dead zone" in the Gulf of Mexico caused directly by monoculture agriculture throughout the Mississippi River Watershed (David et al. 2000, Galloway et al. 2003, Robertson and Vitousek 2009).

Simplified cropping systems with synthetic N fertilizers have limited N sinks in soil organic matter (SOM), including microbial biomass, rapidly destabilizing SOM pools. This results in high N losses (up to 60% of applied fertilizers) and creates a negative feedback loop or "fertilizer treadmill" that requires farmers to keep applying more inorganic N year after year (Drinkwater and Snapp 2007, Gardner and Drinkwater 2009, Schmidt et al. 2011). Further, plant breeding has selected for crop varieties that yield better in response to inorganic N fertilizers, making these varieties less able to access soil organic N sources (Drinkwater and Snapp 2007).

This emphasis on increasing yield over all other ecosystem functions stems from the Green Revolution, largely driven by social and economic constructs that fit within the capitalist model for economic growth. Extensive literature claims that Haber-Bosch derived N fertilizers averted a Malthusian disaster by feeding a growing global population (e.g., Borlaug 2000, Trewavas 2002). This approach claims that the potential consequences of not applying enough fertilizer are too high to risk a transition to ecological nutrient management. However, agroecologists argue that yield is not the best measure of food security (Fischer et al. 2016, Schipanski et al. 2016). Even though yields for many crops have increased over time, rates of food insecurity have not declined. For example, many monoculture grain crops are used for biofuels and animal feed, with only a portion of the original calories making their way into human diets (e.g., Cassidy et al. 2013). Alternatively, diversified, smallholder agriculture is more likely to contribute nutritious food products directly to local and regional markets, improving access to diverse foods and food security. Therefore, Diversified farming systems have the potential to feed a growing population without causing the environmental and societal damage associated with industrial farming (Badgley et al. 2007, Schipanski et al. 2016, Blesh et al. 2019).

For example, increasing plant diversity in agroecosystems can replace chemical inputs by providing ecosystem functions that provide the same, or likely more, services (Isbell et al. 2017, Tamburini et al. 2020). Even small increases in species diversity can have a significant impact on ecosystem functioning (e.g., SOC, N cycling, microbial biomass, weed suppression) (Drinkwater et al. 1998, McDaniel et al. 2014, Tiemann et al. 2015, Blesh 2017). Mixing a few cover crop species with different functional traits that maximize ecosystem functions can support management goals such as drought tolerance, pest management, improved N cycling, and cash crop quality and yield (Snapp et al. 2005, Davis et al. 2012, King and Blesh 2018). Farmers can increase the chances that these ecosystem functions will be present in their agroecosystems by picking species based on known functional traits and positive relationships between species, such as the legume-grass symbiosis (Blesh 2017). Legumes can reduce or replace conventional fertilizer inputs through BNF carried out by symbiotic bacteria, balancing N inputs from BNF with N exported in harvested crops, reducing N losses compared to synthetic N fertilizers (Blesh and Drinkwater 2013). As spatial and temporal functional trait diversity increases on farms from monocultures to more complex rotations with cover crop mixtures, multifunctionality increases beyond optimizing for yield and the need to apply external inputs declines (e.g., Tiemann et al. 2015, Blesh 2017).

This message about ecological nutrient management as a viable alternative to industrial input intensive practices is drowned out by a few global agribusinesses (e.g., Bayer and Cargill), which are becoming increasingly consolidated globally, supported by the neoliberal ideals of free markets and globalization (Hendrickson 2015). Agrochemical companies have successfully spread maladaptive practices in the name of progress. For instance, despite scientific evidence that suggests otherwise, Bayer promises framers that genetically modified seeds will increase farm profits by increasing yield. However, these seeds are not adapted to local soil and climate conditions and require expensive chemical inputs (e.g., fertilizer, glyphosate) and irrigation to produce promised yields (Stone 2007). Almost overnight, generations of local agricultural knowledge were replaced with expensive inputs that had to be re-purchased every year, leaving farmers in debt and agricultural lands highly degraded. The economic burden of this input intensive system will increase as fossil fuel prices rise, increasing the cost of fertilizer (Woods et al. 2010).

Wendell Berry (1984) argues that agrochemical companies advertised their products as infallible solutions to complex, local problems that, historically, have been solved through local knowledge networks, drawing on experiential knowledge passed down through generations. This is an example of propagation of maladaptive practices that ignore ecological science and ultimately degrade the land and hurt farmers and consumers (Berry 1984). After several generations of participation in this input-driven system, traditional social and environmental knowledge has been mostly lost in many Midwest farming communities. However, hope remains in that a small number of farmers have held onto the knowledge needed to implement ecological nutrient management practices and are bringing it back to their communities through farmer networks (Laforge and McLachlan 2018, Strube 2019).

5.4.2 Take farmer perceptions and concerns into account when designing field experiments

The Cover Crop Champions program, evaluated in Chapter 4, provides an example of a well-organized network of farmers in the U.S. Midwest working hard to take back their autonomy over their land by diversifying their rotations and reducing their reliance on chemical inputs. Also in the Midwest, Practical Farmers of Iowa helps fight maladaptive practices by building local, farmer networks around a shared goal of solving on-farm environmental problems with fewer inputs while protecting downstream environments (Bell 2004, Warner 2007).

These Midwestern farmer networks are still relatively small compared to larger global networks like *La Vía Campesina.* The peasant communities who participate in this movement have small, diversified, low-input farms that are often located on land that was degraded by Green Revolution technologies. Peasants are taking back the land from agribusiness through peasant to peasant – *campesino a campesino* – networks sharing knowledge and using collective power to bring back agroecology (Rosset and Martinez-Torez, 2013). While farm workers unions and associations in the U.S. participate in the *La Vía Campesina* movement, conventional grain farmers who control the majority of farmland in the Midwest rely on local and regional farmer networks in the U.S. that have made less progress towards adopting agroecological principles. Midwest grain farmers own large swaths of land, are relatively well off financially, and are actively participating in the industrial agriculture system. Farmer-farmer networks in the

Midwest primarily focus on solving long-term soil management problems within the context of industrial agriculture.

While innovative groups of farmers and farming communities in the Midwest have made some progress, there is still much work to be done. Farmer networks must bring together the social and environmental resources that farmers need to start making changes on their farms, removing barriers to cover crop adoption. Social support from peers within farming communities is critical for increasing adoption of ecological nutrient management practices. Further, as Practical Farmers of Iowa has modeled, peer-peer learning should be combined with collaboration with researchers to develop context specific agroecological practices based on sound science (Holt-Gimenez 2006).

However, farmer-led agroecology initiatives in both developing and industrialized countries often struggle to find ecologically informed research that engages farmers. Diversified farmers are often looking for a different kind of research than what Land Grant universities and agricultural companies are producing (Warner 2007). Researchers in agroecology and related fields can help improve knowledge of complex, context specific agroecological practices by working with farmers to answer farmer-inspired research questions. To improve the quality of the research on ecological nutrient management in Michigan, I used a mixed methods approach integrating social and natural science research methods to study cover crops in a way that was meaningful to farmers working to diversify their rotations with cover crops. Through interviews with two dozen farmers in Michigan, I identified cover crop species that were most likely to be used in the region (cereal rye and crimson clover) and identified uncertainty around N use efficiency as a major concern when using legume cover crops as an alternative to fertilizers. This farmer input was foundational in the design of my field experiments. Future ecological nutrient management research should use similar approaches to design on-farm field experiments that serve the needs of local farmer networks to help them adapt their management to improve resilience to climate change and become economically independent of expensive external inputs.

5.4.3 Maintain and expand long-term datasets to evaluate ecological nutrient management practices

Kellogg Biological Station's Main Cropping System Experiment (MCSE) was established in 1989, providing 30 years of data including soil properties, N_2O emissions, N leaching, cover crop and crop C and N and biomass/yield. This long-term dataset allowed us to construct a six-year, N mass balance which spanned two full crop rotations, finding that N inputs from red clover BNF approximately balanced N removal in harvested crops (corn, soy, wheat). This result was expected, given the long history of ecological nutrient management in this cropping system. Increasing temporal functional diversity in the organic agroecosystem in the MCSE, by adding a red clover cover crop after wheat and a cereal rye cover crop after corn, has significantly improved soil quality compared to the conventional treatment managed with synthetic N fertilizer inputs. Policy makers and farmers need this kind of long-term data to inform decision making. The MCSE provides compelling evidence that diversifying crop rotations with cover crops can restore fertility by building SOM pools in degraded soils and lead to high N use efficiency over time on sandy loam soils in Michigan. However, the outcomes at this site may not be generalizable outside of the upper Midwest or to farms with different soil types, necessitating the expansion of long-term experiments, both across the country and on working farms, to evaluate how N balances and N cycling processes change over time with increasing crop diversity in agroecosystems.

5.4.4 Co-design experiments with farmers on working farmland

Future experiments should assess how reducing inputs by diversifying organic crop rotations impacts N_2O emissions over at least one or two full crop rotations. N_2O emissions are influenced by a wide range of factors including the soil microbial community (particularly the presence of nitrifying (e.g., *Nitrosomonas*) and denitrifying (e.g., *Pseudomonas*) bacteria and nitrous oxide reductase (*nosZ*) enzymes), temperature, precipitation, disturbances (such as tillage), soil type and porosity, soil water, SOM quantity and quality (e.g., POM, MAOM), and NH_4^+ and NO_3^- availability (Firestone and Davidson 1989, Robertson and Groffman 2015). These factors vary widely over space and time making it difficult to draw generalizable conclusions about how management practices influence N_2O emissions (Robertson et al. 1999). Further, cover crop performance (i.e., BNF rates, biomass production, and trait variation) can differ significantly across soil fertility and precipitation gradients (Wilke and Snapp 2008, Blesh 2019, Garcia et al. 2020). Chapter 2 found preliminary evidence that a combination of cover crop performance (i.e., above-ground biomass and total N inputs) and soil fertility significantly impacted N_2O emissions. However, this evidence was not generalizable after comparing just two sites with contrasting soil fertility for one year. Both field studies in this dissertation took place at research sites that provided relatively controlled conditions and were logistically ideal for conducting experiments. Future work on this subject should be conducted on working farms across a range of soil fertilities to capture real-world variability.

As a companion to the two experiments reported in Chapters 2 and 3, I conducted a field experiment across a soil fertility gradient on working grain farms in southeast Michigan ranging from low fertility soils on fields with no history of cover crops to high fertility soils that had been cover cropped and grazed for decades. This study was unfortunately omitted from this dissertation due to serious data collection constraints imposed by the COVID-19 pandemic during the spring of 2020, which prevented us from collecting cover crop and N mineralization data from all 10 field sites originally enrolled in the experiment. Despite these limitations, we were able to collect complete data on 4 of the 10 field sites which I will describe briefly here.

I found that underlying soil conditions varied significantly across farms and that those soil conditions explained variation in cover crop production, soil N mineralization rates, and corn yield. For example, cover crop biomass was significantly higher in the mixture than in other treatments ($P < 0.0001$) and the C:N of the aboveground cover crop biomass was significantly higher in the rye treatment than in the clover or mixture treatments across farms ($P < 0.0001$). Further, we found that soil properties such as particulate organic matter (POM) and micronutrient concentrations influenced rye C:N and clover biomass across farms. For example, as POM concentrations increased, rye C:N decreased, likely reflecting N availability from microbial turnover of SOM. I found different patterns of N mineralization across each farm with no significant differences between cover crop treatments, corroborating famer input from interviews that N release from organic N sources represents a large source of management uncertainty.

Cover crops had positive or neutral impacts on corn yields on all but one field. The latter field demonstrated the negative impact that cover crops can have on corn production with unfavorable spring weather conditions (i.e., early spring flooding followed by drought conditions). This field also had the lowest soil fertility, with cover crops having less of an impact on corn production in higher fertility fields. I developed both farm level and overall recommendations from these on-farm results and shared them with all farmers who participated in the study. This preliminary experiment suggests that detailed information about baseline soil properties could guide farmer decisions about which cover crop species to plant to increase cover crop biomass production and potential yield benefits. More data collection would be needed over a larger number of fields varying in soil fertility conditions and over multiple years to adequately develop such a model.

5.4.5 Provide farmers with resources to overcome structural constraints

Cover cropping as an ecological nutrient management tool could have a significant impact on the sustainability of agriculture in the U.S. Midwest if adoption increased. However, Cover Crop Champions in my case study reported that even when they were able to change perceptions about cover crops, knowledge, funding, and equipment remained common obstacles to adoption. To increase adoption, research and development efforts must shift from their current focus on large-scale commodity production, to ecological practices (Vanloqueren and Baret 2009; DeLonge et al. 2016; FAO et al. 2021). For example, farmers should have access to multiple options for buying and planting cover crop seed. These could include access to low-cost grain drills and contracted airplanes for flying on seed. Further, access to roller crimpers, which mechanically kill cover crops in the spring without disturbing the soil, would allow no-till farmers to terminate their cover crops without using herbicides. Reducing tillage can further tighten N cycles by reducing soil disturbance, which improves aggregate stability and can reduce gaseous losses. Local, state, and federal agencies must all work together with farmers to supply the resources necessary for widespread adoption of cover crops.

5.4.6 Summary of Future Needs

In summary, future cover crop studies should be co-designed with farmers to consider key social factors including farmers' perceptions and attitudes towards cover crops. Experiments should span a wide range of environmental, economic, and social conditions to better understand how cover crops respond to real-world variability. Further, to advance our knowledge of episodic N_2O emissions in agroecosystems that rely on legume N sources, future experiments should increase sampling frequency following soil disturbance events, such as tillage, and following rain events, especially after extended dry periods. Specifically, these studies should measure the effects of cover crop mixture establishment and evenness on N_2O emissions across a soil fertility gradient defined by particulate organic matter fractions. Given that soil fertility changes slowly, these experiments should span years to decades, building long-term data sets to inform soil management practices that reduce reliance on fertilizers and contributions to climate change.

Programs, such as Cover Crop Champions, that facilitate farmer-farmer education to increase use of diversification and soil conservation practices (e.g., crop rotation complexity, cover crops, no-till) should also be evaluated over longer periods of time (i.e., 5-10 years). For example, farmers should be interviewed or surveyed about their land management practices (e.g., cover crops, tillage, fertilizer rates) before, during, and after participation in these education programs to evaluate how their interactions with other farmers affect long-term land management decisions.

In conclusion, an interdisciplinary approach to conducting cover crop research in partnership with farmers can address both the social and ecological factors that mediate on-farm decision-making and management of cover crops and the resulting impacts on the N cycle. By more comprehensively accounting for the complex factors that impact N management, this research can help farmers overcome structural constraints to adopting ecological nutrient management practices to build a more sustainable and resilient food system.

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Appendices

Appendix 1: Supplemental Material for Chapter 2

Table A 1. Means (standard error) for above ground biomass, biomass nitrogen (N), and biological nitrogen fixation (BNF) by species across treatments at *CF* (A) and *KBS* (B).

A.

CF	***All Cover Crops***		***Clover***			***Rye***		***Weeds***	
	Biomass (*kg* ha^{-1})	*Biomass N* (*kg N* ha^{-1})	*Biomass* (*kg* ha^{-1})	*Biomass N* (*kg N* ha^{-1})	*BNF* (*kg N* ha^{-1})	*Biomass* (*kg* ha^{-1})	*Biomass N* (*kg N* ha^{-1})	*Biomass* (*kg* ha^{-1})	*Biomass N* (*kg N* ha^{-1})
Rye	7709.1 (387.2)	98.6 (4.6)				7250.9 (341.7)	89.2 (7.6)	458.2 (201.3)	9.4 (4.1)
Clover	4845.8 (477.9)	121.2 (14.4)	4294.6 (680.5)	106.7 (19.2)	46.2 (8.3)			551.2 (284.3	14.5 (6.5)
Mixture	6392.4 (205.8)	131.3 (14.4)	3371.9 (702.6)	83.3 (20.7)	52.7 (13.1)	2863.5 (495.4)	43.9 (6.6)	157.0 (70.4)	4.1 (1.8)
Fallow	2774.5 (245.1)	59.0 (7.9)						2774.5 (245.1)	59.0 (7.9)

B.

KBS	***All Cover Crops***		***Clover***			***Rye***		***Weeds***	
	Biomass (*kg* ha^{-1})	*Biomass N* (*kg N* ha^{-1})	*Biomass* (*kg* ha^{-1})	*Biomass N* (*kg N* ha^{-1})	*BNF* (*kg N* ha^{-1})	*Biomass* (*kg* ha^{-1})	*Biomass N* (*kg N* ha^{-1})	*Biomass* (*kg* ha^{-1})	*Biomass N* (*kg N* ha^{-1})
Rye	2842.8 (212.2)	31.9 (1.4)				2367.7 (161.8)	25.4 (0.5)	475.2 (89.9)	6.5 (1.1)
Clover	3972.1 (579.7)	80.8 (13.5)	2963.9 (654.8)	67.5 (14.0)	29.2 (6.0)			1008.2 (90.4)	13.3 (1.2)
Mixture	4219.1 (297.2)	73.4 (5.8)	2310.0 (380.7)	50.6 (7.0)	32.1 (4.4)	1148.9 (300.9)	13.1 (3.6)	760.3 (43.3)	9.6 (0.6)
Fallow	2005.8 (387.9)	26.0 (6.6)						2005.8 (387.9)	26.0 (6.6)

Table A 2. Sensitivity analysis for the *CF* site where we estimated %Ndfa at 40, 50, 60, and 70 for the clover grown alone and in mixture.

Treatment	***Block***	***BNF (N kg ha^{-1}) @ 40 %Ndfa***	***BNF (N kg ha^{-1}) @ 50 %Ndfa***	***BNF (N kg ha^{-1}) @ 60 %Ndfa***	***BNF (N kg ha^{-1}) @ 70 %Ndfa***
Clover	**1**	22.6	28.3	35.1	39.6
	2	44.1	55.1	68.5	77.2
	3	43.9	54.9	68.1	76.8
	4	60.1	75.2	93.3	105.2
	Mean (std. error)	**42.7 (7)**	**53.3 (10)**	**66.3 (12)**	**74.7 (13)**
Mixture	**1**	33.1	41.4	49.6	57.9
	2	32.7	40.9	49.0	57.2
	3	54.0	67.5	81.0	94.5
	4	13.5	16.8	20.2	23.6
	Mean (std. error)	**33.3 (8)**	**41.7 (10)**	**50.0 (12)**	**58.3 (14)**

Appendix 2: Supplemental Material for Chapter 3

Table A 3. Species composition, separated by rye, clover, and weeds, of the mixture treatments by block replicate and means (standard error) for each species.

Rep	% Rye	% Clover	% Weeds
1	7.5	71.9	20.7
2	29.2	51.8	19.0
3	26.7	57.9	15.5
4	47.3	35.1	17.5
Mean (std. error)	**27.7 (7.1)**	**54.2 (6.6)**	**18.1 (0.9)**

Figure A 1. Kellogg Biological Station Long Term Ecological Research site Main Cropping System Experiment plot map from 2020 when the experiment was conducted in the northern section of the T4 plots in replicates 1-4.

Appendix 3: Supplemental Material for Chapter 4

A3.1 Semi-Structured Interview Guide: Farmer Champions

Introductions: I'd like to talk with you about your experience in the cover crop champions program and how you think your participation in the program has impacted your community. May I record our conversation? I will not share it with anyone outside of the research team and will keep all the information confidential. I am planning to use what we learn from these phone calls to inform my research on the CCC program, and to learn from you to improve the program for future years. Feel free to skip any questions that you aren't comfortable answering or stop at any time.

1. Why do you grow cover crops? What got you started?

   When did you start?

   Has it been continuous?

2. Questions about cover crop management:
   a. How many acres of row crops did you plant in total on your farm last year?
   b. How many acres do you plant cover crops on?
   c. Which cover crops do you grow?
      i. How many?
      ii. Species?
   d. What impact have cover crops had on your farming operation?

i. What have you observed? Prompts: soil, pest/weeds, cash crop performance?

ii. How do you think cover crops have made an impact?

e. Do you face any barriers on an annual basis to planting cover crops?

i. Can you describe the most challenging one in more detail?

3. How did you find out about the program? Why did you decide to apply for the Cover Crop Champions program?
4. What is one of the first things you say when you meet a farmer at an outreach event?
5. What are examples of messaging about cover crops that you perceive to have been:
   a. most effective? How do you know? What indications did you receive that they were most effective?
   b. least effective? How do you know? What indications did you receive that they were least effective?
6. Did you attend CCC orientation? If no, skip to 8.
7. What are a few important things that you learned during CCC orientation?
   a. Do you apply all of these lessons/methods?
   b. Which method did you think was most helpful?
8. Did the orientation and other training materials change how you communicate about cover crops with other farmers?
   a. If Yes: How? In what way?
   b. If No: Why didn't anything change?
9. What activities did you carry out as a Cover Crop Champion?
10. What outreach activities did you engage in?

11. How did you communicate with farmers in your capacity as a champion?
12. How many people attended your outreach events?
13. Did participating in this program change how you communicate with other farmers about cover crops and other conservation practices when you are not acting in your role as a champion?
14. How did your "outreach champion" impact the quality of the outreach programs you conducted during your time as a cover crop champion?
15. What other sources of knowledge did you draw on to impact your work as a cover crop champion?
16. Do you have examples of farmers giving you feedback after outreach activities?
    a. What type of feedback?
    b. Negative?
    c. Positive?
17. Do you have any specific examples of farmers you've interacted with changing their perceptions of cover crops?
    a. What, in particular, do you think helped to change their perceptions?
18. Do you know if anyone has adopted cover crops as a result of participating in any of your outreach events?
19. How do you define success of a cover crop champion?
20. What do you think made you successful in your role as a champion?
    a. skills you learned through training?
    b. skills you had previously?
    c. skills you learned along the way through trial and error?

    d. funding?

21. What are components of outreach that you feel you could use more guidance on from the program?
22. Based on your experience working with farmers, what else could be added to CCC orientation?
23. Did you learn anything along the way to improve your outreach methods that you would like to share with others?
24. Are you still in contact with farmers you worked with while you were a champion?
    a. I am looking to interview more farmers who are interested in cover crops and who have adopted cover crops. Would you be willing to share any names/numbers with me so I can talk with them about their experience cover cropping?
    b. I am looking for several farmers in your community who range from thinking about growing cover crops, to having successfully adopted cover crops recently.
25. Is there anyone who was particularly stubborn who changed their mind?

Think about any follow up questions, then thank the farmer for their time.

A3.2 Semi-Structured Interview Guide: Outreach Champions

Introductions: I'd like to talk with you about your experience in the cover crop champions program and how you think your participation in the program has impacted your community. May I record our conversation? I will not share it with anyone outside of the research team and will keep all the information confidential. I am planning to use what we learn from these phone calls to inform my research on the CCC program, and to learn from you to improve the program for future years. Feel free to skip any questions that you aren't comfortable answering or stop at any time.

1. How did you find out about the program? Why did you decide to participate in the Cover Crop Champions program?
2. Tell me a little about your team. Why did you decide to work with X Farmer Champion?
3. What skills did you bring from your personal/professional career to the program?
4. Did you attend the Cover Crop Champions Orientation?
    a. What did you learn?
5. What outreach and messaging methods did you encourage your farmer champion to use?
    a. Can you provide some specific examples?
    b. Did they follow through?
6. Which methods turned out to be the most successful?
7. How do you define success for a cover crop champion?
8. What do you think is the most important personality trait of a successful cover crop champion?
9. Would you participate again with another farmer in the future?
10. Do you see the program having a long-term impact on your community?

In what way? Prompts: environment, economy, farmer networks

11. Have you kept in touch with any of the farmers who participated in your outreach activities since you participated as an outreach champion in 2017?
    a. What kind of interactions do you have with them?
12. Do you have any specific examples of farmers you've interacted with changing their perceptions of cover crops?
    a. What, in particular, do you think helped to change their perceptions?
13. I am looking to interview more farmers not associated with the cover crop champions program who are interested in cover crops and who have adopted cover crops since participating in outreach activities.
    a. I am looking for several farmers in your community who range from just thinking about growing cover crops, to having successfully adopted cover crops recently.
    b. Would you be willing to share any names with me so I can talk with them about their experience with cover cropping?
    c. Is there anyone who was particularly stubborn who changed their mind?

Think about any follow up questions, then thank them for their time.

A3.3 Semi-Structured Interview Guide: Target Farmers

Introduction: I'd like to talk with you about your experience growing cover crops and your interactions with X Farmer Champion. May I record our conversation? I will not share it with anyone outside of the research team and will keep all the information confidential. I am planning to use what we learn from these phone calls to inform my research on the CCC program, and to learn from you to improve the program for future years. Feel free to skip any questions that you aren't comfortable answering or stop at any time.

1. How did you get started growing cover crops?
    a. For how long?
    b. How did you find out about cover cropping?
    c. What species of cover crops do you grow?
    d. Do you participate in cost share programs?
2. When you have questions, who do you call?
    a. Have you tried something new that has or hasn't worked?
3. What are important things you have learned while cover cropping?
    a. Have you shared this information with your neighbors?
4. Do other farmers come to you to ask questions about cover cropping?
    a. Do people ever stop by and ask about your farm?
5. Has anyone else adopted cover crops after interacting with you?
6. Anything else you would like to share?

Table A 4. Codebook for question: "How are CCCs sharing information about cover crops (e.g., language choice, messaging methods)?"

Parent Code	Child Code
Farmer use of cover crops on their farm	Reason for growing cover crops
	Acres of cover crops
	Species of cover crops
	Barriers
	Impact
Initial interest in program	Find out?
	Decide to apply?
First thing you say when meeting a farmer?	
Messaging about cover crops	Most effective?
	Least effective?
	Learned through CCC training
	Learned previously
	Learned along the way
Orientation	Attendance?
	New knowledge
	New communication methods
	Application
Webinars	Attendance?
	New knowledge
	New communication methods
	Application
Change in communication style/method	
Active Information Sharing as a CCC	Field Day
	Formal Speaker Event/Panel
	Phone Calls
	Informal chats with neighbors
	Radio Interviews
	YouTube videos
	Articles
Active Information Sharing after being a CCC	Field Day
	Formal Speaker Event/Panel
	Phone Calls
	Informal chats with neighbors
	Radio Interviews
	YouTube videos
	Articles
Farmer Networks	Practical Farmers of Iowa
	Fox Demo Farms
	IDEA Farm Network

Table A 5. Codebook for question: "In what ways have farmer perceptions, and willingness to adopt cover crops, changed as a result of participating in CCC outreach activities?"

Parent Code	**Child Code**
As a result of CCC activities:	
Change in non-CCC Farmer Perceptions as a result of CCC activities	Basic understanding of cover crops
	Reduced skepticism about growing cover crops
	Perception of barriers
	Perception of benefits
	General increased interest
Change in non-CCC Farmer Perceptions as a result of champion interacting with farmers outside of CCC	Basic understanding of cover crops
	Reduced skepticism about growing cover crops
	Perception of barriers
	Perception of benefits
	General increased interest
Willingness to Adopt	
Actual Adoption	
Farmers calling to ask questions	