COVER CROPPING FOR SUSTAINABLE CO-PRODUCTION OF BIOENERGY, FOOD, FEED (BFF) AND ENHANCEMENT OF ECOSYSTEM SERVICES (ES)

by

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A Thesis

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Master of Science

Department of Agronomy

West Lafayette, Indiana

December 2020

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Dedicated to my family and friends.
Without you, I would not be where I am today.

ACKNOWLEDGMENTS

I would like to wish my sincerest gratitude and thankfulness to my major professor Dr. Jeffrey Volenec as well as Dr. Sylvie Brouder. Your willingness to teach and guide me through my master's thesis is something I will never forget or take for granted. I am thankful for your patience and persistence as well as your willingness to take a chance on a student without a traditional agricultural education background. I will take the lessons learned from both of you and do my best to apply it toward further advancements in the field of agriculture. Thank you for making my time at Purdue University enjoyable and proving yet again that Purdue University and its faculty can be an utmost wealth of knowledge and an invaluable resource to students like myself. I trust that your impact on me over the course of my master's program has well prepared me for a future in not only in the agricultural field, but life as well.

I would like to thank my committee members Dr. Shalamar Armstrong and Dr. Lori Hoagland for your added advice and guidance to my master's thesis project. Your renewed perspective on agronomic concepts challenged me to think beyond my comfort zone and view agronomic cropping systems in a different light. I appreciate your willingness to work with my major professor and I and your collaboration on this project. I am thankful to have had the experience of working alongside and learning from each of you.

I would also like to thank Dr. Marc Milne who mentored me from the directionless freshman university football player with sub-par grades into a successful graduate with a Bachelor of Science degree, multiple scientific publications, and numerous research awards while attending the University of Indianapolis. It was this success that further enabled me to enter into a Purdue University master's program in the first place. I am very thankful today to look back and know that most of those accomplishments may have never happened had it not been for the genuine guidance provided by an outstanding educator. Dr. Milne is a living testimony of the positivity that can evolve from a dedicated teacher who puts forth the extra effort on even the most difficult to reach students.

I would like to thank Mitch, Dakoda, Amanda, Nathan, Daniel, Sarah, Nick, Matt, Paula, Josh and Sam for their friendship throughout graduate school. You all have made my experience as a Purdue University Boilermaker, one that I will never forget. I am incredibly thankful to call you all friends.

I would also like to thank all of the members of the Volenec and Brouder laboratories for their assistance in gathering and analyzing the data needed for this project. Without your help, agronomic knowledge, and persistence, this project would surely not have been completed. Thank you to Niki, Mel, and Suzanne as well as numerous undergrads for all you did to help me along the way. I truly appreciate it.

Outside the realm of academia, I would first and foremost like to acknowledge my faith in Jesus Christ and humbly admit that without my faith in God I would have never been able to accomplish this feat. Secondhandedly to my faith, I would like to thank my fiancé Allyson for her endless support throughout this journey. Thank you for being there throughout the long nights, the frustrations, and countless challenges. As far as I am concerned this degree belongs to you just as much as myself, because without you I would not have had the opportunity to even pursue this dream. There is no doubt in my mind that I would not have made it through this experience without your love and encouragement throughout this entire process. You have been with me through the highs and lows and there's no one else I'd rather have by my side. Thank you for all you did to keep me going and challenging me each day to be a better version of myself.

Lastly, but most definitely not least, I would like to thank my friends and family for challenging me to pursue my dream and for their love and support throughout not only graduate school, but throughout my entire education and life. I am a firm believer that no man can be a failure so long as he has friends and family, and for that, I am eternally thankful.

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ABSTRACT

Increasing food, feed, fiber, biofuel production on decreasing amounts of arable land while simultaneously enhancing ecosystem services is challenging. Strategic inclusion of winter rye (*Secale cereale*) for biomass, silage, grain and Kura clover (*Trifolium ambiguum*) living mulch into existing Midwestern cropping systems may offer alternative economic income for farmers without displacing or reducing yields of primary crops. Research was conducted at the Purdue Water Quality Field Station (WQFS) where net balances of water, carbon, nitrogen, and radiation can be measured, and greenhouse gas (GHG) emissions are monitored. The agronomic performance of a corn-soybean rotation and continuous corn (controls) were compared to novel systems that included the use of rye cover cropping and Kura clover co-cropping. Rye was harvested for biomass/silage at heading immediately followed by corn or soybean planting. Continuous corn receiving 69 kg N ha^{-1} was planted into an establishment of Kura clover sod. Controls included these same systems without the rye or clover. GHG samples were taken via the static chamber method and tile-drained water sub-samples were collected, analyzed for nitrate, and load losses calculated. Biomass composition was determined and used to calculate herbage theoretical ethanol (EtOH) yields. Cereal rye did not significantly decrease corn or soybean grain yield. Averaged across years, Kura clover significantly depressed corn grain yields by nearly 70%. Kura clover significantly reduced flow-weighted tile drainage nitrate (NO_3^-) concentrations, however cereal rye did not. Reductions in flow-weighted tile drainage nitrate (NO_3^-) concentrations were found to largely occur during Quarter two (April, May, June). Cover crops did not significantly reduce annual tile drained NO_3^- load losses in most cases, however, they did significantly reduce annual N_2O emissions. Cumulative annual CH_4 emissions were not significantly altered. Annual CO_2 emissions were higher after the introduction of Kura clover and not significantly altered following the introduction of cereal rye. Averaged across years, theoretical ethanol yields in the Kura clover system produced 2,752 L EtOH ha^{-1}, whereas EtOH production in cereal rye systems ranged from 3,245 to 4,210 L EtOH ha^{-1}. Theoretical ethanol yields of continuous corn and rotational controls ranged from 2,982 to 3505 L EtOH ha^{-1} for these same systems without the cereal rye of Kura clover. These data suggest that a multipurpose approach to

cover crop inclusion can provide both environmental and economic advantages worthy of consideration.

CHAPTER 1. REVIEW OF LITERATURE

1.1 Context: Global Population Growth and Food Security

During the past half-century, the need for increased production of biofuels, food, and feed (BFF) with continually diminishing resources has been approached with inefficient consideration toward protecting our nation's ecosystems and the services they provide. The challenge of increasing food/feed production on decreasing amounts of arable land, the need for alternative energy sources, and the enhancement of ecosystem services are three crucial aspects to our society's productivity and sustainability. The term "sustainable intensification" has been defined as biological regulation within agroecosystems to achieve increased agronomic productivity and to also provide ecosystem services, somewhat contradictory goals (Doré et al., 2011). Ecosystem services (ES) are namely the conditions and processes through which natural ecosystems and their species help to sustain and improve human life (Daily, 1997). Success stories of true sustainable intensification of agroecosystems that also maintain high agronomic productivity are few and far between.

Currently, there are many available opportunities within preexisting agricultural lands and cropping systems for significant sustainable intensification of land use that have yet to be refined (Keating et al., 2010). These areas consist of un- or underutilized niches especially in regard to the extended fall-winter-spring fallows that exist in current Midwestern cropping systems. These fallow periods can be exploited and used to introduce additional/alternative solutions to present day agricultural inefficiencies. Utilizing these previously unused niches would enable substantial opportunities for increased enhancement of global food and energy security. This goal can likely be accomplished through the addition of new crops added to previously existing Midwestern monocrop agro-ecosystems.

Previously, the intensification of agriculture has widely relied on optimizing the productivity of monocropping systems. Typically, in monocrop systems, crop diversity is reduced to one or a few crop species that are usually genetically homogeneous throughout. Therefore, due to lack of diversity, external inputs must be supplied in sizeable quantities to achieve desired yields of the monocrop species (Malézieux et al., 2009). Past economic success of monocropping systems, especially in the Midwest, is well-documented. However, history has shown that the overuse of any monocropping system can have detrimental ecological repercussions (Malézieux et al., 2009). The highly productive Midwestern "cornbelt" is arguably one of the most economically important agricultural ecosystems in the world. This makes the need for enhanced co-production of BFF in this geographical area tremendously important. Fortunately, opportunities for improvement are plentiful.

Agricultural intensification is needed to feed the growing population. According to Gerland et al. (2014), the world population is predicted to be 9.6 billion in 2050 and nearly 10.9 billion by the year 2100 (in accordance to medium-fertility projections). This projection would suggest an increase of nearly 1.9 billion people by 2050 and almost 3.2 billion by 2100. Similar projections have been made taking different fertility and life expectancy projections into account. With limited options to bring new land into production, agriculturalists look to intensify production on the arable land already dedicated to agriculture. However, concerns have heightened about long-term stability and the environmental repercussions resulted from the intensification of agriculture (Matson et al., 1997). Concerns at the local scale include decreased soil fertility, dwindling biodiversity, and increased erosion. On the regional scale, agricultural intensification has negatively influenced groundwater quality via pollution and aided in the eutrophication of both rivers and lakes. These negative consequences even extend to the global scale as atmospheric components and climate are being affected (Matson et al., 1997).

A novel and improved form of agriculture, "Sustainable Intensification" (SI) could be implemented in order to achieve sustainability, while also meeting the demand of a growing population. Baulcombe et al. (2009) defined SI as a form of agricultural production where yields can be increased, while avoiding negative environmental impacts and without increasing land use. In addition to SI's previously stated benefits, SI could also encourage shifts towards greener economies and subsequent benefits from progress in other areas rather than just agriculture (Pretty and Bharucha, 2014 and sources therein). Disregarding the sustainability of our current agroecosystems and the effects they have on the surrounding ecosystems could spell disaster for the future generations.

In recent decades agriculture has stepped up to meet the demands of a rapidly increasing global population. In turn, the expansion of agricultural land for food production has become one of the largest alterations to the global environment (Matson et al., 1997). These adverse environmental effects have been propelled by high agricultural demands and expectations set forth in order to feed this increasing population.

Today, current agricultural management practices include intentionally maintaining agroecosystems in a simplified, nutrient-enhanced, and disturbed state. This is achieved by the use of monocropping, externally supplied fertilizers, and mechanical disturbances such as tillage. The limiting factors of most agricultural systems require supplementation including water, minerals fertilizers, and pesticide applications (Tilman, 1999). This helps to achieve agricultural intensification and amplified yields, while regrettably decreasing biodiversity and thus limiting ecosystem service benefits. Today, many farming practices damage the environment and are a major source of greenhouse gases (GHG) (Garnett et al., 2013). Although the complications of high productivity associated with agriculture are glaringly evident, the solutions to these issues are incredibly complex.

According to the United Nations Food and Agriculture Organization for the years (2012–2014) nearly 795 million people (roughly 1 out of every 9 persons) were not able to have access to enough food (Sharma et al., 2018). With an increasing human population and over 795 million humans who already live daily facing hunger, there is tension among agriculturalists who are forced to face difficult decisions. The issue lies between either limiting the use of external inputs in order to abide by environmental ambitions, which may decrease yields significantly, or pursuing the goals set forth to feed the growing population with less regard to sustainability targets. Thus, the broader goal in this study is to locate inefficiencies in current cropping systems that can be enriched upon or replaced with improved/innovative methodologies. If achieved, these alterations could ultimately offset the previously needed external inputs without decreasing yields and could simultaneously enhance our ES.

1.2 Annual Row Crops

1.2.1 Overview: Production and Trends

A major system in North American agriculture is the corn/soybean *(Zea mays/Glycine max)* rotation that predominates as the primary rotation sequence in the United States Corn Belt. Decades of use have proved this system to be beneficial for numerous reasons. Porter et al. (1997) found corn yields were highest in the first-year corn cropping sequences and in corn/soybean rotations as opposed to five other cropping sequences that were studied over a combined twenty different environments. There was also a noticeable advantage found where 1st year soybean yields were greatest compared to continuous soybean systems. However 1st year soybean yields were not different as compared to yields of any of the rotational systems when averaged across all years in the eleven year study (Porter et al., 1997).

The United States is globally the number one producer of corn. The US has produced 35.43% of the overall global corn production from the years 2007-2017 (FAO STAT, 2007-2017). The United States is also the number one producer of soybeans globally. The US has produced 34.39% of the overall global soybean production worldwide from the years 2007-2017 (FAOSTAT, 2007-2017). Of the large amount of corn and soybean production that occurs in the United States, roughly 85% of these totals are produced in the Midwestern ‘Corn Belt’. Within the ‘Corn Belt’, corn/soybean rotations are the predominant cropping system (≈65%) and continuous corn systems result in the remaining (≈35%) (Grassini et al., 2014).

The majority of today’s crop varieties are genetically altered to give crops resistance to various pests (e.g. *Diabrotica virgifera virgifera* (Western corn rootworm), *Ostrinia nubilalis* (European corn borer), *Diatraea grandiosella* (Southwestern corn borer)) and herbicide treatments (e.g. glyphosate, dicamba, glufosinate) which are used to decrease competition from weed species (USDA-ERS, 1996-2018). The percentage of US soybeans planted that include a herbicide-tolerance (HT) trait rose and then plateaued around 94% in the year 2014. Currently approximately 90% of corn acres in the United States are planted using HT varieties. Various crop varieties with insect-resistant properties have genes from a soil bacterium, *Bacillus thuringiensis* (Bt), which produces proteins that are insecticidal. The advent of these varieties and their adoption began around 1996. In 2018, just twenty-two years later, roughly 82% of US corn acres were planted with Bt traits. A combination of HT and Bt traits in a single crop variety are considered “stacked” and therefore have a combination of herbicide tolerance and insecticidal properties. In 2018, nearly 80% of the US corn acres were planted with stacked genetic traits (USDA-ERS, 1996-2018).

In modern agriculture, management practices used for annual row crop systems are often driven by water and temperature requirements of the crops being grown. Due to advancements in technology, field size widely ranges, however most individual fields are >50 ha and crop production is highly advanced using large scale machinery that reduces overall labor inputs (Grassini et al., 2014).

Ray et al. (2013) assessed that global corn and soybean yields are increasing annually at a rate of 1.6% and 1.3%, respectively, however an ~2.4% annual increase (non-compounding) of grain yields would be needed in order to meet increasing food demands before mid-century. At their current rates the global production of these crops would increase by ~67% and ~55% correspondingly by the year 2050. These increases fall below the projected demand for crop production by the 2050 mark (Ray et al., 2013).

It is generally understood that agriculture is in need of intensification in order to meet increased demands for a growing global population. This need will challenge even the most successful row crop systems to achieve higher yields no matter how effective they are in their current state. The way agriculturalists are pursuing increased production must evolve over time to not only meet demands, but also to ensure sustainability.

1.2.2 Nitrogen Management Guidelines for Research Site (Purdue ACRE)

Camberrato and Nielsen, (2014) used field-scale trials in multiple locations around the state of Indiana to summarize how corn yield responds to nitrogen (N) fertilizer during a long-term study. This was in part due to the movement from yield-based N recommendations to data-driven recommendations based on field-scale trials (Camberrato and Nielsen, 2014). It is important to note that the most common forms of N fertilizer applied in a corn/soybean rotation and continuous corn systems include anhydrous ammonia (82 % N), urea (46 % N), urea-ammonium nitrate (UAN, 28 to 32 % N), as well as ammonium nitrate comprised of 34% N and ammonium sulfate (21% N) (Vitosh et al., 1995). Man-made fertilizer, decomposed crop residues, and soil organic matter are various sources of plant available N (NO_3^- and NH_4^+) a corn crop may utilize. Soil types vary throughout the state, resulting in different N supply and N loss potentials for various locations. This adds to the need for N rates to be tailored to different environments (Camberrato and Nielsen, 2014).

This study found that the 10-year agronomic optimum nitrogen rate for the West Lafayette, Indiana research site was 221 kg N ha^{-1}, however the rates at this location varied by year ranging from 146 to 294 kg N ha^{-1}. This range was likely a result of variation in weather, soil N supply, and fertilizer N losses (Camberrato and Nielsen, 2014). Sidedress N applications using liquid UAN were primarily used for these trials. These recommended N application rates may have differing results if early pre-plant applications of liquid UAN or anhydrous ammonia are used, meaning if the application timing is altered, the rate of N applied may need adjusted. Similarly, varied results may be observed if anhydrous ammonia is applied in the fall. This is due to the risk of N loss occurring from the timing of those applications, as fall applied N has more time to leach through the soil profile before the subsequent crop has an opportunity to use the applied N (Camberrato and Nielsen, 2014).

1.2.3 Effect on ecosystem services

Large-scale agriculture has pioneered forward with sizeable advancements in regard to yield and overall production, however these improvements do not come without a cost. The monoculture corn and corn/soybean rotational systems that are largely in place for United States agriculture has serious consequences to the environment and on ES that are necessary for the well-being and longevity of the human race. Over recent decades, agriculture has improved production drastically thanks to the use of high-performing crop cultivars, improvements in irrigation techniques, fertilization recommendations, and increased control. However, the intensification of agriculture and land modification has altered biotic relations and trends of resource availability in ecosystems, which, in turn, can result in damaging consequences on local, regional, and global scales (Matson et al., 1997).

Daily (1997) defines ecosystem services as “the conditions and processes through which natural ecosystems, and the species that make them up, sustain and fulfill human life. They maintain biodiversity and the production of *ecosystem goods*, such as seafood, forage, timber, biomass fuels, natural fiber, and many pharmaceuticals, industrial products, and their precursors.” Conversely, because the importance of a species or community varies radically across crops and regions, large-scale universal guidelines about what is determined as an ES is virtually non-existent (Zhang et al., 2007). Literature suggests that ecosystems can include, but are not limited to, providing various services such as water quality protection, purification of air, carbon sequestration, partial stabilization of climate, generation and renewal of soil, nutrient cycling regulation, and pollination (Daily, 1997; Doré et al., 2011). Nonetheless, agricultural production can have a negative impact on the environment through a multitude of means. Some of the most noteworthy include eutrophication of waterways and oceans, increased GHG emissions, decreased biodiversity, increased land use change, nutrient runoff, and soil erosion. These instances where agricultural production causes adverse effects on the environment can be deemed ecological disservices (Matson et al., 1997).

Two of the main ecosystem disservices that agriculturalists are currently attempting to mitigate are the degradation of water quality and increasing GHG emissions induced by agriculture. Nutrients lost from agroecosystems can contaminate waterways and ultimately lead to hypoxic "dead zones" downstream. The term "dead zone" is in reference to the threat imposed on marine life and fisheries (Hunter et al., 2017). In the United States the hypoxic zone in Northern Gulf of Mexico is a major environmental calamity. This hypoxic zone is fed via the Mississippi–Atchafalaya River Basin system. The two primary nutrients responsible for this are agriculturally contributed N and P (Hunter et al., 2017). In 2019 the United States Environmental Protection Agency (US EPA) reported that the hypoxic zone in the Gulf of Mexico measured 18,005 square km which was larger than the 5,000 square kilometer goal set forth by the US EPA Hypoxia Task Force (US EPA, 2019). According to the (Change, I.C., 2014), 11 to 13% of the world's total anthropogenic GHG emissions arise due to agricultural production, while another 12% are contributed from indirect emissions as a result of land-use change in the agriculture and forestry sectors. In order to reach goals of reducing agricultural GHG contributions by the year 2050 to mitigate climate change, strategic changes would need to occur, as GHG emissions as a direct result of agriculture are steadily climbing (Hunter et al., 2017). On the contrary, global agriculture also possess the potential to sequester carbon from the atmosphere and can be managed to help aim at reductions in atmospheric CO_2 emissions (Hutchinson et al., 2007).

1.3 Traditional Cover Crops: Double and Relay Cropping

1.3.1 Overview

The fundamental principle behind using cover crops is to reduce fallow periods in cropping systems by incorporating additional plant species that provide added benefits, via covering the soil, hence the name "cover crops". Any living ground cover that is commonly killed before the subsequent crop is planted or planted into is considered to be a cover crop (Hartwig and Ammon, 2002). Undisturbed natural systems usually lack lengthy fallow periods due to a portion of the plants continually growing throughout the year as long as the soil is not frozen. These extended periods of growth allow for plants to cover the soil, scavenge nutrients, transpire water, encourage increased soil fauna activity, and fix carbon (Kaspar et al., 2011). When not harvested or removed, cover crops are left as a residue on the soil surface or tilled into the soil.

Agricultural cropping systems used to grow food and fiber crops traditionally have plants growing for a relatively small proportion of time (~four to six months out of the year) with the remaining months (late fall, winter, early spring) left fallow leaving soil barren and subject to exposure of the natural elements such as wind and water. Even throughout the growing season of the primary crop, there are still areas of the field that remain fallow for the entire year, due to crops being planted in rows with some barren space between individual plant and rows. Thus, soil can be left unprotected and susceptible to erosion, organic matter and nutrients may be lost or not replenished, runoff increases, and overall productivity of the soil can decrease (Kaspar et al., 2011). There are a multitude of plant species available for implementation as a cover crop. The determining factors for species selection frequently depends on the climate of the given area, the potential growth and subsequent benefits derived from the cover crop, the type of cash crop rotation, the residue quality of the cover crop, and the time between the main cash crops (Bakker et al., 2016). These undesirable effects of modern cropping systems are some of the driving forces that influence agriculturalists to look for novel and innovative management decisions, such as cover crops, that have both short- and long-term benefits.

Though sometimes misleading, the idea and exercise of cover cropping is by no means novel. Sister crops grown to enhance the growth of cultivated foodstuffs is a practice that has been in place for over a thousand years. Native Americans were perhaps the first to use this system whereby multiple crops were grown together for their biodiverse cooperation that aided in productive farming practices and replenishment of the soil (Groff, 2015). During the nineteenth century, cover crops were commonly used under the alias of “green manure” which often referred to plants grown for the production of improved soil fertility. Legumes such as hairy vetch, peas, and lupins were often grown in row crop systems to replenish soil N levels, while grass crops were used to reduce erosion (Groff, 2015). It is documented in the United States that cover cropping had occurred approximately two hundred years prior to World War II. However, after World War II, the rise of simplistic and relatively cheap man-made N fertilizers using the Haber-Bosh process spurred a decreased need for manure crop use. Cover crop usage was almost nonexistent from the mid 1960’s through the 1980’s. Farmers slowly begin experimenting with cover crops again around the 1990's when the USDA implemented the Sustainable Agriculture and Research Education (SARE) program that provided farmers funding to test sustainability concepts on their farms (Groff, 2015).

While the idea of using cover crops is a longstanding idea, the driving forces of adoption may be slowly changing direction as producers expand their focus from traditional benefits (N_2 fixation, soil conservation, weed/pest management) to look to include additional multifunctional benefits (reduction in GHG emissions, reductions in tile drained NO_3^- concentrations, soil carbon sequestration, livestock feed, biofuel production, and additional soil health benefits). These multifunctional benefits aim to have positive impacts on environmental quality and ecosystem services (Blanco-Canqui et al., 2015). The amount of ES that can be provided by cover crops is due, in part, to their multifunctionality. These ES may include, but are not limited to: reduced wind/water erosion, improved soil bulk density, improved soil structure, decreased compaction, increased soil organic carbon, improved nutrient cycling/availability and microbial activity, and improved water infiltration properties (Kaye and Quemada, 2017).

1.3.2 Advantages and Disadvantages

Due to the extensive use of cover crops, the advantages and disadvantages of their implementation in various systems have shown both success and various pitfalls. Some of the longstanding known benefits of cover crops include erosion control, reduction in surface water pollution, fixation of atmospheric N (legumes) , supplemental organic matter, greater soil productivity, weed control, pest suppression, recycling of unused soil N, improved cash crop productivity, improved soil and water quality, as well as overall improved soil structure and tilth (Hartwig and Ammon, 2002; Snapp et al., 2005; Roesch-McNally et al., 2018).

Among the many benefits of cover crops also lies inherent challenges as well. These concerns primarily center around issues such as reduced income if competition/interference occurs with cash crops, slow soil warming in the spring, increased costs, challenges predicting N mineralization potential, and overall added production expenses (Snapp et al., 2005). A common theme with various cover crop species is that the potential for unfavorable effects on corn/soybean yields when there is a decrease in precipitation due to competition for water. This observation of reduced grain yields due to cover crop depleting soil moisture has been well documented (Feyereisen et al., 2013; Martin et al., 1999). This is due to the preceding cover crop species infringing upon the stored water availability for the subsequent corn/soybean crop (Kaye and Quemada, 2017). For these reasons it is likely that cover crop benefits tend to be more pronounced and less risky to achieve in irrigated cropping systems or areas where the succeeding crop is not in a water-limited region (Snapp et al., 2005). Another major challenge is predicting the optimal time for proper termination of the cover crop to decrease or eliminate the potential for competition and negative effects to occur between the cover and cash crops. Cover crop termination dates can have effects on seedling diseases, growth and yield of the subsequent crop (Bakker et al., 2016).

1.3.3 Cover Crops in Annual Monocrop Production

Traditional cover cropping systems involve a double or relay cropping system that allows for the growth of the cover crop to be grown either preceding or succeeding the growth and harvest of a cash crop. This process allows for limited impact on the cash crop's traditional growing season (Berti et al., 2015). There are two main requirements for a successful multi-cropping sequence such as cover cropping; adequate time must be available for both crops to reach their desired level of maturity and there must be sufficient water available to produce both crops consecutively. In some geographical areas (primarily Northern regions), growing seasons are not long enough to ensure adequate time for two crops to reach their desired levels of maturity, while in other areas (primarily Southern regions) water may be the limiting resource. Therefore, geographical areas such as the Midwest might provide a specific niche for relay cropping sequences to be adopted (Berti et al., 2015).

When cover crops are used in rotation with cash crops, the cover crop will either need to be harvested or terminated as to reduce the amount of competition for resources such as water, sunlight, and nutrients between the different crops. Termination can be achieved through chemical or mechanical means (tillage, mowing, crimping) or by some combination of the two (Mirsky et al., 2009). Different cover crops require various "kill dates" so as to limit any negative effects on the subsequent crop. The overarching goal of cover crop termination is to minimize the amount of soil moisture loss, while simultaneously increasing the amount and availability of the soil N in the system, which is aimed at improving subsequent crop yields. On the contrary, cover crops can also be managed to help moderate excess moisture in cropping systems as well. The goal is to avoid any negative management strategies that could harm subsequent yields (Wortman et al., 2012). Yield influencing factors such as soil moisture availability, levels of weed suppression, cover crop N content, soil N content, and crop N uptake have been shown to be affected by both the timing and termination method chosen (Mirsky et al., 2009; Parr et al., 2011; Wortman et al., 2012).

Agriculturalists must attempt to match the peak benefits desired from the winter cover crops with optimal spring cash crop planting dates, a task that has proven difficult in many regions of the United States (Parr et al., 2011). Traditionally, prolonged growth of cover crop species corresponds to the increased benefits/services they provide (prolonged soil erosion control, increased biomass production, additional N fixation, etc.). However, these added benefits do not come without an effect to the subsequent corn/soybean crop. The potential for cover crops to reduce available soil moisture for the cash crop is cited as one of the top concerns among most farmers (Wortman et al., 2012).

If herbicides are not used, cover crops are typically terminated using tillage, or mowing when tillage is not desirable (Mirsky et al., 2009). Mowing has particular drawbacks such as regrowth of the cover crop which may ensue if mowing takes place before the cover crop reaches the proper maturity and uneven distribution of mowed residue depending on the type of mowing equipment used (Creamer and Dabney, 2002; Mirsky et al., 2009).

Tillage used for termination has obvious drawbacks as this practice disturbs the soil structure. In further efforts to reduce tillage, alternative cover crop termination techniques such as the roller-crimper have arisen. The roller-crimper creases the stems of cover crops, while leaving the roots and soil undisturbed below the soil surface. Effectiveness of this method is related to the growth stage of the cover crop, which for cereal crops such as cereal rye (*Secale cereale* L.) corresponds to anthesis (Ashford and Reeves, 2003; Mirsky et al., 2009; Parr et al., 2011). Ashford and Reeves (2003) discovered that using a roller-crimper at anthesis accompanied by herbicides (1/2 the recommended rate) was effective at terminating black oat (*Avena strigose)*, wheat (*Triticum aestivum)*, and cereal rye at a 94% success rate. Using a roller-crimper alone at anthesis could decrease costs by as much as \$26.28 ha^{-1} while providing the same kill rate as a full herbicide rate. (Carrera et al., 2004) determined that termination of cover crops using mechanical means (roller crimper and flail-mowing) were just as effective as using herbicides. The overarching goal is to choose a cover crop species that has active growth until terminated, does not compete with the cash crop species for light, and that the system has an adequate soil water supply in order to lessen the competition for water between the cover crop and primary crop (Blanco-Canqui et al., 2015).

1.3.4 Cover Crop Adoption

Although the introduction of cover crops has proven benefits within various cropping systems, their integration can still be perceived as a risky endeavor in certain geographical areas due to the availability of competition between the cover crop and cash crop (Roesch-Mcnally et al., 2018). There are multitudes of factors that influence cover crop adoption; inability, unwillingness, and ignorance are the largest contributors. Inability to adopt cover crops largely ascends from thin economic margins while unwillingness most frequently arises from incentives that are unattractive due to low profitability (Nowak, 1992).

It has been suggested that policy interventions should be set in place to help alleviate some of the barriers that prevent cover crop adoption. These interventions could take the form of cost-sharing programs that may enable more financial freedom to allow farmer experimentation to take place. These include the creation of markets for cover crop products that would make them more economically suitable for implementation, additional economic incentives such as decreased costs for implementation, and increased flexibility of government programs such as crop insurance (Roesch-Mcnally et al., 2018). Any of the previous suggestions could potentially alleviate some of the barriers that famers cite as reasons for not adopting cover crops. It is also noteworthy to consider that if cover crop implementation is beneficial beyond the farm gate, it may be rational to support the idea of public subsidies, which allow for societal benefits, such as ecosystem services, to be tapped into as a public good (Snapp et al., 2005).

While some programs have financial incentives to encourage producers and land managers to use cover crops for the purpose of enhanced ES, these payments typically do not counterweigh the additional costs (Singer et al., 2007; Feyereisen et al., 2013). Wade et al. (2015) determined that various conservation practice adoption rates can vary by crop and region. It was concluded that approximately 4 % of farmers had adopted cover crops on at least some portion of their fields and that cover crops were only used on 1.7 % of total cropland in the US (6.8 million acres in total during the years 2010-2011). This low rate of adoption is likely due to challenges with profitability and the potential for competition among the cover crop and cash crop species. Cover crop adoption was lowest in the Heartland states, which includes Indiana (Wade et al., 2015). This highlights the opportunity for greater cover crop adoption.

1.4 Cereal Rye

1.4.1 Species Background

The origin of rye is not fully known, however it has been speculated that cultivation of this crop arose in southwestern Asia similar to that of many other grain-crops such as barley (*Hordeum vulgare*), wheat, and oats (*Avena sativa*) (Bushuk, 2001). Rye is primarily grown as a winter cereal. This crop's high tolerance to infertile soils, drought, sandy soils, acidic conditions, salt, and its extreme overwintering ability is unmatched compared to other small grain cereal crops (Geiger and Miedaner, 2009)**.** Compared to that of other crops, rye has relatively few improved cultivars due to the difficult nature of breeding. This is primarily due to the fact that rye is self-pollinating which discourages genetically pure cultivars (Bushuk, 2001).

There is a plethora of uses for cereal rye. Some of the most notable include using the green plant material as livestock pasture, as a green-manure crop, and as a source of bioenergy production. The mature crop can be utilized for both grain and straw. Cereal rye grain is most commonly used in flour, livestock feed, alcohol distilling and numerous other baked goods for human consumption (Bushuk, 2001; Geiger and Miedaner, 2009). Rye can be cultivated in areas that are generally not conducive to other cereal crops due to its extreme winter hardiness and its ability to grow in sandy and low fertility soils (Bushuk, 2001). Cereal rye has the distinctive capability to have considerable growth in the late fall, and resume growth rather quickly in the early spring months. Even though the uses of cereal rye are both numerous and diverse, rye acreage was nearly cut in half between 1995 to 2005 (Geiger and Miedaner, 2009).

1.4.2 Implementation: Advantages and Disadvantages

As a cover crop, the benefits of including cereal rye are numerous, however some of the most notable include improved water quality, erosion control, soil moisture conservation, and increased landscape-level water productivity. Additional benefits such as increased net biomass production, weed control, bioenergy potential, and potential to offer ES co-benefits such as water quality protection, freshwater provisioning, erosion control, flood and nutrient cycling regulation, and overall improved soil health are well cited (Ruffo et al., 2004; Eckert, 2013; Patel, 2016; Tumbalam et al., 2016; Jean et al., 2017; Korucu et al., 2018; Lyons et al., 2019). Some of the leading disadvantages of rye cover crops cited for causing subsequent crop yield depression include allelopathy which is a chemical inhibition of plant growth from one plant to another via growth inhibitors, soil moisture depletion, and soil N depletion (Feyereisen et al., 2013).

Certain characteristics of cereal rye that make it a suitable candidate as a cover crop include its ability to rapidly accumulate biomass via extensive tiller structures during the cool weather conditions of the fall and early spring, robust germination, and its familiarity and availability throughout agricultural communities (Feyereisen et al., 2013). Winter cover crops such as cereal rye have the ability to influence soil NO_3^- levels, while also affecting the overall water budget. The scavenging potential of cereal rye allows for decreased leaching and improved water quality which reduces soil NO_3 concentrations and helps to synchronize some of the opposing factors during the water-recharge period of the fall and spring (Meisinger et al., 1991). However, trapping excess N and water is not a simplistic process that does not come without various challenges.

As mentioned previously, cover crops may reduce subsequent cash crop yields during water-limited conditions due to the competition for this resource. Likewise, N availability can also be a concern with non-leguminous crops. Crandall et al. (2005) determined that winter rye cover crops decreased corn biomass and attributed this to decreased soil N availability to the corn. This decrease in soil N was increased as rye termination timing was delayed, and soil N was used for cover crop biomass accumulation. However, Kuo and Jellum (2000) found that over the span of a nine-year study, corn biomass increased with repeated seasons of cereal rye winter cover crops due to increased available soil organic N which was associated with decomposing rye biomass.

This study concluded that continual use of cereal rye as a winter cover crop can improve soil N availability in the soil.

Mineralization rates of cover crop residues are difficult to manage and predict. The amount of N mineralized from cover crop residue relies heavily on the biomass C:N ratio and the prevailing environmental conditions (Kuo and Sainju, 1998). Kuo and Jellum (2000) confirmed that high C:N ratios lowered the cumulative N mineralization. Specifically, this study found that cover crops such as hairy vetch (average C:N of <10) had higher amounts of N mineralization as compared to rye (average C:N of 26). Averaged across years the amount of available N in rye residue was 49 ± 20 kg N ha^{-1} (mean ± standard deviation). Clearly, more information than just the C:N ratio is needed to accurately predict N mineralization (Kuo and Jellum, 2000).

Jahanzad et al. (2016) found that cover crop residue mineralization was faster when residues were buried rather than remaining on the soil surface. This was due to the chopping and accelerated decay of plant material that is associated with tillage. This study concluded that residue decomposition rates could be predicted using initial N concentration and C:N ratio. However, these authors also determined that cover crop residues with greater concentrations of lignin and cellulose were associated with decreased N release.

Termination timing plays a large role in these processes as well. Termination of any cover crop is often dependent on the producer's goals. Krueger et al. (2011) determined the effect of harvest vs terminated rye cover crops on soil moisture, subsequent corn yields, and soil NO_3-N in rain fed environments. Soil moisture following rye that was harvested was 16% lower than that of the soil moisture on control plots and those where rye was terminated and left as ground cover. Available soil NO_3-N was decreased in rye treatments compared to the control. Soil NO_3-N was reduced 35% in killed rye plots and 59% in harvested rye plots immediately following the termination of rye. Corn biomass yields were decreased only in treatments where the rye was harvested. This demonstrates the depletion of soil resource NO_3-N and water that can negatively impact subsequent corn biomass yields (Krueger et al., 2011).

1.4.3 Cereal Rye for Biofuel Production

As previously mentioned, cover crops are typically killed via mechanical or chemical means and left on the soil surface or incorporated depending on the goal of the producer or land manager. However, there is also a potential to harvest the cover crop for additional uses such as bioenergy production. Harvesting cover crops for biomass could simultaneously increase profits for farmers, reduce land competition, and decrease the risks associated between biomass production infringing on food production goals (Kemp, 2011; Tumbalam et al., 2016).

Feyereisen et al. (2013) quantified the opportunity of harvesting cellulosic biomass on land that was currently in corn-soybean rotation, with an emphasis on minimal interference on the current grain production system in place. A spatial analysis of the United States was conducted which determined that 7.44 Mha currently dedicated to continuous corn and 31.7 Mha in corn-soybean rotation are fit for winter rye production. Furthermore, this study determined that 112 to 151 Tg of rye biomass could be harvested from the suitable land base within 14 to 7 days before the spring planting of the subsequent crop planting (Feyereisen et al., 2013).

1.4.4 Effect on Ecosystem Services

One of the many benefits of a cereal rye cover crop is to scavenge and trap N that would otherwise exit the system via leaching, volatilization, and runoff. Korucu et al. (2018) determined that a cereal rye cover crop reduced surface nutrient loss of NH_4-N from 2.8 to 0.4 kg ha^{-1}, a nearly 86% reduction. This same study also showed that cereal rye cover crops reduced runoff by nearly 65% which highlights rye's potential for water management. NO_3 -N levels in the soil, leaching of nutrients, and groundwater quality can be influenced by winter cover crops such as winter rye. This is due to winter cover crops having the ability to alter the soil NO_3 -N concentration during the soil water-recharge season by scavenging up N that otherwise might be lost (Meisinger et al., 1991). Grass species used as cover crops have the potential to be two-to-three times more efficient at reducing the amount of N leached when compared to legumes likely due to their inability to fixate N and ability to rapidly accumulate biomass. This scavenging potential can help decrease the overall mass of N leached as well as the concentration of the leachate by anywhere from 20 to 80% (Meisinger et al., 1991).

Martinez-Feria et al. (2016) found that rye cover crops reduced NO_3^- losses by 20% and tile drain flow by nearly 12%, however corn yields were also reduced 6%. This study also highlighted that there were minimal effects on soil temperature, and water shortages that reduced grain yields were only detected during drought years. It is pertinent to note that the inclusion of winter rye as a cover crop will only reduce N leaching if extra N fertilizer is not applied when the cover crop is planted (Snapp et al., 2001). Winter rye has also been examined as a means of reducing GHG emissions, a degradation mechanism that reduces ecosystem services. Parkin et al. (2016) monitored a corn-soybean rotation with and without rye cover crops and found that, over a 10-year period, N_2O emission means were lower in cover crop treatments, however the differences were not significant. This was determined to be partially due to environmental influences on GHG emissions, including year-to-year variation in precipitation. Similar results in a North Dakota study found that the incorporation of a cereal rye cover crop into the fallow period of a dryland cropping sequence under no-till management practices yielded no net GHG reduction benefits (Liebig et al., 2010).

1.5 Perennial Living Mulches

1.5.1 Overview

Cover crops that are planted either before or with a main cash crop and maintained throughout the growing season as living ground cover are considered living mulches (Hartwig and Ammon, 2002). Perennial living mulches (PLM) are a form of cover crop that incorporates a perennial species grown both during and after the cash crop's growing season. Growth of PLMs are suppressed in ways to reduce their competition with the cash crop during its growing season and do not need reseeded on an annual basis (Kaspar et al., 2011). This is different than the traditional use of annual cover crops, which are most commonly terminated before the beginning of the cash crop's growing season (Hartwig and Ammon, 2002).

Before planting and following harvest of the cash crop these PLM species remain in situ rather than a fallow period. This type of system allows for year-round cover and extended periods of growth that often allows for more efficient resource acquisition to take place. In most systems the perennial species is strip killed either prior to or directly after the cash crop is planted through the use of mechanical tillage or various herbicides, although management may vary depending on species used and the overall needs of the cropping system. Suppression of the cover crop is typically via a no-till or minimal-till management strategy into which the following crop is planted (Hartwig and Ammon, 2002).

Orchards and vineyards often incorporate living mulches into their management regime. However, if PLMs are restricted to fallow spaces between crop rows and are suppressed during the cash crops growing season, it is also possible to incorporate PLMs into annual cropping systems (Hartwig and Ammon, 2002; Kaspar et al., 2011). Positive attributes of perennial living mulch species include perenniality which eliminates the need for reseeding, weed control, slowly degrading mulch, reduced competition with the cash crop, N_2 fixation and nutrient scavenging potential (Hartwig and Ammon, 2002; Paine and Harrison, 2018). Nicholson and Wien (1983) found that clovers and shorter, less vigorous turfgrasses were more suitable as PLMs.

1.5.2 Advantages and Disadvantages

Perennial mulch systems offer a multitude of benefits including extended erosion control, improved system level radiation/water use efficiencies, increased soil organic matter, upfront establishment that decreases annual input costs, enhanced yields, weed control/suppression, and supply of crop available N (Hall et al., 1984; Echtenkamp and Moomaw, 1989; Hartwig and Ammon, 2002; Sawyer et al., 2010). A previous study determined that when legume PLMs were adequately suppressed with herbicides, corn grain yields were not significantly reduced and PLMs were more successful at impeding erosion than surfaces with corn stover residues alone (Hall et al., 1984).

One of the major drawbacks of this system is that the living mulch has the potential to compete with the cash crop for soil moisture and nutrients (Echtenkamp and Moomaw, 1989; Martin et al., 1999). This competition for resources has also been cited as a major concern for delayed development and reduction of yield of the cash crop (Martin et al., 1999). An additional challenge highlighted in a previous study determined perennial living mulches that rapidly disintegrated tended to result in subsequent weed issues (Echtenkamp and Moomaw, 1989). Although the intrinsic benefits of PLMs do exist, these benefits cannot be attained without deep understanding of how cropping systems function in order to avoid or minimize the inherent disadvantages that may accompany them.

1.5.3 Perennial Living Mulches in Annual Monocrop Production

As previously mentioned, one of the key shortcomings of PLMs is that the living mulch has the potential to compete with the cash crop for soil moisture and nutrients (Echtenkamp and Moomaw, 1989; Martin et al., 1999). To implement a successful living mulch into an annual monocropping sequence, the amount of competition must be reduced so the growth and development of the annual cash crop is normal. Just as well, the suppression of the mulch crop should not be excessive or else the mulch may not recover resulting in loss of stand (Affeldt et al., 2004).

Martin et al. (1999) reported that corn yields were reduced 39 to 72% for living mulch plots when only one method of mulch suppression (roto-tillage or herbicide treatment) was used. After reporting delayed corn emergence and development, Martin et al. (1999) determined that due to the apparent competition, the use of PLMs should be limited to cool-season crops when implemented for cooler, temperate climates. Competition for N was found to be dependent on the species that was selected. Crop yields were higher in plots that used legumes rather than grass species for the living mulch (Hartwig and Ammon, 2002).

1.6 Kura Clover

1.6.1 Species Background

Trifolium ambiguum M. Bieb has numerous aliases such as Kura (named after the Kura river in Georgia, USSR), Honey, Caucasian, or Pellet clover (Speer and Allinson, 1985). Kura clover is a relatively short, herbaceous, rhizomatous, perennial species that has an extensive multi-branched taproot (Speer and Allinson, 1985; Seguin, 1999; Seguin et al., 2013). Kura clover was first introduced into the United States in 1911 as a forage legume, however its developmental period in becoming a genuine choice as a forage crop arrived when a suitable *Rhizobium* strain capable of inoculating the legume was discovered in 1954 (Taylor and Smith, 1997; Erdman and Means, 2010).

Kura clover is capable of producing high-quality forage, however, establishment can be challenging due to poor seedling vigor which is thought to be a response of poor stress tolerance at the seedling stage when subjected to harsh environmental conditions (Seguin et al., 2013).

Establishment success has been inconstant, and frequently poor, which is thought to diminish its use as a forage crop (Speer and Allinson, 1985). A study conducted by (Seguin et al., 2013) determined the clover forage and seed yield was amplified when establishment was accompanied with the use of a pre-plant herbicide. It was concluded that this establishment method reduced the majority of the competition among weeds and companion crops, however, more research on Kura clover establishment is needed (Seguin et al., 2013).

1.6.2 Kura Clover as a Living Mulch for Corn Production

Orchards, vineyards, small grains, and corn production systems have incorporated use of ground covers. Specifically, crops such as corn that have a high requirement for N are grown with legumes that have N-fixation potential, which in turn can help supply a portion of the corn's N needs (Hartwig and Ammon, 2002). Zemenchik et al. (2000) determined that a Kura clover PLM incorporated into corn production and adequately suppressed with herbicides is viable, causing little reduction in grain or whole-plant yields of corn. Clover stands recovered within 12 months to full production potential without the need for reestablishment. Decreased corn plant populations and delayed development were cited as inherent risks during cool and wet springtime conditions (Zemenchik et al., 2000; Affeldt et al., 2004; Sawyer et al., 2010). Zemenchik et al. (2000) and Affeldt et al. (2004) concluded that additional chemical suppression of Kura clover may be needed in order to prevent delayed corn development when it is planted in cool springs. When water is limiting planting drought-tolerant corn hybrids maintains high corn yields, while simultaneously allowing regrowth and survival of the living mulch species (Ziyomo et al., 2013). Corn grain yield and plant population were greatest when herbicides were band-applied than broadcast to suppress Kura clover (Zemenchik et al., 2000; Ziyomo et al., 2013).

As previously mentioned, one of the motives for implementing a PLM using a legume is the potential to fix atmospheric N into plant available N. A study conducted by (Albrecht et al., 2009) determined that Kura clover residues were mineralized and provide N adequate for corn production where grain yields ranged from 12.3 to 13.2 Mg ha^{-1}. This study suggested that there was no advantage to applying more than 22.4 kg ha^{-1} of N fertilizer to this mulch-cropping system, an amount that could be supplied feasibly as a starter fertilizer application. In contrast, Sawyer et al. (2010) found no additional plant available N was supplied from a Kura clover mulch intercropped with corn. This study also revealed that the Kura clover intercropped system (with no N applied) was not able to produce comparable corn yields to that of the adequately fertilized (180 kg N ha^{-1}) Kura clover and non-Kura clover treatments. When competition between Kura clover and corn was effectively controlled, corn plant populations, grain yields, growth, and N status were similar to systems without Kura clover (Sawyer et al., 2010). However, existing literature agrees that close monitoring, adequate suppression of the perennial species, and good establishment and management of the annual row crop are needed in order to produce the potential benefits that this system has to offer including the potential to meet the N needs of a successful corn crop (Affeldt et al., 2004; Sawyer et al., 2010; Ziyomo et al., 2013). The aforementioned sources and their results suggest that implementing a Kura clover PLM is achievable in geographical locations that are not readily water-limited and that are conducive to ample corn production.

1.6.3 Kura Clover for Biofuel Production

In addition to the numerous benefits aforementioned, Kura clover could be investigated as a potential source of biomass for biofuel production. Although little is known with respect to Kura clover being harvested for biofuel production, the idea of using a perennial mulch in a corn stover cellulosic biofuel production system has been evaluated. Bartel et al. (2017) compared the use of Kentucky bluegrass (*Poa pratensis*) and creeping red fescue (*Festuca rubra*) PLMs to traditional corn-based cropping systems used for cellulosic biofuel production. This system involves removing corn stover to be used in biofuel production. However, removing stover that would otherwise remain in the field, can cause issues of decreased organic matter, soil erosion, and increased nitrate leaching. The incorporation of PLMs is able to mitigate some of these potential issues; however, this has only been achieved with variable and significant yield reductions to the subsequent corn crop. This highlights the potential for a Kura clover-corn PLM to be used in biofuel production systems. However, more research is needed to determine how PLM-corn systems can be done without decreasing grain yields. Research also is needed to determine if Kura clover biomass is a viable source of biofuel material.

1.6.4 Effect on Ecosystem Services

One of the many benefits of a Kura clover PLM is the fixation of atmospheric N to plant available N that may reduce the need to apply fertilizer N that can be detrimental to the environment and reduces the net energy balance of corn production systems. Hartwig and Ammon, (2002) suggested that although legumes are often defined by their N fixation potential, these plants tend to scavenge available soil N rather than fixing N. This suggests that Kura clover may be used as a means of reducing N leaching through the soil profile in addition to providing other co-production benefits like soil erosion control, weed control, N credit, and pest management.

Although there are inherent agronomic benefits of a Kura clover PLM, results of this system on ES is conflicting. Sawyer et al. (2010) found that NO_3-N levels in the upper 15 cm of the soil profile post corn harvest were not significantly reduced by a Kura clover PLM. Their study also found similar soil profile NO_3-N levels in the spring prior to planting corn, indicating that the Kura clover PLM would not be effective at reducing the potential leaching of soil NO_3-N. In contrast, Albrecht et al. (2009) found that a Kura clover-corn PLM reduced soil NO_3-N deep in the soil profile when compared directly to monocultured corn. These authors associated the decreased NO_3-N leachate levels to the deep root system that Kura clover possess. These roots would have the ability to capture some of the NO_3-N that may have otherwise leached out of the soil profile completely. It is important to note that these two studies varied in their approach which could be part of the inconsistency between studies. Observation depth of soil samples for NO_3^-N concentrations, differences in N rates applied, and overall grain yields achieved varied among these two studies (Albrecht et al., 2009; Sawyer et al., 2010).

Due to having continuous ground cover, reduced or no-till is needed to maintain PLM species which in turn has reduced GHG emission benefits. Al-Kaisi and Yin, (2005) found that within corn-soybean rotations with less intensive tillage practices, soil CO_2 emissions were generally lower, being reduced between 19 and 41%, with the greatest differences observed directly following tillage procedures. Furthermore, CO_2 emissions were 24% lower in corn-soybean rotations when no-till with residue was implemented. Al-Kaisi and Yin, (2005) further determined that decreased tillage resulted in reduced soil C pools by 22 to 66%. This reduction in mineralizable C was hypothesized to be a partial factor in the reduced CO_2 emissions observed particularly in the no tillage treatments. Continuing with these findings suggest that a Kura clover PLM may have the potential to reduce GHG emissions through the reduction of tillage and increased surface residues that are not rapidly mineralizable with the absence of tillage (Al-Kaisi and Yin, 2005). However, Turner et al. (2016) found that in regard to N_2O gas, Kura clover may be less successful at reducing emissions. They observed higher GHG emissions even when fertilizer rates were reduced 43% in the Kura clover PLM. The authors concluded that this increase in N_2O emissions is a trade-off due to the management of the living mulch system. Any reduction of N_2O emissions observed during the pre-plant period (due to the N scavenging potential of the legume) are offset by the post-anthesis mineralization of the Kura clover which increases N_2O emissions following strip tillage (Turner et al., 2016). In addition, it is important to note the high N content of the Kura clover residue may drive increase N_2O emissions following suppression. More studies are needed to further understand and manage the effects of a Kura clover living much on GHG emissions.

1.7 Summary

Meeting the high expectations of society focused on simultaneous intensification of agriculture and ES will require the development of novel agricultural systems (Doré et al., 2011). In order to achieve sustainable requirements of food, fiber, feed, and bio-based products in a rapidly growing world, innovative cropping systems are a necessity. Double, relay, and inter-cropping systems are viable options to produce crops dedicated for diverse purposes within a single growing season on the same area of land without the fear of infringing on food security (Berti et al., 2015).

As previously stated, cover crops are now being integrated into cropping systems for expanded benefits beyond those traditionally sought, including enhancement of various ecosystem services (reduced wind/water erosion, improved soil bulk density, improved soil structure, decreased compaction, increased soil organic carbon, improved nutrient cycling/availability and microbial activity, and improved water infiltration properties) (Kaye and Quemada, 2017). Using cover crops and PLMs to obtain these additional ES benefits could be a potential way to address some of the opposition between sustainable intensification and agricultural intensification goals, while simultaneously maintaining farm profitability. Due to the aforementioned benefits and capabilities of both cereal rye cover cropping and Kura clover PLM systems, these systems may be suitable for the sustainable coproduction of biofuels, food, feed, and the enhancement of various ecosystems.

CHAPTER 2. COVER CROPPING FOR SUSTAINABLE CO-PRODUCTION OF BIOENERGY, FOOD, FEED (BFF) AND ENHANCEMENT OF ECOSYSTEM SERVICES (ES)

2.1 Introduction

The overall goal of this project is to design new and/or improved management practices for traditional Midwestern cropping systems to be both intensified and diversified. Our goal is to improve sustainable bioenergy and the enhancement of ecosystem services such as water/nutrient use efficiencies, increased soil aggregate stability, improved nutrient cycling, and soil tilth without reducing production of feed/food/forage crops. Strategic inclusion of winter rye (*Secale cereale*) for biomass/silage/grain and Kura clover (*Trifolium ambiguum*) as a living mulch into existing Midwestern cropping systems may offer alternative income for farmers without displacing or reducing maize or soybean yield. This study compared the agronomic and environmental performance of a corn-soybean rotation and continuous corn (controls) to novel systems that included rye cover cropping and Kura clover co-cropping. Rye was harvested for biomass/silage at heading immediately followed by corn or soybean planting, while Kura clover was assessed for yield in the fall prior to corn/soybean harvests. Greenhouse gas (GHG) samples were taken via the static chamber method and tile-drained water was collected, analyzed for nitrate, and nutrient load losses were calculated. Biomass composition was determined and used to calculate theoretical ethanol (EtOH) yields.

We hypothesize that inclusion of rye for biomass/grain and Kura clover as a PLM into existing Midwestern cropping systems will: offer additional economic income for farmers without displacing or reducing yields of existing food/feed crops; increase landscape-level water productivity and nutrient use efficiencies, and offer ES co-benefits such as landscape/regional water quality protection and management (freshwater provisioning, erosion, flood and nutrient cycling regulation) and improved soil health. This hypothesis addresses the known challenges of competition between food/feed and bioenergy production, the lack of diversity in Midwestern cropping systems, the degradation of ES, and declining soil health associated with monoculture systems. The objective of this study includes using field-scale experimentation at the Purdue University Water Quality Field Station (WQFS) research facility for advanced cropping systems (integration of cereal rye as a cover crop and Kura clover as a PLM) to: (1) measure the biofuel, food, and feed co-productivity potentials, ecosystem impacts on nutrient use, GHG emissions, water use/quality, and soil tilth, and (2) calculate theoretical ethanol and biodiesel yields of these newly implemented cropping systems.

2.2 Materials and Methods

2.2.1 Field site

This study was conducted at the Purdue University WQFS located in West Lafayette, IN (40° 29’55” N; 86° 59’ 53” W) on a gradually sloped 4-hectare (ha) site. This experimental site was developed in 1992 and is located within the Purdue University Agronomy Center for Research and Education (ACRE). This facility enables researchers and agriculturalists alike to pinpoint best agricultural practices that reduce the contamination of water from applied agricultural inputs. The WQFS facility is unique in that it allows researchers to evaluate cropping systems on their agronomic, economic, and environmental performances. This experimental site is predominately a Mollisol soil order. The dominant soil at this location is a Drummer (fine-silty, mixed, mesic Typic Endoaquoll) with the addition of some Raub-Brenton complex, 0 to 1 percent slopes (fine-silty, mixed, mesic Typic Argiudoll). More detailed soil descriptions can be found in Appendix A (Soil Description 1 and Soil Description 2). Hourly temperature and precipitation data were recorded at the Purdue WQFS field station using an on-site weather station. Annual mean precipitation for this location ranged from 973 mm to 1559 mm (Table 2.1), while annual mean temperature ranged from 11 °C to 13 °C (Table 2.2) during the study years (2017, 2018, and 2019). Temperature and precipitation data were primarily sourced from the on-site WQFS weather station. When necessary, gaps due to lost data were filled using weather stations at the nearby Indiana State Climate office’s weather station (Purdue ACRE; ~3 km South of the WQFS) and a National Oceanic and Atmospheric Administration weather station (West Lafayette, 6NW, IN; ~11 km Southwest of WQFS). Data sourced from: (https://ag.purdue.edu/indiana-state-climate/) and (https://w2.weather.gov/climate/xmacis.php?wfo=ind).

Table 2.1. Monthly mean precipitation for the study years 2017 to 2019. Data were primarily sourced from the on-site WQFS weather station. Gaps due to lost data were filled using weather stations at the nearby Indiana State Climate office's weather station (Purdue ACRE) and a National Oceanic and Atmospheric Administration weather station (West Lafayette, 6NW, IN). Data sourced from: (https://ag.purdue.edu/indiana-state-climate/) and (https://w2.weather.gov/climate/xmacis.php?wfo=ind).

Month	**2017**	**2018**	**2019**	**Monthly Avg.**	**30 Year Avg. (1989-2019)**
	----------Mean Precipitation (mm) ----------				
Jan	125	24	41	63	75
Feb	33	147	57	79	61
Mar	110	72	101	94	93
Apr	109	73	151	111	111
May	155	124	148	143	122
Jun	255	181	97	177	124
Jul	213	108	37	119	113
Aug	198	187	61	149	87
Sep	71	115	68	84	86
Oct	97	157	65	106	81
Nov	186	82	39	102	87
Dec	7	80	110	66	78
Yearly Sum	1559	1349	973	1293	1118

Table 2.2. Monthly mean temperatures for the study years 2017 to 2019. Data were primarily sourced from the on-site WQFS weather station. Gaps due to lost data were filled using weather stations at the nearby Indiana State Climate office's weather station (Purdue ACRE) and a National Oceanic and Atmospheric Administration weather station (West Lafayette, 6NW, IN). Data sourced from: (https://ag.purdue.edu/indiana-state-climate/) and (https://w2.weather.gov/climate/xmacis.php?wfo=ind).

Month	**2017**	**2018**	**2019**	**Monthly Avg.**	**30 Year Avg. (1989-2019)**
	---------- Mean Temperature (°C) ----------				
January	0	-5	-4	-3	-2
February	5	1	-1	2	0
March	6	3	7	5	6
April	13	7	17	13	12
May	16	22	23	20	18
June	23	23	25	23	22
July	23	23	25	24	24
August	21	23	22	22	24
September	19	21	21	20	20
October	14	12	13	13	13
November	6	2	2	4	6
December	-2	1	2	0	1
Yearly Avg.	12	11	13	12	12

2.2.1 WQFS Design and BFF Treatments

The WQFS is comprised of forty-eight research plots that are 10 x 48.5 m permitting use of farm-scale equipment. Within each plot lies a 10 x 24 m lysimeter made with Bentonite clay walls that isolate a known tile-drained area. Adjacent to lysimeters lies companion tiles which simulate a tile drainage spacing of 10 m. These companion tiles are in place to minimize the cross contamination of water and nutrients from outside the lysimeter to the water and nutrients within the lysimeter area. These companion tiles also serve a dual purpose to ensure that regardless of treatment, plots experience similar soil moisture conditions. Within the lysimeter lies a 10-cm-diameter tile line that is perforated only the length of the lysimeter and is buried 90 cm below the soils surface. Subsurface tile drainage water flows to the basement of a small building ("hut" henceforth) equipped with data- and water-collection equipment. Each of the eight huts receive flow from six tile lines (plots). Water from each tile flows over a separate calibrated tipping bucket system and the number of tips used to determine water flow. The volume of water exiting the tile, along with concentration of N in the tile effluent, permits determination of N load loss from each plot. These data inform environmental performance of cropping systems, as well as water and nutrient use efficiencies. The experimental design was a randomized complete-block design (RCBD) with 4 replicates (blocks). This study examined seven of the existing twelve treatments (Table 2.3).

Table 2.3. List of Biofuel, Food, and Feed (BFF) treatments at the Purdue Water Quality Field Station (WQFS) field site. The following table shows the crop species included in the treatment, plot numbers, current N management and the presence of tillage. Cropping system abbreviations are as listed parenthetically and illustrate the rotation of the system, whether there is a presence of cover crops, and the total annual N rate. Highlighted treatments denote treatments that only receive N in corn years of the corn/soy rotation.

Cropping system (Trt. No.)	System Abbreviation	Tile No.	N Management	Tillage
Continuous corn + rye (3)	CC-CR-192	12, 23, 30, 46	179 kg ha^{-1} preplant + 13 kg ha^{-1} starter = 192 kg ha^{-1}	No
Continuous corn + Kura (5)	CC-KC-69	6, 16, 29, 39	56 kg ha^{-1} sidedress + 13 kg ha^{-1} starter = 69 kg ha^{-1}	No
Continuous corn control (12)	CC-192	3, 21, 31, 41	179 kg ha^{-1} preplant + 13 kg ha^{-1} starter = 192 kg ha^{-1}	Yes
Corn-soy control (6)	CS-170	5, 13, 35, 40	157 kg ha^{-1} preplant + 13 kg ha^{-1} starter = 170 kg ha^{-1}	Yes
Soy-corn control (7)	SC-170	8, 20, 27, 47	157 kg ha^{-1} preplant + 13 kg ha^{-1} starter = 170 kg ha^{-1}	Yes
Corn-soy + rye (8)	CS-CR-148	2, 14, 33, 45	135 kg ha^{-1} sidedress + 13 kg ha^{-1} starter = 148 kg ha^{-1}	No
Soy-corn + rye (9)	SC-CR-148	9, 19, 34, 48	135 kg ha^{-1} sidedress + 13 kg ha^{-1} starter = 148 kg ha^{-1}	No

Cropping system abbreviations are as follows: CC-192 (Tilled, continuous corn receiving 192 kg N ha^{-1} every year), CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1} every year), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1} every year), SC-CR-148 (soybean component of a no-till soybean/corn rotation with cereal rye, soybean planted Year 1 (2017) with no N, corn Year 2 (2018) receiving 148 kg N ha^{-1}), CS-CR-148 (corn component of a no-till corn/soy rotation with cereal rye, corn planted Year 1 receiving 148 kg N ha^{-1}, soybean Year 2 with no N), CS-170 (corn component of tilled corn/soybean rotation, corn planted in Year 1 receiving 170 kg N ha^{-1}, soybean Year 2 with no N), SC-170 (soybean component of tilled, soybean/corn rotation, soybean in Year 1 with no N, corn planted Year 2 receiving 170 kg N ha^{-1}). The different N rates on the corn-soy rotations (148 vs 170 kg N ha^{-1}) reflect the current recommendations when N is applied pre-plant (170 kg N ha^{-1}) versus side dressed after rye removal and corn planting (148 kg N ha^{-1}). However, it is important to note that this N recommendation is typically used for CS rotational systems and was not altered for the addition of rye to the system.

2.2.2 Treatment Management

Cropping systems in this study consisted of the seven aforementioned treatments. Treatment controls consisted of both a continuous corn cropping system as well as a CS and SC rotation so that both species of the rotation would be present each year. These control cropping systems did not include cover crops. Novel cropping systems were developed by incorporating cover crops. This included a continuous corn-cereal rye system, a continuous corn-Kura clover system, a corn/soybean-cereal rye system and a soybean/corn-cereal rye rotation. The N source was 28% urea ammonium nitrate (UAN) injected approximately 10 cm below the soil surface. In addition to preplant or side-dress N applications, a starter fertilizer application was applied to all treatments that had corn planted as the summer annual crop (Table 2.4). The starter fertilizer in 2017 and 2019 was applied at a rate of 13 kg ha^{-1} N in the form of liquid 19-17-0 (N - P_2O_5 - K_2O) at planting. Starter fertilizer in 2018 was applied at a rate of 13 kg ha^{-1} N in the form of liquid 10-34-0 (N- P_2O_5 - K_2O) applied at planting. In all years, starter fertilizer was banded 5 cm to the side of the corn seed and 5 cm deep. No N fertilizer was applied to soybean plots (Table 2.5). Planting dates for both corn and soybean plots ranged from May 10th to June 11th. Harvest dates for corn ranged from October 17th to November 7th, whereas soybean harvest dates ranged from October

24th to October 29th. Corn varieties, tillage, seeding rates and type of N fertilizer applied by treatment are recorded in Table 2.4. Soybean varieties, tillage, and seeding rates by treatment are presented in Table 2.5. Corn was planted using a John Deere Max Emerge 2 Vacumeter 6 row planter (John Deere, Johnston, IA). It is important to note that soybeans were drilled in 2017 using a 19 cm John Deere 750 with an ~4.5 m wide drill with ~19 cm spacing, whereas in 2018 and 2019 soybeans were planted using an ~6 m wide Kinze 3500 planter (Kinze Manufacturing, Williamsburg, IA) with ~38 cm spacing.

Table 2.4. Corn hybrids sowed, seeding rates, planting/harvest dates, tillage and N applications for treatments planted with corn during each experimental year.

Year	Cropping System	Variety†	Target Seeding Rate (seeds ha^{-1})	Completion Dates of Field Operations			
				Planting Date	Harvest Date	Tillage	Fertilizer N application
2017	CS-170 CC-192	DKC 56-53	81,545	May 30th	Oct 17th	Disk (5/18) Chisel (11/29)	Preplant (5/30) Starter (5/30)
2017	CC-CR-192	DKC 56-53	81,545	May 30th	Oct 31st	Disk (5/18)	Preplant (5/30) Starter (5/30)
2017	CS-CR-148	DKC 56-53	81,545	May 30th	Oct 17th	Disk (5/18)	Starter (5/30) Sidedress (6/22)
2018	SC-170 CC-192	DKC 63-60	81,545	May 10th	Oct 18th	Disk (5/8)	Preplant (5/10) Starter (5/10)
2018	CC-KC-69	DKC 63-60	81,545	May 10th	Oct 18th	None	Starter (5/10) Sidedress (6/7)
2018	CC-CR-192	DKC 63-33	81,545	May 24th	Oct 23rd	None	Preplant (5/24) Starter (5/24)
2018	SC-CR-148	DKC 63-33	81,545	May 24th	Oct 18th	None	Starter (5/24) Sidedress (6/7)

Table 2.4 continued

2019	CS-170 CC-192	DKC55-53	81,545	June 11th	Nov 7th	Disk (6/4)	Preplant (6/4) Starter (6/11)
2019	CC-KC-69 CS-CR-148	DKC55-53	81,545	June 11th	Nov 7th	None	Starter (6/11) Sidedress (6/27)
2019	CC-CR-192	DKC55-53	81,545	June 11th	Nov 5th	None	Preplant (6/4) Starter (6/11)

†DKC indicates seed cultivars sourced from the Dekalb seed company. Cropping system abbreviations are as follows; CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1} every year), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1} every year), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted Year 1 with no N, corn Year 2 receiving 148 kg N ha^{-1}), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted Year 1 receiving 148 kg N ha^{-1}, soybean Year 2 with no N), CC-192 (Tilled, continuous corn receiving 192 kg N ha^{-1} every year), CS-170 (Tilled corn/soybean rotation, corn planted in Year 1 receiving 170 kg N ha^{-1}, soybean Year 2 with no N), SC-170 (Tilled, soybean/corn rotation, soybean in Year 1 with no N, corn planted Year 2 receiving 170 kg N ha^{-1}).

Table 2.5. Soybean cultivars sowed, seeding rates, planting/harvest dates, tillage and N applications for treatments planted with soybeans during each experimental year.

Year	Cropping System	Variety†	Target Seeding Rate (Seeds ha^{-1})	Dates of Major Field Operations			
				Planting Date	Harvest Date	Tillage	Fertilizer N application
2017	SC-CR-148	P34T07	444,790	May 31^{st}	Oct 29^{th}	Disk (5/18)	none
2017	SC-170	P34T07	444,790	May 31^{st}	Oct 29^{th}	Disk (5/18) Chisel (11/29)	none
2018	CS-170	B6366LL	370,657	May 10^{th}	Oct 24^{th}	Disk (5/8)	none
2018	CS-CR-148	C23548LL	370,657	May 24^{th}	Oct 24^{th}	none	none
2019	SC-170	P31A22X	429,962	June 11^{th}	Oct 25^{th}	Disk (6/4)	none
2019	SC-CR-148	P31A22X	429,962	June 11^{th}	Oct 25^{th}	none	none

†P indicates seed sourced from the Pioneer Hi-Bred seed company. C indicates seed sourced from the Credenz soybean cultivar line offered by BASF. B indicates seed sourced from the Becks Hybrids seed company.

2.2.3 Soil Sampling: Collection, Processing, Analysis

As a baseline soil sample for this study, deep soil cores (9 cores per plot) were obtained in fall of 2017. Cores were taken to a 120-cm depth, separated into five depth increments: 0-15 cm, 15-30 cm, 30-60 cm, 60-90 cm, and 90-120 cm and air dried at room temperature (~ 21º C) for approximately 2 weeks. These samples were ground to pass a 2-mm sieve and subsamples sent to A&L Great Lakes Laboratory (3505 Conestoga Drive, Fort Wayne, Indiana) to be analyzed for organic matter, available phosphorus, exchangeable potassium, magnesium, calcium, soil pH, buffer pH, cation exchange capacity, and percent base saturation of cation elements. These routine fertility assays conducted by A&L Great Lakes aligned with regional recommendations (North Central Regional Research Publication No. 221, 1998). Furthermore it is important to note the protocol for phosphorus analysis was the Mehlich 3/ICP procedure which uses couple plasma spectrometry (ICP, Chalmers and Handley, 2006).

In addition to these baseline deep soil cores, standard soil samples (9 cores per plot) at a 0-20 cm depth were collected using hand probes from each plot. These soil samples were collected in the fall of 2017, 2018, and 2019 following grain harvest. These samples were process for analysis as described above and sent to A&L Great Lake Laboratory for the same analyses. Similarly, these 0-20 cm soil samples also were ground to the same fineness (ground to pass a 2-mm sieve) and analyzed for soil N concentration using a flash combustion elemental analyzer (Flash EA 1112 Series, Thermo Fisher Scientific, The Netherlands; Nelson and Sommers, 1996).

2.2.4 Established Plant Populations/Stover Collection: Processing and Analysis

Plant population counts were made for each plot at the V6 and V5 growth stages for corn and soybeans, respectively. Soybean populations in 2017 were measured using a 1-m-diameter ring (hula hoop) randomly placed at three locations in each plot. Soybean populations in 2018 and 2019 were estimated by placing a meter stick in three random locations within each plot and counting plants per row for both rows adjacent to the meter stick. Corn populations were determined by placing a 5.3-m-long chain between two rows of corn and counting plants in both rows adjacent to the chain. These measurements were taken three times within each plot and counts averaged on a per-plot basis.

Corn stover samples were collected from 10 whole-plant samples from rows immediately adjacent to the 6-center rows of each plot later harvested for yield with a combine. These whole-plant corn samples were collected at physiological maturity (R6). Likewise, 10 whole-plant soybean samples were collected from rows directly adjacent to the 5.5 m pass in the middle of each plot that was harvested with the combine. Soybean plants were collected at the R6 growth stage in order to acquire leaves prior to leaf drop at R8 (full maturity). Both corn and soybean 10-plant samples were collected as close as possible to the rows harvested with a combine, and away from the plot border in order to avoid edge effects. Plants were hand-cut just above the soil surface. Corn ears were immediately removed from whole-corn plants and air dried for one week before being shelled (Ariculex SCS-2, Ontario, Canada). Corn cob plant material was not analyzed in this study. The wet weight of the remaining corn stover and whole soybean plants (including soybean pods) was recorded. Plant material was run through a forage chopper and subsamples were collected. Plant subsamples were dried at 60 °C for at least 48 h, reweighed, and dry weight recorded. The dry plant material was ground to pass a 1-mm sieve and analyzed for N concentration using a flash combustion elemental analyzer (Flash EA 1112 Series, Thermo Fisher Scientific, The Netherlands; Nelson and Sommers, 1996). Plant N concentrations and tissue masses were used to calculate N mass per plant, which along with plant population estimates, was used to estimate N returned to the field for each system after grain was harvested.

2.2.5 Corn and Soybean Grain Harvest: Processing and Analysis

Corn grain yields at R6 were determined by mechanically harvesting the center 6 rows of the 12-row plots using a John Deere 3300 plot combine with a 3 row combine head (John Deere, Johnston, IA). Soybeans were harvested using the same John Deere 3300 plot combine (John Deere, Johnston, IA) with an ~3 m bean head. Soybeans were picked by mechanically harvesting the center 5.5-m of each plot when soybeans reached R8. Harvested grain from each plot was weighed using a Parker weigh wagon (Unverferth Manufacturing Co., Kalinda OH). Grain moisture levels were determined using a Dickey John GAC 3000 meter (Dickey John Corp., Aurora, IL). Grain yields were corrected to 155 g/kg (15.5%) for corn and 130 g/kg (13%) for soybeans. Grain subsamples were oven dried at 60 °C, weighed and ground to pass a 1-mm sieve using an industrial food processor (RSI 2Y-1, Robot Coupe USA). Ground grain were analyzed for C and N concentrations using a flash combustion elemental analyzer (Flash EA 1112 Series, Thermo Fisher Scientific, The Netherlands; Nelson and Sommers, 1996).

2.2.6 Quantifying Water Flow/ Nutrient Load losses

As previously stated, six tile lines enter each hut basement and flow volumes determined using tip counts from the tipping buckets and the known volume per tip. The following equation was used to determine daily flow volumes: Daily flow volume (L/ha/day) = [(tips/day) x (L/tip)]/(lysimeter area (ha))]. Number of tips was recorded automatically using CR 10 data loggers (Campbell Scientific, Logan UT, USA) on an hourly basis. Data loggers were powered by 12-volt marine batteries connected to solar panels. Data were recorded and stored in the Purdue University Research Repository (PURR; https://purr.purdue.edu/).

A flow-proportionate sample of every other tip was captured in a polypropylene 20-L bucket. Samples were retrieved daily and frozen at -4 °C until analyzed. For analysis, water samples were thawed and filtered through Whatman #2 (~8 μm) filter paper (Whatman plc. Maidstone, UK). Colorimetric analysis for nitrate concentrations was conducted using a Seal AQ2 discrete analyzer. Daily load loss of NO_3-N was calculated by multiplying nitrate and nitrite concentrations by their corresponding tile flow volumes. Thus, the following equation was used to derive daily load loss of N: Daily load loss (kg/ha) = ([*Conc. analyte* (mg/L)] x [*Flow* (L/ha)] x [(1 kg/1,000,000 mg)]). Water data in this study was recorded from January 1st, 2017 through December 31st, 2019 for a total of 730 total days.

2.2.7 Gap Filling and Estimation of Missing Water Data

A total of 28 tiles were to be used in this study (7 treatments x 4 reps), but tile flow was not always consistent within treatments. Therefore, function of tile lines was assessed by aligning precipitation events with flow data in order to identify irregularities in the rate and duration of tile line flow. We applied the "<5% rule" to eliminate tiles/plots with unusually low flow. In this study, there was 3 years of data observed (365*3 = 1095 days), therefore any tile with less than 54 non-zero flow days (1095 x 0.05) was deemed "non-functioning" and thus omitted from the study. This resulted in 5 tile lines/plots being removed from the water data analysis. Omitted tiles and their respective treatments are stated as follows: (Tile 23) cereal rye continuous corn (CC-CR-192);

(Tile 29) Kura clover continuous corn (CC-KC-69), (Tile 5) and (Tile 13) corn/soybean rotational control (CS-170); and (Tile 2) cereal rye corn/soybean (CS-CR-148) treatment. For reference the mean flow volumes across all treatments and years for functioning tiles in this study was approximately 1357 kl water ha $^{-1}$ yr $^{-1}$.

Missing N concentration and flow data occasionally occurred due to unanticipated issues in the field (flooding, data logger failure, etc.) and laboratory (lost sample, equipment error, etc.). When flooding occurred, water completely submerged the calibrated tipping bucket systems resulting in loss of water flow data and sometimes prevented water sample collection resulting in loss of N concentration data. In cases where there was missing NO_3-N concentration data, N concentrations were estimated using a previously established decision rule (Greve, 2019; Welikhe, 2018). This decision rule determined that missing concentration data could be gap-filled using N concentration data acquired within four days (plus or minus) of the missing sample date. On some occasions, gaps of missing concentration values appeared between two present values that both fit the four-day rule. In these instances, the two present values that fit this rule were averaged and data was gap filled with the average of the two values. Unlike NO_3^- concentration data, missing flow data were not estimated due to the less predictable pattern of tile drainage volume. Water flow data from data loggers were compared to manual field logs to identify gaps in the water flow data record. After analyzing each of the 28 tile lines in this study it was determined that less than 1% of flow data was missing during the duration of this study. Supplemental data is provided in the appendix (Table A1.1). Water data was deposited in PURR - WQFS project.

2.2.8 Greenhouse Gas Collection and Analysis

Greenhouse gas measurements were taken weekly from April through October on each plot using the static chamber technique (Parkin et al., 2010). Start dates for greenhouse gas measurements ranged from April 4th (2019) to May 3rd (2017), while end dates ranged from November 16th (2018) to December 5th (2017). Total weeks sampled ranged from 22 weeks to 26 weeks (Table 2.6). Custom-made metal chamber frames (base) (74.9 cm long by 36.8 cm wide) were pounded into soil surface and leveled. To determine chamber volumes, frame height from the soil surface and to the top interior edge of the frame was recorded at both ends of each inserted frame. Frames were removed prior to major field operations (tillage, planting, harvesting), and immediately reset in the field as described. Depending on the time of year, plant material growing within the frame was cut to enable the chamber head to be placed on top of the frame. This resulted in the termination of some plants (corn, soybean, rye) while only suppressing the growth of other species (Kura clover). Prior to GHG measurements, the channel in the metal frame was filled with water to provide an air-tight seal between the lid and the frame. Gas samples were removed through the chamber head using a hypodermic needle attached to a syringe (Monoject 35 mL Syringe, Tiger Medical, NJ). The needle was pierced through a rubber septum (Dichtshelben/septa N17, Macheret-Nagel, Duren, Germany) inserted on the top of each chamber head and 30 mL (~30 cm^3) of gas was removed from the chamber and transferred to a septum-sealed gas sample vial (Headspace Screw Vial, ~20 mL, clear, 22X75MM, Thermo Fisher Scientific, The Netherlands). Gas samples were removed from each chamber following installation of the chamber head at 0, 15, and 30 minutes. This time series was used to calculate GHG flux over time. Furthermore, the evaluation of slopes was used to understand sample precision and accuracy. Soil moisture readings were taken in four locations around each gas chamber using a calibrated soil moisture probe (Field Scout -TDR 300, Spectrum Technologies, Aurora, IL). Soil temperature readings were taken in two locations around each gas chamber using two soil temperature thermometers (Digital Soil Thermometer 6300, Spectrum Technologies, Aurora, IL).

Gas samples were then transported back to Purdue University where they could be analyzed using gas chromatography (GC System 7890A, Agilent Technologies, Wilmington DE) using the Chapter 3 USDA-ARS GRACEnet Project Protocols for chamber-based trace gas flux measurements (Parkin and Venterea, 2010). Machine detection limits were as follows: N_2O (0.128 mg L^{-1}), CH_4 (0.178 mg L^{-1}), CO_2 (33.7 mg L^{-1}). Sampling times were recorded in the field which allowed for hourly ambient air temperatures to be sourced from the onsite WQFS weather station at a later date. Gaps in the WQFS records, due to lost data, were filled using weather stations at the nearby Indiana State Climate office's weather station (Purdue ACRE) https://ag.purdue.edu/indiana-state-climate/. Linear regression was used to calculate a slope of the gas concentrations over time (0, 15, and 30 min). Correction factors were used to properly convert and interpolate the concentration of gas (ppm) to a volume (Table 2.7). Individual GHG sample fluxes were then calculated by multiplying the slope of the observed gas to the height of the static chamber. These hourly flux data were then used to derive cumulative emissions over the experimental period. This was done by using linear interpolation and numerical integration via the trapezoid method, a method used widely in the GHG community (Hernandez-Ramirez et al., 2009;Jarecki et al., 2008).

Table 2.6. Start and end dates for greenhouse gas measurements taken over the course of this study. 2017 is used as a baseline for the 2018-2019 study years. Table includes total range of weeks sampled throughout the season.

Year	Start Date	End Date	Total Weeks Sampled
2017	May 3rd	December 5th	22
2018	April 18th	November 16th	23
2019	April 4th	November 20th	26

Table 2.7. Conversion factors used in greenhouse gas (GHG) calculations accompanied with verbiage explaining their role in overall GHG flux.

Correction Factor	Description	Equation Used
N_2O correction factor	Uses the ideal gas law (PV=nRT) and the air temperature to calculate the sample-specific correction factor to convert ppm(volume) to ng N/cm3. P=pressure in atm at the WQFS altitude of 215 meters, V=volume of the GHG, n=the number of moles of gas, R=the ideal gas law constant (0.08206 in units of L atm Mol^{-1} ^{o}K), and T is temperature in ^{o}K or (273+Temp ^{o}F). The atomic weight of N is 14 and there are 2 Ns per molecule	(g/mol) x Atmos * (1L/10^6 uL) * (mol K/L Atmos) * (1/K) * (10^9ng/g) * (1L/10^3 cm3) **OR** (14*2) * 0.9788 / 1000000 / 0.082057 / (273+C15)*1000000
CH_4 & CO_2 correction factor	Used when quantities of CO_2 are higher than N_2O and there is only 1 C per molecule	(g/mol) x Atmos * (1L/10^6 uL) * (mol K/L Atmos) * (1/K) * (10^6ug/g) * (1L/10^3 cm3) OR (12) * 0.9788 / 1000000 / 0.082057 / (273+C15)*1000

2.2.9 Cover Crop Management, Biomass Harvest: Processing and Analysis

The Kura clover perennial living mulch (PLM) was seeded at 5.8 kg ha^{-1} using a 1.5 m Brillion Sure Stand Seeder (Brillion Iron Works, Inc., Brillion, WI) in June of 2017 (Table 2.8). Kura clover was left to establish except for one mowing event in late September of 2017 (Table 2.8). Both mowing and herbicide applications were used as means of reducing competition between the PLM and corn crop. Thus, mowing was implemented at an ~10 cm height. Glyphosate was mixed at a 2.5 oz rate per gallon water and sprayed at a volume of 61.86 gallons ha^{-1}. This application was band applied (~15-20 cm) over the corn rows using a fan nozzle attached to a Roundup 190327 Backpack Sprayer™ (The Fountainhead Group Inc., New York Mills, NY) immediately following corn planting. In some cases, such as 2019, a second glyphosate application was applied in order to suppress overly competitive Kura clover stands (Table 2.8).

Cereal Rye was planted annually directly following the corn/soybean harvest. Cereal rye was seeded at 76 kg ha^{-1} using a TYE grain drill (AGCO, Duluth, GA) set to ~19 cm spacing. Cereal rye was allowed to grow extensively into the spring until reaching the heading growth stage. This allowed for increased biomass accumulation prior to harvest of the cereal rye cover crop.

Due to the nature of the different cover crop species used in this study, harvest timing and method varied between Kura clover and cereal rye cover crops (Table 2.8). Cereal rye was harvested when the majority of the stand reached the "heading" growth stage. For cereal rye plots the centermost 1.2 m was harvested using a plot forage harvester (Wintersteiger Cibus, Salt Lake City, UT, USA). For Kura clover, yield was determined by taking a subsample of biomass from a known area within two random locations of each plot. Due to variations in equipment used to harvest the biomass the area varied from 0.5 to 0.16 m^2 in the years 2018 and 2019 respectively. Wet weights of subsamples of both cereal rye (~300 to 500 g) and Kura clover (~50 to 200 g) were recorded. Samples were dried at 60 °C to constant weight, reweighed, and percent moisture used to adjust plot weights to biomass yield/ha on a dry matter basis. The dry plant material was ground to pass a 1-mm sieve and analyzed for N concentration using a flash combustion elemental analyzer (Flash EA 1112 Series, Thermo Fisher Scientific, The Netherlands: Nelson and Sommers, 1996). Dry matter yield and tissue N concentrations were used to calculate N removal in aboveground biomass.

Table 2.8. Cover crop varieties sowed, seeding rates, planting/harvest dates for each cover crop treatment during the experimental years.

Year	Cropping System	Variety	Seeding Rate (kg ha^{-1})	Dates of Major Field Operations		
				Planting Date	Harvest Date	Maintenance
2017	CC-KC-69	VNS†	5.8	6/1/17	10/18/18 11/7/19	Mowed: 9/28/17 Sprayed: 5/11/18 Mowed: 6/3/19 Sprayed: 6/12/19 Sprayed:7/2/19
2017	SC-CR-148 CS-CR-148	VNS†	76.2	10/20/17	5/23/18	NA
2017	CC-CR-192	VNS†	Initial: 76.2 Replant: 49.3	Initial: 11/1/17 Replant: 11/17/17	5/23/18	NA
2018	SC-CR-148 CS-CR-148 CC-CR-192	VNS†	76.2	10/24/18	6/4/19	Sprayed: 6/7/18
2019	SC-CR-148 CS-CR-148 CC-CR-192	VNS†	76.2	11/18/19	NA	NA

† VNS indicates seed where the variety was not stated.

2.2.10 Determination of Theoretical Ethanol (EtOH) Yields

Cover crop biomass and corn and soybean stover was analyzed for fiber composition using a forage analyzer (Fiber Analyzer A2000, ANKOM Technology, Macedon NY). Subsamples of plant material were weighed, placed in filter bags (F57 Filter Bags, ~25 µm porosity, ANKOM Technology, Macedon NY) and sealed using a heat sealer (1915 Heat Sealer, ANKOM Technology, Macedon NY). Samples were analyzed for neutral detergent fiber (NDF) using the Ankom Method 13 provided in the appendix (Table A1.2) which removes cell contents and pectin, leaving hemicellulose, cellulose, and lignin. After calculating NDF concentrations, the NDF residue was analyzed for acid detergent fiber (ADF) in accordance with Ankom Method 12 located in the appendix (Table A1.2), which separates hemicellulose from cellulose and lignin. Following the ADF analysis, residue was analyzed for acid detergent lignin (ADL) using the Ankom Method 8 provided in the appendix (Table A1.2) which removed the cellulose leaving lignin and insoluble minerals. Finally, this residue was ashed at 550-600 °C in order to determine the acid-insoluble ash concentration using the Ankom Ashing Procedure 034 located in the appendix (Table A1.2).

Fiber Composition Calculations

Hemicellulose concentration = NDF – ADF

Cellulose concentration= ADF-Lignin concentration

Lignin concentration=ADF-Cellulose concentration

Plant tissues were also analyzed for nonstructural carbohydrates (CHO). For clover, corn, and soybean stover, sugars were extracted from 30 mg of plant tissue with 800 mL/L ethanol and sugars quantified using anthrone (Koehler, 1952). Starch in the ethanol-extracted residue was gelatinized, suspended in buffer, and hydrolyzed to glucose using amylase and amyloglucosidase as previously described (Berg et al., 2009). Glucose oxidase (glucose Trinder, Sekisui Diagnostics L.L.C., Exton, PA) was used to determine glucose concentrations. Starch was estimated by multiplying glucose concentration by 0.9. Rye tissues were analyzed for water-soluble carbohydrates, including sugars and fructans, by extracting 30 mg of tissue with room temperature deionized water as described by Smith and Grotelueschen (1966) and Smith (1969). Water-soluble carbohydrates were analyzed using anthrone as described. The 2019 herbage composition was predicted using 2018 herbage composition data. This decision was made due to limited access to laboratory facilities during the worldwide COVID19 pandemic. Due to restrictive limitation to laboratory space, analysis of 2019 herbage composition could not take place. Thus, 2018 herbage concentration values were used with the 2019 yield values to calculate 2019 herbage theoretical ethanol yield values. Bioenergy production per ha (L of ethanol ha^{-1}) was estimated using ethanol conversion factors for starch, sugars, cellulose, and hemicellulose (Long, 2015 and sources therein) (Table 2.9). These individualized equations and their conversion factors are stated below. Together these individual parts sum together for the total herbage theoretical EtOH yield (HTEY henceforth).

Theoretical EtOH Calculations

$HTEY_{Su} = [(\text{sugar, kg ha}^{-1}) \times (0.51 \times 0.95)] \div 0.789$

$HTEY_{St} = [(\text{starch, kg ha}^{-1}) \times (0.51 \times 0.95) \times (1.11)) \div 0.789]$

$HTEY_{C} = [(\text{cellulose, kg ha}^{-1}) \times (0.51 \times 0.95) \times (1.11 \times 0.9)) \div 0.789]$

$HTEY_{H} = [(\text{hemicellulose, kg ha}^{-1}) \times (0.51 \times 0.79) \times (1.136 \times 0.853)) \div 0.789]$

Total $HTEY = HTEY_{Su} + HTEY_{St} + HTEY_{C} + HTEY_{H}$

Table 2.9. Conversion factors used in theoretical ethanol calculations accompanied with verbiage explaining their role in herbage theoretical ethanol yields.

Conversion Factor	Description
0.51	conversion factor for each gram of glucose and xylose to each gram of EtOH via the fermentation process, this value is assumed to be indistinguishable for both glucose and xylose and is expressed as 0.51 g EtOH g^{-1} glucose or xylose
0.95	conversion efficiency of glucose to EtOH during the fermentation process.
0.79	the conversion efficiency of xylose to EtOH during the fermentation process.
0.789	the density of EtOH at 20ºC which is 0.789 kg L^{-1}
1.11	conversion factor used for the hydrolysis of anhydroglucose in starch which is 1.11 g g^{-1}
1.136	conversion factor used for the hydrolysis of anhydroxylose in hemicellulose to free xylose which is 1.136 g g^{-1}
0.90	the recovery efficiency of glucose from cellulose during the hydrolysis of cell wall polymers to their corresponding simple sugars
0.853	the recovery efficiency of xylose from hemicellulose during the hydrolysis of cell wall polymers to their corresponding simple sugars

2.2.11 Statistical Analysis

The experimental design was a randomized complete block design (RCBD) with 4 replicates (blocks) per treatment. Univariate descriptive statistics were used in the analysis of this data to characterize the plot-to-plot variation within each system and to confirm tile function over the 2-year study period. Any non-functioning tiles were omitted from this study. General Linear Models (GLM) and Analysis of Variance (ANOVA) were used to identify significant main effects and interactions (Table 2.10). Analyses were conducted at both the system level (sum of total annual system productivity; e.g., total biomass yield of corn and rye) and as individual system components (e.g., rye yield in corn vs rye yield in soybean; corn yields with and without Kura, etc). Annual data were analyzed by year rather than by season. In addition, greenhouse gas and water data were broken down further into trimester and quarterly analyses respectively. Where F-tests were significant, Tukey's Honest Significant Difference (HSD) was used to identify difference between treatment means. Statistical analyses were performed using RStudio version 1.3.1056.

Table 2.10. General linear models (GLM)s designed for analysis of variance (ANOVA) with various facets of the study.

Model Definition	**Description**
(Corn Grain Yield ~ Treatment + Year + Rep + Year:Treatment)	analyzes mean corn grain yield among treatments and between years
Corn Grain N Concentration ~ Treatment + Year + Rep + Year:Treatment)	analyzes mean corn grain N concentration among treatments and between years
(Soybean Grain Yield ~ Treatment + Year + Rep + Year:Treatment)	analyzes mean soybean grain yield among treatments and between years,
Soybean Grain N Concentration ~ Treatment + Year + Rep + Year:Treatment)	analyzes mean soybean grain N concentration among treatments and between years
(Cover Crop Yield ~ Treatment + Year + Rep + Year:Treatment)	analyzes mean cover crop biomass yields: cereal rye and Kura clover were analyzed together
(Cover Crop N Concentration ~ Treatment + Year + Rep + Year:Treatment)	analyzes mean cover crop biomass N concentrations among treatments and between years
Cover Crop Biomass N Content ~ Treatment + Year + Replication + Year:Treatment)	analyzes mean cover crop biomass N content, cereal rye and Kura clover analyzed together
Corn Stover N Concentration. ~ Treatment + Year + Rep + Year:Treatment)	analyzes mean corn stover N concentration among treatments and between years
Soybean Stover N Concentration ~ Treatment + Year + Rep + Year:Treatment)	analyzes mean soybean stover N concentration among treatments and between years
(Annual Flow ~ Treatment + Year + Rep+ Year:Treatment)	Analyzes mean tile drained flow volumes among treatments and between years
(Annual Tile Drained N Concentration ~ Treatment + Year + Rep + Year:Treatment)	Analyzes mean tile drained NO_3 concentrations among treatments and between years

Table 2.10 continued

(Load ~ Treatment + Year + Rep + Year:Treatment)	Analyzes mean tile drainage NO_3 loads among treatments and between years
(Treatment Total HTEY ~ Treatment + Year + Rep + Year:Treatment)	Analyzes mean total herbage theoretical ethanol yields (HTEY) among treatments and between years
(Cover crop HTEY, ~ Treatment + Year + Rep + Year:Treatment)	Analyzes mean herbage theoretical ethanol yields (HTEY) of just cover crops, among treatments and between years
(Corn/Soy HTEY ~ Treatment + Year + Rep + Year:Treatment)	Analyzes mean herbage theoretical ethanol yields (HTEY) of just corn/soybean, among treatments and between years
(N_2O.Loss.kg.ha ~ Treatment + Year + Rep + Treatment:Year)	Analyzes annual cumulative loss of N_2O among treatments and between years
(CH_4.Loss.kg.ha ~ Treatment + Year + Rep + Treatment:Year)	Analyzes annual cumulative loss of CH_4 among treatments and between years
(CO_2.Loss.kg.ha ~ Treatment + Year + Rep + Treatment:Year)	Analyzes annual cumulative loss of CO_2 among treatments and between years
(N_2O.Loss.kg.ha ~ Treatment + Year + Trimester + Rep + Year:Trimester + Year:Treatment + Trimester:Treatment + Year:Trimester:Treatment)	Analyzes cumulative loss of N_2O by trimester among treatments and between years
(CH_4.Loss.kg.ha ~ Treatment + Year + Trimester + Rep + Year:Trimester + Year:Treatment + Trimester:Treatment + Year:Trimester:Treatment)	Analyzes cumulative loss of CH_4 by trimester among treatments and between years
(CO_2.Loss.kg.ha ~ Treatment + Year + Trimester + Rep + Year:Trimester + Year:Treatment + Trimester:Treatment + Year:Trimester:Treatment)	Analyzes cumulative loss of CO_2 by trimester among treatments and between years

2.3 Results and Discussion

2.3.1 Weather and Climate

Weather influences yield and the year-to-year variability in yield of field-scale agronomic studies, including results of this research. Monthly mean temperatures were recorded and averaged across the three study years (2017, 2018, 2019) and ranged from -3 to 24 °C. Across study years annual mean temperatures ranged from 11 to 13 °C (Table 2.2). In 2017, January and February were noticeably warmer than the 30-year mean for their respective months. July through September of 2017 was somewhat cooler than the 30-year mean, a period critical to crop growth and development, and reproduction. Cooler temperatures during these months can be associated with reduced heat stress during the critical pollination/grain-fill periods and increased grain yields.

January of 2018 was much colder than the 30-year mean as was March and April averaging 3 to 5 °C cooler than the average temperature of the 30-year means for those respective months. Cooler springtime temperatures can slow soil warming which further has the potential to effect early-season crop development. This effect of slow soil warming can be further exacerbated in systems with overwintering perennial cover crops such as cereal rye and PLMs such as Kura clover that cover the soil's surface increasing albedo from plant tissues and reducing radiation absorption by soil (Kaye and Quemada, 2017). May and June were somewhat warmer in 2018 than the 30-year mean. October and November of 2018 were slightly cooler than average.

January and February of 2019 were cooler than average when compared to the 30-year mean as well. March through July of 2019 was substantially warmer for all months when compared to respective 30-year averages. Increased temperatures from March to June could affect biomass accumulation among cover crop species in early spring, while simultaneously enhancing soil temperatures and effecting early development for both corn and soybeans. This increased temperature, however, for late June and into July of 2019 (accompanied with decreased precipitation in those months) might have affected developing grain crops during critical reproductive growth stages.

Yearly precipitation totals for the three years ranged from 973 to 1559 mm in the years 2019 and 2017 respectively (Table 2.1). Averaged over years the monthly precipitation totals ranged from 63 to 177 mm in the months of January and July respectively. Significant departure from the 30-year mean precipitation occurred within individual years. In 2017, May to August were drastically wetter than their respective 30-year means. This wet spring delayed initial planting dates towards the end of May. November of 2017 was wet followed by a dry December, which might have affected rye establishment.

Excluding July, the months of June to October of 2018 had substantially higher precipitation than their respective 30-year means. Receiving above-average rainfall during these months, which are critical to growth and development of corn and soybeans, is noteworthy.

In 2019, the months of April and May were noticeably wetter than their respective 30-year means which delayed planting. June through November of 2019 had substantially decreased precipitation compared to the 30-year means. Traditionally this summer period in Indiana corresponds to a crucial growth and development period for annual grain crops such as corn and soybeans. The total precipitation during June and July of 2019 was reduced to 97 and 37 mm, respectively, while the three-year average for these months were 177 mm in June and 119 mm in July. This shortage of rainfall can be especially detrimental in co-cropping systems where species compete for limited water. In this study, the continuous corn planted into a Kura clover sod likely had heightened competition for water which may have ultimately affected grain yields in this treatment (Ziyomo et al., 2013). Overall precipitation and temperature are large drivers in corn and soybean grain yields as well as biomass acquisition among cover crops and PLMs and influenced overall performance of each of the systems in this study.

2.3.2 Soil Fertility

Following the baseline deep core (0-120 cm depth) soil sample, soil OM content in the 0 to 15 cm increment was found to be lowest in the cereal rye-corn/soy rotation (CS-CR-148) system (40 g kg^{-1}) and increased to 48 g kg^{-1} in the cereal rye-continuous corn (CC-CR-192) system. These levels are considered adequate to high (Gerber et. al, 2012) and consistent with previous field site-specific literature (Greve, 2019). It is important to note that the OM content in 2017 is more reflective of past treatments rather than the novel cropping systems implemented in the spring of 2017. Soil P concentrations in the 0 to 15 cm increment ranged from 21 to 32 mg kg^{-1} with the highest concentrations found in the corn/soybean control (CS-170) treatment. Soil K concentrations ranged from 115 mg kg^{-1} to 128 mg kg^{-1} for the corn/soybean control (CS-170) and soybean/corn control (SC-170) plots, respectively. For the given soil type, these P and K levels are considered adequate for both corn and soybean production and do not indicate any limitation to yield, according to the Purdue University Corn and Soybean Field Guide (Gerber et al., 2012). Magnesium and Ca concentrations ranged from 699 to 843 mg kg^{-1} and 2919 to 3386 mg kg^{-1} respectively. Slight differences between treatments in the starting year of this study reflect previous treatments and are not representative of changes caused by the BFF study treatments and thus are treated as a baseline. The reported values (Table 2.11**)** are all considered to fall within the "maintenance" nutrient regime (Vitosh et al., 1995). Soil pH was relatively consistent among all treatments and ranged from 6.0 to 6.9. The CEC ranged from 23.2 to 26.4 meq 100 g^{-1} for the Kura clover/continuous corn treatment (CC-KC-69) and soybean/corn control (SC-170) systems respectively. Across the three continuous corn treatments, soils of the Kura clover/continuous corn treatment (CC-KC-69) had the lowest CEC of the three systems. This same Kura clover/continuous corn (CC-KC-69) system had a pH level of 6.9 which is likely important for dinitrogen fixation of the Kura clover component of the system (Franco and Munns, 1982) (Table 2.11). Initial organic matter content was lower in the Kura clover/continuous corn treatment (CC-KC-69) when compared to the cereal rye/continuous corn (CC-CR-192) system. The Kura clover/continuous corn (CC-KC-69) treatment had higher soil Mg concentration than the continuous corn control (CC-192) treatment initially, while P, K, and Ca levels were relatively homogenous across three continuous corn treatments.

Among like rotational systems, organic matter content was not substantially different. Initial soil Mg content was lower in corn/soybean control (CS-170) when compared to cereal rye-corn/soybean rotation (CS-CR-148). Soil pH was slightly higher in the cereal rye-corn/soybean rotation (CS-CR-148) system compared to the corn/soybean control (CS-170) control, while no major differences were found among initial CEC for these respective systems. No major soil test differences were found when the soybean/corn rotational control (SC-170) and cereal rye/soybean-corn (SC-CR-148) treatments were compared. Overall, most of the soil test differences among these systems were small and unlikely to limit growth and yield of corn, soybean, cereal rye, or Kura clover. It is important to note that these subtle differences are either attributed to previous treatments that took place in the same plot locations in years prior to 2017 or reflect indigenous plot-to-plot variability, however it is crucial to note the differences in the baseline soil test data so as to not confound any differences found among subsequent years of soil test data.

Table 2.11. Data for deep core soil test analyses for soils sampled in fall 2017 by cropping system. Cores were randomly taken from nine locations in each plot to a depth of 120 cm and separated into five segments (0 to 15, 15 to 30, 30 to 60, 60 to 90, and 90 to 120 cm). Data represent the mean ± standard error of four replications. Background shading used to differentiate between cropping systems for ease of observation.

Table 2.11 continued

Cropping System λ	Depth	O.M. †	P	K	Mg	Ca	Soil pH	CEC§
	cm	g kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	n/a	meq 100 g^{-1}
(CC-192)	0-15	44±2	31±6	125±5	699±22	2977±140	6.0±0.1	25.2±1.5
	15-30	39±2	12±2	105±3	745±34	3281±251	6.3±0.1	26.5±2.0
	30-60	24±1	3±1	115±3	780±30	3023±168	6.7±0.1	23.3±0.7
	60-90	15±1	3±1	129±7	811±39	2700±138	7.0±0.0	20.7±1.0
	90-120	12±1	5±1	126±21	696±89	2455±282	7.4±0.2	18.4±2.1
(CC-CR-192)	0-15	48±3	30±8	121±9	788±69	3088±136	6.1±0.4	26.3±1.0
	15-30	41±4	14±3	109±3	793±26	3467±178	6.3±0.2	27.8±2.0
	30-60	24±1	2±1	109±7	790±21	3095±99	6.9±0.1	23.1±0.9
	60-90	14±1	2±1	105±16	740±51	2790±279	7.5±0.3	20.5±1.4
	90-120	9±2	4±1	93±23	643±101	3017±785	7.8±0.3	20.7±3.1
(CC-KC-69)	0-15	41±2	26±6	127±9	802±32	3049±136	6.9±0.2	23.2±1.6
	15-30	33±1	9±1	101±5	763±46	3156±225	6.7±0.1	23.7±1.5
	30-60	22±1	1±0	112±3	781±42	2890±141	6.9±0.2	22.2±0.9
	60-90	12±1	2±1	90±9	634±52	3228±589	7.7±0.2	21.7±2.9
	90-120	8±1	3±2	79±10	514±56	3785±833	8.0±0.2	23.4±4.3
(CS-170)	0-15	43±3	32±7	115±9	705±58	2919±196	6.2±0.2	24.2±1.3
	15-30	38±4	16±6	102±6	692±46	2944±227	5.9±0.1	25.9±1.8
	30-60	26±2	4±2	109±5	730±22	2965±186	6.4±0.1	23.9±1.5

Table 2.11 continued

	60-90	14±0	2±1	108±10	767±38	2694±150	7.2±0.2	20.3±1.0
	90-120	11±1	6±4	112±21	763±57	2972±390	7.8±0.3	21.6±2.0
(SC-170)	0-15	47±3	25±4	128±6	843±51	3386±193	6.5±0.2	26.4±1.4
	15-30	40±4	12±2	112±7	802±41	3470±307	6.2±0.2	27.8±2.4
	30-60	23±2	3±1	103±10	743±43	2998±295	6.7±0.1	22.8±2.5
	60-90	13±1	2±1	90±20	727±58	2576±299	7.6±0.3	19.3±2.0
	90-120	10±1	5±3	83±19	542±90	3345±464	7.9±0.3	21.5±1.7
(CS-CR-148)	0-15	40±4	21±5	119±10	822±58	3170±181	6.8±0.3	24.5±1.6
	15-30	38±4	11±3	112±3	862±31	3460±181	6.7±0.2	26.4±1.7
	30-60	23±3	1±0	95±8	846±20	3138±256	7.2±0.2	23.3±1.6
	60-90	13±2	1±0	95±20	677±111	3978±607	7.7±0.3	25.8±2.1
	90-120	11±1	4±1	101±21	654±116	3760±1184	7.8±0.3	24.5±5.1
(SC-CR-148)	0-15	42±3	24±8	121±4	788±41	3199±164	6.8±0.4	24.7±1.2
	15-30	38±3	15±6	114±6	784±32	3241±58	6.5±0.3	25.6±1.2
	30-60	21±1	3±2	111±5	783±37	2977±89	6.9±0.3	23.2±1.6
	60-90	13±1	2±1	93±16	656±74	3355±550	7.8±0.3	22.5±2.5
	90-120	8±1	4±3	71±7	460±39	3525±818	8.1±0.3	21.6±3.9

† Organic matter percentage converted to g kg^{-1}, § Cation Exchange Capacity,† Cropping systems abbreviated as: CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1}), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1}), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted first, receiving 148 kg N ha^{-1} during corn years), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted first, receiving 148 kg N ha^{-1} during corn years), CC-192 (Tilled, continuous corn receiving 192 kg N ha^{-1}), CS-170 (Tilled corn/soybean rotation, corn planted first, receiving 170 kg N ha^{-1} during corn years), SC-170 (Tilled, soybean/corn rotation, soybean planted first, receiving 170 kg N ha^{-1} during corn years).

Routine soil sampling (0 to 20 cm) was conducted by cropping system annually in the fall of each year to monitor soil OM, P, K, Mg, Ca, pH, and CEC (Table 2.12). Similar to the 2017 “deep core” soil tests, the 0-20 cm cores from 2017 were also used as baseline data to identify any key soil fertility differences among treatments before implementation of the new cropping systems. The Kura clover-continuous corn (CC-KC-69) system had noticeably lower soil organic matter content when compared to the continuous corn control (CC-192) and cereal rye continuous corn (CC-CR-192) treatment. Although there were no major differences in soil P levels, there was a slight reduction in soil K levels where the Kura clover-continuous corn (CC-KC-69) treatment had lower soil K content than the continuous corn control (CC-192). Soil Mg and Ca levels in 2017 were markedly higher in the cereal rye-continuous corn (CC-CR-192) system when compared to the continuous corn control (CC-192). Soil pH levels were higher in the Kura clover-continuous corn (CC-KC-69) system when compared to the continuous corn control (CC-192) and cereal rye-continuous corn (CC-CR-192) treatments. Baseline CEC levels were lower in the Kura clover-continuous corn (CC-KC-69) system when compared to the cereal rye-continuous corn (CC-CR-192) treatments, however they were not markedly different than the continuous corn control (CC-192). These findings are similar to those discussed in the deep core soil tests. As evidence from the soil test data, these cropping systems had generally similar baseline soil test results which were all conducive to sufficient corn and soybean growing conditions (Gerber et al., 2012; Greve, 2019). This is likely due to previously routine soil fertility maintenance at the WQFS field site.

After the introduction of cover crops into the novel BFF treatments, soil samples were again taken and analyzed in the fall of 2018 and 2019 (Table 2.12). These soil test results showed that soil OM remained relatively unchanged across all treatments. Soil P levels remained relatively unchanged in the three continuous corn systems as well. It is noteworthy to mention the cereal rye-continuous corn (CC-CR-192) treatment received P fertilizer (67.2 kg P ha^{-1}) in May of 2018 as part of routine fertility maintenance on the Purdue WQFS site. Soil K content slightly decreased over time within the continuous corn control (CC-192) treatment. Soil Mg and Ca content decreased in all three of these systems. Soil pH was relatively constant among all three systems, while CEC showed a slow decrease over time for both the control and two treatments. Although small changes were observed all of these soil test levels are considered adequate for corn and soybean growth (Gerber et al., 2012).

As previously mentioned, routine annual soil sampling from the 0 to 20 cm depth occurred in fall of 2017 and was repeated in fall of 2018 and 2019 in order to monitor soil fertility using the recommended sampling depth for Indiana. In 2017, soil test results of the soybean/corn control (SC-170) and cereal rye-soybean/corn (SC-CR-148) were similar (Table 2.12). When comparing the corn/soybean control (CS-170) and cereal rye-corn/soybean (CS-CR-148) systems, soil pH was relatively similar in 2017 when compared to the control. Again, these levels are considered suitable for the proper growth of corn and soybeans in accordance with local extension guidelines (Gerber et. al, 2012).

In both years following the introduction of cover crops soil organic matter content stayed relatively unchanged between the soybean/corn control (SC-170) and cereal rye-soybean/corn (SC-CR-148) systems as well as between the corn/soybean control (CS-170) and cereal rye-corn/soybean (CS-CR-148) systems (Table 2.12). Soil P levels were not found to be substantially different among the soybean/corn control (SC-170) and cereal rye-soybean/corn (SC-CR-148) systems after the introduction of cereal rye cover crops. There was no significant difference in soil P between the cereal rye-corn/soybean rotation (CS-CR-148) when compared to the corn/soybean control system (CS-170) in 2017, prior to cover crop introduction. Soil K, Mg, and Ca levels slightly decreased with time across all four rotational systems and there were no substantial differences between the soybean/corn control (SC-170) and cereal rye-soybean/corn (SC-CR-148) systems or the corn/soybean control (CS-170) and cereal rye-corn/soybean (CS-CR-148) systems. These reported parameters (Table 2.12) are all considered to fall within the “maintenance” nutrient regime for the species included in this project (Vitosh et al., 1995), however it is important to note that soil K levels were nearing a level that may be considered somewhat low. Soil pH was not noticeably changed among the soybean/corn control (SC-170) and cereal rye-soybean/corn (SC-CR-148) systems after the introduction of cereal rye cover crops. Soil pH remained relatively constant over time in the corn/soybean rotational control (CS-170) as well as the cereal rye-corn/soybean (CS-CR-148) treatment. Soil CEC in the soybean/corn control (SC-170) somewhat decreased, while somewhat increased in the cereal rye- soybean/corn (SC-CR-148) system which lessened the minute gap between the two systems. Soil CEC in the corn/soybean control (CS-170) and cereal rye-corn/soybean (CS-CR-148) systems were not noticeably different after the introduction of cover crops. Although small numerical changes in these aforementioned soil attributes can be noted, overall, there were no substantial changes in soil test values after the introduction of these novel cropping systems. This is not entirely surprising due to the fact that these novel cropping systems were only "fully implemented" for two full growing seasons. Among all soil attributes, levels are considered adequate (Gerber et. al) and consistent with previous field site-specific literature (Greve, 2019).

Table 2.12. Annual fall soil test analyses as influenced by cropping system. Cores (0 to 20 cm) were randomly taken from nine locations in each plot. Data represent the mean ± standard error of four replications. Background shading used to differentiate between treatments for ease of observation.

Table 2.12 continued

Year	Cropping System λ	Plots Sampled	O.M. †	P	K	Mg	Ca	Soil pH	CEC§
			g kg-1	mg kg-1	mg kg-1	mg kg-1	mg kg-1	NA	meq 100 g-1
2017	(CC-192)	3,21,31,41	47±3	30±8	159±9	709±16	3165±95	5.9±0.2	27.2±1.4
2018	(CC-192)	3,21,31,41	48±3	30±8	153±5	714±20	3248±177	6.0±0.1	27.4±1.5
2019	(CC-192)	3,21,31,41	48±6	37±10	128±5	668±25	2909±136	5.8±0.1	25.9±1.4
2017	(CC-CR-192)	12,23,30,46	49±2	25±4	144±9	791±28	3453±145	5.9±0.2	30.2±1.6
2018	(CC-CR-192)	12,23,30,46	51±3	28±4	137±7	769±40	3377±155	6.0±0.1	28.8±1.3
2019	(CC-CR-192)	12,23,30,46	48±4	37±10	128±5	668±25	2909±136	5.8±0.1	25.9±1.4
2017	(CC-KC-69)	6,16,29,39	41±2	22±4	138±9	773±27	3193±122	6.4±0.1	25.8±1.0
2018	(CC-KC-69)	6,16,29,39	45±2	21±3	144±7	770±32	3152±204	6.4±0.2	25.4±1.5
2019	(CC-KC-69)	6,16,29,39	42±3	33±9	130±8	741±23	2970±147	6.5±0.2	23.3±1.7
2017	(SC-170)	8,20,27,47	45±5	21±5	158±9	829±35	3565±194	6.2±0.2	29.0±1.6
2018	(SC-170)	8,20,27,47	47±4	21±5	152±8	797±21	3441±174	6.3±0.2	27.7±1.8
2019	(SC-170)	8,20,27,47	47±4	28±6	133±7	756±34	3182±204	6.4±0.3	25.6±1.4
2017	(SC-CR-148)	9,19,34,48	43±4	23±7	155±10	791±42	3388±233	6.4±0.3	21.6±5.4
2018	(SC-CR-148)	9,19,34,48	45±3	21±6	145±5	766±28	3347±140	6.5±0.3	26.5±1.4

Table 2.12 continued

2019	(SC-CR-148)	9,19,34,48	45±4	26±7	131±8	750±28	3166±141	6.6±0.3	24.5±1.5
2017	(CS-170)	5,13,35,40	42±4	25±7	139±5	739±55	3136±217	6.1±0.1	26.1±1.8
2018	(CS-170)	5,13,35,40	45±3	24±5	136±8	694±46	3055±233	6.2±0.2	20.2±5.0
2019	(CS-170)	5,13,35,40	42±3	29±6	116±10	672±44	2843±220	5.9±0.1	24.9±1.6
2017	(CS-CR-148)	2,14,33,45	40±3	16±3	149±2	776±39	3254±111	6.5±0.2	25.8±1.1
2018	(CS-CR-148)	2,14,33,45	42±3	14±3	140±8	762±45	3131±160	6.7±0.3	24.3±1.4
2019	(CS-CR-148)	2,14,33,45	39±3	18±2	122±7	757±39	3087±137	6.6±0.1	23.9±1.2

† Organic matter percentage converted to g kg^{-1}, § Cation Exchange Capacity,† Cropping systems abbreviated as: CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1}), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1}), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted first, receiving 148 kg N ha^{-1} during corn years), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted first, receiving 148 kg N ha^{-1} during corn years), CC-192 (Tilled, continuous corn receiving 192 kg N ha^{-1}), CS-170 (Tilled corn/soybean rotation, corn planted first, receiving 170 kg N ha^{-1} during corn years), SC-170 (Tilled, soybean/corn rotation, soybean planted first, receiving 170 kg N ha^{-1} during corn years).

Fall soil samples (0 to 20 cm) were also analyzed for total N concentration (Table 2.13). Across all treatments and years, soil N concentration ranged from 1.64 g kg^{-1} in the cereal rye-corn/soybean (CS-CR-148) treatment in 2017 to 2.26 g kg^{-1} in the cereal rye/continuous corn (CC-CR-192) system in 2019. Based on these results, within-system soil N concentrations were generally the same. Between year means for soil N concentration ranged from 2.13 to 2.16 g kg^{-1} for the cereal rye/continuous corn (CC-CR-192) system, 1.70 to 1.96 g kg^{-1} for the Kura clover/continuous corn (CC-KC-69) system, 1.71 to 1.93 g kg^{-1} for the corn/soybean control (CS-170), 1.88 to 2.01 g kg^{-1} for soybean/corn control (SC-170), 1.64 to 1.78 g kg^{-1} for cereal rye/continuous corn (CC-CR-192), 1.77 to 1.93 g kg^{-1} for cereal rye-soybean/corn system (SC-CR-148), and 1.97 to 2.12 g kg^{-1} for continuous corn control (CC-192).

When comparing like systems, soil N content was observed to be noticeably different between the Kura clover/continuous corn (CC-KC-69) which averaged 1.82 g kg^{-1} across experimental years and the cereal rye/continuous corn (CC-CR-192) and continuous corn control (CC-192) systems which averaged 2.21 and 2.06 g kg^{-1} respectively. It is important to note the initial low soil N concentration within the Kura clover/continuous corn (CC-KC-69) may be attributed to no fertilizer N input into these plots in the Kura establishment year (2017). This is in agreement with similar studies which found Kura clover can and will scavenge N if available in the soil (Albrecht et al., 2009). The years following 2017 within this system showed slightly increased levels of soil N concentrations. Soil N concentrations among rotational systems were also observed. Averaged across years, soil N concentrations in the corn/soybean rotation (CS-170) were similar to those in cereal rye-corn/soybean (CS-CR-148) treatment. There were no noteworthy differences in soil N concentration between the soybean/corn rotation (SC-170) and the companion cereal rye-soybean/corn rotation (SC-CR-148) treatments. It is important to note that within systems across years, soil N concentrations appeared to be equal or higher following the soybean crop rather than corn. Similar site-specific findings have been highlighted which suggest that high residual NO_3^- accumulation following corn can have a carryover effect throughout the fall into the following spring prior to the period to which soybean N demand typically occurs. This can result in higher soil N concentrations in soybean crop years (Hernandez-Ramirez et al., 2011). Furthermore, our results agree with others who have documented that the inclusion of soybean into rotation can increase soil N concentration via N fixation and rapid mineralization of soybean stover (Ding et al., 1998; Gentry et al., 2001). These same impacts are cited as means of increasing soil N through the inclusion of Kura clover (Albrecht et al., 2009).

Table 2.13. N concentrations from fall soil samples. Nine cumulative cores were taken from each plot in random locations at a depth of 0-20 cm. Data in the following table are mean ± standard error across the four replications per each treatment. Background shading denotes soybean years in rotational cropping systems.

	Cropping System†						
Year	(CC-192)	(CC-CR-192)	(CC-KC-69)	(CS-170)	(SC-170)	(CS-CR-148)	(SC-CR-148)
	------------------- Soil N g kg^{-1} -------------------						
2017	1.97±0.19	2.13±0.17	1.70±0.12	1.71±0.20	1.88±0.21	1.64±0.15	1.77±0.18
2018	2.09±0.17	2.24±0.13	1.96±0.13	1.93±0.18	2.01±0.19	1.78±0.14	1.90±0.19
2019	2.12±0.20	2.26±0.16	1.82±0.15	1.82±0.16	1.99±0.18	1.70±0.13	1.93±0.20
Trt Ave.	2.06±0.10	2.21±0.08	1.82±0.08	1.82±0.10	1.96±0.10	1.71±0.08	1.87±0.10

† Cropping systems abbreviated as: CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1}), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1}), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted first, receiving 148 kg N ha^{-1} during corn years), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted first, receiving 148 kg N ha^{-1} during corn years), CC-192 (Tilled, continuous corn receiving 192 kg N ha^{-1}), CS-170 (Tilled corn/soybean rotation, corn planted first, receiving 170 kg N ha^{-1} during corn years), SC-170 (Tilled, soybean/corn rotation, soybean planted first, receiving 170 kg N ha^{-1} during corn years).

2.3.3 Corn/Soybean Grain Yield

Grain yields of corn and soybeans were evaluated separately. Mean corn grain yield across all years and treatments ranged from 0.9 to 13.3 Mg ha^{-1} (Figure 2.1). In 2017, prior to the introduction of cover crops, mean corn grain yields ranged from 10.9 to 12.3 Mg ha^{-1} in corn/soybean control (CS-170) and cereal rye/continuous corn (CC-CR-192) treatments respectively (this range does not include the CC-KC-69 treatments as 2017 was the establishment year for Kura clover and no corn crop was sown). In 2018, the first year following cover crop introduction, mean corn grain yields ranged from 6.4 to 13.3 Mg ha^{-1} in the Kura clover/continuous corn (CC-KC-69) and cereal rye/continuous corn (CC-CR-192) treatments respectively. In 2019, mean corn grain yields ranged from 0.9 to 11.3 Mg ha^{-1} in the Kura clover/continuous corn (CC-KC-69) and cereal rye/continuous corn (CC-CR-192) treatments respectively.

The general linear model and ANOVA for corn grain yield determined that the independent factors of Year and Cropping System as well as the Year x Cropping System interaction were significant ($P<0.001$) (Figure 2.1). The significant Year effect is expected with year-to-year variation in temperature and precipitation (Table 2.1 and Table 2.2), while the significant Year x Cropping System interaction results from the substantial year-to-year differences in grain yields of the Kura clover/continuous corn (CC-KC-69) treatment that did not occur with the other continuous corn treatments (CC-192 and CC-CR-148). Post-hoc analysis using the Tukey-Kramer HSD pairwise comparisons revealed no significant differences among treatments on corn grain yield in 2017 (baseline year). In the 2018 and 2019 growing seasons, there was a significant ($P \leq 0.001$) difference between the continuous corn control (CC-192) and cereal rye/continuous corn (CC-CR-192) systems when both were compared to the Kura clover/continuous corn (CC-KC-69) system. However, grain yields of the continuous corn control (CC-192) and cereal rye/continuous corn (CC-CR-192) treatments were similar both years. Likewise, inclusion of the rye cover crop did not alter corn grain yield in the corn-soybean rotation systems (Figure 2.1). Corn grain yields among the soybean/corn control (SC-170) and cereal rye-soybean/corn treatment (SC-CR-148) were also not significantly different during the 2018 and 2019 growing seasons.

As stated previously, the main risk of incorporating cover crops into corn production systems is grain yield reduction. Our results indicated that the incorporation of cereal rye into a continuous corn cropping system did not significantly reduce corn grain yield. Previous studies suggest that documented yield depression has been attributed to several factors including allelopathy, soil moisture depletion, and soil N depletion (Feyereisen et al., 2013). The timing of rye termination plays a large role in these processes as well. Delayed termination of rye until heading in this study could allow for increased landscape-level water productivity and nutrient use efficiencies, a concept that had also been cited as positive attribute of cereal rye cover crops (Meisinger et al., 1991). For example, in regard to the negative effects of excess precipitation in early springtime of 2018 (Table 2.1) we speculate these potential negative impacts of cereal rye inclusion may have been outweighed by the benefit of cereal rye utilizing excess water which may have enabled earlier access to field operations. Although not statistically significant, corn grain yields were lower in 2019 when planting was delayed until early June and precipitation in summer months was reduced (Table 2.1). Although soil moisture data was not directly analyzed in this study, previous studies have highlighted instances where soil moisture was depleted and grain yields were reduced with the incorporation of cereal rye, especially when rye termination was delayed and harvested as grain (Krueger et al. 2011). It is crucial to note that this decrease in corn grain yield in the cereal rye-corn/soybean rotation treatment (CS-CR-148) in this case is likely not an outcome of changes in soil moisture. It is imperative to note that the comparison between the cereal rye-corn/soybean rotation treatment (CS-CR-148) and the corn/soybean control (CS-170) although similar, do include different N rates. Since there was not a statistically significant reduction in yield for the cereal rye continuous corn (CC-CR-192) treatment as compared to the continuous corn control (CC-192), the data suggest that a reduction in available N in the cereal rye-corn/soybean rotation treatment (CS-CR-148) may be a more prominent factor for the reduction in grain yield as opposed to other factors such as soil moisture depletion.

Adopting Kura clover into corn production as a PLM species has its own set of challenges. Our results contradict results found by Zemenchik et al. (2000) which determined that a Kura clover PLM incorporated into corn production and adequately suppressed with herbicides is viable, and causes little reduction in grain or whole-plant yields of corn. It is important to note that the most successful corn grain yields in the study conducted by Zemenchik et al. (2000) were those where Kura clover was completely terminated rather than just suppressed or band-killed, methods that are more similar to those used in this study. These differences may be justification for the differences in observations. However, other studies (Affeldt et al., 2004; Sawyer et al., 2010) indicate that Kura clover as a PLM reduced corn grain yields by decreasing corn plant populations and delaying plant development, especially during cool and wet springtime conditions. These inherent challenges are similar to those observed in this study. It is important to note that both these previously mentioned studies as well as this study did not physically harvest the Kura clover biomass which would act as an additional mode of suppression that may limit the competition between the corn and Kura clover crops. These pitfalls hold true to those found in this study. Although not statistically analyzed, corn plant populations were somewhat reduced in both springs following planting. Reduction in plant population is another driver in the observed reduction in corn grain yields. In addition, studies (Ziyomo et al., 2013) indicate that Kura clover may reduce corn yield especially in water-limited circumstances similar to those of the 2019 growing season of this study (Table 2.1).

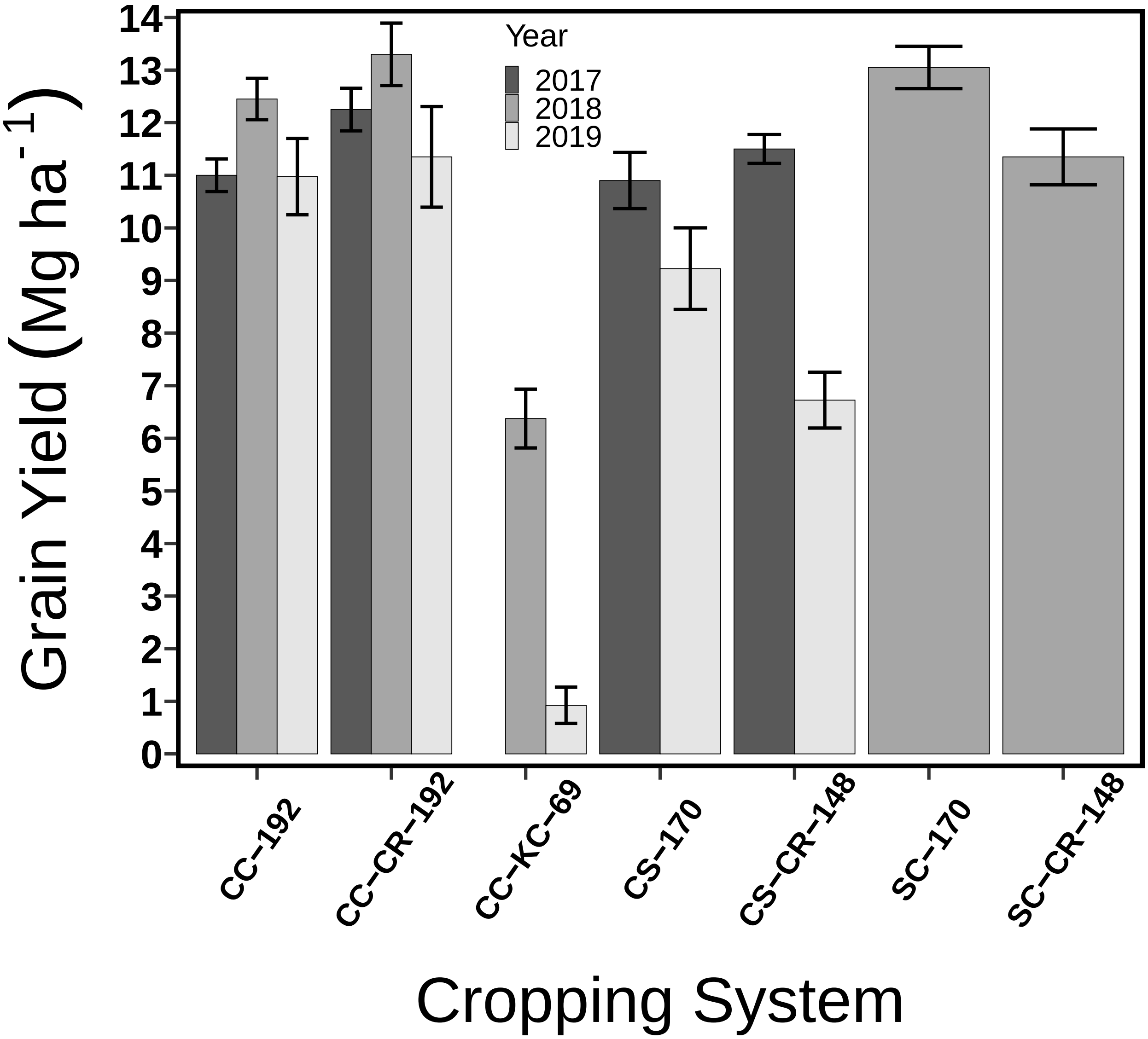

Figure 2.1. Mean ± standard error corn grain yields across years and as influenced by cropping system. Data are presented across the three study years. Corn grain yield values are presented at 15.5% moisture.

Soybean grain yield across all years and treatments ranged from 2.6 to 3.8 Mg ha^{-1} (Figure 2.2). In 2017, prior to the introduction of cover crops, soybean grain yields ranged from 3.7 to 3.8 Mg ha^{-1} in soybean/corn rotation control (SC-170) and rye-soybean/corn (SC-CR-148) treatments respectively. In 2018, the first year following cover crop introduction, soybean grain yields ranged from 3.1 to 3.5 Mg ha^{-1} in the cereal rye-corn/soybean rotation (CS-CR-148) and the corn/soybean control (CS-170) treatments, respectively. Soybean grain yields in 2019 ranged from 2.6 to 2.9 Mg

ha^{-1} in the cereal rye-soybean/corn (SC-CR-148) and soybean/corn control (SC-170) treatments, respectively.

Analysis of variance (ANOVA) revealed that the Year effect was significant, and the Cropping System and the Year x Cropping System interaction effects were not significant. As previously stated, weather differences (Table 2.1 and Table 2.2) of these years would be expected to impact grain yields. As an example, years such as 2017 which offered milder temperatures and more summer precipitation which are more conducive to higher grain yields as compared to years such as 2019 where growing season temperatures were higher and precipitation later in the season was limited. Our results agree with findings of others who have reported that soybean grain yield was not significantly affected when cereal rye was added to rotational cropping systems (Ruffo et al., 2004). It is important to note that the study conducted by Ruffo et al. (2004) did not allow cereal rye to reach the heading growth stage and the cereal rye biomass was killed using herbicides rather than being harvested such as in this study. In soybean-rye cover crop systems, soil moisture depletion and allelopathy could limit growth and yield as in corn (Feyereisen et al., 2013), but because soybeans can fix their own N, depletion of soil N by the rye would be of less concern. Weather conditions were cited as the largest drivers of soybean grain yield and thus explains the lack of soybean grain yield reduction following the introduction of cereal rye (Ruffo et al., 2004 and sources therein). Furthermore, these results highlighting no significant reduction in soybean yields may be attributed to the indeterminant nature of soybeans which allows them to flourish even in somewhat shortened growing seasons.

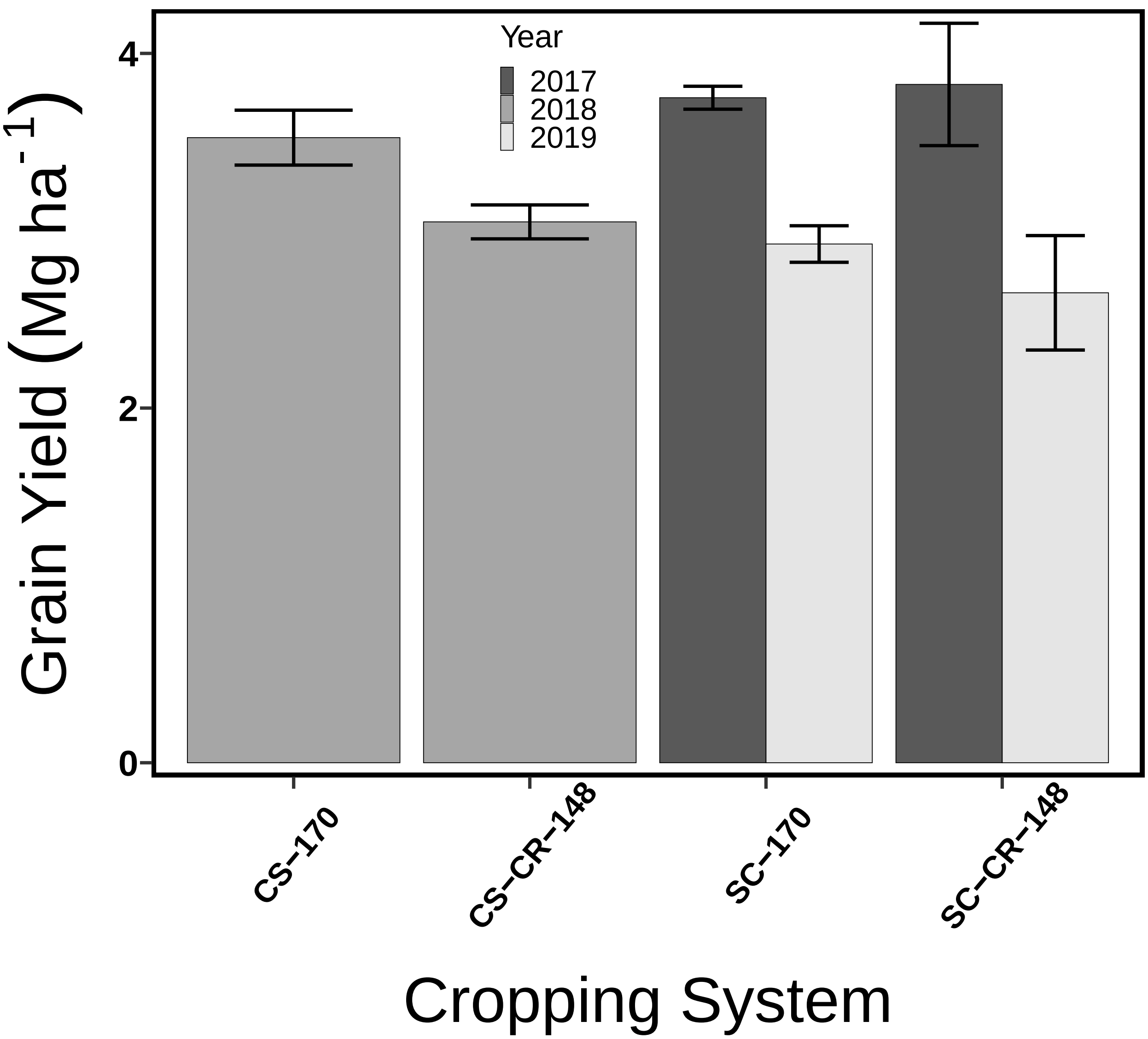

Figure 2.2. Mean ± standard error soybean grain yields across years and as influenced by cropping systems. Data are presented across the three study years. Soybean grain yield values are presented at 13% moisture.

2.3.4 Corn and Soybean Grain N Concentrations

Statistical analyses were conducted on corn and soybean grain N concentrations separately. Corn grain N concentration across all years and treatments ranged from 10.4 to 13.4 g N kg^{-1} dry matter (DM) (Table 2.14). Analysis of variance (ANOVA) revealed significant effects of Cropping System and Year, but no Year x Cropping System interaction. Corn grain was not available for analysis in 2017 for the Kura clover/continuous corn (CC-KC-69) system as this was the establishment year for the clover living mulch. Post-hoc analysis using the Tukey-Kramer HSD pairwise comparisons revealed there were no significant differences of corn grain N concentrations across all years and within all cropping systems of the pairwise comparisons that are of interest. It is important to note that there were significant pairwise comparisons among some treatment comparisons, however they were not among “like systems” and are of limited to no interest in this study. Furthermore, these significant pairwise comparisons of the non-interest comparisons are likely the driver for the significant independent factors from the overall ANOVA. All grain N concentrations were considered adequate as confirmed by a study conducted by Brouder et al. (2000). A study conducted by Vyn et al. (2000) found non-legume cover crops such as wheat and cereal rye did not increase the N availability to succeeding corn crops. However, Ahmadi et al. (1993) found that decreases in corn grain N concentration did not directly correlate to decreased grain yields and that typically grain N concentration was conserved when other portions of the plant, such as the leaf material, were often affected first.

Mean soybean grain N concentration across all years and treatments ranged from 56.4 to 62.6 g N kg^{-1} dry matter (DM) (Table 2.14). Analysis of variance (ANOVA) revealed that the Cropping System main effect on soybean grain N concentration was significant, whereas the Year and Year x Cropping System interaction effects were not significant. Post-hoc analysis using the Tukey-Kramer HSD pairwise comparisons revealed there was significantly higher soybean grain N concentrations in the cereal rye-corn/soybean (CS-CR-148) treatment as compared to the corn/soybean rotational control (CS-170). It is important to note that this is likely due to a growth dilution effect as the corn/soybean rotational control (CS-170) out yielded the cereal rye-corn/soybean (CS-CR-148) treatment (Figure 2.2) as opposed to an effect from the addition of cereal rye. There is little known literature on the effects Kura clover PLM and cereal rye cover crop on grain N content in soybeans.

Table 2.14. Grain N concentrations for corn and soybeans as influenced by cropping system. Data are means ± standard errors of four replications. Background shading denotes soybean years in rotational cropping systems.

	Cropping System†						
Year	(CC-192)	(CC-CR-192)	(CC-KC-69)	(CS-170)	(SC-170)	(CS-CR-148)	(SC-CR-148)
	---------- N concentration g kg^{-1} ----------						
2017	11.2±0.4	11.1±0.3	NAλ	10.7±0.3	56.9±1.3	10.8±0.5	56.4±1.2
2018	12.8±0.3	12.5±0.5	12.1±0.2	58.2±1.1	12.2±0.1	62.6±0.7	13.4±0.4
2019	12.2±0.2	11.6±0.9	11.0±0.5	11.7±0.1	57.5±0.6	10.4±0.4	59.2±0.4
Corn Ave.	12.1±0.3	11.7±0.4	11.6±0.3	11.2±0.2	12.2±0.1	10.6±0.3	13.4±0.4
Soy Ave.	NA	NA	NA	58.2±1.1 **B**	57.2±0.7	62.6±0.7 **A**	57.8±0.8

λ denotes the establishment year of Kura Clover where no summer annual was planted/harvested. ϑ denotes that yield from this treatment was derived from 10 ear samples as opposed to combine yield data. This is due to the fact that this treatment is harvested as a residue removal plot. † Cropping systems abbreviated as: CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1}), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1}), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted first, receiving 148 kg N ha^{-1} during corn years), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted first, receiving 148 kg N ha^{-1} during corn years), CC-192 (Tilled, continuous corn receiving 192 kg N ha^{-1}), CS-170 (Tilled corn/soybean rotation, corn planted first, receiving 170 kg N ha^{-1} during corn years), SC-170 (Tilled, soybean/corn rotation, soybean planted first, receiving 170 kg N ha^{-1} during corn years).

2.3.5 Corn and Soybean Stover Yields

Similar to corn and soybean grain yield, corn and soybean stover yields were statistically analyzed separately. Analysis of variance (ANOVA) indicated that the main effects of Cropping System, Year, and the Cropping System x Year interaction were significant (P<0.01) for corn stover biomass yields. Post-hoc analysis using the Tukey-Kramer HSD pairwise comparisons revealed there were no significant differences among corn stover biomass yields in the 2017 growing season (Table 2.15). In the year 2018 corn stover yield was significantly ($P \leq 0.05$) lower in the Kura clover/continuous corn (CC-KC-69) system when compared to the continuous corn control (CC-192). However, cereal rye did not significantly depress corn stover yield in both the continuous corn or rotational systems in 2018. In 2019, corn stover yield was significantly ($P \leq 0.05$) lower in the Kura clover/continuous corn (CC-KC-69) system when compared to the continuous corn control (CC-192) as well as the cereal rye continuous corn (CC-CR-192) system. The negative effect that Kura clover seemed to induce on corn stover yields in both 2018 and 2019 is likely due to competition between the Kura clover PLM and the corn crop. Competition among these two species may have been heightened in years such as 2019 where wet springtime conditions would have enabled Kura clover to thrive even with suppression and outcompete the corn crop, a trend observed in numerous studies (Zemenchik et al., 2000; Affeldt et al., 2004; Sawyer et al., 2010). This rough spring start followed by summer months with decreased precipitation (Table 2.1) may have limited the corn's ability to accrue stover biomass. Similar to 2018, 2019 did not exhibit depressed corn stover yields following the introduction of cereal rye for either the continuous corn or rotational systems. Previous studies determined that decreased corn stover yields following cereal rye can be attributed to decreased soil N availability due to the cover crop scavenging available N and converting it to cover crop biomass accumulation (Crandall et al., 2005). However, other studies have highlighted a positive relationship between adopting cereal rye cover crops and subsequent corn crop biomass yields, stating that cover crop residues decompose and provide available soil organic N which can become available for the corn crop (Kuo and Jellum, 2000). It is important to note that in the previously mentioned study these effects were observed over the span of a nine-years as compared to this study which only observed the effect of cereal rye for two growing seasons. In addition, this study terminated the cereal rye cover crop leaving it in situ whereas in this study cereal rye biomass was harvested off.

The ANOVA for soybean stover biomass yield determined that only the Cropping System main effect was significant. Averaged across years, soybean stover biomass yields were significantly higher in the corn/soybean (CS-170) control as compared to the cereal rye corn/soybean (CS-CR-148) treatment. Although not statistically analyzed, soybean plant populations tended to be decreased following cereal rye. This may be due to challenges planting soybeans into cereal rye stubble using somewhat outdated planting equipment. However, it is unclear if more modern equipment would have diminished this effect entirely or if this is an inherent challenge when planting soybeans following cereal rye that has been allowed to grow to the heading growth stage. Previous studies such as Eckert (1988), highlighted decreases in soybean stand being the largest contributors to decreased soybean plant biomass yields. One important disparity to highlight however is that most studies which observe the effects of soybean biomass yields following cereal rye do not harvest the rye biomass off therefor leaving it in situ. Among controls, soybean stover biomass yields were significantly higher in the corn/soybean (CS-170) control as compared to the soybean/corn (SC-170) control. It is important to note that this comparison of the rotational controls is not entirely objective, due to the fact that a soybean crop was only present in one of these systems during each growing season.

Table 2.15. Stover biomass yield from corn and soybean as influenced by cropping system. Data are the mean ± standard error of four replications. Background shading denotes soybean years in rotational cropping systems. Means followed by the same letter are not significantly different at P<0.05 using a Tukey-Kramer HSD test.

	Cropping System†						
Year	CC-192	(CC-CR-192)	(CC-KC-69)	(CS-170)	(SC-170)	(CS-CR-148)	(SC-CR-148)
	---------- Mg dry matter ha^{-1} ----------						
2017	7.1±0.4	7.2±0.2	NAλ	7.0±0.2	6.4±0.8	7.4±0.7	6.1±0.7
2018	10.2±0.2	8.5±0.3	6.9±0.5	12.5±1.4	9.7±0.6	7.1±0.4	8.3±0.9
2019	7.1±0.4	6.4±0.5	2.3±0.7	7.1±0.2	8.2±0.6	5.7±0.6	6.8±0.5
Corn. Ave.	8.2±0.5	7.4±0.3	4.6±1.0	7.1±0.1	9.7±0.6	6.6±0.5	8.3±0.9
Soy Ave.	NA	NA	NA	12.5±1.4	7.3±0.6	7.1±0.4	6.5±0.4

λ denotes the establishment year of Kura Clover where no summer annual was planted/harvested.

† Cropping systems abbreviated as: CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1}), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1}), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted first, receiving 148 kg N ha^{-1} during corn years), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted first, receiving 148 kg N ha^{-1} during corn years), CC-192 (Tilled, continuous corn receiving 192 kg N ha^{-1}), CS-170 (Tilled corn/soybean rotation, corn planted first, receiving 170 kg N ha^{-1} during corn years), SC-170 (Tilled, soybean/corn rotation, soybean planted first, receiving 170 kg N ha^{-1} during corn years.

2.3.6 Corn and Soybean Stover N Content

Corn and soybean stover N content was statistically analyzed separately. Analysis of variance (ANOVA) indicated that the main effects of Cropping System, Year, and the Cropping System x Year interaction were significant (P<0.01) for corn stover N content. Post-hoc analysis using the Tukey-Kramer HSD pairwise comparisons revealed there were no significant differences in corn stover N content in the 2017 growing season (Table 2.16). In 2018, corn stover N content was significantly ($P \leq 0.05$) higher in the continuous corn control (CC-192) when compared to the Kura clover/continuous corn (CC-KC-69), cereal rye continuous corn (CC-CR-192), and soybean/corn (SC-170) systems. In 2019, corn stover N content was similar for all control or control/treatment comparisons. The disparities observed in total stover N content are likely driven by differences in overall corn biomass production as discussed previously (Table 2.15).

Analysis of variance (ANOVA) indicated that the main effects of Cropping System and Year significantly (P<0.01) affected soybean stover N content while the Cropping System x Year interaction did not. Averaged across years, soybean stover N content was significantly higher in the corn/soybean (CS-170) control as compared to the cereal rye corn/soybean (CS-CR-148) treatment. This is likely due to the differences in overall biomass production as previously discussed (Table 2.15). Likewise, soybean stover N content was significantly higher in the corn/soybean (CS-170) control as compared to the soybean/corn (SC-170) control. It is important to note that this comparison is not entirely reasonable due to the fact that a soybean crop was only present in one of these systems each year. Therefore, any affect that year may have had on stover N content such as differences within the growing season, may have been the reason for this disparity.

Table 2.16. Stover N content from corn and soybean as influenced by cropping system. Data are the mean ± standard error of four replications. Background shading denotes soybean years in rotational cropping systems. Means followed by the same letter are not significantly different at *P*<0.05 using a Tukey-Kramer HSD test.

	Cropping System†						
Year	CC-192	(CC-CR-192)	(CC-KC-69)	(CS-170)	(SC-170)	(CS-CR-148)	(SC-CR-148)
	---kg N ha^{-1}---						
2017	58.1±4.1	59.2±1.3	NAλ	59.7±3.6	157.9±20.5	60.7±4.1	159.9±17.6
2018	131.2±2.7	83.4±7.3	58.3±8.7	325.1±33.0	92.2±9.5	191.5±16.1	75.4±12.4
2019	47.3±5.5	47.9±5.5	17.8±5.0	50.0±2.3	230.7±11.8	40.5±4.6	201.9±15.4
Corn. Ave.	78.8±11.5	63.5±5.3	38.0±9.0	54.8±2.7	92.2±9.5	50.6±4.8	75.4±12.4
Soy Ave.	NA	NA	NA	325.1±33.0	194.3±17.6	191.5±16.1	180.9±13.4

λ denotes the establishment year of Kura Clover where no summer annual was planted/harvested.

† Cropping systems abbreviated as: CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1}), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1}), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted first, receiving 148 kg N ha^{-1} during corn years), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted first, receiving 148 kg N ha^{-1} during corn years), CC-192 (Tilled, continuous corn receiving 192 kg N ha^{-1}), CS-170 (Tilled corn/soybean rotation, corn planted first, receiving 170 kg N ha^{-1} during corn years), SC-170 (Tilled, soybean/corn rotation, soybean planted first, receiving 170 kg N ha^{-1} during corn years.

2.3.7 Corn and Soybean Stover N Concentrations

Similar to corn and soybean grain yield, corn and soybean stover N concentrations were statistically analyzed separately. Analysis of variance (ANOVA) indicated that the main effects of Cropping System, Year, and the Cropping System x Year interaction were significant ($P<0.001$) for corn stover N concentrations. Post-hoc analysis using the Tukey-Kramer HSD pairwise comparisons revealed there were no significant differences among corn stover N concentrations in the 2017 growing season (Table 2.17). In 2018 corn stover N concentration was significantly ($P \leq 0.05$) lower in the cereal rye/continuous-corn treatment (CC-CR-192) treatment when compared to the continuous corn control (CC-192). Likewise, corn stover N concentration was significantly ($P \leq 0.05$) lower in the Kura clover/continuous corn (CC-KC-69) system when compared to the continuous corn (CC-192) control. In 2019, there were no significant differences in corn stover N concentration among like-systems. These results are in agreement with previous studies that highlighted later cereal rye kill dates, similar to those in this study, can result in low corn plant N content (Crandall et al., 2005). There are no published reports on the potential effects Kura clover has on corn stover N concentrations.

The ANOVA for soybean stover N concentration determined that only the Year main effect was significant. Large differences in weather among years (Table 2.1 and Table 2.2) is likely a factor underpinning these effects. Disparities among years like 2017 where precipitation (Table 2.1) and temperature (Table 2.2) were milder during the growing season, as compared to years like 2019 where average temperatures were higher (Table 2.2) and water was limited during the growing season (Table 2.1) are likely the drivers at play. Previous research found that hot and dry conditions decreased soybean stover N concentration as much as 30-40% (Streeter et al., 2003).

Overall, these results suggest that the inclusion of cereal rye and Kura clover resulted in significantly decreased stover N concentrations only in corn and only in some years. It is also important to note that corn stover N concentration appeared to decrease as planting date was delayed. For instance, in 2018 corn stover N concentrations were found to be higher in the continuous corn control (CC-192) when compared to the cereal rye/continuous-corn treatment (CC-CR-192) treatment which has a lag in planting date by 14 days. Corn planting in the cereal rye/continuous-corn treatment (CC-CR-192) treatment was delayed due to the growth of the cereal rye which was allowed to reach heading before being harvested. With similar instances being cited in literature, this 14-d planting delay, along with the N-scavenging potential of cereal rye may have depleted soil N and reduced stover N concentrations for corn (Krueger et al., 2011). Soybean stover N concentrations were not significantly affected by inclusion of cereal rye. Furthermore, rotational systems that included cereal rye were less likely to have significantly decreased N concentrations in corn stover. This could be attributed to the N fixation potential and N credit given from the inclusion of soybeans in rotation which in turn could have supplied N to cereal rye leaving more soil N for the subsequent corn crop (Ruffo et al., 2004). We might expect to see a similar legume response in the Kura clover system, however the decrease in corn stover N concentration observed in the Kura clover/continuous corn (CC-KC-69) treatment could instead be due to the much lower N rate applied to this treatment (69 kg N ha^{-1}) as the year-to-year difference within this treatment is marginal.

Table 2.17. Stover N concentrations from corn and soybean as influenced by cropping system. Data are the mean ± standard error of four replications. Background shading denotes soybean years in rotational cropping systems. Means followed by the same letter and within like systems (continuous corn versus rotational systems) are not significantly different at *P*<0.05 using a Tukey-Kramer HSD test.

	Cropping System†						
Year	CC-192	(CC-CR-192)	(CC-KC-69)	(CS-170)	(SC-170)	(CS-CR-148)	(SC-CR-148)
	---g N kg^{-1} dry matter---						
2017	8.2±0.5 **A**	8.2±0.2 **A**	NAλ	8.5±0.5 **B**	24.8±1.0 **C**	8.3±0.5 **B**	26.3±0.9 **C**
2018	12.8±0.3 **A**	9.8±0.6 **B**	8.3±0.7 **B**	26.2±0.5 **C**	9.5±0.7 **D**	27.1±0.8 **C**	9.0±0.9 **D**
2019	6.6±0.4 **A**	7.4±0.5 **A**	7.8±0.5 **A**	7.0±0.3 **B**	28.2±1.1 **C**	7.0±0.2 **B**	29.7±0.8 **C**
Corn. Ave.	9.2±0.8 **A**	8.5±0.4 **A**	8.1±0.4 **A**	7.8±0.4 **B**	9.5±0.7 **B**	7.7±0.3 **B**	9.0±0.9 **B**
Soy Ave.	NA	NA	NA	26.2±0.5 **A**	26.5±0.9 **A**	27.1±0.8 **A**	28.0±0.8 **A**

λ denotes the establishment year of Kura Clover where no summer annual was planted/harvested.
† Cropping systems abbreviated as: CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1}), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1}), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted first, receiving 148 kg N ha^{-1} during corn years), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted first, receiving 148 kg N ha^{-1} during corn years), CC-192 (Tilled, continuous corn receiving 192 kg N ha^{-1}), CS-170 (Tilled corn/soybean rotation, corn planted first, receiving 170 kg N ha^{-1} during corn years), SC-170 (Tilled, soybean/corn rotation, soybean planted first, receiving 170 kg N ha^{-1} during corn years.

2.3.8 Cover Crop Biomass Yields

Cover crop biomass yield across all years and treatments ranged from 2.3 to 5.2 Mg ha^{-1} (Table 2.18). In 2018, the first year of cover crop introduction cover crop biomass yields ranged from 2.7 to 4.2 Mg ha^{-1} in the cereal rye and Kura clover continuous corn systems (CC-CR-192/CC-KC-69) and the cereal rye-corn/soybean rotational treatment (CS-CR-148) respectively. In 2019, mean cover crop biomass yields ranged from 2.3 to 5.2 Mg ha^{-1} in the cereal rye-soybean/corn (SC-CR-148) and Kura clover/continuous corn (CC-KC-69) treatments respectively.

Analysis of variance (ANOVA) revealed a significant Cropping System x Year interaction for cover crop biomass yields. Tukey-Kramer HSD pairwise comparisons indicated no significant differences among any of the treatments on cover crop biomass yields in 2018. However, in the 2019 Kura clover biomass yield of Kura clover/continuous corn (CC-KC-69) system was significantly ($P \leq 0.01$) higher than the rye in the cereal rye/continuous corn (CC-CR-192) and the cereal rye-soybean/corn rotation (SC-CR-148). The disparity of Kura clover yield across the two-year period is not only a likely factor of change in seasonal variation of temperature and precipitation (Table 2.1 and Table 2.2), but also likely due to the greater establishment of Kura clover in 2019. A thin stand due to < 1 year of establishment time accompanied with the first chemical suppression in 2018 may have resulted in decreased Kura biomass yields for the 2018 season. Similar Kura clover stands and biomass accumulation issues regarding Kura clover yields have been found in previous studies. In a study conducted by Zemenchik et al. (2000), Kura clover yields took nearly twelve months to fully recover from herbicide suppression. As previously mentioned, it can take up to three years for Kura clover stands to reach full established potential. Rye biomass yields in the cereal rye-continuous corn (CC-CR-192) system also significantly ($P \leq 0.05$) outperformed rye biomass yield in the cereal rye-soybean/corn rotation (SC-CR-148) (Table 2.18). Rather than a difference originating due to rotational corn/soybean compared to continuous corn, this disparity is most likely attributed to differences in N rate applied in the growing season. Similar responses have been found in literature, citing higher N rates resulting in increased cereal rye biomass yields (Lyons et al., 2019). However, all remaining pairwise comparisons among cover crop treatments were not significantly different. It is notable that Kura clover was only grown in companion with corn so there is no comparison against Kura clover yields based on different cash crops.

Table 2.18. Biomass yields of cover crops as influenced by cropping system. Cereal rye was harvested at anthesis directly before planting the summer annual (corn/soybeans), while Kura clover was harvested prior to the fall corn harvest. Background shading denotes the lone Kura clover cropping system. Data are means ± standard errors of four replications. Means followed by the same letter are not significantly different at $P<0.05$ using a Tukey-Kramer HSD test.

	Cropping System†			
Year	(CC-CR-192)	(CC-KC-69)	(CS-CR-148)	(SC-CR-148)
	------------------------------------ Biomass Yield Mg DM ha^{-1} ------------------------------------			
2018	2.7±0.4	2.7±0.3	4.2±0.9	3.3±0.5
2019	4.4±0.6 **AB**	5.2±0.4 **A**	2.4±0.3 **B**	2.3±0.5 **B**
Trt Ave.	3.5±0.5	4.0±0.5	3.3±0.5	2.8±0.4

† Cropping systems abbreviated as: CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1}), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1}), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted first, receiving 148 kg N ha^{-1} during corn years), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted first, receiving 148 kg N ha^{-1} during corn years).

2.3.9 Cover Crop Biomass N Content

Analysis of variance (ANOVA) revealed significant (P<0.001) Treatment, Year, and Treatment x Year interaction effects for cover crop biomass N content. As discussed before, the significant year effect is not surprising, as previously mentioned, weather and precipitation can affect crop growth from year to year and cause variation. In the case of biomass N content, biomass yield is a major driver. Meaning years such as 2018, with higher precipitation and milder temperatures during the growing season, may be the reason for higher cover crop biomass N content values which result due to more favorable growing season conditions. The Treatment x Year interaction is likely due to the variation in N content found in the Kura clover continuous corn treatment between the two years (Figure 2.3). The variation within this system is no doubt this same byproduct of year-to-year variation in precipitation and temperature (Table 2.1 and Table 2.2), but also due to differences in overall biomass production from year to year (Table 2.18). Noticeable differences in Kura clover biomass were mentioned in the Cover Crop Biomass Yield section (Section 2.3.8) which hinted at differences in yield being attributed largely to minimal establishment time, suppression techniques, and suppression recovery periods, which are patterns consistent with observations found in literature (Zemenchik et al. 2000).

Tukey-Kramer HSD pairwise comparisons indicated statistically significant (P<0.001) differences in both the 2018 and 2019 growing seasons. In 2018, the Kura clover continuous corn (CC-KC-69) treatment had significantly higher cover crop N content when compared to all three cereal rye treatments. In the 2019 growing season Kura clover continuous corn (CC-KC-69) had significantly (P<0.001) higher N content when compared to all three cereal rye systems. However, cereal rye cover crop N content was not significantly different among rotational or continuous corn systems. The difference in overall N content between Kura clover and cereal rye species is by no means surprising. The ability for legumes, such as Kura clover, to convert atmospheric N to organic N via biological N fixation is well documented as the reason legumes tend to have higher N concentration levels than grass species like cereal rye. This is well-documented and explains the disparity among species (Moore, 1974). Current literature suggests that even a thin stand of most clover species can have total legume N content around 56 kg N ha^{-1} and suggest that full establishment of clover stands can have N contents ranging from 160 to 225 kg N ha^{-1} (Jennings, 2010). A similar trend was displayed in these results regarding N content of the Kura clover increasing as establishment time is increased, likely due to increased biomass accumulation.

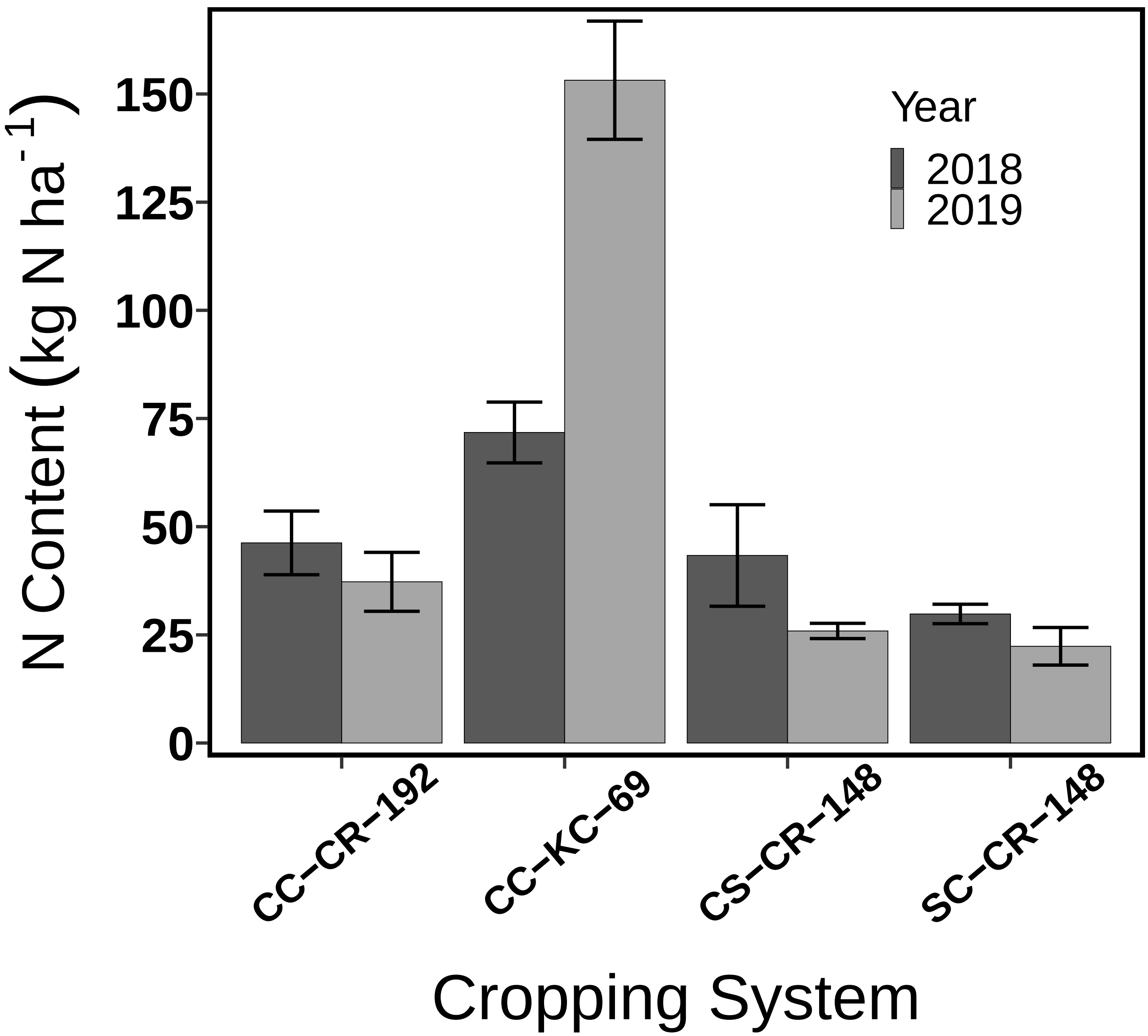

Figure 2.3. Biomass N content of cover crops as influenced by cropping system. Data are means ± standard errors of four replications.

2.3.10 Cover Crop Biomass N Concentrations

Analysis of variance (ANOVA) revealed significant (*P*<0.001) Treatment as well as the Treatment x Year interaction effects for cover crop biomass N concentrations. Tukey-Kramer HSD pairwise comparisons indicated significantly higher cover crop biomass N concentrations in the cereal rye continuous corn (CC-CR-192) treatment as compared to the rye-corn/soybean (CS-CR-148) rotation and cereal rye-soybean/corn (SC-CR-148) rotation in 2018 (Table 2.19). In 2019, cover crop biomass N concentrations among cereal rye cover crops were similar regardless of cropping system. The increased level of biomass N found within the cereal rye-continuous corn treatment in 2018 (CC-CR-192) may have been attributed to higher N rate (192 kg N ha^{-1} applied to the preceding corn crop during the 2017 growing season). This treatment had the largest external supply of N to the system out of all of the cropping systems. This higher N rate could have resulted in a byproduct of unused N from the previous corn crop which fed the scavenging cereal rye crop, something that has been well documented (Ruffo et al., 2004; Eckert, 2013; Patel, 2016; Korucu et al., 2018; Lyons et al., 2019). Kura clover biomass N concentrations from the lone Kura clover treatment (CC-KC-69) were significantly higher than all pairwise comparisons against cereal rye biomass N concentrations regardless of cropping system (Table 2.19). These results are expected when comparing a legume to a cereal (Thomas and Asakawa, 1993).

Table 2.19. Biomass N concentrations of cover crops as influenced by cropping system. Shaded cells denote the Kura clover cropping system. Data are means ± standard errors of four replications. Means followed by the same letter within rows are not significantly different at P<0.05 using a Tukey-Kramer HSD test.

	Cropping System†			
Year	(CC-CR-192)	(CC-KC-69)	(CS-CR-148)	(SC-CR-148)
	---- g N kg^{-1} Dry Matter ----			
2018	17.0±1.5 **A**	26.8±0.4 **B**	10.2±1.0 **C**	9.3±0.9 **C**
2019	8.3±0.5 **A**	29.2±0.9 **B**	11.1±0.9 **A**	9.8±1.0 **A**
Trt Ave.	12.7±1.8	28.0±0.7	10.6±0.7	9.6±0.6

† Cropping systems abbreviated as: CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1}), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1}), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted first, receiving 148 kg N ha^{-1} during corn years), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted first, receiving 148 kg N ha^{-1} during corn years).

2.3.11 Herbage Theoretical Ethanol Yields

Herbage theoretical ethanol yield (HTEY) was statistically analyzed at a cropping system level which included both stover from the cash crop (corn or soybean) along with biomass from the cover crop (cereal rye or Kura clover). It is important to note that not all plant residues were physically removed from plots, however theoretical ethanol yields were calculated assuming both the cash crop (corn or soybean) and cover crop (cereal rye or Kura clover) plant stover/biomass was removed entirely from the field and used for the purpose of ethanol production. Analysis of variance (ANOVA) indicated that Cropping System, Year, and the Cropping System x Year interaction effects significantly (P<0.001) affected total HTEY. The significant effect Year had on HTEY can be explained by the year-to-year variation within season in terms of precipitation and temperature (Table 2.1. and Table 2.2). HTEY is largely driven by biomass production, therefore growing seasons that exhibit patterns conducive to increased biomass yields, such as nominal precipitation and temperature (2018), may lead to improved HTEY production. The Cropping System x Year interaction could be a result of the rotational systems with different crops grown each year being observed over a two-year period. For instance, in the cereal rye-soybean/corn (SC-CR-148) treatment where corn was analyzed in 2018 and soybeans in 2019 (Table 2.20). This significant interaction could also reflect disparities among cover crop HTEY yields which varied from year to year.

Tukey-Kramer HSD pairwise comparisons revealed similar total HTEY for all continuous corn treatments in 2018 (Table 2.20). Likewise, total HTEY values were similar across all rotational corn/soybean treatments in 2018. In 2019, HTEY of the Kura clover continuous corn (CC-KC-69) system was significantly (P<0.001) lower than that of the continuous corn-cereal rye (CC-CR-192) treatment. The disparities of HTEY between these systems is likely driven by differences in the overall biomass production of each system (Table 2.18). Previous studies indicate that biofuel production potential is largely driven by biomass yield (Feyereisen et al., 2013; Tumbalam et al., 2016, Woodson and Jablonowski, 2008), a trend similarly observed in this study. Few published studies have compared biomass production of Kura clover to cereal rye or other cover crops due to the fact that most cover crops are not harvested for biomass (Heggenstaller, 2008). All other pairwise comparisons of the continuous corn systems were not significantly different from one another. Likewise, all pairwise comparisons among the rotational systems in 2019 were not significantly different from one another.

Treatment means were also calculated and averaged across the two-year sampling period. Tukey pairwise comparisons revealed that within the continuous corn systems, the cereal rye-continuous corn (CC-CR-192) system outperformed the Kura clover/continuous corn (CC-KC-69) system in HTEY production (Table 2.20). As previously mentioned, the differences among these treatments are largely a function of differences in overall biomass yield rather than differences in concentration values of sugar, starch, cellulose, hemicellulose, etc. of the biomass per se. All other pairwise comparisons among continuous corn systems were not significant. Likewise, Tukey HSD pairwise comparisons were not significant among rotational system means.

After total HTEY was analyzed at the system level, a general linear model was generated to observe differences in herbage theoretical ethanol yield (HTEY) at the crop-species level within the various cropping systems. Cover crop and cash crop HTEY were analyzed separately (Table 2.21). Analysis of variance (ANOVA) revealed that the Cropping System x Year interaction effect was significant. The significant Cropping System x Year interaction is most likely attributed to the noticeable disparities of biomass yield within the treatments between 2018 and 2019. Tukey HSD comparisons revealed that the cereal rye cover crop from the cereal rye-continuous corn (CC-CR-192) system produced significantly (P<0.001) more HTEY than the Kura clover cover crop in the Kura clover continuous corn (CC-KC-69) system in 2018. Part of this difference most likely arose from the decreased Kura biomass production of the developing clover stand during the 2018 growing season. Establishment of Kura clover has been cited as notoriously difficult and somewhat time consuming, with some studies citing multiple years needed for full biomass potential to be realized (Bartel et al., 2017; Sawyer et al., 2010; Seguin et al., 1999). All other cover crop pairwise comparisons were not significantly different from one another in 2018 or 2019.

Differences in total theoretical ethanol yield (HTEY) for corn and soybeans within the various cropping systems were statistically analyzed. Analysis of variance (ANOVA) revealed significant Year and Cropping System main effects as well as the Cropping System x Year interaction significantly affected HTEY among corn and soybeans. The significant effect that Year had on cash crop HTEY is not surprising as year to year variation in crop growing conditions can vary. The interaction between Cropping System and Year is likely a result of variation in species (corn vs soybean) HTEY which varied between the 2018 and 2019 within systems. Tukey HSD comparisons revealed that in 2018, corn HTEY was significantly lower in the Kura clover continuous corn (CC-KC-69) system as compared to the cereal rye-continuous corn (CC-CR-192) treatment. Corn and soybean HTEY were also found to be significantly lower in the cereal rye-corn/soybean rotation (CS-CR-148) treatment as compared to the corn/soybean control (CS-170). In 2019, corn and soybean HTEY was noted to be significantly higher in the continuous corn control (CC-192) as compared to the Kura clover-continuous corn (CC-KC-69) system. It was also noted that in 2019, the cereal rye-continuous corn treatment (CC-CR-192) produced significantly more corn HTEY as compared to the Kura clover-continuous corn treatment (CC-KC-69). Overall, these significant differences likely arose from decreased production of cash crop HTEY within the Kura clover-continuous corn (CC-KC-69) treatment. Over the study years, this system generally performed poorly with regard to corn stover and grain yield (Figure 2.1). This decreased corn biomass production within a Kura clover living mulch system has been well noted in literature (Zemenchik et al., 2000; Affeldt et al., 2004; Sawyer et al., 2010) and could have ultimately been a driving factor in decreased HTEY within the system.

Corn and soybean grain were not directly measured for their theoretical ethanol yield potential. This was due to the objective of this study, which focused on additional means to meet alternative energy demands without reducing current day grain production being used to meet food/feed demands. However, current conversion factors are 0.441 L EtOH per kg dry corn grain (Long, 2015, Bothast and Schlincher, 2005) and 0.207 L biodiesel per kg dry soybean grain (Gray, 2006). Thus, averaged across treatments and years, theoretical ethanol yields from corn grain ranged from to 413 to 5877 L EtOH ha^{-1} while theoretical biodiesel yields from soybean grain ranged from 548 to 773 L biodiesel ha^{-1}. Just as biomass yield is the primary driver HTEY, grain yield would be the driving factor in the overall production of corn and soybean grain biofuel production. Thus, statistical differences among treatments would likely be similar to those found in the analysis of grain yields (Figure 2.1 and Figure 2.2).

Table 2.20. Total Herbage Theoretical EtOH Yields (HTEY) across the two study years. HTEY averages for corn/soybean and cover crop summed for total HTEY yield by cropping system. Background shading denotes soybean years in rotational cropping systems. Data presented are data are means ± standard errors of eight replications across two years. Means followed by the same letter across rows are not significantly different at P<0.05 using a Tukey-Kramer HSD test.

	Cropping System†						
Year	(CC-192)	(CC-CR-192)	(CC-KC-69)	(CS-170)	(SC-170)	(CS-CR-148)	(SC-CR-148)
	---------- L EtOH ha $^{-1}$ ----------						
2018	4130±100	4350±216	3387±218	3134±359	3902±238	3302±380	4594±487
2019	2880±161	4070±211 **A**	2117±227 **B**	2863±62	2062±153	3188±282	2570±298
Trt Ave.	3505±252 **AB**	4210±149 **A**	2752±281 **B**	2998±176	2982±372	3245±220	3582±465

† Cropping systems abbreviated as: CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1}), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1}), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted first, receiving 148 kg N ha^{-1} during corn years), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted first, receiving 148 kg N ha^{-1} during corn years), CC-192 (Tilled, continuous corn receiving 192 kg N ha^{-1}), CS-170 (Tilled corn/soybean rotation, corn planted first, receiving 170 kg N ha^{-1} during corn years), SC-170 (Tilled, soybean/corn rotation, soybean planted first, receiving 170 kg N ha^{-1} during corn years.

Table 2.21. Total Herbage Theoretical EtOH Yields (HTEY) across the two study years. HTEY averages for plant material summed for yearly total HTEY yield by cropping system. Data presented are data are means ± standard errors of eight replications across two years.

		Source of HTEY					
		Corn Stover	Soybean Stover	Cereal Rye	Kura Clover	Yearly Total HTEY	Mean Total HTEY
Year	Cropping System†	----------L EtOH ha $^{-1}$----------					
2018	CC-192	4130±100	NA	NA	NA	4130±100	3505±252
2019	CC-192	2880±161	NA	NA	NA	2880±161	
2018	(CC-CR-192)	3433±106	NA	917±130	NA	4350±216	4210±149
2019	(CC-CR-192)	2592±200	NA	1477±218	NA	4070±211	
2018	(CC-KC-69)	2779±185	NA	NA	609±62	3387±218	2752±281
2019	(CC-KC-69)	934±271	NA	NA	1182±80	2117±227	
2018	(CS-170)	NA	3134±359	NA	NA	3134±359	2998±176
2019	(CS-170)	2863±62	NA	NA	NA	2863±62	
2018	(SC-170)	3902±238	NA	NA	NA	3902±238	2982±372
2019	(SC-170)	NA	2062±153	NA	NA	2062±153	
2018	(CS-CR-148)	NA	2306±242	1537±315	NA	3302±380	3245±220
2019	(CS-CR-148)	1765±97	NA	882±105	NA	3188±282	
2018	(SC-CR-148)	3342±332	NA	1252±191	NA	4594±487	3582±465
2019	(SC-CR-148)	NA	1712±157	856±166	NA	2570±298	

† Cropping systems abbreviated as: CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1}), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1}), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted first, receiving 148 kg N ha^{-1} during corn years), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted first, receiving 148 kg N ha^{-1} during corn years), CC-192 (Tilled, continuous corn receiving 192 kg N ha^{-1}), CS-170 (Tilled corn/soybean rotation, corn planted first, receiving 170 kg N ha^{-1} during corn years), SC-170 (Tilled, soybean/corn rotation, soybean planted first, receiving 170 kg N ha^{-1} during corn years. λ Denotes combined corn and soybean stover from rotational systems.

2.3.12 Tile Drained Flow, Nitrate-N Concentrations, and Nitrate-N Load Losses

Annual tile drained flow was statistically analyzed across cropping system and years. Analysis of variance (ANOVA) highlighted significant (P<0.05) Cropping System and Rep (P<0.01) main effects for annual tile drained water flow. However, the Year and Cropping System x Year interaction effects were not significant. Tukey-Kramer HSD pairwise comparisons were used to further identify significant differences among specific pairwise comparisons. These pairwise comparisons indicated there was not a significant difference in annual flow volumes among any of the three control cropping systems. Likewise, Tukey HSD disclosed there was not a significant difference in annual tile drainage flow volumes in any of the treatment/control comparisons. These results were contradictory to those found in literature which cited Kura clover as a perennial living mulch decreased drain flow rates (Ochsner et al., 2018). One potential reason for the contrast in the Oshner et al. (2018) study and the results observed here is the vigor of the Kura clover stand. Improved stands in other studies may have had deferring results to those observed here, meaning additional growing seasons may be needed to allow the Kura clover PLM to reach its full stand potential. Improved Kura clover stand may allow for increased rates of evapotranspiration which in turn might reduce tile drain flow rates. These results are also contradictory to those found in literature which stated findings of reduced tile drainage flow volumes when cereal rye was incorporated as a cover crop (Martinez-Feria et al., 2016). It is important however to note that the cereal rye in the Martinez-Feria et al. (2016) study was killed at an early vegetative growth stage and was not harvested off of the plots as was done in this study. Furthermore, our results compliment site-specific research conducted at the Purdue WQFS site, where a culmination of historical water data was analyzed. Greve (2019) found that cropping system did not significantly affect tile drainage flow volumes among cropping systems at the Purdue WQFS site. However, it is crucial to mention the study conducted by Greve (2019) was a long-term study and did not include the newly implemented cropping systems used in this study. The mean annual tile drainage flow volumes observed in this study align with those found by Greve (2019) and tend to illustrate similar patterns based off of tile line function rather than cropping system effects. It is important to note that some of the variation exhibited among tiles may be due to inherent tile-to-tile differences that have existed for years prior, rather than explicitly due to effects from the newly implemented treatments used in this study. Further investigation into an analysis of flow among functioning tiles should be performed to help identify specific influences on flow.

To further understand tile drainage flow volume, and the response during various times during the growing season, annual flow data was broken down into quarters and analyzed statistically. As previously stated, this project sought to understand the effect cover crops had on water flow/quality, thus quarterly breaks were chosen because they correspond with the periods of time cover crops had the most influence on water flow/quality. Analysis of variance (ANOVA) highlighted significant (P<0.001) Cropping System, Year, Quarter, and Rep main effects as well as Year x Quarter interaction effects for quarterly flow data. However, the Cropping System x Quarter, and Cropping System x Year x Quarter interactions were not significant. The significant effect Year had on tile drainage flow volumes is due to fluctuation in temperature and precipitation from year to year (Table 2.1 and Table 2.2). For obvious reasons, precipitation is the main driver of tile drainage flow volumes. Therefore, years such as 2017, where precipitation exceeded the 30-year mean, will tend to exhibit larger flow volumes, regardless of treatment, when compared to years such as 2019, where annual precipitation values fall below the 30-year mean. Temperature is also a factor in tile-drainage flow. Increased ambient air temperatures tends to increase processes such evaporation which then decreases the amount of water that finds itself into agricultural tile lines. Furthermore, colder temperatures in the winter months tend to result in the increased likelihood of frozen tile lines which also decrease flow volumes. The significant effect Quarter played on tile drainage flow volumes is due to varied periods of increased precipitation within the different study years (Table 2.1). For example, precipitation totals in Quarter 4 of 2018 were roughly 319 mm whereas precipitation totals of Quarter 4 of 2019 were down to approximately 214 mm (Table 2.1). Variation among quarters such as this is likely the result of this significant Quarter effect on tile drainage flow volumes. Furthermore, the significant Year x Quarter interaction effect was likely due to the differences from year-to-year variation when precipitation fluctuated with the various quarters (Table 2.1). When cereal rye was added to continuous corn systems, there was no significant difference in quarterly tile drainage flow volumes. Similarly, there were no significant differences in quarterly tile drainage flow volumes in continuous corn systems after the addition of Kura clover. These results contradict similar work which stated that the inclusion of cover crops increased evapotranspiration, thus resulting in reduced cumulative flow volumes at different parts of the growing season (Daigh et al., 2014). A potential reason our observations differ to those of Daigh et al. (2014) could be due to differences in cover crop species management, geographical location, and differences in observed growing season precipitation amounts.

Table 2.22. Tile drained flow volumes as influenced by cropping system and averaged across the three study years. Data are means ± standard errors of functioning tile lines.

Cropping System†							
(CC-192)	(CC-CR-192)ϑ	(CC-KC-69)ϑ	(CS-170)λ	(SC-170)	(CS-CR-148)ϑ	(SC-CR-148)	3-Year Mean Precipitation
-- kl water ha $^{-1}$ yr $^{-1}$ --							mm
845.5±222.2	1424.8±167.8	1850.5±279.7	530.9±251.4	1911.1±385.2	1684.5±331.6	1258.9±442.9	1294

† Cropping systems abbreviated as: CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1}), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1}), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted first, receiving 148 kg N ha^{-1} during corn years), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted first, receiving 148 kg N ha^{-1} during corn years), CC-192 (Tilled, continuous corn receiving 192 kg N ha^{-1}), CS-170 (Tilled corn/soybean rotation, corn planted first, receiving 170 kg N ha^{-1} during corn years), SC-170 (Tilled, soybean/corn rotation, soybean planted first, receiving 170 kg N ha^{-1} during corn years). ϑ Denotes cropping systems where one tile was omitted as 'non-functioning' thus resulting in an average of three replications as opposed to four. λ Denotes cropping systems where two tiles were omitted as 'non-functioning' thus resulting in an average of two replications as opposed to four.

Table 2.23. Tile drained flow volumes as influenced by cropping system and quarter. Data are means ± standard errors of functioning tile lines averaged across the three study years.

	Quarter 1	**Quarter 2**	**Quarter 3**	**Quarter 4**
Cropping System	-- kl water ha $^{-1}$ quarter $^{-1}$ --			
CC-192	273.4±65.3	405.8±123.0	63.1±32.7	129.6±81.9
CC-CR-192	523.4±34.3	631.5±447	78.9±45.9	239.6±99.1
CC-KC-192	753.8±125.87	771.9±185.2	83.5±45.2	279.4±126.4
CS-170	168.9±82.3	290.8±152.3	41.9±40.7	68.6±47.2
CS-CR-148	560.7±123.5	806.5±185.7	84.7±59.8	298.6±137.6
SC-170	611.1±135.4	898.1±265.0	89.4±53.6	344.4±140.2
SC-CR-148	397.7±155.1	631.9±245	52.2±32.2	229.7±146.2

When flow-weighted annual tile drained NO_3^- N concentrations were statistically analyzed across cropping system and years, analysis of variance (ANOVA) exposed significant (P<0.001) Cropping System, Year, Rep, main effects while the Cropping System x Year interaction was not significant. Tukey-Kramer HSD pairwise comparisons were used to gauge how flow-weighted NO_3^- N concentration differentiated among cropping systems (Table 2.24). Averaged across years, flow-weighted NO_3^- N concentrations were not significantly different among any of the rotational controls or rotational treatment/control comparison. Similarly, there was not a significant difference in flow-weighted NO_3^- N concentrations among the continuous corn (CC-192) control and the cereal rye continuous corn (CC-CR-192) system. This alludes to the idea that cereal rye was not entirely successful at reducing flow-weighted NO_3^- N concentrations in rotational cropping systems over this study's two-year study period. However, although not statistically significant, the cereal rye corn/soybean (CS-CR-148) and cereal rye soybean/corn (SC-CR-148) systems exhibited a downward trend in favor of reduced tile drainage NO_3^- N concentrations as compared to their respective controls. It is unclear if these reduced flow-weighted NO_3^- N concentrations were reduced as an effect of the cereal rye cover crop or due to the reduced N application rates of these systems. Therefore, these results somewhat agree with previous literature where cereal rye reduced N runoff and leachate (Korucu et al., 2018; Meisinger et al., 1991; Snapp et al., 2001). Meisinger et al., (1991) suggested that rye could decrease the concentration of N in drainage water and the mass of N leached by 20 to 80%. Furthermore, Snapp et al. (2001) indicated that the inclusion of winter rye as a cover crop will only reduce N leaching if extra N fertilizer is not applied when the cover crop is planted; the same methodology was used in this study. This approach is consistent with other literature which cited reduced N leaching in continuous corn/Kura clover mulch systems where no additional N was applied (Ochsner et al., 2018). Averaged across years, flow-weighted NO_3^- N concentrations were significantly lower in the Kura clover continuous corn (CC-KC-69) system as compared to both the cereal rye continuous corn (CC-CR-192) system and the continuous corn (CC-192) control. These results agree with literature that supports the idea that even though clover species are legumes and can fix atmospheric N into plant-available N, they often times tend to scavenge available soil N rather than undergoing N fixation (Hartwig and Ammon, 2002). Similarly, Albrecht et al. (2009) found that Kura clover used as a perennial living mulch (PLM) reduced soil NO_3-N deep in the soil profile when compared directly to monocultured corn systems. Albrecht et al. (2009) attributed these decreased NO_3-N leachate levels to the Kura clover's deep root system. These lengthy roots may have captured NO_3-N that may have otherwise leached out of the soil profile and into the tile drained water. However, other studies suggested that Kura clover as a PLM does not significantly reduce soil NO_3-N levels (Sawyer et al., 2010). It is important to note the external N fertilizer applied in the Sawyer et al. (2010) study consisted of six different N rates whereas this study only had one N rate (69 kg N ha^{-1}). In this study, the observed reduction in tile drained NO_3-N may also be due to the reduced N rate received in the Kura clover-continuous corn (CC-KC-69) treatment. These results suggest Kura clover may be effective at reducing tile drained NO_3^- N concentrations however, results are varied. Additional observations across more growing seasons may be needed to add detail to some of the patterns observed in this study. Overall, these results coincide with long-term, site-specific study that found cropping system significantly affected flow-weighted tile drainage NO_3^- N concentrations (Greve, 2019). However, it is important to note that the treatments in this study include cover crops (cereal rye and Kura clover) which have not been previously implemented in past studies such as those conducted by Greve (2019) at the WQFS field site.

To further understand flow-weighted tile drained NO_3^- N concentrations and how they responded during various times during the growing season within different treatments, annual flow-weighted NO_3^- N concentration data was broken down into quarters and statistical analyses were conducted in a manner similar to water flow data. Analysis of variance (ANOVA) highlighted significant ($P<0.001$) Cropping System, Year, Quarter and Rep main effects as well as Year x Quarter, Cropping System x Year, Cropping System x Quarter, and Cropping System x Year x Quarter interaction effects for quarterly flow-weighted NO_3^- N concentration data. The significant effect Quarter played on tile drainage NO_3^- N concentrations is most likely due to natural fluctuations in precipitation (Table 2.1), such as increased precipitation in spring months, as excess water is necessary for NO_3^- N to be flushed from the soil. Furthermore, the effect Quarter played on tile drainage NO_3^- N concentrations may also be due to certain management practices, such as N fertilization of plots, which occurs at roughly the same time (Quarter two) each year. The significant Year x Quarter interaction effect was likely due to the differences from year to year when precipitation was higher or lower in the various quarters of calendar year (Table 2.1). As previously mentioned, an obvious example of this occurred in Quarter four where precipitation values in 2018 were markedly higher (319 mm) as compared to those in 2019 (214 mm). The significant Cropping System x Quarter x Year interaction is caused by differences among quarterly data from year to year within the different cropping systems (Table 2.25). As indicated above, Kura clover significantly reduced tile drainage NO_3^- N concentrations and cereal rye may be responsible for a downward trend in tile drainage NO_3^- N concentrations. Furthermore, these reductions in flow-weighted NO_3^- N concentrations likely affected quarterly data due to the timing of cover crops growth/uptake and in the case of cereal rye, termination. Tukey HSD pairwise comparisons were used to investigate significant differences in the Cropping System x Quarter interaction (Table 2.25). It is important to note beforehand, that these Cropping System x Quarter pairwise comparisons were averaged across the three study years. In Quarter one, tile drainage NO_3^- concentrations were similar among the continuous corn (CC-192) control and corn/soybean (CS-170) soybean/corn (SC-170) controls. In Quarter two, there was not a significant difference in tile drained NO_3^- N concentration among the continuous corn (CC-192) control and the corn/soybean (CS-170) and soybean/corn (SC-170) controls. Tile drainage NO_3^- N concentrations were significantly lower in the Kura clover cereal rye (CC-KC-69) as compared to the continuous corn (CC-192) control for Quarter two, a period of time where the control cropping system was primarily fallow and subject to leaching of soil NO_3^- N. The potential for Kura clover to scavenge excess N is something that has been cited in previous literature (Albrecht et al., 2009; Hartwig and Ammon, 2002). These results agree with these literature sources and highlight that decreased N leaching potential during Quarter two is likely due to the presence of Kura clover living mulch. Dissimilarly, NO_3^- N concentration was not significantly lower in the cereal rye continuous corn (CC-CR-192) system as compared to the continuous corn (CC-192) control for Quarter two. There were no significant quarterly differences between these two systems. Although not statistically significant, it is notable to mention the downward trend observed in flow-weighted NO_3^- N concentrations among all three of the cereal rye cropping systems as compared to their controls for Quarter two especially. This observation of reduced NO_3^- N in Quarter two is important to note as it suggests the active growth of the cereal rye during the early spring months may have contributed to reduced NO_3^- N concentrations via scavenging excess N. Previous studies, Korucu et al. (2018) and Meisinger et al. (1991), found that cereal rye reduced NO_3^- concentration levels in the soil and tile drained water during the soil recharge period of the spring months, a pattern that aligns with the observations seen in this study. These results suggest that reduced N rates and incorporation of cereal rye and Kura clover have the potential to significantly reduce tile drainage NO_3^- N concentration values.

Table 2.24. Annual flow-weighted tile drained NO_3^- N concentrations as influenced by cropping system. Data are means ± standard errors of functioning tile lines. Background shading denotes soybean years in rotational cropping systems. Means followed by the same letter and within like systems (continuous corn versus rotational systems) are not significantly different at *P*<0.05 using a Tukey-Kramer HSD test. Vertical black line indicates the separation between Tukey-Kramer comparisons.

	Cropping System†						
Year	(CC-192)	(CC-CR-192)ϑ	(CC-KC-69)ϑ	(CS-170)λ	(SC-170)	(CS-CR-148)ϑ	(SC-CR-148)
	mg L^{-1}						
2017	8.2±0.9	10.6±0.9	5.0±1.6	11.1±2.5	8.4±1.1	9.9±0.6	8.3±1.3
2018	9.8±3.3	7.6±0.8	2.2±0.6	6.0±4.2	9.1±2.0	4.2±1.0	2.8±0.6
2019	6.5±1.7	4.3±0.5	2.6±0.7	8.7±1.6	6.3±0.9	3.6±0.3	3.8±0.9
Trt Ave.	8.1±1.2 **A**	7.5±1.0 **A**	3.2±0.7 **B**	8.6±1.6	7.9±0.8	5.9±1.1	5.0±0.9
Corn Ave.	8.1±1.2	7.5±1.0	3.2±0.7	9.9±1.4	9.1±2.0	6.8±1.4	2.8±0.6
Soy Ave.	NA	NA	NA	6.0±4.2	7.4±0.8	4.2±1.0	6.0±1.1

† Cropping systems abbreviated as: CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1}), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1}), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted first, receiving 148 kg N ha^{-1} during corn years), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted first, receiving 148 kg N ha^{-1} during corn years), CC-192 (Tilled, continuous corn receiving 192 kg N ha^{-1}), CS-170 (Tilled corn/soybean rotation, corn planted first, receiving 170 kg N ha^{-1} during corn years), SC-170 (Tilled, soybean/corn rotation, soybean planted first, receiving 170 kg N ha^{-1} during corn years). ϑ Denotes cropping systems where one tile was omitted as 'non-functioning' thus resulting in an average of three replications as opposed to four. λ Denotes cropping systems where two tiles were omitted as 'non-functioning' thus resulting in an average of two replications as opposed to four.

Table 2.25. Flow-weighted tile drained NO_3^- N concentrations as influenced by cropping system and quarter. Data are means ± standard errors of functioning tile lines averaged across the three study years.

	Quarter 1	**Quarter 2**	**Quarter 3**	**Quarter 4**
Cropping System	------------ mg L^{-1} quarter $^{-1}$ ------------			
CC-192	6.9±1.2	9.7±2.3	5.6±2.2	3.4±1.4
CC-CR-192	6.6±0.8	7.3±1.3	7.5±3.5	6.0±1.4
CC-KC-192	3.2±0.6	4.0±0.9	1.8±1.0	0.6±0.2
CS-170	6.2±2.3	11.2±1.7	6.0±3.5	4.9±1.8
CS-CR-148	5.6±1.1	5.4±1.2	6.1±2.8	4.3±1.5
SC-170	8.4±1.0	8.9±1.5	2.7±1.1	4.2±1.2
SC-CR-148	5.7±1.2	5.4±0.9	2.0±1.1	2.0±0.6

Annual load losses were statistically analyzed across cropping systems and years (Table 2.26). Analysis of variance (ANOVA) revealed significant (*P*<0.001) Year and Rep main effects for tile drained nitrate (NO_3^-) load loss. The significant effect Year had on tile drainage load loss is largely a result of annual fluctuations in precipitation and temperature (Table 2.1 and Table 2.2). It was determined that Cropping System did not significantly affect tile drained NO_3^- load loss (Table 2.26). These results are somewhat contradictory to those in previous studies which have highlighted significantly decreased NO_3^- loads after the implementation of cereal rye cover crops (Kaspar et al., 2007). Variations in geography, temperature/precipitation, and cover crop management are likely the reasoning for varied observations within the literature. This study indicated no significant difference in tile drained NO_3^- load losses of continuous corn systems with the inclusion of Kura clover, however, results trended toward decreased NO_3^- load losses with the inclusion of Kura clover. Although there is sufficient literature on the effects Kura clover has on soil NO_3^- concentrations (Albrecht et al., 2009; Sawyer et al., 2010), there is little known literature on the effects Kura clover on tile drained NO_3^- load loss. Additionally, what literature is available focused on continuous corn and Kura clover perennial living mulch receiving no additional input of fertilizer N (Ochsner et al., 2018) which further significantly reduced grain yields.

Analysis of variance (ANOVA) highlighted significant (*P*<0.001) Cropping System, Year, Quarter, and Rep main effects as well as the Year x Quarter interaction effects for quarterly load loss data. This ANOVA indicated that the Cropping System x Quarter, Cropping System x Year, and Cropping System x Year x Quarter interaction effects were not significant (*P*≥0.05) (Table 2.27). As previously mentioned, the significant effect Quarter played on tile drained load loss is most likely due to periods of increased precipitation within years at certain times of the calendar year (Table 2.1) as well as fertilizer N applications occurring in the spring. Both can affect tile drainage flow volumes and NO_3^- concentrations, the two variables that factor into NO_3^- load loss. Within season (quarterly) differences of load have been previously observed and are well noted throughout literature. For instance, it has been previously noted that quarterly load loss decreases in late Quarter two and primarily in Quarter three as plants become more established and scavenging potential as well as increased evapotranspiration play roles in reducing load loss (Tomer et al., 2003). The significant Year x Quarter interaction effect was likely due to instances within cropping systems where quarterly load loss levels varied from year to year within cropping systems. As previously stated, there was not a significant Cropping System x Quarter interaction effect which alludes to the observation that cover crop treatments did not significantly alter quarterly tile drained load losses when compared to their system controls.

Table 2.26. Tile drained NO_3^- load loss as influenced by cropping system. Data are means ± standard errors of functioning tile lines averaged across study years. Means followed by the same letter and within like systems (continuous corn versus rotational systems) are not significantly different at $P<0.05$ using a Tukey-Kramer HSD test. Vertical black line indicates the separation between Tukey-Kramer comparisons.

	Cropping System†							
	(CC-192)	(CC-CR-192)ϑ	(CC-KC-69)ϑ		(CS-170)λ	(SC-170)	(CS-CR-148)ϑ	(SC-CR-148)
	----------NO_3^- Load Loss kg N ha^{-1} yr^{-1}----------							
Trt Ave.	8.5±3.0	11.7±2.7	5.6±1.3		6.2±3.5	14.1±2.5	10.0±3.0	6.7±2.9
Corn Ave.	8.5±3.0	11.7±2.7	5.6±1.3		7.8±5.1	12.4±4.0	12.1±4.3	4.5±3.6
Soy Ave.	NA	NA	NA		3.1±3.1	14.9±3.4	5.9±2.1	7.8±4.1

† Cropping systems abbreviated as: CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1}), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1}), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted first, receiving 148 kg N ha^{-1} during corn years), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted first, receiving 148 kg N ha^{-1} during corn years), CC-192 (Tilled, continuous corn receiving 192 kg N ha^{-1}), CS-170 (Tilled corn/soybean rotation, corn planted first, receiving 170 kg N ha^{-1} during corn years), SC-170 (Tilled, soybean/corn rotation, soybean planted first, receiving 170 kg N ha^{-1} during corn years). ϑ Denotes cropping systems where one tile was omitted as 'non-functioning' thus resulting in an average of three replications as opposed to four. λ Denotes cropping systems where two tiles were omitted as 'non-functioning' thus resulting in an average of two replications as opposed to four.

Table 2.27. Tile drained NO_3^- N load loss as influenced by cropping system and quarter. Data are means ± standard errors of functioning tile lines averaged across the three study years.

	Quarter 1	**Quarter 2**	**Quarter 3**	**Quarter 4**
Cropping System	---------- NO_3^- Load Loss kg N ha^{-1} $quarter^{-1}$ ----------			
CC-192	2.2±0.6	4.0±1.5	0.8±0.4	1.5±1.2
CC-CR-192	3.3±0.3	5.6±2.1	1.3±0.8	1.5±0.5
CC-KC-192	2.0±0.3	3.0±1.0	0.2±0.1	0.3±0.1
CS-170	1.1±0.5	4.0±2.4	0.7±0.7	0.5±0.3
CS-CR-148	2.6±0.5	5.1±2.0	1.3±1.0	1.0±0.3
SC-170	4.3±0.9	6.9±1.6	0.5±0.3	2.3±1.0
SC-CR-148	1.7±0.6	4.2±2.1	0.2±0.2	0.6±0.4

2.3.13 Annual Greenhouse Emissions

The three greenhouse gasses (GHG) of focus in this study (nitrous oxide, methane, carbon dioxide) were statistically analyzed at the cropping system level. It is important to note prior to discussing GHG results, that plants were not terminated within GHG chambers, however if growth of the plant species prohibited closure of the GHG chamber, then plants were cut to fit within the chamber headspace. This resulted in the termination of some plants (corn, soybeans, cereal rye) while allowed for other species (Kura clover) to survive throughout the sampling period. Observed results may have been influenced by living plants within respective chambers. The continuation of this study will aim to terminate all plant growth within the GHG chambers at a uniform time across all treatments.

Analysis of variance (ANOVA) indicated that Cropping System alone significantly (P<0.001) affected annual loss of nitrous oxide (N_2O) while the Year, Rep, and the Cropping System x Year interaction effects were not significant. Tukey-Kramer HSD pairwise comparisons disclosed that averaged across the three study years, the loss of N_2O in the continuous corn control (CC-192) system was significantly greater than the loss of N_2O in both the corn/soybean rotational control (CS-170) and the soybean/corn rotational control (SC-170) (Figure 2.4). However, there was not a significant difference in loss of N_2O among the corn/soybean rotational control (CS-170) and soybean/corn rotational control (SC-170). Averaged across the study years, N_2O emissions were significantly lower in the cereal rye-continuous corn (CC-CR-192) and Kura clover continuous corn (CC-KC-69) systems when compared to the continuous corn (CC-192) control. These results are contradictory to Turner et al. (2016) which found that in regard to N_2O gas, Kura clover may be less successful at reducing emissions. Turner et al. (2016) observed higher GHG emissions even when fertilizer rates were reduced 43% in the Kura clover PLM. Furthermore, this increase in N_2O emissions was a trade-off due to the management of the living mulch system. Any reduction of N_2O emissions observed during the pre-plant period (due to the N scavenging potential of the legume) are offset by the post-anthesis mineralization of the Kura clover which increases N_2O emissions following strip tillage (Turner et al., 2016). Our results may differ Turner et al. (2016) due, in part, to the tillage used in their study instead of the no-till system used in this study. Likewise, N_2O emissions were significantly greater in the cereal rye-continuous corn (CC-CR-192) system when compared to the Kura clover continuous corn (CC-KC-69) system. There was not a significant difference in N_2O loss among the corn/soybean (CS-170) control and the cereal rye corn/soybean (CS-CR-148) treatment. Likewise, there was not a significant difference in N_2O loss among the cereal rye soybean/corn (SC-CR-148) treatment and the soybean/corn rotational control (SC-170). These results align well with Parkin et al. (2016) who monitored a corn-soybean rotation with and without rye cover crops and found that over a 10-year period N_2O emissions tended to be lower in cover crop treatments, although the differences were not statistically significant. This was determined to be partially due to strong influences on GHG emissions, such as the observed high year-to-year variations in precipitation. However, Parkin et al. (2016) stated that loss of N_2O from corn treatments were significantly higher than from the soybean treatments which agrees with the trends found in this study. This was believed to be an effect of corn receiving N fertilizer which resulted in higher N_2O emissions as compared to soybeans. On the contrary, Phillips et al. (2009) found N_2O losses were similar between both corn and soybean systems; a finding that could be due to treatments which were different than those used in this study as well as fertilizer management differences which ultimately sought to highlight that fertilizer application timing effects total net GHG emissions. These results suggest Kura clover and cereal rye are effective at significantly reducing N_2O emissions in continuous corn systems and tend to reduce levels of N_2O in rotational systems, although this reduction is not statistically significant.

To further understand loss of N_2O response during various times of the growing season within cropping systems, annual N_2O loss data was analyzed by trimester and similar statistical analyses conducted (Table 2.28). Trimester data was broken down as follows: Trimester one (Dec-March), Trimester two (April-July), Trimester three (August-November). It is important to note Trimester one contained minimal data as this period in time was not routinely sampled for GHG collection. Analysis of variance (ANOVA) highlighted significant ($P<0.001$) Cropping System, Year, and Trimester main effects as well as Cropping System x Year and Cropping System x Trimester interaction effects for Trimester N_2O emissions data. The significant Cropping System x Year interaction is likely due to differences in N_2O emission loss from year to year within cropping systems. This could likely be due to the nature of the rotational systems and the different species (corn versus soy) and N rate applied from year to year. The significant effect Trimester played on N_2O loss is most likely due to fluctuations in precipitation and temperature within years (Table 2.1 and Table 2.2), as well as major field operations such as tillage, which can increase N_2O emission loss and typically occur during routine timeframes annually (Table 2.1 and Table 2.2).

Tukey HSD pairwise comparisons were used to investigate significant differences within the Treatment x Trimester interaction. It is important to note that these Cropping System x Trimester pairwise comparisons were averaged across the three study years. There were no significant differences in N_2O loss among any of the cropping systems in Trimester one. This is not surprising as previously stated, little to no data collection took place over this time period. However, there were a few differences within Trimester two among the different cropping systems. Field operations that would be taking place at the time of Trimester two observations, including but not limited to, spring tillage, planting, and harvesting of cover crops. In Trimester two, Tukey comparisons indicated significantly higher N_2O loss in the continuous corn (CC-192) control treatment as compared to both the soybean/corn (SC-170) control and corn/soybean (CS-170) control. Likewise, N_2O loss in the continuous corn (CC-192) control treatment was significantly higher than that of both the cereal rye continuous corn (CC-CR-192) treatment and the Kura clover continuous corn (CC-KC-69) system. However, there was not a significant difference in N_2O loss between the Kura clover continuous corn (CC-KC-69) and cereal rye corn/soybean (CC-CR-192) treatment in Trimester two. Integration of cereal rye appeared to affect trimester N_2O loss data, especially within Trimester two. These results contradict previous studies which highlighted that cereal rye did not significantly decrease N_2O loss (Liebig et al., 2010; Parkin et al., 2016). Though, it is crucial to mention that insignificance in these studies were deemed partially due to environmental influences on GHG emissions, including year-to-year variation in precipitation. Similar to Trimester one, there were no significant pairwise comparison among cropping systems in Trimester three that would be of interest to this study. It is important to note that while Trimester two is correlated with early spring and mid-summer growth of the corn/soybean crop, in contrast, Trimester three was correlated to the later growth and maturation of annual crops, harvest of corn/soybeans, fall seeding of cereal rye cover crops, and minimal decomposition of corn/soybean residues.

Without further investigation it is not entirely clear that the differences among in-season N_2O emissions within these cover cropping systems is caused solely by the cover crop itself or rather the overall system's design. This includes type of tillage, decreased fertilizer N inputs, increased residue with cover crops, or some combination of these factors. Overall, all treatments and control systems experienced the largest loss of N_2O in Trimester two, followed by Trimester three, with Trimester one having minimal loss. These trends within Trimester two are similar to those found by Almaraz et al. (2009) which highlighted a correlation to peak flux of N_2O in the spring which was largely attributed to spring precipitation events. This additional spring precipitation may have led to more water filled pore spaces thus inducing an anaerobic environment conducive to denitrification and thus increasing flux of N_2O. Spring tillage is also a large contribution to N_2O loss from soil. While the downward trend in N_2O loss in Trimester three is likely due to decreased soil disturbance, and uptake of fertilizer N by the corn crop. Overall, the range of mean seasonal flux of N_2O from this study ranged from < 1 to 5.3 kg ha^{-1} yr^{-1} which is on par with results from other site-specific studies at the Purdue WQFS where Hernandez-Ramirez et al. (2009) found that seasonal flux of N_2O ranged from <1 to between 3 and 5 kg ha^{-1} yr^{-1}.

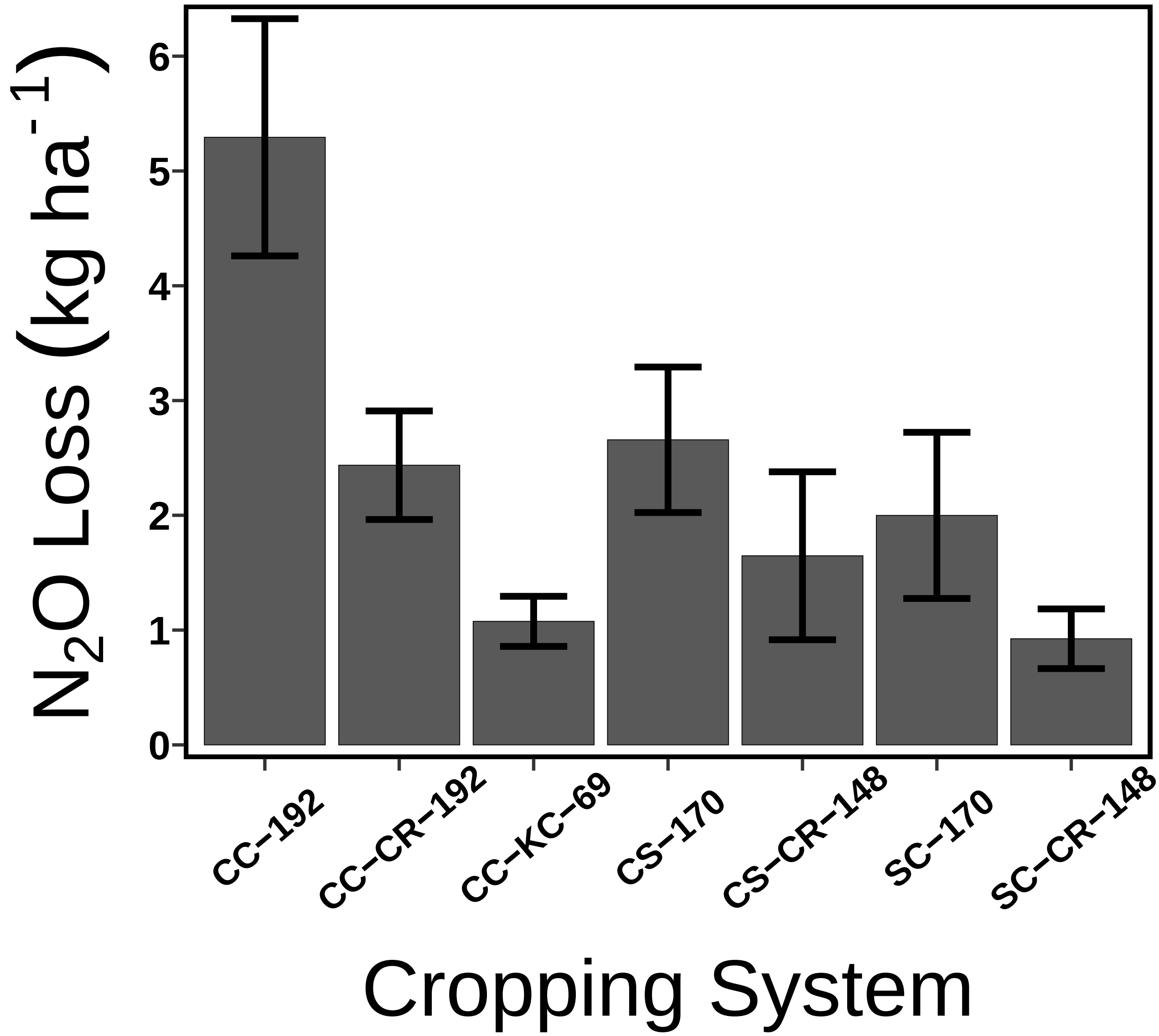

Figure 2.4. Annual N_2O loss as influenced by cropping system. Data are means ± standard errors of four replications averaged across the three study years.

Table 2.28. Flux of N_2O (kg ha^{-1} $trimester^{-1}$) as influenced by Cropping System and Trimester, broken down by trimester and averaged across years. Data are means ± standard errors. Trimester data was broken down as follows: Trimester one (December-March), Trimester two (April-July), Trimester three (August-November). It is important to note Trimester one contained minimal data as this period in time was not routinely sampled for GHG collection. Background shading used to differentiate between cropping systems for ease of observation.

Cropping System	Trimester	N Observations	Flux of N_2O
			---------------kg ha^{-1}---------------
CC-192	1	8	0.00±0.00
CC-192	2	12	4.63±1.01
CC-192	3	12	0.66±0.20
CC-CR-192	1	8	0.00±0.00
CC-CR-192	2	12	2.18±0.44
CC-CR-192	3	12	0.26±0.09
CC-KC-69	1	8	0.00±0.00
CC-KC-69	2	12	0.82±0.15
CC-KC-69	3	12	0.26±0.10
CS-170	1	8	0.00±0.00
CS-170	2	12	2.36±0.59
CS-170	3	12	0.30±0.13
CS-CR-148	1	8	0.00±0.00
CS-CR-148	2	12	1.40±0.72
CS-CR-148	3	12	0.24±0.08
SC-170	1	8	0.00±0.00
SC-170	2	12	1.46±0.55
SC-170	3	12	0.54±0.23
SC-CR-148	1	8	0.00±0.00
SC-CR-148	2	12	0.73±0.25
SC-CR-148	3	12	0.19±0.05

† Cropping systems abbreviated as: CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1}), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1}), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted first, receiving 148 kg N ha^{-1} during corn years), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted first, receiving 148 kg N ha^{-1} during corn years), CC-192 (Tilled, continuous corn receiving 192 kg N ha^{-1}), CS-170 (Tilled corn/soybean rotation, corn planted first, receiving 170 kg N ha^{-1} during corn years), SC-170 (Tilled, soybean/corn rotation, soybean planted first, receiving 170 kg N ha^{-1} during corn years).

Analysis of variance (ANOVA) indicated that Cropping System, Year, Rep, and the Cropping System x Year interaction did not significantly affect annual methane (CH_4) loss (Figure 2.5). Liebig et al. (2010) reported that the inclusion of a cereal rye cover crop into the fallow period of a dryland cropping sequence under no-till management practices yielded no net GHG (CH_4, CO_2, and N_2O) reduction benefits. Similarly, Abagandura et al. (2020) highlighted that CH_4 fluxes were similar regardless of treatment across a study period. The trends of CH_4 uptake among these systems appeared regardless of the nature of these systems (tillage vs no-tillage, differences in N rate, differences in crop species). This is largely due to the fact that major biological source of CH_4 gas in cropping systems results from anaerobic decomposition of plant material while the sink of CH_4 gas is oxidation via methanotrophic bacteria under aerobic conditions (Ussiri et al., 2009).

To further understand CH_4 uptake response during various times during the growing season within cropping systems, annual CH_4 uptake data was analyzed by Trimester and similar statistical analyses were conducted (Table 2.29). Trimester data was broken down as follows: Trimester one (Dec-March), Trimester two (April-July), Trimester three (August-November). It is important to note Trimester one contained minimal data as this period in time was not routinely sampled for GHG collection. Analysis of variance (ANOVA) highlighted significant (P<0.05) Trimester and Rep main effects. The Cropping System and Year, main effects as well as Year x Trimester, Cropping System x Year, Cropping System x Trimester, and Cropping System x Year x Trimester interaction effects were not significant. The significant effect that Trimester had on CH_4 uptake was likely caused by fluctuations in temperature and precipitation (Table 2.1 and Table 2.2). These results are similar to those found by Lehman and Osborne (2013) which highlighted CH_4 flux increased linearly with increase soil moisture and temperature. Furthermore, early spring precipitation may have led to more water filled pore spaces thus inducing an anaerobic decomposition of organic matter further increasing flux of CH_4. Overall, these results coincide with previous studies which indicated that residue management and cover cropping did not affect CH_4 loss (Wegner et al., 2018). The range of mean seasonal flux of CH_4 from this study ranged from -0.33 to -0.13 kg ha^{-1} yr^{-1} which is similar to results from site-specific studies at the Purdue WQFS where Hernandez-Ramirez et al. (2009) found that seasonal flux of CH_4 ranged from -0.13 to 0.33 kg ha^{-1} yr^{-1}. Although there are some minor disparities due to Hernandez-Ramirez et al. (2009) having treatments that were net emitters of CH_4 it is important to note the treatments in that study included manure applications that were not present in this study.

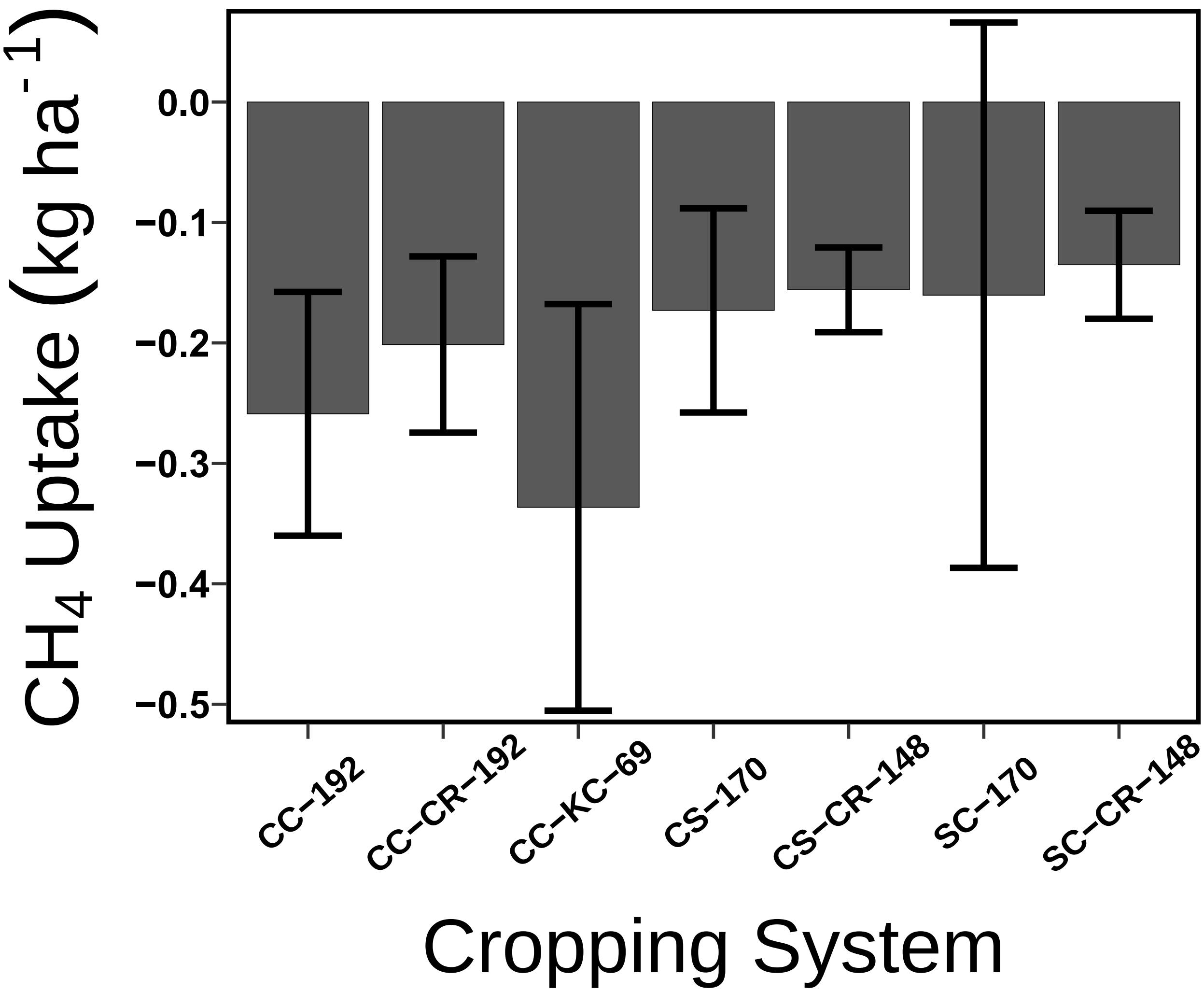

Figure 2.5. Annual CH_4 uptake as influenced by cropping system. Data are means ± standard errors of four replications averaged across the three study years.

Table 2.29. Flux of CH_4 (kg ha^{-1} $trimester^{-1}$) as influenced by Cropping System and Trimester, broken down by trimester and averaged across years. Data are means ± standard errors. Trimester data was broken down as follows: Trimester one (December-March), Trimester two (April-July), Trimester three (August-November). It is important to note Trimester one contained minimal data as this period in time was not routinely sampled for GHG collection. Background shading used to differentiate between cropping systems for ease of observation.

Cropping System	Trimester	N Observations	Flux of CH_4
			---------------kg ha^{-1}---------------
CC-192	1	8	0.00±0.00
CC-192	2	12	-0.12±0.09
CC-192	3	12	-0.14±0.04
CC-CR-192	1	8	0.00±0.00
CC-CR-192	2	12	-0.11±0.03
CC-CR-192	3	12	-0.09±0.06
CC-KC-69	1	8	0.00±0.00
CC-KC-69	2	12	-0.11±0.04
CC-KC-69	3	12	-0.22±0.13
CS-170	1	8	0.00±0.00
CS-170	2	12	-0.03±0.07
CS-170	3	12	-0.14±0.03
CS-CR-148	1	8	0.00±0.00
CS-CR-148	2	12	-0.08±0.03
CS-CR-148	3	12	-0.07±0.02
SC-170	1	8	0.00±0.00
SC-170	2	12	-0.21±0.09
SC-170	3	12	0.05±0.19
SC-CR-148	1	8	0.00±0.00
SC-CR-148	2	12	-0.05±0.03
SC-CR-148	3	12	-0.09±0.03

† Cropping systems abbreviated as: CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1}), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1}), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted first, receiving 148 kg N ha^{-1} during corn years), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted first, receiving 148 kg N ha^{-1} during corn years), CC-192 (Tilled, continuous corn receiving 192 kg N ha^{-1}), CS-170 (Tilled corn/soybean rotation, corn planted first, receiving 170 kg N ha^{-1} during corn years), SC-170 (Tilled, soybean/corn rotation, soybean planted first, receiving 170 kg N ha^{-1} during corn years).

Analysis of variance (ANOVA) indicated that Cropping System and Year significantly ($P<0.001$) affected loss of carbon dioxide (CO_2) (Figure 2.6). However, the Year, Rep, and Cropping System x Year interaction effects were not significant. The significant effect Year played on CO_2 loss is most likely due to varied precipitation and temperature across years (Table 2.1 and Table 2.2). The significant effect Cropping System had on CO_2 loss is likely due to differences in crop species planted, presence/absence of cover crops, and system difference in tillage.

Tukey-Kramer HSD pairwise comparisons revealed there was not a significant difference in CO_2 emissions between the continuous corn (CC-192) control and the corn/soybean (CS-170) and soybean/corn (SC-170) controls when averaged across years (Figure 2.6). Similarly, there was not a significant difference in CO_2 emissions between the corn/soybean (CS-170) and soybean/corn (SC-170) controls. However, CO_2 emissions in the Kura clover continuous corn (CC-KC-192) system were significantly greater than those in the continuous corn (CC-192) control. Similarly, CO_2 emissions in the Kura clover continuous corn (CC-KC-192) system were significantly greater than those in the cereal rye-continuous corn (CC-CR-192) system. The increased flux of CO_2 from this cover cropping system somewhat contradicts previous literature. Paustian et al. (1997) found GHG emissions are generally lower in systems with covered soil, decreased tillage, increased crop residue, and increased use of perennial forages (especially legumes). A potential explanation for this disparity is that the Kura clover living mulch does not cover all of the soil surface due to the strip killed rows to which corn was planted into. This may explain some of the variable results between Paustian et al. (1997) and the results observed in this study. However, CO_2 emissions in the continuous corn (CC-192) control were not significantly different when compared to the cereal rye-continuous corn (CC-CR-192) system. Among the two rotational controls, there was not a significant difference in CO_2 loss. Likewise, there was not a significant difference in CO_2 loss between the corn/soybean (CS-170) control and the cereal rye corn/soybean (CS-CR-192) treatment or between the cereal rye soybean/corn (SC-CR-170) treatment and the soybean/corn rotational (SC-170) control. Finally, there was not a significant difference in CO_2 emission loss among the cereal rye corn/soybean (CS-CR-192) and cereal rye soybean/corn (SC-CR-170) treatments. These results among the cover crop treatments compared to their controls are contradictory to similar system studies which determined that reduced tillage and increased crop residue cover are effective at reducing CO_2 emissions. Al-Kaisi et al. (2005) found that as tillage intensity was reduced and crop residue was increased, CO_2 emissions decreased by 19 to 41%. The results highlighted here within the Kura clover continuous corn (CC-KC-69) system seem to contradict the previously known literature. One explanation for this disparity may be due to living Kura clover plants within the gas chamber. The increased CO_2 loss in the Kura clover system observed in this study may be somewhat confounded due to respiration of the living plants, rather than from the soil alone.

To further understand CO_2 emission response during various times during the growing season and within different treatments, annual CO_2 loss data was analyzed by Trimester and similar statistical analyses conducted (Table 2.30). Trimester data was broken down as follows: Trimester one (Dec-March), Trimester two (April-July), Trimester three (August-November). It is important to note Trimester one contained minimal data as this period in time was not routinely sampled for GHG collection. Analysis of variance (ANOVA) highlighted significant (P<0.001) Cropping System, Year, and Trimester main effects as well Year x Trimester, Cropping System x Year and Cropping System x Trimester interaction effects for Trimester CO_2 loss data. The significant effect Trimester played on CO_2 loss is likely due to variation in precipitation and temperature within years at certain times of the calendar year and was an expected effect (Table 2.1 and Table 2.2). This Trimester effect might also be influenced from major field operations such as tillage, which can increase peak CO_2 emission loss and typically occur during routine timeframes annually (Table 2.4 and Table 2.5). The significant Year x Trimester interaction effect is likely the result of variation in Trimester data from year to year.

Tukey HSD pairwise comparisons were used to investigate significant differences in the Treatment x Trimester interaction. It is important to note beforehand that these Cropping System x Trimester pairwise comparisons were averaged across the three study years. There were no significant differences in CO_2 loss among any of the control treatment in any of the three trimesters (Table 2.30). Furthermore, there were no significant differences in trimester pairwise comparisons among any of the rotational systems or between any of the rotational control/treatment comparisons. However, there were significant differences within trimester data within the continuous corn systems. In Trimester two and three, CO_2 loss was significantly higher in the Kura clover continuous corn (CC-KC-69) treatment compared to the continuous corn (CC-192) control. This pattern of heightened CO_2 loss in trimester two and three can easily be tracked back to the management of this system. In Trimester two, strip killing of the Kura clover occurs resulting in rapid decomposition of the legume. In Trimester three, Kura clover undergoes fall senescence which also contributes to the pool of decomposing plant material which. Furthermore, in Trimester three, CO_2 loss was significantly higher in the Kura clover continuous corn (CC-KC-69) treatment compared to the cereal rye continuous corn (CC-CR-192) system. These results are contradictory to previous studies which have identified that intercropping significantly reduced GHG (CO_2 and N_2O) emissions. Dyer and Echarte (2012) determined CO_2 fluxes across an array of different intercropping systems were significantly reduced as compared to corn and soybean single crop cropping systems. The disparity in results between Dyer and Echarte (2012) and these observations are likely due to differences in intercropping species and differences in suppression techniques. All other trimester pairwise comparisons among control and treatment/control comparisons were not significant from one another. Overall, the mid-season peak (Table 2.30) of CO_2 loss was similarly highlighted by Almaraz et al. (2009) and was largely attributed to increased temperatures in summer months. Almaraz et al. (2009) also noted that conventionally tilled plots had greater peaks of CO_2 as compared to no-till plots, but these differences were only noticeable directly after spring disking and thus differences between different tillage systems were small following corn establishment. Generally, Trimester three revealed decreased CO_2 emissions by all systems. This is likely due to decreased soil disturbance as well as cooling temperatures into late fall and early winter (Table 2.2). Future investigation into a more segmented time series may be needed to tease out more of these nuances in this study. Overall, the range of mean seasonal flux of CO_2 from this study ranged from 2.7 to 5.1 Mg ha^{-1} yr^{-1} which is similar to results found from other site-specific studies at the Purdue WQFS where Hernandez-Ramirez et al. (2009) found that seasonal flux of CO_2 was roughly 4.4 Mg ha^{-1} yr^{-1} across all treatments.

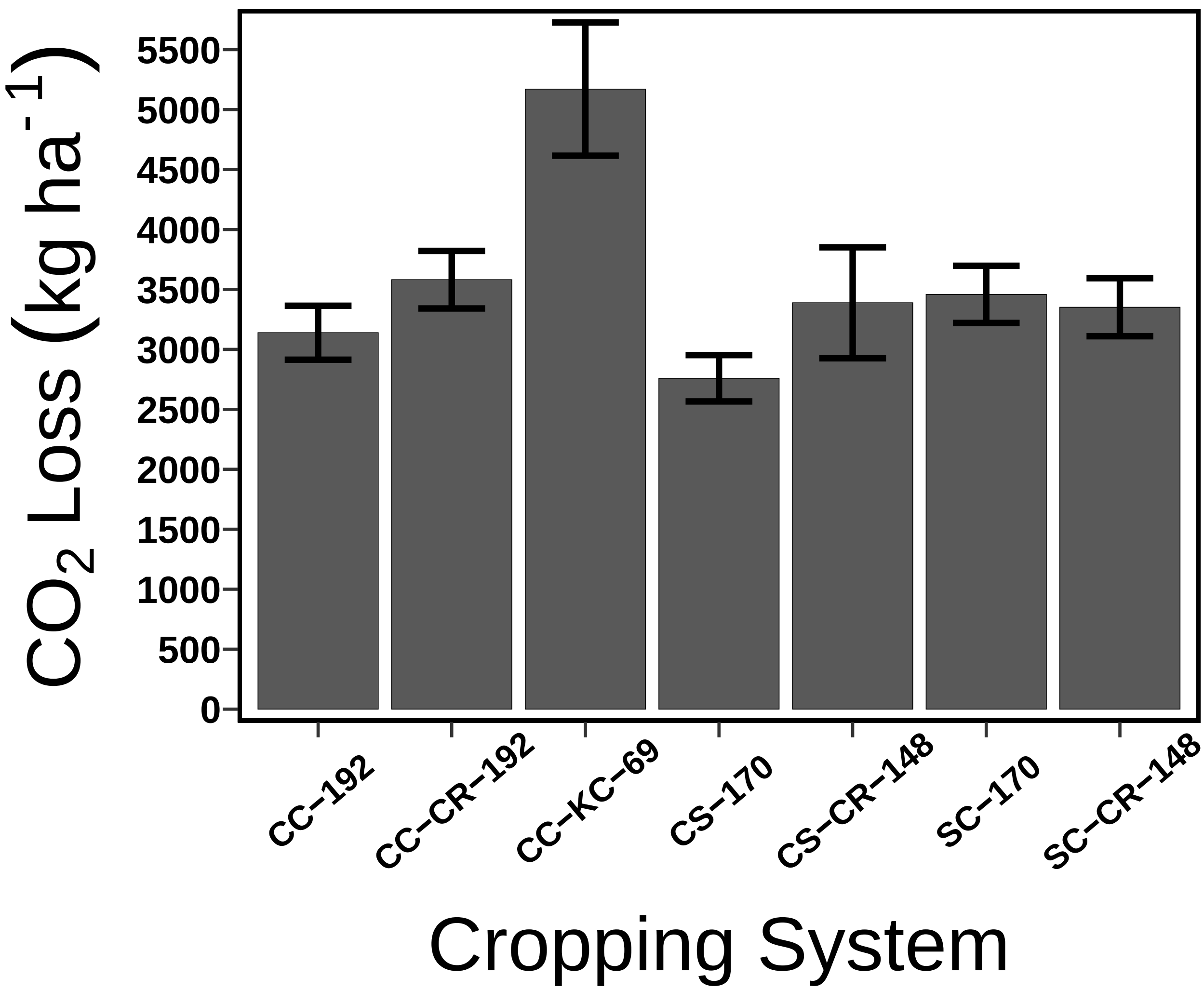

Figure 2.6 . Annual CO_2 loss as influenced by cropping system. Data are means ± standard errors of four replications averaged across the three study years.

Table 2.30. Flux of CO_2 (kg ha^{-1} $trimester^{-1}$) as influenced by Cropping System and Trimester, broken down by trimester and averaged across years. Data are means ± standard errors. Trimester data was broken down as follows: Trimester one (December-March), Trimester two (April-July), Trimester three (August-November). It is important to note Trimester one contained minimal data as this period in time was not routinely sampled for GHG collection. Background shading used to differentiate between cropping systems for ease of observation.

Cropping System	Trimester	N Observations	Flux of CO_2
			---------------kg ha^{-1}---------------
CC-192	1	8	2.5±1.7
CC-192	2	12	1774.9±129.2
CC-192	3	12	1362.5±151.7
CC-CR-192	1	8	10.4±3.7
CC-CR-192	2	12	2347.4±177.4
CC-CR-192	3	12	1227.2±133.8
CC-KC-69	1	8	10.1±3.3
CC-KC-69	2	12	2834.3±331.0
CC-KC-69	3	12	2329.4±330.4
CS-170	1	8	2.5±1.1
CS-170	2	12	1610.3±135.2
CS-170	3	12	1147.2±87.9
CS-CR-148	1	8	6.6±2.1
CS-CR-148	2	12	1960.8±251.8
CS-CR-148	3	12	1423.7±232.4
SC-170	1	8	1.3±0.6
SC-170	2	12	1865.0±113.4
SC-170	3	12	1593.1±181.0
SC-CR-148	1	8	8.3±2.9
SC-CR-148	2	12	1989.2±161.7
SC-CR-148	3	12	1356.8±137.8

† Cropping systems abbreviated as: CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1}), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1}), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted first, receiving 148 kg N ha^{-1} during corn years), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted first, receiving 148 kg N ha^{-1} during corn years), CC-192 (Tilled, continuous corn receiving 192 kg N ha^{-1}), CS-170 (Tilled corn/soybean rotation, corn planted first, receiving 170 kg N ha^{-1} during corn years), SC-170 (Tilled, soybean/corn rotation, soybean planted first, receiving 170 kg N ha^{-1} during corn years).

2.3.14 Summary and Conclusions

The objective of this study was to use field-scale experimentation at the Purdue WQFS research facility to evaluate novel cropping systems (integration of cereal rye as a cover crop and Kura clover as a perennial living mulch) by measuring the herbage theoretical ethanol and grain co-productivity potentials, ecosystem impacts on nutrient use, greenhouse gas (GHG) emissions, water use/quality, and soil tilth of these newly implemented cropping systems. This was implemented by observing weather and climate patterns, overall soil fertility, corn and soybean grain yields, corn and soybean grain N concentrations, corn and soybean stover N concentrations, cover crop biomass N concentrations, cover crop biomass N content, herbage theoretical ethanol yields of cover crop/corn/soybean, tile drained flow, flow-weighted tile drainage NO_3^- concentrations, and annual flux of greenhouse gasses (N_2O, CH_4, CO_2) (Table 2.31).

Table 2.31. Partial N balance as influenced by cropping system. Data presented are means averaged across the two study years where cover crops were in place (2018 and 2019).

	Nitrogen Pools						
	N Fertilizer	Removed Grain	Corn/Soybean Stover Returned	Rye or Kura Clover Biomass	N_2O Loss	NO_3^- Loss	Partial N Balance
Cropping System†	---Kg N ha^{-1} yr^{-1}---						
CC-192	192	146.3	89.2	NA	5.6	6.8	122.5
(CC-CR-192)	192	150.3	65.6	41.8	2.0	7.0	56.5
(CC-KC-69)	69	43.5	38.0	112.5	1.4	6.7	-57.1
(CS-170)	170	134.0	71.1	NA	3.6	7.0	96.5
(CS-CR-148)	148	111.3	57.9	27.9	1.2	4.9	60.6
(SC-170)	0	187.1	277.9	NA	0.9	7.4	82.5
(SC-CR-148)	0	174.2	196.7	32.9	0.5	4.4	-15.3

† Cropping systems abbreviated as: CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1}), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1}), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted first, receiving 148 kg N ha^{-1} during corn years), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted first, receiving 148 kg N ha^{-1} during corn years), CC-192 (Tilled, continuous corn receiving 192 kg N ha^{-1}), CS-170 (Tilled corn/soybean rotation, corn planted first, receiving 170 kg N ha^{-1} during corn years), SC-170 (Tilled, soybean/corn rotation, soybean planted first, receiving 170 kg N ha^{-1} during corn years).

Conclusions from this study include, but are not limited to:

- Cereal rye and Kura clover did not alter observed soil characteristics (Organic matter, phosphorus, potassium, magnesium, calcium, soil pH, and cation exchange capacity)
- Soil N concentrations were not significantly affected after the introduction of cereal rye and Kura clover.
- Cereal rye did not significantly alter corn grain yield, while Kura clover did significantly decrease corn grain yields.
- There were no significant differences of corn grain N concentrations across all years and within all cropping systems before and after cover crop systems were implemented.
- Soybean grain yield was not significantly affected when cereal rye was added to rotational cropping systems.
- Inclusion of cereal rye did not significantly affect soybean grain N concentrations
- Inclusion of cereal rye and Kura clover significantly decreased stover N concentrations only in corn and only in some years.
- Biomass yield of cereal rye was not significantly different than that of Kura clover until 2019, where Kura clover biomass out yielded all cereal rye treatments.
- Kura clover biomass N concentrations were significantly higher than all cereal rye crops regardless of system or year.
- Cereal rye cover crop N content was not significantly different among rotational or continuous corn systems.
- Kura clover cover crop N content was significantly higher than all cereal rye cover crops in all years.
- Total herbage theoretical ethanol yield (HTEY) was not significantly different across rotational cropping systems in any of the study years.
- Total HTEY was not significantly different in continuous corn systems in 2018, however in 2019, HTEY of the Kura clover continuous corn (CC-KC-69) system was significantly lower than that of the continuous corn-cereal rye (CC-CR-192) treatment.
- Annual and quarterly tile drainage flow volumes were significantly affected by treatment; however, Kura clover and cereal rye did not seem to significantly alter tile drainage flow volumes.
- Kura clover was effective at reducing flow-weighted tile drained NO_3- N concentrations

    - Analysis by Quarter revealed that Kura clover was effective at reducing tile drained NO_3^- N concentrations especially in Quarter two.
- Although not statistically significant, the introduction of cereal rye tended to reduce annual and quarterly flow-weighted tile drained NO_3^- N concentrations.
- Cereal rye and Kura clover did not significantly reduce annual or quarterly tile drained NO_3^- N load losses
- Averaged across years, N_2O emissions were significantly reduced in continuous corn systems that included cereal rye as a cover crop or and Kura clover as a mulch crop.
    - Within-year analysis revealed that cereal rye and Kura clover in continuous corn systems reduced N_2O loss data especially in Trimester two (May, June, July and August).
- Cereal rye did not significantly reduce N_2O emissions in rotational systems.
- Averaged across years, annual CH_4 emissions were not significantly affected by treatment.
- Averaged across years, CO_2 emissions in the Kura clover continuous corn (CC-KC-69) system were significantly greater than those of the continuous corn (CC-192) control, especially in Trimester two (May, June, July, August) and Trimester three (September, October, November, December).
    - CO_2 emissions were significantly higher in the continuous corns systems for Trimester two and three when Kura clover was included in the system.
- Annual CO_2 emissions in cereal rye rotational treatments were not significantly different than their controls.

Although there are numerous potentially positive attributes among these novel cropping systems, the efficacy of their ability to cohesively enhance the production of biofuels, food and feed while simultaneously improving ecosystem services is not entirely certain. Future work is needed to supplement the results observed in these two preliminary study years.

A few limitations occurred during the duration of this study. Data loggers used to calculate water flow volumes would temporarily crash, causing gaps in water flow data. These gaps in the data record were generally identifiable, however no effort was made to gap fill missing water data, as tile drained flow patterns can be unpredictable. This could have resulted in an underestimation of overall cropping system N load losses. Further limitations to this study included year to year duplication of concentration values for plant tissues. This decision was made due to limited access to laboratory facilities during the global COVID19 pandemic. Due to restrictive limitation to laboratory space, analysis of 2019 herbage composition could not take place. Thus, 2018 herbage concentration values were applied to 2019 yield values, which were used to calculate overall 2019 herbage theoretical ethanol yield values. Although yield is the major driver in these equations, it is important to note that this estimation could have altered final results in total herbage theoretical ethanol yields among cropping systems. Lastly, this entire study was conducted at one location with only two years of treatment data. To further study these cropping systems and their effect on the observed parameters, multiple locations across numerous years would add robustness to the results stated above, although it is notable to mention the uniqueness of the Purdue water quality field station is what made this study possible and may not be easily adapted to numerous locations/environments without sufficient funding.

2.3.15 Future Considerations

Within years there were some notable differences in seasonal precipitation and temperatures. Thus, if one of the two years of cover crop implementation data is skewed, the results we obtained may be somewhat skewed from what a true average over many years could be. To gain a better grasp of how these novel cropping systems perform from year to year, additional study years may be needed to substantiate these findings. In addition, specific datasets such as tile drainage flow, tile drainage NO_3^- load losses, greenhouse gas fluxes, and herbage theoretical ethanol yields should be investigated using linear regression to derive correlation coefficients to further better understand the tradeoffs within some of the intervariable relationships. More intricate time series trends should be investigated to observe patterns of peak influence during the growing season among the various ecosystem service effects. More experimentation is needed to determine an effective method of corn management in a Kura clover perennial living mulch system that simultaneously increases corn yield while avoiding complete termination of the clover. Targeted corn hybrid selection may be needed to locate hybrids that better match delayed planting dates due to late spring cereal rye harvests. Improved planter technology might be needed to improve corn/soybean stand emergence. Additionally, other crop species, especially rye varieties bred specifically for their use as a cover crop, should be investigated to meet the multipurposed goals of this study. Lastly, a theoretical economic analysis should be conducted to verify the legitimacy of these cropping systems and to understand if government subsidies will be needed to generate positive return on investments.

APPENDIX A

Soil Description 1

Location: Agronomy Research Center, Water Quality Field Station.

Tipton till plain physiographic region, S 1/2, E V2, SW %, NW %, Sec 28, T24N, R5W.

Landform, depression

Soil Delineation, Drummer Soils

Soil series of pedon, description fits Drummer

Soil Classification, fine—silty, mixed, mesic Typic Endoaquoll

Drainage Class, poorly drained

A1-0 to 5 cm; black (IOYR 2/1) silty clay loam; moderate fine and medium granular structure; firm; many fine and medium roots; clear smooth boundary.

A2-5 to 12 cm; black (IOYR 2/ 1) silty clay loam; moderate medium and thick platy structure; firm; many fine and medium roots; clear smooth boundary.

A3—12 to 29 cm; black (IOYR 2/1 silty clay loam; moderate fine and medium subangular blocky structure; firm; many fine and medium roots; gradual wavy boundary.

A4—29 to 42 cm; very dark gray (IOYR 3/ 1) silty clay loam; moderate medium subangular blocky structure; firm; many fine and medium roots; gradual wavy boundary.

Bg1-42 to 51 cm; dark grayish brown (2.5Y 4/2) silty clay loam; few medium distinct light yellowish brown (2.5Y 6/4) mottles; moderate medium subangular blocky structure; firm; few fine roots; common fine and medium pores; thin continuous dark grayish brown (2.5Y 4/2) clay films on faces of peds; many thin continuous black (IOYR 2/ 1) organic coatings on faces of peds and on surfaces of root channels; gradual wavy boundary.

Bg2-51 to 70 cm; grayish brown (2.5Y 5/2) silty clay loam; few medium distinct olive yellow (2.5Y 6/6) mottles; moderate medium prismatic structure parting to moderate medium subangular blocky; firm; few fine roots; common fine and medium pores; thin continuous very dark grayish brown (IOYR 3/2) clay films on faces of peds; many thin continuous black (IOYR 2/ 1) organic coatings on faces of peds and on surfaces of root channels; gradual wavy boundary.

Bg3-70 to 82 cm; grayish brown (2.5Y 5/2) silty clay loam; common medium prominent brownish yellow (IOYR 6/8) mottles; weak medium prismatic structure parting to moderate medium subangular blocky; firm; many black (IOYR 2/ 1) organic coatings on surfaces of root channels.

2Bg4—82 to 113 cm; grayish brown (2.5Y 5/2) clay loam; many medium prominent brownish yellow (IOYR 6/8) mottles; moderate medium subangular blocky structure; firm; patchy grayish brown (2.5Y 5/2) clay films on surfaces of peds; many black (IOYR 2/ 1) organic coatings on surfaces of root channels; thin continuous carbonate coatings on faces of peds.

201 13 to 147 cm; light olive brown (2.5Y 5/4) loam and clay loam; common medium distinct gray (2.5Y 6/ 1) mottles; massive structure; strongly effervescent.

<u>Soil Description 2</u>

Location: Agronomy Research Center, Water Quality Field Station.

Tipton till plain physiographic region, S 1/2, E 1/2, SW %, NW %, Sec 28, T24N, R5W.

Landform, swell

Soil Delineation, Raub—Brenton Complex 0 to 1 percent slope

Soil Series, more like Throckmorton soil with a mollic epipedon

Soil Classification, fine—silty, mixed, mesic Typic Argiudoll

Drainage Class, moderately well drained

Al-O to 8 cm; black (10YR 2/ 1) silty clay loam; weak fine granular structure; firm; many fine and medium roots; clear smooth boundary.

A2-8 to 20 cm; black (10YR 2/1) silty clay loam; weak medium platy structure; firm; many fine and medium roots; clear smooth boundary.

A3-20 to 37 cm; dark brown (10YR 3/3) silty clay loam; few medium faint dark yellowish brown (10YR 4/4) mottles; moderate medium subangular blocky structure; firm; many fine and medium roots; thin patchy black (10YR 2/ 1) clay films on faces of peds; gradual wavy boundary.

Bt1-37 to 60 cm; dark yellowish brown (10YR 4/4) silty clay loam; common medium distinct brownish yellow (10YR 6/6) mottles; moderate medium subangular blocky structure; firm; many fine roots; thin continuous light olive brown (2.5Y 5/4) clay films on faces of peds; thin continuous black (10YR 2/ 1) organic coatings on surfaces of root channels; gradual wavy boundary.

Bt2-6O to 84 cm; dark yellowish brown (10YR 4/4) silty clay loam; common medium distinct brownish yellow (10YR 6/6) mottles; moderate medium subangular blocky structure; firm; thin continuous dark grayish brown (1 OYR 4/2) clay films on faces of peds; gradual wavy boundary.

2Bt3-84 to 104 cm; brown (10YR 5/3) clay loam; common medium prominent brownish yellow (10YR 6/8), and common fine faint grayish brown (10YR 5/2) mottles; weak medium subangular blocky structure; firm; few fine black (10YR 2/ 1) manganese accumulations; continuous carbonate coatings on faces of ped; gradual wavy boundary.

2C-104 to 180 cm; light olive brown (2.5Y 5/4) loam; common medium distinct brownish yellow (10YR 6/6), and common medium distinct gray (2.5Y 5/ 1) mottles; massive structure; continuous carbonate coatings on faces of fractures; gradual wavy boundary; strongly effervescent.’

Table A1.1 .Instances where flow was visually confirmed from tile lines that contradict non-flow data records from the calibrated tipping bucket systems used at the Purdue University WQFS.

Year	Observed Flow Not Recorded by TB Sensors†	Total Projected Observations
2017	25	10,220
2018	36	10,220
2019	17	10,220
Total	78	30,660

†TB abbreviation stands for the tipping buckets used to count flow during flow events. Instances of observed flow not recorded were counted by individual tile line.

Table A1.2. ANKOM procedures used for analysis of cover crop biomass composition

Procedure	Description	Hyperlink
ANKOM Method 13	Neutral Detergent Fiber in Feeds - Filter Bag Technique (for A2000 and A2000I	https://www.ankom.com/sites/default/files/document-files/Method_13_NDF_A2000.pdf
ANKOM Method 12	Acid Detergent Fiber in Feeds - Filter Bag Technique (for A2000 and A2000I)	https://www.ankom.com/sites/default/files/document-files/Method_12_ADF_A2000.pdf
ANKOM Method 8	Method 8 – determining Acid Detergent Lignin in beakers	https://www.ankom.com/sites/default/files/document-files/Method_8_Lignin_in_beakers_0.pdf
ANKOM Ashing Procedure 034	Ashing Procedure 034	https://www.ankom.com/sites/default/files/document-files/AS034_Ashing_Procedure.pdf

Table A1.3. Non-flow-weighted tile drained NO_3^- N concentrations as influenced by cropping system. Data are means ± standard errors of functioning tile lines. Background shading denotes soybean years in rotational cropping systems. Means followed by the same letter and within like systems (continuous corn versus rotational systems) are not significantly different at $P<0.05$ using a Tukey-Kramer HSD test. Vertical black line indicates the separation between Tukey-Kramer comparisons.

	Cropping System†						
Year	(CC-192)	(CC-CR-192)ϑ	(CC-KC-69)ϑ	(CS-170)λ	(SC-170)	(CS-CR-148)ϑ	(SC-CR-148)
	mg L^{-1}						
2017	11.6±1.1	11.2±1.1	5.0±2.4	11.9±0.2	9.3±1.5	9.9±1.5	10.2±1.9
2018	15.9±1.7	8.1±0.7	2.9±1.2	9.6±3.2	13.6±1.7	4.4±1.3	4.7±0.7
2019	6.6±1.8	5.5±1.3	2.1±1.0	5.7±0.9	5.6±0.7	5.5±1.8	3.5±0.4
Trt Ave.	11.4±1.4 **A**	8.3±1.0 **A**	3.4±0.9 **B**	9.0±1.4 **AB**	9.5±1.2 **A**	6.6±1.1 **AB**	6.1±1.2 **B**
Corn Ave.	11.4±1.4	8.3±1.0	3.4±0.9	8.8±1.8	13.6±1.7	7.7±1.4	4.7±0.7
Soy Ave.	NA	NA	NA	9.6±3.2	7.4±1.0	4.4±1.3	6.9±1.5

† Cropping systems abbreviated as: CC-CR-192 (No-till, continuous corn with cereal rye receiving 192 kg N ha^{-1}), CC-KC-69 (No-till continuous corn with Kura clover receiving 69 kg N ha^{-1}), SC-CR-148 (No-till soybean/corn rotation with cereal rye, soybean planted first, receiving 148 kg N ha^{-1} during corn years), CS-CR-148 (No-till corn/soy rotation with cereal rye, corn planted first, receiving 148 kg N ha^{-1} during corn years), CC-192 (Tilled, continuous corn receiving 192 kg N ha^{-1}), CS-170 (Tilled corn/soybean rotation, corn planted first, receiving 170 kg N ha^{-1} during corn years), SC-170 (Tilled, soybean/corn rotation, soybean planted first, receiving 170 kg N ha^{-1} during corn years). ϑ Denotes cropping systems where one tile was omitted as 'non-functioning' thus resulting in an average of three replications as opposed to four. λ Denotes cropping systems where two tiles were omitted as 'non-functioning' thus resulting in an average of two replications as opposed to four.

Table A1.4. Non-flow-weighted tile drained NO_3^- N concentrations as influenced by cropping system and quarter. Data are means ± standard errors of functioning tile lines averaged across the three study years.

	Quarter 1	**Quarter 2**	**Quarter 3**	**Quarter 4**
Cropping System	---------- mg L^{-1} quarter $^{-1}$ ----------			
CC-192	9.2±1.1 **A**	16.3±2.8 **A**	15.6±1.5 **A**	7.1±1.4
CC-CR-192	7.4±0.6	8.9±1.4 **B**	15.8±2.4	6.4±1.3
CC-KC-192	2.8±0.3 **B**	4.7±0.9 **B**	4.3±1.8 **B**	1.0±0.3
CS-170	8.9±1.1	12.1±1.7	18.0±4.5	5.6±1.0
CS-CR-148	5.9±0.5	6.5±1.4	9.2±2.2	7.2±3.8
SC-170	8.4±0.6	14.1±2.4 **A**	5.1±1.1	5.6±0.9
SC-CR-148	6.3±0.8	7.3±1.0 **B**	6.2±1.6	3.1±0.6

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