Management Considerations for Maize in Kura Clover Living Mulch

A DISSERTATION
SUBMITTED TO THE FACULTY OF THE
UNIVERSITY OF MINNESOTA
BY

Jonathan Robert Alexander

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF SCIENCE

John M. Baker and Rodney T. Venterea

May 2022

ACKNOWLEDGEMENTS

My sincere gratitude goes to my advisors, colleagues, and peers who have supported me during my time at the University of Minnesota. My appreciation for their guidance and mentorship cannot be overstated.

ABSTRACT

Kura clover living mulch (KCLM) can be incorporated into upper-Midwestern row-crop production systems to provide perennial living groundcover during vulnerable spring and fall fallow periods. The cool season legume crop takes advantage of an extended growing season to increase carbon capture, provide habitat and nutrition to soil biota, and reduce nutrient loss relative to monocrop maize. These advantages, as well as observed improvements in water infiltration and reductions of soil and nitrate loss, may help to mitigate regionally important environmental impacts from intensive agricultural production systems in the upper Midwest. Designing KCLM systems for upper-Midwestern row-crop production requires consideration of the current production needs and management strategies. The objectives of this research were to (i) determine the effect of row establishment method and fertilizer N rate on maize yield and nitrogen use efficiency, (ii) identify the effect of row establishment method and fertilizer N source on the spatiotemporal distribution of clover and maize roots, and (iii) describe the effect of fertilizer N rate on the spatial and temporal distributions of clover biomass inputs, soil N concentrations, and nitrous oxide emissions within a KCLM for maize. These questions were addressed with two field experiments conducted at the Rosemount Research and Outreach Center in Rosemount, MN and one experiment conducted at Rosemount and the Arlington Research Station in Arlington, WI. The results of this research indicate that optimal row establishment methods during drought conditions depend on the severity of moisture stress, tillage intensity is positively correlated with clover root loss, maize roots were isolated to the tilled row zone to a depth of 0.20 m, and N_2O emissions are alarmingly high from the interrow zone under normal growing conditions. The conclusions of this work are that aboveground clover biomass inputs provide a relatively small amount of N to the row crop and that N contributions are sourced from disturbed roots during row establishment. This indicates that N management in a KCLM-maize cropping system is highly dependent on stand history. Finally, 85% of N_2O was emitted from the interrow zone in a KCLM under normal weather conditions. This finding indicates that hotspots of microbial denitrification are a potentially significant contributor to N_2O emissions in KCLM systems, and that mitigation strategies may depend on clover

residue management. Overall, KCLM systems may benefit maize producers in the upper Midwest, but care must be taken to mitigate known management challenges. Further research should be conducted to define remaining management questions and to identify strategies to mitigate high N_2O emissions from these systems.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AIC	Akaike information criterion
BIC	Bayesian information criterion
BH	Banded herbicides
C3	Dominant photosynthetic pathway of non-grass plants
C4	Dominant photosynthetic pathway of grasses
DAP	Days after planting
DFR	Distance from row
DOY	Day of year KCLM
DSCI	Drought severity and coverage index
KCLM	Kura clover living mulch
NB	Nitrogen balance; total crop N uptake - fertilizer N
NUE	Nitrogen use efficiency; total crop N uptake/fertilizer N
RZT	Rotary zone tillage
SD	Standard deviation
ST	Shank-type strip tillage

CHAPTER 1: MAIZE PRODUCTION IN A KURA CLOVER LIVING MULCH UNDER DROUGHT CONDITIONS

1.1. Synopsis

Row establishment for maize production in a kura clover living mulch (KCLM) is important to reduce competition between the living mulch and maize seedlings. Methods that have been used include banded herbicide and various forms of zone tillage. This study investigated the effect of row-establishment and fertilizer N rate treatments on maize yield and nitrogen use efficiency in a KCLM system in Arlington, WI, and Rosemount, MN, during the 2021 growing season. Row establishment treatments included rotary zone tillage (RZT), shank strip-tillage (ST), and banded herbicides (BH), each evaluated at six N rates from 0 to 225 kg N ha^{-1}. Challenging environmental conditions were experienced during the study period, which included extended periods of moderate and severe drought. The Arlington and Rosemount sites received 64 and 63% of the normal precipitation between 20 Apr and 31 Oct 2021, but poor distribution and higher temperatures at Rosemount elevated drought severity indices at that site. Grain yields at Rosemount were 3.6 Mg ha^{-1}, while Arlington produced yields that matched expectations for the area (10 Mg ha^{-1}). Row establishment affected maize grain yield differentially at each site, where, under severe drought conditions, BH maximized maize yield, while under moderate drought conditions, the higher level of tillage (RZT) maximized maize yield. These responses are most likely attributed to reduced moisture loss in the row-zone of the BH treatment and the greater level of clover suppression in the RZT treatment and their interactions with specific weather conditions at the research sites. Results from this study indicate that row establishment methods in a KCLM cropping system should be considered with spring environmental conditions and the expected weather outlook.

1.2. Introduction

Kura clover living mulch (KCLM) is a perennial cover cropping system that can be used within maize production systems as a soil and water conservation tool (Zemenchik et al., 2000). Kura clover's robust root system and vigorous spring growth reduces soil erosion and takes up excess moisture and nutrients when conventionally managed fields lie fallow (Ochsner et al., 2010, 2017; De Baets et al., 2011; Siller et al., 2016; Baker et al., 2021). These attributes provide environmental benefits relative to conventionally managed maize during wet or non-moisture limiting spring conditions. However, there is limited information regarding the effect of agronomic management of KCLM systems on crop performance during drought.

In a KCLM, the row-zone must be freed of clover and weeds to reduce the competition of the living mulch with emerging maize seedlings. The literature describes two main approaches to row establishment: strip-tillage and herbicides. Most of the early work optimizing KCLM management in Wisconsin utilized herbicides (Zemenchik et al., 2000; Albrecht and Sabalzagaray, 2006). Affeldt et al., (2004) utilized herbicide suppression that included preplant broadcast glyphosate and dicamba, banded dicamba and clopyralid. This suppression regime resulted in no difference between maize yield in monocrop and KCLM treatments. Later work in Minnesota utilized approaches that relied on strip tillage. Dobbratz et al., (2019) tested two levels of tillage intensity against banded glyphosate row establishment treatments and found that strip tillage increased maize emergence, hastened crop development, reduced competition from clover, and increased maize grain yield relative to banded herbicides. These results were attributed to reduced inter-species competition under strip tillage treatments.

While both banded herbicides and strip tillage are useful in managing competition between the KCLM and the row crop, each relies on different modes of action that influence the soil environment in different ways. It is well known that tillage influences water use efficiency due to its effects on available energy, available water, and the rate of exchange between the soil and the atmosphere (Hatfield et al., 2001; Irmak et al., 2019). Ghaffarzadeh et al., (1997) found that maize yield responded differentially to no-till and

strip tillage in a wet and dry growing season in a strip intercropping system, where no-till increased yields under moisture limited conditions, and strip till maximized yields during a wet year. In that study, soil water content was affected by tillage in the dry year, while differences were less pronounced and confined to upper soil depths in the wet growing season. Ochsner et al., (2010) measured soil moisture levels for maize under KCLM and conventional management. They found that the KCLM induced a soil water deficit of ~50 mm in the KCLM in early June, which is the time that corresponds with peak clover biomass (unpublished data). However, that study observed higher than normal precipitation in each of the three growing seasons.

Identifying relationships between management and maize yield in a KCLM under drought conditions is timely, as climate change is projected to increase the frequency and severity of drought (Polasky et al., 2021). These relationships, in combination with other remaining management questions related to fertilizer N management will provide necessary information for growers managing these systems under uncertainty. This research aimed to determine the influence of row-establishment technique and fertilizer N rate on maize yield and crop efficiency metrics of maize grown in a KCLM. Because of the unique growing conditions experienced during the 2021 growing season, results will be presented with emphasis on drought intensity and its influence on cropping system performance under row-establishment and fertilizer N treatments.

1.3. Materials and Methods

Field experiments were conducted during the 2021 growing season at the University of Wisconsin - Arlington Research Station in Arlington, WI, and the University of Minnesota - Rosemount Research and Outreach Center in Rosemount, MN. Soils at the Arlington site were a Saybrook silt loam (Fine-silty, mixed, superactive, mesic Oxyaquic Argiudolls), and soils at the Rosemount site were a Waukegan silt loam (Fine-silty over sandy or sandy-skeletal, mixed, superactive, mesic Typic Hapludolls). Kura clover stands were more than 3 years old at each site and the clover was managed as a living mulch for maize production in the previous growing season. While each site was

managed under the same cropping system in the previous year, the specific management of the plot area differed, where the Rosemount site received shank strip tillage, broadcast glyphosate application at 2.2 kg a.e. ha^{-1} and no fertilizer N, and the Arlington site received broadcast glyphosate at 4.5 kg a.e. ha^{-1}, broadcast glufosinate at 2.5 kg a.e. ha^{-1}, and broadcast urea at 240 kg N ha^{-1}.

The experiment investigated two factors, row establishment method and fertilizer N rate, and their effects on maize yield and nitrogen use efficiency. At both sites, individual plots measured 3 by 12 m and were laid out in a split-plot design with four replications, with row establishment as the main plot and fertilizer N rate as the split-plot treatments. Row establishment treatments included rotary zone tillage (RZT), shank strip-tillage (ST), and banded herbicides (BH), and were chosen based on previous work and their practicality for farmers managing a KCLM (Affeldt et al., 2004; Alexander et al., 2019a). Additional details regarding the row establishment treatments are provided in Table 1.1. Within row establishment treatments, fertilizer N was applied at 0, 45, 90, 135, 180, and 225 kg N ha^{-1} as SuperU, urea fertilizer with nitrification and urease inhibitors, banded in the center of the row with an EarthWay garden seeder at a 50 mm depth in the RZT and ST treatments and on the surface in the BH treatment (Koch Agronomic Services, Wichita, KS, USA; EarthWay, Bristol, IN, USA). Row establishment and fertilizer treatments were applied on 7 May at the Arlington site and 11 May at the Rosemount site. The maize hybrid DEKALB DK47-54RIB was seeded on 0.76 m rows at 89,000 seeds ha^{-1} on 8 May and 13 May at the Arlington and Rosemount sites, respectively. Glyphosate herbicide was broadcast at 1.4 kg ha^{-1} on each plot area on all treatments one week after planting to kill weeds in the row zone and to chemically suppress clover growth during the establishment period. Each site received 100 kg P_2O_5 ha^{-1}, 114 kg K_2O ha^{-1}, and 39 kg SO_4-S ha^{-1} as broadcast triple-superphosphate (0-45-0) and sulfate of potash (0-0-50-17) 10 d after planting.

Grain, cobs, and stover were harvested on 7 Oct and 21 Oct at the Arlington and Rosemount sites, respectively. Maize ears were picked and stover was cut from a 3 m long row in each plot. Maize stover was weighed and subsampled before the subsamples were chopped into 20-50 mm pieces, bagged, and weighed at the field moisture level.

Maize ears and stover subsamples were dried at 60° C until reaching a constant mass. Grain was shelled from the cob and each product was weighed before being subsampled and pulverized. Dry stover subsamples were weighed to determine field moisture content that could be related to the whole sample mass before a subsample was collected and pulverized. Pulverized grain, cob, and stover subsamples were analyzed for N content with the Dumas dry combustion method in an elemental combustion analyzer (Elementar GmbH, Langenselbold, DE) (Bremner and Mulvaney, 1982). Yield and N content data were used to calculate total crop N uptake, N balance (NB = total crop N uptake - fertilizer N) and nitrogen use efficiency (NUE = total crop N uptake/fertilizer N), which have potential to indicate differences in in-season soil N contributions from the living mulch (Congreves et al., 2021).

Dependent variables were tested for normality and homogeneity of variance by visual inspection of QQ plots and residual vs fitted plots, respectively (Kutner et al., 2004). When data from both sites were tested, several of the dependent variables violated one or more assumptions of ANOVA, and data transformations were unable to satisfy the required conditions. Because of this, data were separated between sites, retested, and when needed, appropriate data transformations were made to satisfy assumptions of normality and homogeneity of variance. Data were then fit with linear mixed-effects models in the *lme4* package (Bates et al., 2014) in R (R Core Team, 2020), where row-establishment method and fertilizer N rate were fixed effects, and the main plot nested within block was the distribution of random error (Langhans et al., 2005). Models were tested for and met the assumption of homogeneity of variance by visual inspection of residual vs fitted plots (Kutner et al., 2004). Mixed models were then tested with the type III F test with Kenward-Roger estimated denominator degrees of freedom using the *car* package in R. When $P \leq 0.05$, treatment means were separated using the *emmeans* (Lenth et al., 2018) and *multcomp* (Fox and Weisberg, 2019) packages in R, and results were presented using compact letter display (Piepho, 2018).

Weather data were collected from the UW Arlington Farm weather station of the National Weather Service and the US-Ro6 weather station of the AmeriFlux network (Novick et al., 2018), for the Arlington and Rosemount sites, respectively. Drought

severity and coverage index (DSCI), which represents an integrated value of drought severity for a region ranging from 0 to 500, was collected from the US Drought Monitor for Columbia County, WI, and Dakota County, MN, for the Arlington and Rosemount sites, respectively (National Drought Mitigation Center et al., 2021).

1.4. Results

1.4.1. Environmental conditions

Growing conditions were challenging at the two sites during the 2021 growing season. The Arlington and Rosemount sites received 64 and 63% of the 1991-2020 average precipitation between 20 Apr and 31 Oct, respectively (Figure 1.1). Arlington and Rosemount were also hotter than normal and accumulated 108 and 122% of the 1991-2020 average growing degree days between 20 Apr and 31 Oct, respectively (Figure 1.2). Despite similar amounts of precipitation across sites, the distribution of rainfall varied and above average air temperature increased the relative drought severity at the Rosemount site. These conditions were reflected in the DSCI, which categorized the Rosemount and Arlington sites under severe and moderate drought conditions, respectively, for most of the growing season.

1.4.2. Arlington

At Arlington, row establishment treatments influenced grain yield, grain N, stover N, total N, NB, and NUE (Table 1.2). Grain yield and Grain N were greatest in the RZT treatment and lowest in the BH treatment, while ST was not significantly different than RZT or BH treatments ($p < 0.05$). Stover N was greatest in the RZT treatment, lowest in the ST treatment, and the BH treatment was not significantly different than the RZT and ST treatments. Total N uptake, NB, and NUE was greater in the RZT treatment than the ST and BH treatments.

Grain N, stover yield, stover N, total N, NB, and NUE were significantly affected by fertilizer N rate (Table 1.2). Grain N was lowest in the unfertilized treatment and

greatest in the 90 – 180 kg N ha^{-1} treatments. Stover yield was lowest in the unfertilized treatment and greatest in the 135 and 225 kg N ha^{-1} treatments. Total N was greater in the 90 – 225 kg N ha^{-1} treatments than the unfertilized treatment. N Balance and NUE were maximized in the low N rate treatments and followed a downward trend with increasing fertilizer N rates.

1.4.3. Rosemount

At Rosemount, row establishment treatments significantly affected grain yield, grain N, total N uptake, and NUE (Table 1.3). For each of these response variables, the BH treatment was significantly greater than the RZT treatment and the ST treatment was not significantly different than the BH or RZT treatments at the $p < 0.05$ level. Nitrogen Balance was also affected by the row establishment treatment, where the BH and ST treatments took up a larger fraction of the applied fertilizer N than the RZT treatment.

Stover yield, stover N, total N uptake, NB, and NUE were affected by fertilizer N rate treatments (Table 1.3). Stover yield was greatest in the 135 and 180 kg N ha^{-1} treatments, lowest in the unfertilized plots, and the 45, 90, and 225 kg N ha^{-1} treatments were not significantly different than the highest and lowest yielding treatments. Stover N was greatest in the 135 - 225 kg N ha^{-1} treatments, lowest in the unfertilized treatment, and the 45 – 90 kg N ha^{-1} treatments were not different than the 0 and 180 kg N ha^{-1} treatments. Similarly, total N uptake was lowest in the unfertilized treatment, greatest in the 135 – 225 kg N ha^{-1} treatment, and the 45 – 90 kg N ha^{-1} treatments were not significantly different from high and low uptake treatments. Nitrogen balance and NUE were maximized at the low N rate treatments and followed a negative trend with increasing fertilizer N application. The NB was positive in the 0 - 45 kg N ha^{-1} treatments and was -149 at the highest N rate.

1.5. Discussion

Results from these experiments highlight the risks of utilizing a KCLM system for maize production in rainfed cropland in the upper Midwest. While the Arlington site had

acceptable grain yields at all fertilizer N rates despite moderate drought conditions during some periods of the growing season, the Rosemount site nearly experienced a total crop failure. Differences in crop performance between sites is almost certainly a result of moisture stress. While the Arlington and Rosemount sites received a similar amount of total rainfall, the distribution varied. Rainfall was relatively well distributed over the growing season at the Arlington site, but the Rosemount site received most of its precipitation in May and early Sep.

The lack of precipitation during critical development periods at the Rosemount site impacted maize yield and profitability. The Rosemount site received very little (< 16 mm) precipitation in the 23 days before and 4 days after planting and entered a severe drought (DSCI > 200) on 27 Jul that ended on 24 Aug. These periods of extended moisture stress likely reduced stand count and slowed seedling development and stunted maize plants between the V6 and VT growth stages, when they determined the number of ears, kernel rows, and kernels per row (Darby and Lauer, 2013). The moisture limited conditions continued into maize silking, which is a critical time period for pollination and seed set. Drought conditions in late vegetative stages and silking reduce yield potential by 3 and 7% d^{-1}, respectively. The underdeveloped maize plants nearly died in two of the four blocks at the Rosemount site.

Moisture stress may have also been influenced by soil quality and site history. While grain yields across all plots at Rosemount yielded 3.6 Mg ha^{-1}, the plots that were less affected by drought yielded 5.3 Mg ha^{-1}, which is not significantly different than conventionally managed plots adjacent to the study area, which yielded 5.2 (SD = 0.6) Mg ha^{-1} (unpublished data). Differences in crop development and maturity between blocks within the plot area were apparent early in the growing season, and without rainfall in early Sep, it is likely that the most severely affected plots would not have reached physiological maturity. The highest and lowest yielding plots at the Rosemount site were spatially correlated and differences in crop performance are likely attributed to soil depth and texture, which are well known to exacerbate the effects of drought (Jia et al., 2013). Because of the variability in soil properties across the plot area, we cannot say

conclusively whether the KCLM exasperated or helped the maize crop respond to drought conditions.

Differences in moisture stress between the two sites may have also been influenced by initial clover stand health. Kura clover growth is most vigorous from mid-April to late-May and typically accumulates ~2 Mg biomass ha^{-1} during that time. Ochsner et al., (2010), analyzed soil water content and estimated evapotranspiration and found that a KCLM system imposed a water deficit of 37 – 50 mm in May relative to monocrop maize. Living mulch management imposes prolonged stress on kura stands and depletes belowground clover biomass reserves (unpublished data). While both sites were used as a living mulch in the previous year, the management of the sites may have favored increased relative clover health at the Rosemount site. Specifically, the Arlington site received broadcast glyphosate at 4.5 kg a.e. ha^{-1} and glufosinate at 2.5 kg a.e. ha^{-1} while the Rosemount site was managed with strip tillage and broadcast application of glyphosate at 2.2 kg a.e. ha^{-1}. The additional broadcast herbicide application may have increased the suppression of clover in the interrow and reduced water use at the Arlington site.

Row establishment treatments influenced maize performance at both sites. At Arlington, the RZT treatment outperformed the ST and BH treatments, and at Rosemount, the BH treatment outperformed the ST and RZT treatments. While this experiment did not collect in-season soil moisture, clover biomass, or crop development data, the literature suggests that these observed treatment differences could be attributed to moisture conservation, inter-species competition, and unique weather conditions during the study period. Conservation tillage management, including no-till, is known to increase soil moisture availability and reduce evapotranspiration during the early growing season (Blevins et al., 1983). This period was critical at the Rosemount site because of dry planting conditions, whereas Arlington received rain the day before tillage and planting. Because soil moisture at Arlington was not limiting as seedlings emerged, the more intensive mechanical suppression of clover from the RZT treatment likely increased the rate of emergence and development of maize at this site (Dobbratz et al., 2019). The Arlington site entered a moderate drought on 8 Jun, when maize was at the V4 stage that

lasted until 20 Jul, when maize was entering the VT stage, and the better developed and more deeply rooted plants may have been able to better withstand the moderate moisture stress during that period. The Rosemount site did not receive significant rainfall in the 23 days preceding and 7 days after planting. The low moisture in the seed zone of the RZT treatment likely hampered maize emergence and development relative to ST and undisturbed BH treatments due to water conservation of the less intensive tillage. The Rosemount site entered a moderate drought on 8 Jun, when maize was at the V3 stage, and a severe drought on 27 Jul, when maize was at the V6-V8 stage, that lasted until 24 Aug. Crops were severely stunted by moisture stress and poorly established maize nearly died due to the prolonged drought conditions.

Fertilizer N rate treatments did not affect maize performance at either site. Kura clover living mulch systems have been found to reduce fertilizer N requirements for maize in previous research, however, because of the unique environmental conditions during the 2021 growing season, conclusions regarding the N management of KCLM-maize systems will not be made based on the observed data. Nitrogen efficiency metrics did not reveal differences in the N contributions from row-establishment treatments. This result is expected, as grain yields at both sites were unresponsive to fertilizer N rate treatments. These metrics might be useful in future N rate research to determine estimates of in-season N contributions from the KCLM or other legume inter-cropping systems under N limited conditions.

1.6. Conclusions

Despite the unique environmental conditions, these findings are important to highlight the risks associated with the use of cover crops and living mulches during moderate to severe drought conditions in rainfed maize systems in the upper Midwest. While maize performance was adequate at the Arlington site, the uneven distribution of rain and excessive heat at Rosemount resulted in a near crop failure. In years with abnormally dry spring conditions, the results indicate that the banded herbicide treatment can be used to suppress clover growth in the row-zone and conserve soil moisture and

reduce production costs. In years with favorable planting conditions, row-establishment with tillage can incorporate clover residues, reduce N requirements, and increase grain yields for maize production in a KCLM. In a world where the frequency and severity of drought is projected to increase, having the tools to manage conservation cropping systems based on weather forecasts will be important to mitigate challenging climatic conditions.

Table 1.1. Row establishment treatments.

Treatment	Photo	Description
Rotary zone tillage (RZT) (Northwest Tillers, Yakima, WA, USA)		Rotating groups of tines mulched a 0.30 m wide band to a 0.15 m depth every 0.76 m. Fully incorporated surface clover residues and displaced established roots and rhizomes. The resulting seedbed was soft which may cause planting depth issues depending on soil conditions..
Shank-type strip tillage (ST) (Orthman Manufacturing, Lexington, NE, USA)		A straight coulter sliced the soil before a shank with a 50 mm wide foot tilled to 0.25 m depth. Two wavy colters set 0.10 m on each side of the shank sliced the soil and clover roots and contained the displaced soil before a rolling basket prepared a 0.20 m wide seedbed. Surface clover residues were mostly unincorporated but were buried by soil and roots displaced by the shank.
Banded herbicides (BH)		Herbicides were applied as a mix of 0.56 kg a.e. ha^{-1} of dicamba (2-(1-[([3,5-difluorophenylamino]carbonyl)-hydrazono]ethyl)-3-pyridinecarboxylic acid), and 0.05 kg a.e. ha^{-1} clopyralid (3,6-dichloro-2-pyridinecarboxylic acid) to kill the clover in a 0.30 m wide band spaced every 0.76 m.

Table 1.2: Treatment means for maize grain yield, N uptake, and efficiency metrics using banded herbicide (BH), strip-tillage (ST) or rotary-zone tillage (RZT) as row establishment methods at varying fertilizer N rates (kg N ha^{-1}) in KCLM plots in Arlington, WI.

	Grain yield	**Grain N**	**Stover yield**	**Stover N**	**Total N**	**NB**	**NUE**
	Mg ha^{-1}	kg ha^{-1}	Mg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg N	kg N_{crop} kg N_{fert}^{-1}
Row treatment							
BH	9.1b†	112b	7.74	50.1ab	162b	47.5b	1.61b
ST	9.7ab	121ab	7.54	44.1b	165b	53.0b	1.66b
RZT	11.3a	140a	8.64	54.3a	194a	81.2a	2.01a
N rate							
0	9.1	103b	6.97b	36.0c	138b	138.8a	
45	9.6	115ab	7.64ab	44.3bc	159ab	114.2b	3.54a
90	10.4	134a	7.83ab	49.2ab	183a	91.6c	2.02b
135	10.5	134a	8.67a	56.8a	191a	55.6d	1.41c
180	10.2	131a	8.09ab	49.5ab	181a	0.74de	1.00d
225	10.2	130ab	8.65a	61.2a	191a	-37.7e	0.83e
				P > F			
Row treatment	0.04	0.01	0.05	0.03	< 0.01	< 0.01	0.02
Fertilizer N rate	0.30	< 0.01	< 0.01	<0.001	< 0.001	< 0.001	< 0.001
Row x N rate	0.80	0.79	0.15	0.09	0.44	0.44	0.37

† Within a column for each fixed effect, means followed by the same letter are not significantly different at $p \leq 0.05$.

Table 1.3: Treatment means for maize grain yield, N uptake, and efficiency metrics using banded herbicide (BH), strip-tillage (ST) or rotary-zone tillage (RZT) as row establishment methods at varying fertilizer N rates (kg N ha^{-1}) in KCLM plots in Rosemount, MN.

	Grain yield	**Grain N**	**Stover yield**	**Stover N**	**Total N**	**NB**	**NUE**
	Mg ha^{-1}	kg ha^{-1}	Mg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg N	kg N_{crop} kg N_{fert}^{-1}
Row treatment							
BH	4.48a†	58.3a	4.39	34.7	93.0a	-19.5a	1.01a
ST	3.22ab	42.6ab	3.66	32.8	75.4ab	-37.1a	0.77ab
RZT	2.68b	32.5b	3.59	33.3	65.8b	-83.6b	0.63b
N rate							
0	2.56	30.2	2.69b	18.8c	48.9b	46.6a	
45	3.68	44.1	4.05ab	29.7bc	73.8ab	18.3a	1.64a
90	3.47	42.7	3.71ab	28.0bc	70.7ab	-26.3b	0.79b
135	3.46	47.0	4.39a	43.6a	90.6a	-59.3c	0.67b
180	4.13	54.0	4.38a	40.0ab	94.0a	-99.6d	0.52bc
225	3.46	48.7	4.04ab	41.6a	90.3a	-149.3e	0.40c
				P > F			
Row treatment	0.04	0.02	0.14	0.85	0.02	<0.001	0.03
Fertilizer N rate	0.22	0.07	0.02	< 0.001	< 0.001	<0.001	<0.001
Row x N rate	0.18	0.33	0.74	0.98	0.44	0.49	0.22

† Within a column for each fixed effect, means followed by the same letter are not significantly different at $p \leq 0.05$.

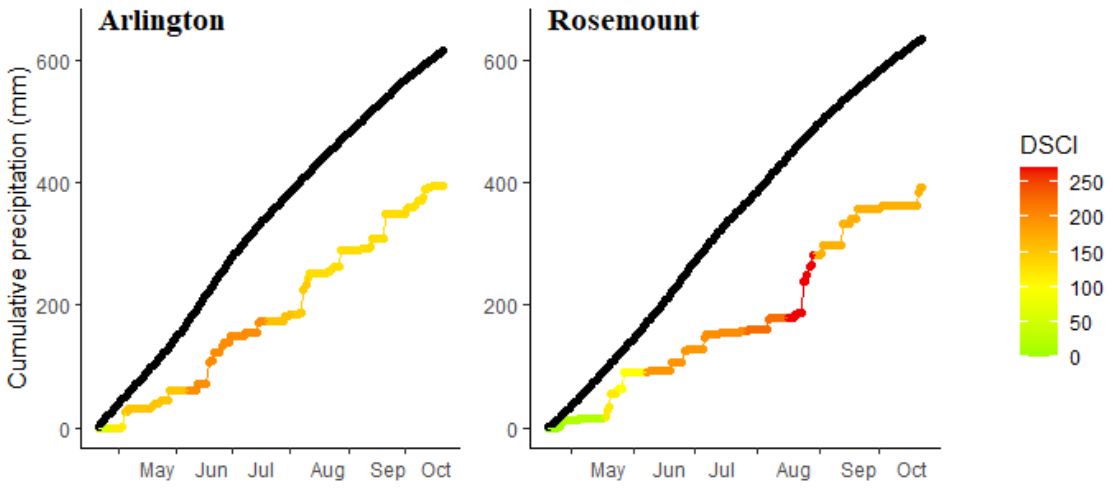

Figure 1.1: Average (1991-2020) (black) and observed (multi-colored) cumulative precipitation at the Arlington and Rosemount sites from 20 Apr to 22 Oct 2021. Colors represent the Drought Severity and Coverage Index (DSCI) for Columbia County, WI (Arlington), and Dakota County, MN, (Rosemount).

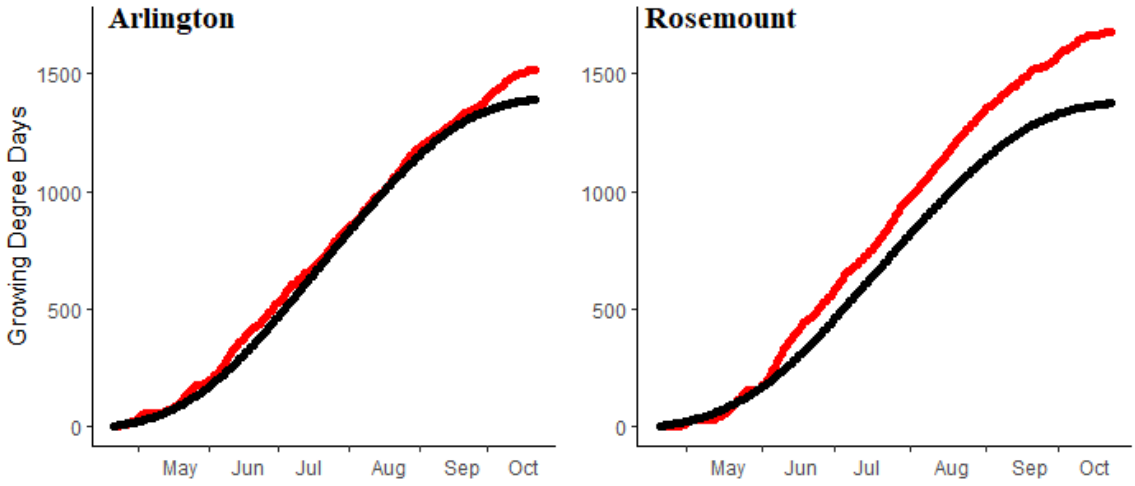

Figure 1.2: Average (1991-2020) (black) and observed (red) growing degree days at the Arlington and Rosemount sites from 20 Apr to 22 Oct 2021.

CHAPTER 2: SPATIOTEMPORAL DISTRIBUTION OF ROOTS IN A MAIZE – KURA CLOVER LIVING MULCH SYSTEM: IMPACT OF TILLAGE AND FERTILIZER N SOURCE

2.1. Synopsis

Maize production in a kura clover living mulch (KCLM) requires agronomic management to mitigate interspecies competition between emerging maize seedlings and vigorous spring clover growth. In a KCLM, the row zone is typically freed of clover with tillage or herbicides before maize is seeded, but banded fertilizer application technologies cannot be easily integrated into these management operations. Banding high rates of nitrogen fertilizers, such as anhydrous ammonia (NH_3), can result in a caustic soil environment that damages plant material. This study aimed to utilize this attribute to damage clover roots near the row zone and provide supplemental clover suppression in combination with a less aggressive and more cost-effective tillage tool. Maize was seeded into a KCLM prepared with either rotary zone tillage (RZT) or shank strip tillage (ST) and fertilized with either ammonium nitrate (NH_4NO_3) or anhydrous ammonia (NH_3) banded at 0.20 m under the tilled zone. End of season crop yields were collected and the spatial and temporal distributions of aboveground clover biomass, total root biomass, and soil N, were sampled at seven times between tillage application and the R4 maize physiological growth stage after field management operations and critical maize development stages. Total root biomass samples were analyzed for their ^{13}C isotope signature to determine the relative abundance of clover (C3) and maize (C4) roots. The study concludes that ST can result in adequate clover suppression and high grain yields regardless of fertilizer N source. Fertilizer N source also did not affect the spatial or temporal distribution of above or belowground clover biomass. Finally, the interactions between the growth of the row crop and the decline of clover biomass over the growing season impacted soil mineral N pools, evident by increasing soil NO_3-N concentrations in the interrow zone, however, these soil N pools were spatially separated from the majority of maize root biomass.

2.2. Introduction

Suppressing spring clover growth in a kura clover (*Trifolium ambiguum* M Beib) living mulch (KCLM) system for maize production is essential to reduce interspecies competition between emerging seedlings and the living mulch. Without adequate suppression, the KCLM can significantly reduce maize stand count, seedling development, grain yield, and profitability (Affeldt et al., 2004; Dobbratz et al., 2019). Clover suppression can be accomplished with mechanical disturbance, such as tillage and mowing (Alexander et al., 2019a), with herbicides (Affeldt et al., 2004), or combinations of these practices (Alexander et al., 2019b).

Previous work has demonstrated that rotary zone tillage (RZT) is a highly effective tool for managing clover competition in the row zone (Alexander et al., 2019a). Rotary zone tillage features rotating groups of tines that mulch 0.30 m wide strips to 0.15 m depth. In comparison to shank strip tillage (ST), which features a straight coulter in-line with a shank that tills to 0.20 m and two wavy coulters spaced 0.10 m on each side of the shank, RZT was found to speed seedling development and increase maize grain yield (Dobbratz et al., 2019). While RZT can be more effective at managing competition than ST, its high cost, high horsepower requirements, slow working speeds, and low availability is prohibitive to many producers.

Combining essential field management passes while effectively suppressing spring clover growth will reduce costs and increase the practicality of managing a KCLM. While we know that ST can reduce yields in a KCLM system relative to RZT, its design has the capability to integrate banded fertilizer application under the tilled row. This cost saving strategy in combination with increased clover suppression by other means may mitigate the risk of interspecies competition while increasing the profitability of and reducing barriers to adoption of KCLM.

Upon application, ammonia-based fertilizers react rapidly with their environment through hydrolysis (Equation 1).

$$NH_3\ (aq) + H_2O \rightleftharpoons\ NH_4^+\ (aq) + OH^-(aq)$$

In a concentrated fertilizer band, the resulting ammonium salt can increase the osmotic potential above 1 MPa, and the hydroxide anion can increase soil pH to above 8 (Blue

and Eno, 1954; Smiley, 1970; Clay et al., 1995). The resulting environment is hazardous to most soil biota, including living plant material (Bittsánszky et al., 2015).

Utilizing ammonia-based fertilizer to damage clover roots near the row zone may reduce inter-species competition during the critical maize seedling stage. To our knowledge, application of NH_3 to kill root biomass and suppress plant growth has not been investigated for use in mixed cropping systems. Work in this area is often concerned with soil conditions and NH_3 management as it relates to seedling injury, which stunts maize development and reduces stand count (Hunter and Rosenau, 1966; Colliver and Welch, 1970; Kovacs et al., 2011). However, we aim to utilize this effect to our advantage as supplemental clover suppression in combination with tillage and fertility management in a KCLM.

This study aims to investigate the combined effects of strip tillage, RZT or ST, and fertilizer source, NH_3 or NH_4NO_3, on maize yield and belowground biomass pools. Targeted sampling of root biomass in combination with ^{13}C isotopic methods to differentiate between clover (C3) and maize (C4) roots (Eleki et al., 2005) will define spatial and temporal distributions of root biomass pools in the KCLM. Management treatments will result in two hypothetical gradients of relative clover suppression, where ST < RZT, and NH_4NO_3 < NH_3. The combined effect of these treatments should therefore result in ST × NH_4NO_3 < RZT × NH_3, with unknown effects between RZT × NH_4NO_3 and ST × NH_3. If effective, this management strategy could simplify the management and increase profitability of KCLM systems by integrating seedbed preparation, clover suppression, and fertilizer application into a single field operation. Additionally, defining general distributions of clover and maize roots in a KCLM system will fill an important knowledge gap regarding the effect of management on belowground biomass pools.

2.3. Materials and Methods

2.3.1. Site description and experimental design

An experiment was conducted during the 2019 growing season to investigate the effect of tillage and fertilizer N source on grain yield and the spatiotemporal distribution

of clover biomass, root biomass, and soil N in a KCLM cropping system. The plots were located at the University of Minnesota's Rosemount Research and Outreach Center on a Waukegan silt loam (fine-silty over sandy or sandy-skeletal, mixed, superactive, mesic Typic Hapludolls). Kura clover was established at the site in 2006 and was used for row crop production for eight of the 12 growing seasons since its establishment. Most recently, the site was used for maize production in 2017 and the clover was cut and harvested as hay twice during the 2018 growing season.

Two main agronomic factors were examined in this experiment: strip tillage type and fertilizer N source. Strip tillage treatments included ST (Orthman Manufacturing, Lexington, NE, USA; Figure 2.1) or RZT (Northwest Tillers, Yakima, WA, USA; Figure 2.1). Split plots were laid out in a randomized complete block design with four replications, where N fertilizer source was the main plot factor and strip-tillage was the split plot factor. Strip tillage treatments were applied to split-plots on 21 May before NH_3 was applied on 31 May and NH_4NO_3 was applied on 4 Jun, each at 168 kg N ha^{-1} in the center of the tilled strip at a 0.20 m depth. Kura clover was cut with a flail mower on 6 Jun before the maize hybrid (Pioneer P8542) with an 85-d relative maturity was seeded at 79,000 seeds ha^{-1} on the center of the tilled and fertilized row on 10 Jun (Pioneer Hi Bred International, Johnston, IA, USA). Chemical clover suppression was achieved by broadcast application of glyphosate on all plots at 1.4 kg a.e. ha^{-1} on 17 Jun at the VE maize physiological growth stage.

2.3.2. Clover biomass and soil sampling

Aboveground clover biomass and soil were sampled at eight timepoints throughout the year; before tillage, after tillage, after fertilizer application, and during maize physiological growth stages VE, V3, V9, VT, and R4. Clover biomass was sampled from 0.5 m^2 quadrats placed between maize rows and dried at 60 ℃ until samples reached a constant mass. Soils were collected with a 50-mm diameter soil corer to 200 mm depth at 0, 125, 250, and 375 mm from the center of the planted row, in a design similar to (Li et al., 2006) (AMS Samplers, American Falls, ID, USA). Soil cores were collected in triplicate and combined into a single composite sample of the three soil cores from the

same relative location to the maize row. Composite samples were stored at 5°C for no more than 4 d before they were weighed, homogenized, and subsampled. Extractable soil N concentrations were obtained using the methods of Mulvaney (1996). Briefly, 38 mL of 2M KCl was added to 10 g of field moist soil and shaken at 120 rpm for 1 h before the slurry was filtered through 1 µm filter paper. The extracts were analyzed for NO_2-N + NO_3-N (NO_3-N) and NH_4-N analysis using the Griess-Ilosvay with Cd reduction, and sodium salicylate-nitroprusside methods, each modified for flowthrough injection analysis, respectively (Lachat, Loveland, CO, USA) (Mulvaney, 1996). A 5-10 g subsample of each composite sample was weighed and then dried at 105 °C until reaching a constant mass to determine the field moisture content. Soil bulk density was calculated by dividing the total sample dry soil mass by the sample volume.

2.3.3. Root analysis

In order to quantify the relative contributions of root biomass from maize and kura clover, a stable C isotopic method was utilized based on the differing C fractionation occurring during photosynthesis pathways in maize (C4) and clover (C3) (Eleki et al., 2005). Briefly, the soil remaining after extraction for mineral N (above) was put into an elutriator which washed the soil from plant roots with a vortex water jet (Smucker et al., 1982). Roots and coarse sediments separated by the elutriator were carefully sifted with a 250-µm sieve, bagged, and dried at 60 °C until samples reached a constant mass. Dried root samples were emptied onto a tray for manual sorting where sand was separated and discarded. Roots were bagged, weighed, and roots from the V3, V9, and R4 growth stages were pulverized with a ball mill and subsequently analyzed for ^{13}C isotope signature by combustion elemental analysis (vario ISOTOPE cube; Elementar Americas Inc., NY, USA) interfaced to an isotope ratio mass spectrometer (IRMS; Elementar Americas Inc., NY, USA, isoprime precisION). Site calibration was performed by creating customized clover and maize root standards by sorting, cleaning, and ball milling the pre-treatment root samples and maize root balls, respectively. Milled standards were mixed at 20, 40, 60, and 80% relative abundance by weight of clover:maize, and the standards and standard mixes were analyzed for ^{13}C isotope signatures in triplicate. The

^{13}C isotope signatures of mixed samples were then compared to the standard curve (R^2 = 0.997) to determine the relative abundance of clover and maize roots (Figure 2.2).

2.3.4. Maize harvest and plant sampling

At physiological maturity, ears were harvested from 3 m in each of the two center rows in each plot. The remaining plant material was cut 0.15 m from its base, weighed, subsampled, chipped with a woodchipper, and bagged. Grain and stover subsamples were weighed, dried at 60 ℃ until samples reached a constant mass, and weighed again to determine the field moisture content. Grain was shelled from the cob and cobs and grain were weighed separately. The moisture of the oven dried grain was tested using an electronic moisture tester, and the moisture reading was added to the calculated field moisture content and used to adjust grain and cob yield to a dry matter basis. Grain, stover, and cobs were subsampled, pulverized, and analyzed for biomass N content with a combustion analyzer (vario MAX cube, Elementar, Langenselbold, DE) (Stevenson, 1996).

2.3.5. Data analysis and statistics

Data were analyzed in three groups to determine treatment differences in seasonal, temporal, and spatiotemporal variables, which represent data that are collected at one timepoint, multiple timepoints, and multiple locations and multiple timepoints, per plot, respectively. Prior to model fitting, data were tested for normality with QQ plots (Kutner et al., 2004). When dependent variables exhibited a non-normal distribution, an appropriate data transformation was done before variables were re-tested. Model parameterization was conducted by comparing the Akaike information criterion (AIC) of models that each excluded one fixed effect. For spatially integrated and spatiotemporal variables, the AIC was minimized by excluding fertilizer source, consequently, we chose to exclude this fixed effect from temporal analyses.

Seasonal variables included maize grain yield, maize stover yield, maize grain N, and crop N uptake. Data were fit with linear mixed effects models using the *lme4* package in R (Bates et al., 2015; R Core Team, 2021), where fertilizer type and tillage

treatment were main effects and the main plot nested within block were the random effects (Bates et al., 2014). Models were tested for and met the assumption of homogeneity of variance by visual inspection of residual vs fitted plots (Kutner et al., 2004). Fixed effects were tested with the type III F test with Kenward-Roger estimated denominator degrees of freedom. When fixed effects were significant at $p < 0.05$, means were separated by Tukey's multiple comparison test with the *emmeans* (Lenth et al., 2018) and *multcomp* (Fox and Weisberg, 2019) packages in R, and results were presented using compact letter display (Piepho, 2018).

Temporal variables included aboveground clover biomass and clover biomass N. Temporal data were fit to linear mixed effects models with repeated measures using the *lme4* package in R, where tillage treatment and days after planting (DAP) were fixed effects and subplot nested within main plot nested within block represented the random slope term (Langhans et al., 2005; Bates et al., 2015). Regression of dependent variables and time were tested as a linear, quadratic, and cubic function of DAP, and the degree of the polynomial was determined by minimizing the AIC of the respective models. Models were tested for homogeneity of variance with residual vs fitted plots (Kutner et al., 2004). Autocorrelation between measurements from the same plot were tested by visual inspection of model residuals by index value and by calculating autocorrelation with the *acf* function in base R. When ANOVA determined significant differences at $p \leq 0.05$, data were evaluated with polynomial regression to provide insight into the effects of treatments based on the mean and shape of the linear relationships (Tremblay et al., 1998).

Spatiotemporal variables included clover root biomass, maize root biomass, soil NH_4-N, soil NO_3-N, soil moisture, and soil bulk density. The assumption that root biomass samples collected before planting contain only clover roots is reasonable, therefore, clover root biomass analyses included data from samples collected after tillage and after fertilizer application, as well as samples that were analyzed for the relative proportions of clover and maize root biomass according to the ^{13}C isotope signature (V3, V9, and R4). Maize root biomass data was only analyzed for data that was estimated by the ^{13}C isotope signature at the V3, V9, and R4 growth stages. Maize root biomass data

included many zero values and data transformations were unable to meet the assumptions of ANOVA. Consequently, a two-part model approach was used (Boulton and Williford, 2018). Part one of the two-part model estimated the probability that a sample contained maize roots and part two fit maize root biomass data on the condition that values were nonzero. Standard and two-part analyses were conducted with linear mixed effects models with repeated measures with the *lme4* package in R, where tillage treatment, distance from row (DFR), and DAP were fixed effects, and subplot, nested within main plot, nested within block represented the random slope term (Bates et al., 2015). Regression of dependent variables and time were tested as a linear, quadratic, cubic, and quartic function of DAP, and the degree of the polynomial was determined by minimizing the AIC or the Bayesian Information Criterion (BIC) under reasonable assumptions of the biophysical functioning of the dependent variable (Aho et al., 2014). For example, the AIC for clover root biomass indicated a cubic relationship over DAP while the BIC indicated a linear response. The regression of the cubic model indicated large temporal variation in clover root biomass, however, it is not likely that this relationship best represents reality, and the model is overfit. The linear response indicated by BIC was better aligned with our understanding of this variable, consequently, that model was chosen (Aho et al., 2014). Discrepancies between AIC and BIC was found for each variable in the spatiotemporal analysis and the more complex models parameterized by AIC included maize root biomass, soil moisture, soil NH_4-N, and soil NO_3-N concentrations, while the BIC was used to parameterize clover root biomass and soil bulk density. After model parameterization, autocorrelation between measurements from the same plot were tested by visual inspection of model residuals by index value and by calculating autocorrelation with the *acf* function in base R. Models were then tested for and met the assumption of homogeneity of variance by visual inspection of residual vs fitted plots (Kutner et al., 2004). Treatment effects were tested with the type III F test with Kenward-Roger estimated denominator degrees of freedom. When fixed effects were significant at $p < 0.05$, means were separated by Tukey's multiple comparison test with the *emmeans* (Bates et al., 2014) and *multcomp* (Tremblay et al., 1998) packages in R, and results were presented using compact letter display (Piepho, 2018). When

ANOVA determined significant differences across DAP at $p \leq 0.05$, data were evaluated with linear regression to provide insight into the effects of treatments based on the mean and shape of the linear relationships.

2.4. Results

2.4.1. Seasonal variables

Seasonal variables included grain yield, stover yield, grain N, and crop N uptake. No statistically significant differences or interactions were found between treatments within these variables (Table 2.1).

2.4.2. Temporal variables

Temporal data included aboveground clover biomass and biomass N. Aboveground clover biomass and biomass N were affected by DAP. Between -12 and 97 DAP, clover biomass and biomass N declined by 5.2 and 0.19 kg ha^{-1} d^{-1} (Table 2.2; Figure 2.3a, 2.3b).

2.4.3. Spatiotemporal variables

Clover root biomass was influenced by the interaction between tillage and DFR and the interaction between tillage and DAP (Table 2.3; Figure 2.4). Averaged over DAP, clover root biomass was greatest in the 250 and 380 mm distances across tillage treatments (Figure 2.4a). Clover root biomass was significantly greater in the ST treatment than the RZT treatment at the 120 mm distance and was minimized across tillage treatments in the 0 mm distance. Across distances, clover root biomass decreased at a greater rate in the RZT treatment than the ST treatment over DAP (Figure 2.4b).

The probability of maize roots in a sample was predicted by the interaction between tillage, DFR, and DAP (Table 2.3; Figure 2.5). The probability of maize roots in a sample had similar slopes over DAP for all treatments and distances except for the 120 mm distance of the RZT treatment, which increased by 71% from 21 – 97 DAP. The probability of roots also increased at similar rates between all distances in the RZT

treatment and the 0 mm distance of the ST treatment, while the probability of roots remained constant or slightly decreased over DAP in the 120 – 380 mm distances of the ST treatment.

Maize root biomass was significantly affected by the interaction between tillage and DFR and the interaction between DFR and DAP (Table 2.3; Figure 2.6). Across DAP, maize root biomass was greatest in the 0 mm distance and the 120 mm distance of the RZT treatment. Maize root biomass was very low across treatments in the 250 and 380 mm distances. Across tillage treatments, maize roots increased quadratically in the 0 mm distance, while maize roots in the 120 – 380 mm distances had no significant increase from 21 – 97 DAP.

Soil NH_4-N was affected by the interaction between DFR and DAP (Table 2.3; Figure 2.7). Soil NH_4-N in the 0 mm distance increased until ~15 DAP before declining and remaining at a low level at 75 DAP. Soil NH_4-N in the 120 – 380 mm distances followed a similar temporal pattern, but NH_4-N concentrations peaked at ~25 kg N ha^{-1}, which is roughly 1/3 the concentration of the peak in the 0 mm distance.

Soil NO_3-N was significantly affected by the interaction between tillage and DFR and the interaction between DFR and DAP (Table 2.3; Figure 2.8). Soil NO_3-N concentration was greatest in the 0 mm distance of the RZT treatment and lowest in the 120 and 250 mm distances of the ST treatment and the 380 mm distance of the RZT treatment. NO_3-N concentrations in the 0 and 380 mm distance of the ST treatment and the 120 and 250 mm distances of the RZT treatment were not significantly different than the other distances. Over the course of the growing season averaged across tillage treatments, soil NO_3-N increased rapidly to ~95 kg N ha^{-1} in the 0 mm distance before declining and remaining at ~25 kg N ha^{-1} at 70 DAP. Soil NO_3-N concentrations in the 120 mm distance exhibited a similar temporal relationship as the 0 mm distance, but the peak concentration reached only 50 kg N ha^{-1}, or half of the concentration in the 0 mm distance. Soil NO_3-N concentrations in the 250 and 380 mm distances remained at a similar level or slightly increased over the growing season and ended the growing season with approximately 40 kg N ha^{-1}.

Soil moisture and bulk density were significantly affected by the main effects DFR and DAP (Table 2.3). Soil moisture averaged across tillage treatments and DAP was significantly greater in the 0 mm distance than the 250 and 380 mm distances, and the 120 mm distance was not different than the high and low moisture areas. Bulk density, averaged across tillage treatments and DAP, was lowest in the 0 mm distance followed by the 120 mm distance, and greatest in the 250 and 380 mm distances. Averaged across tillage treatments and DFR, bulk density increased by 0.085 Mg m^{-3} over the course of the growing season.

2.5. Discussion

This study aimed to reduce the cost of agronomic management for maize production in KCLM by utilizing a less aggressive strip-tillage tool in combination with a caustic fertilizer N source. At the end of the growing season, there were no significant differences in grain yield, stover yield, or crop N uptake between tillage or fertilizer treatments, suggesting that yields can be maximized without rotary zone tillage or anhydrous ammonia. This finding goes against previous research that found differences in crop emergence, crop development, and grain yield by tillage intensity (Dobbratz et al., 2019), and suggests that the level of clover suppression is influenced by the interaction between agronomic management and environmental conditions.

Carbon isotope fractionation of mixed root samples was able to provide estimates of maize root biomass in the mixed cropping system. Across tillage treatments, maize root biomass increased by 15 kg ha^{-1} d^{-1} over the growing season. Maize root biomass at 97 DAP averaged 1,294 kg ha^{-1}, which is equivalent to ~16.4 g $plant^{-1}$, and is on the lower end of maize root biomass estimates at the R4 growth stage (Amos and Walters, 2006). While this estimate lies within the expected range of maize root biomass, the true value is likely higher due to the relatively shallow sampling depth (200 mm) and the sampling location, which was not directly below the crown of the plant (Nicoullaud et al., 1994). While these values likely underestimate the amount of maize root biomass, they are reasonable and promote confidence in the sampling, processing, and analysis of the mixed root samples by C isotope fractionation.

Clover root biomass data provided insights into the relationship between maize and kura clover as a function of tillage in the mixed cropping system. Examination of clover root biomass across row zones shows that the wider working width of the RZT tool reduces root biomass in the 120 mm distance relative to the ST treatment (Figure 2.4a), and also indicates a greater loss of root biomass in the RZT treatment (-70.7 kg ha^{-1} d^{-1}) relative to the ST treatment (-40 kg ha^{-1} d^{-1}) (Figure 2.4b). This finding is nonintuitive: how could RZT affect the *rate* of root loss throughout the growing season? It is likely that this finding is influenced by the method's inability to differentiate between living and dead/dying root fractions. This indicates that the seasonal decline of clover root biomass is attributed to the initial tillage disturbance and subsequent biomass decay rather than a physiological response to low light conditions under the maize canopy.

Maize growth and development coincided with the decline of aboveground clover biomass and increased soil NO_3-N concentration in the interrow space (Figure 2.3;2.8b). While it is likely that increased NO_3-N in the interrow space is linked to aboveground clover biomass inputs, few maize roots are present outside of the 0 and 120 mm distances to utilize the mineralized N (Figure 2.6a). Consequently, maize accessible mineral N from the KCLM is likely dominated by the decomposition of root biomass rather than N inputs provided by the in-season decline of aboveground clover biomass. This finding is consistent with previous research, which attributed increased fertilizer N requirements for second-year maize in a KCLM to decreased clover stand health, vigor, and biomass N reserves in the year following maize production (Alexander et al., 2019a). While these observations provide insight into in-season N contributions from the living mulch, more work is needed to quantify clover biomass inputs from above and belowground pools, mineralization of these biomass pools, and their fertilizer replacement value for maize production in KCLM.

Finally, it is reasonable to assume that loss of clover root biomass negatively affects clover stand health following living mulch management. Kura clover growth during the establishment period has been described to "sleep, creep, and leap", meaning that little growth occurs in the first year after seeding due to large energy partitioning into

belowground root and rhizome structures, but once these biomass pools are established, the clover "leaps" as a vigorous and productive cool-season forage crop (Taylor and Smith, 1997; Seguin et al., 1999). Given this growth pattern and physiology, minimizing the intensity of tillage while achieving a level of clover suppression that does not compete with maize seedlings will increase the long-term health of clover stands under extended row-crop rotations. Identifying and testing agronomic solutions to achieve these goals must still be a priority of KCLM research.

2.6. Conclusions

This study concludes that RZT is not a requirement for adequate clover suppression and high grain yields in a KCLM-maize cropping system regardless of fertilizer N source. This study also found that fertilizer N source does not affect root biomass. This finding goes against our initial hypothesis that NH_3 fertilizers would damage clover roots and increase clover suppression and indicates that fertilizer application at agronomically relevant rates cannot aid in clover suppression. There is, however, evidence that RZT reduces clover root biomass relative to ST, which may reduce clover stand vigor in the following spring without agronomic benefits under the tested conditions. Finally, the decline of aboveground clover biomass and subsequent decomposition contributes soil N to the mixed cropping system, as evidenced by increasing soil NO_3-N in the interrow zone. However, these N inputs are not likely utilized by the row crop, and in-season N contributions to the row-crop likely originate from incorporated aboveground biomass and decomposing roots in the tilled row.

Table 2.1: Treatment means, standard error, and significance of maize yield and N uptake between tillage and fertilizer treatments.

Fixed Effects	**Grain yield**	**Stover yield**	**Grain N**	**Crop N uptake**
	Mg ha^{-1}		kg ha^{-1}	
Tillage				
RZT‡	9.14	4.96	119	178
	(0.27)†	(0.80)	(4.28)	(10.55)
ST§	9.20	5.25	119	180
	(0.34)	(0.77)	(3.46)	(8.83)
Fertilizer				
NH_4NO_3	9.11	4.63	117	172
	(0.25)	(0.75)	(3.53)	(5.13)
NH_3	9.23	5.58	121	187
	(0.36)	(0.74)	(3.88)	(11.34)
Significance				
Fertilizer	0.73	0.24	0.32	0.17
Tillage	0.77	0.68	0.95	0.60
Fertilizer * Tillage	0.22	0.58	0.11	0.08

† Standard error. ‡ Rotary zone tillage. § Shank type strip-tillage.

Table 2.2: Treatment means, standard error, and significance of temporal variables between tillage treatments and days after planting (DAP).

Fixed Effects	Clover biomass	Clover N
	kg ha^{-1}	
Tillage		
RZT‡	781 (69) †	23.4 (1.5)
ST§	916 (69)	26.2 (1.5)
DAP		
x	-5.2 (1.3)	-0.19 (0.03)
Significance		
Tillage	0.22	0.31
DAP	<0.01	<0.001
Tillage * DAP	0.68	0.44

† Standard error. § Rotary zone tillage. ‖ Shank type strip-tillage. ⁋ Days after planting.

Table 2.3: Treatment means, standard error, and significance of spatiotemporal distributions of root biomass, soil N, soil moisture, and soil bulk density between tillage treatments, distance from row (DFR) (mm), and days after planting (DAP).

Fixed Effects	Cloverroot mass	Maize root mass		NH_4-N	$NO3_3$-N	Moisture	Bulk density
			Zero values				
	kg ha^{-1}			kg ha^{-1}		g g^{-1}	Mg m^{-3}
Tillage							
RZT§	12336 (614)‡	985 (131)	0.45* (0.03)	29.4 (4.1)	40.9 (4.1)	0.20 (0.005)	1.21 (0.01)
ST‖	14127 (612)	1099 (130)	0.31 (0.03)	28.3 (4.1)	33.8 (4.1)	0.20 (0.005)	1.22 (0.01)
DFR							
0	2691 (866)	3931 (186)	0.92 (0.05)	53.5 (5.8)†	69.2 (5.8)	0.21 (0.007)a	1.13 (0.01)a
120	10449 (861)	197 (184)	0.42 (0.05)	16.3 (5.8)	29.6 (5.8)	0.20 (0.007)ab	1.18 (0.01)b
250	19715 (861)	27 (184)	0.10 (0.05)	21.3 (5.8)	25.1 (5.8)	0.20 (0.007)b	1.26 (0.01)c
380	20072 (861)	14 (184)	0.08 (0.05)	24.4 (5.8)	25.4 (5.8)	0.19 (0.007)b	1.27 (0.01)c
DAP							
x‖	-55.3	$2.91 * 10^4$	$2.13 * 10^{-3}$	-286.3	-165.5	-0.12	$7.8 * 10^{-4}$
x^2		$-4.39 * 10^3$		-182.5	-245.3	0.52	
x^3				391.3	428.8	-0.07	
x^4				-90.3	-84.8	0.08	
Significance							
Tillage	0.13	0.04	0.62	0.82	0.02	0.92	0.69
Zone	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
DAP	<0.01	<0.0001	<0.01	<0.0001	<0.0001	<0.0001	<0.001
Tillage * Zone	<0.01	0.02	0.45	0.63	0.01	0.09	0.78
Tillage * DAP	0.03	0.18	0.03	0.79	0.60	0.80	0.52
Zone * DAP	0.39	<0.0001	0.27	<0.001	<0.0001	0.97	0.95
Tillage * Zone * DAP	0.55	0.20	0.02	0.98	0.97	0.91	0.96

† Within a column for a given fixed effect, means followed by the same letter are not significantly different at $p \leq 0.05$. ‡ Standard error. § Rotary zone tillage. ‖ Shank type strip-tillage. ⁋ Coefficients of simple or polynomial linear regression. *Means represent probability that the value of maize roots is not equal to 0.

Figure 2.1: Strip tillage after treatment application.

Shank type strip tillage (ST)	Rotary zone tillage (RZT)
	
A straight coulter sliced the soil before a shank with a 50 mm wide foot tilled to 0.20 m depth every 0.76 m. Two wavy coulters set 0.10 m on each side of the shank sliced the soil and clover roots and contained the displaced soil.	Rotating groups of tines mulched a 0.30 m wide band to a 0.15 m depth every 0.76 m. Fully incorporated surface clover residues and displaced established roots and rhizomes.

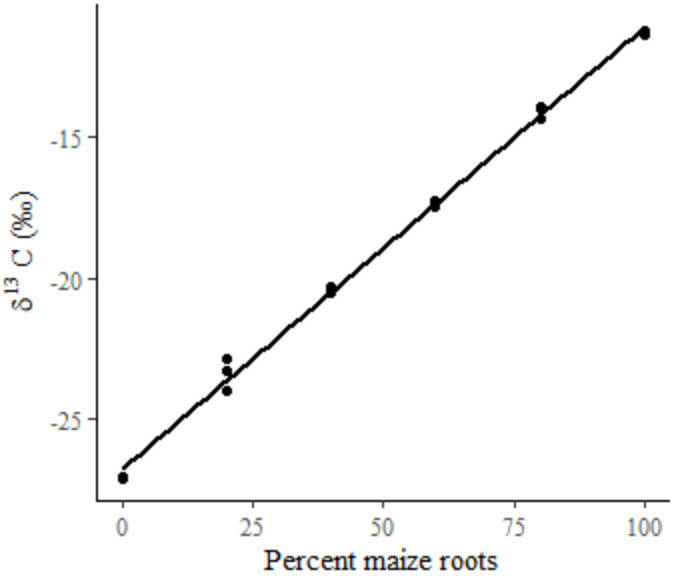

Figure 2.2: Regression of ^{13}C isotope signature and percent maize roots in standard mixes.

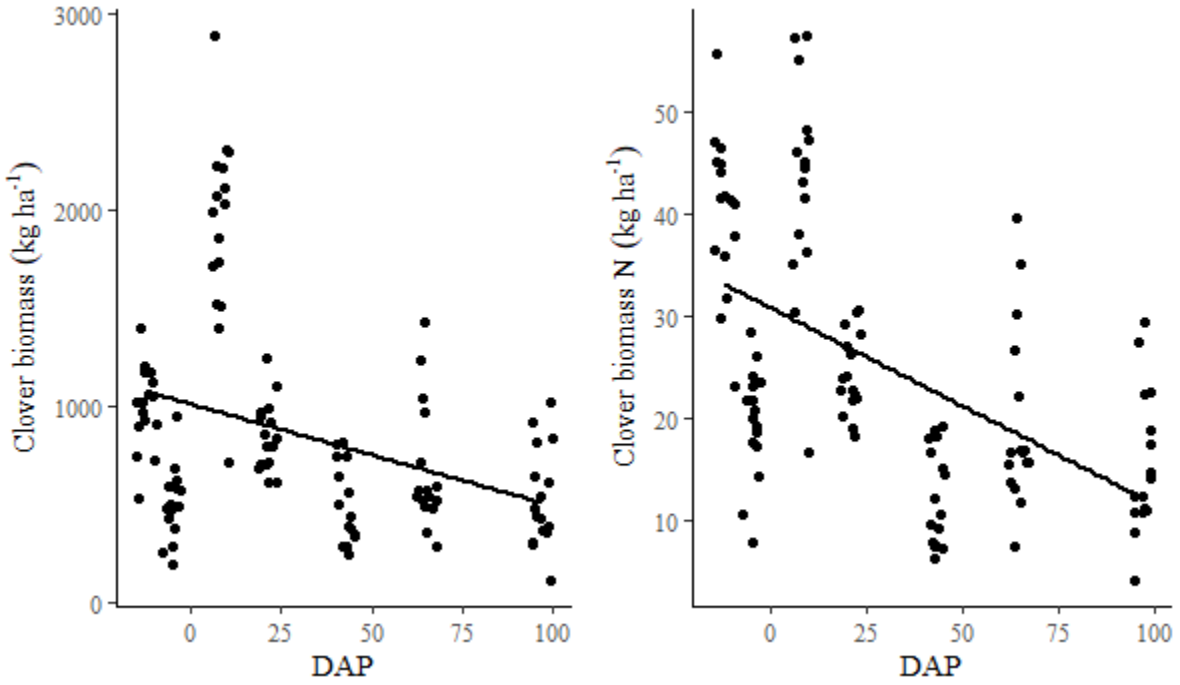

Figure 2.3: Distribution of **a)** clover biomass and **b)** clover biomass N over days after planting (DAP).

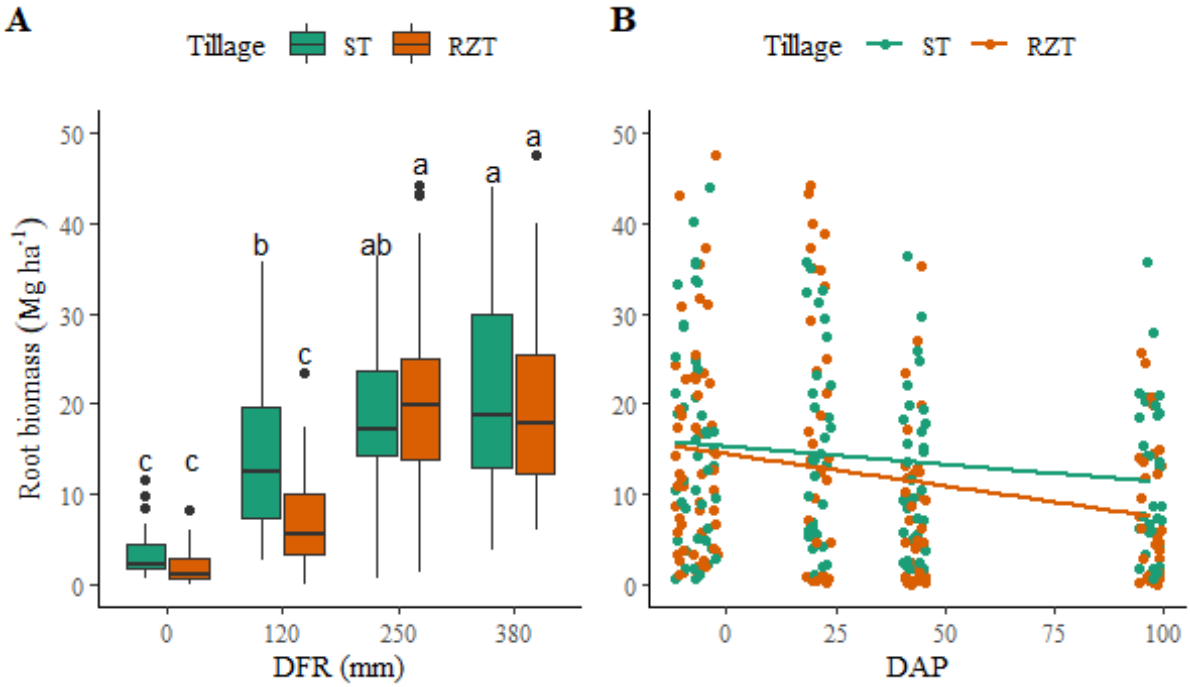

Figure 2.4: Clover root biomass by **a)** the interaction between Tillage and distance from row (DFR), and **b)** the interaction between DFR and days after planting (DAP). Means labelled by the same letter are not significantly different at $p \leq 0.05$.

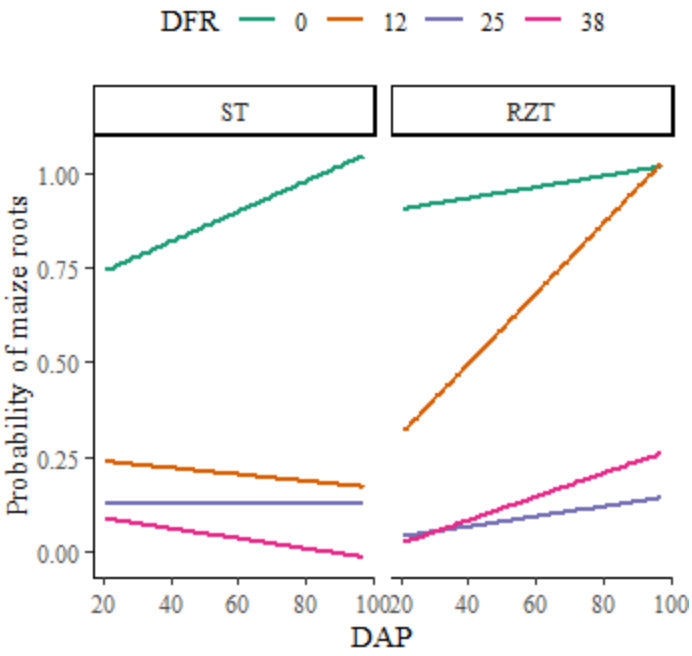

Figure 2.5: The probability of maize roots in a sample by the interaction between Tillage, distance from row (DFR), and days after planting (DAP).

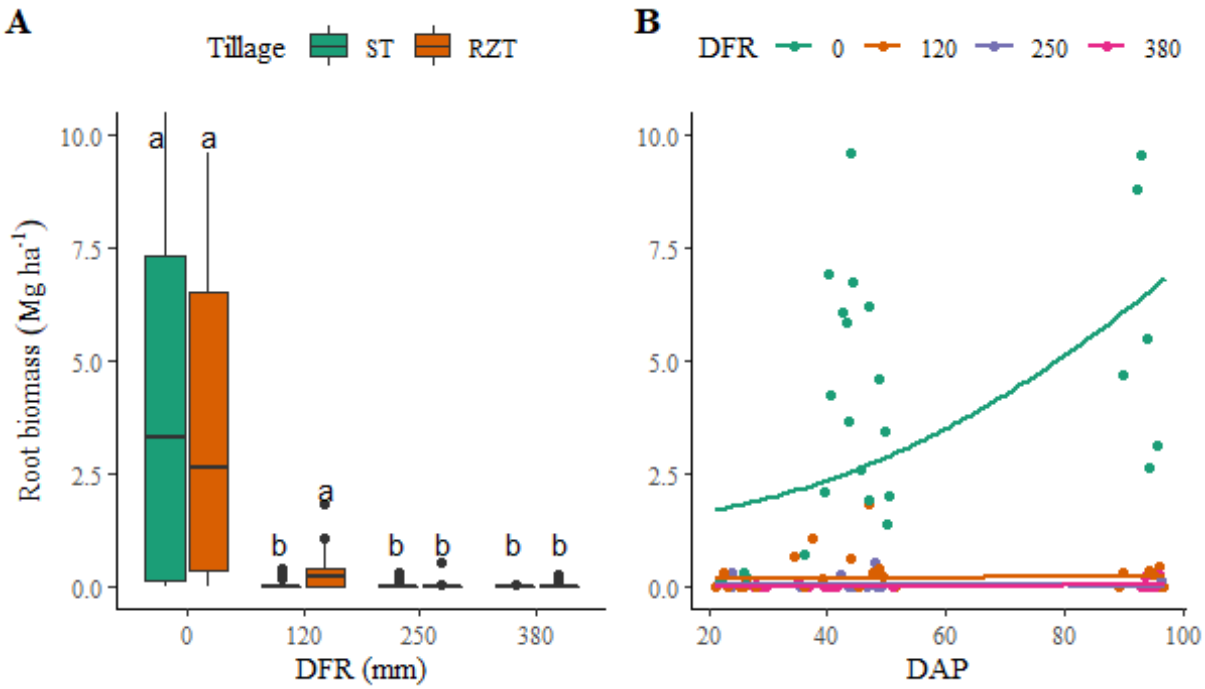

Figure 2.6: Maize roots by the interaction between **a)** Tillage and distance from row (DFR), and **b)** DFR and days after planting (DAP). Means labelled by the same letter are not significantly different at $p \leq 0.05$.

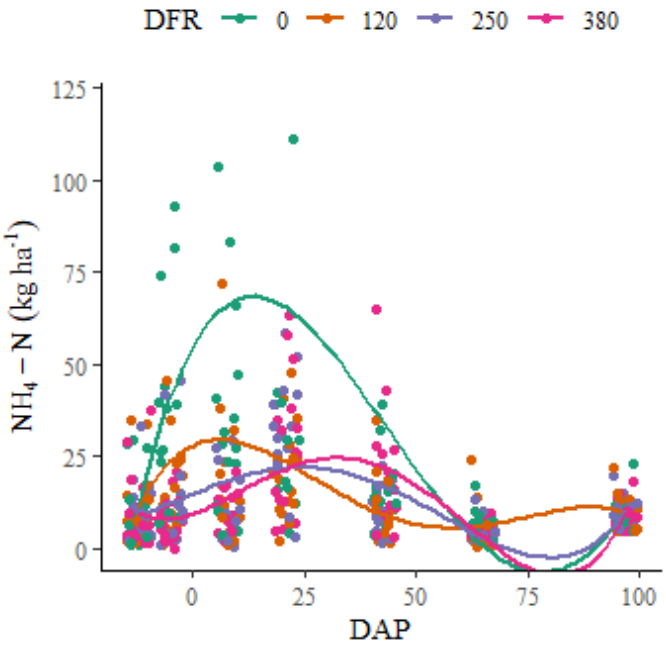

Figure 2.7: Distribution of NH_4-N by the interaction between distance from row (DFR) and days after planting (DAP). Means labelled by the same letter are not significantly different at $p \leq 0.05$.

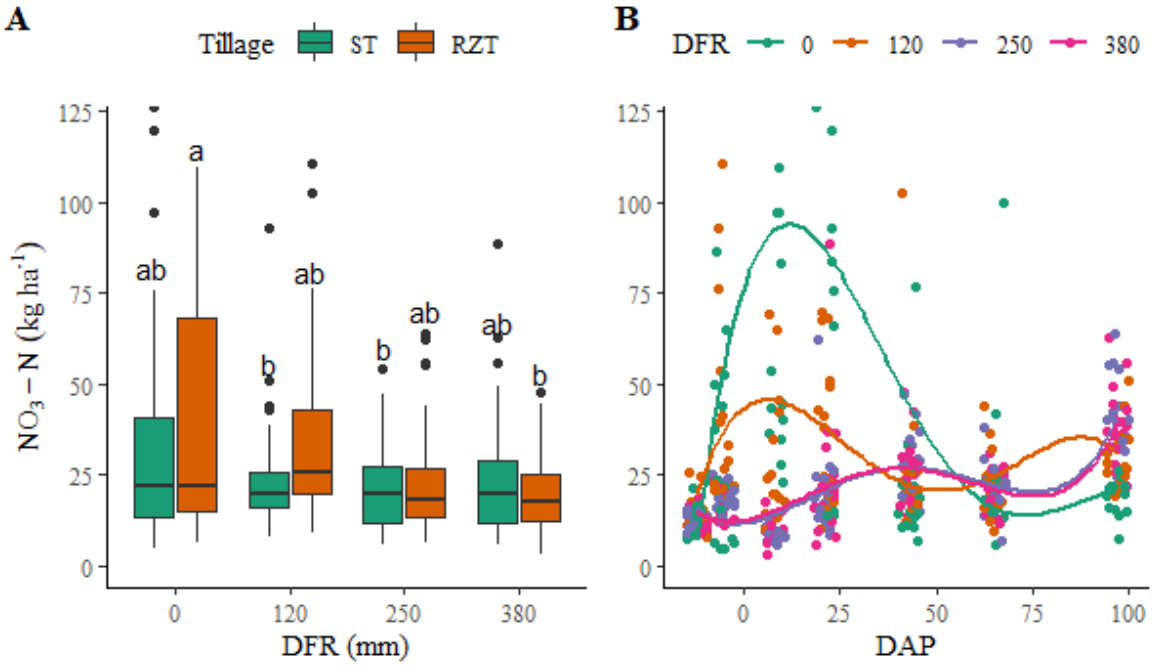

Figure 2.8: Distribution of NO_3-N by **a)** the interaction between distance from row (DFR) and days after planting (DAP), and **b)** the interaction between tillage and DFR. Means labelled by the same letter are not significantly different at $p \leq 0.05$.

CHAPTER 3: BIOMASS INPUTS AND NITROGEN CYCLING IN A KURA CLOVER LIVING MULCH SYSTEM UNDER CONTRASTING WEATHER CONDITIONS

3.1. Synopsis

Kura clover living mulch (KCLM) is thought to provide in-season nitrogen (N) inputs to maize through clover biomass inputs from spring management and the suppression of clover due to low light conditions under the maize canopy. The magnitude and the spatiotemporal distribution of these inputs, however, have not been investigated. This study aimed to determine the effect of fertilizer N application rate on clover biomass production and turnover, changes in soil inorganic N pools, N_2O emissions, and to quantify N cycling processes occurring separately in row versus interrow space within a KCLM system for maize production. The two growing seasons included in this study represent normal environmental conditions and conditions under the extreme case of crop failure due to drought. The results suggest that aboveground clover biomass contributes 80-100 kg N ha^{-1} of mineralized N to the KCLM system, but clover growth under normal conditions recycles most of these N inputs back into clover biomass pools. Maize required fertilizer N application near the grower rate to maximize grain yield under the tested conditions. Nitrous oxide emissions in a KCLM system were primarily from the interrow zone in a normal year, and high variability and the positive correlation between daily N_2O emissions and standing clover biomass suggest hotspots as a likely source of N_2O in these systems.

3.2. Introduction

Kura clover (*Trifolium ambiguum* M. Bieb) can be used within row crop production systems of the Midwestern US as a perennial living cover or living mulch. Kura clover is a low-growing cool-season forage legume whose growth habit is complementary to the dominant warm-season row crops in the Midwest, namely, maize (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.) (Zemenchik et al., 2000). Kura

clover's root and rhizome system stores a large amount of metabolic energy that enables kura to be extremely persistent under stressful environmental conditions and intensive agronomic management (Speer, G. S. and Allinson, 1985). Including kura clover as a living mulch in maize production systems can provide several environmental benefits, such as reduced soil erosion and runoff (Siller et al., 2016; Baker et al., 2021), reduced nitrate leaching (Ochsner et al., 2010, 2017), and increased primary productivity (H. Seran and Brintha, 2010). These benefits have historically come with a yield disadvantage; however, strides have been made in the agronomic management of these systems and recent research has observed yield results on par with conventionally managed maize systems (Affeldt et al., 2004; Ziyomo et al., 2013; Alexander et al., 2019a).

While these benefits promote the use of KCLM for environmental protection and soil quality, many aspects of this cropping system are understudied. The existing literature discusses the potential for KCLM to provide in-season N to the cash crop, however, our best estimation of in-season N credits from KCLM is observed indirectly through N rate research that neglects to quantify temporal distributions of clover biomass and subsequent mineralization of residue inputs (Sawyer et al., 2010; Alexander et al., 2019a). This is an important knowledge gap as producers are highly interested in the potential to cut input costs or utilize KCLM for organic production systems.

Litter bag methods have been used to answer many research questions related to biomass decomposition dynamics (Graça et al., 2005). These methods are used to provide insight on mineralization of organic materials and their effects on nutrient cycling within natural and agricultural systems. While residue decomposition data is important to estimate the timing and magnitude of biomass N inputs in a KCLM, no study has utilized these methods in this cropping system. Coupling the decomposition rates of clover residues with biomass inputs from spring management and in-season shade suppression has the potential to provide more direct estimates of biomass N contributions and may reveal important insights into nutrient management of maize in KCLM.

Agriculture as a tool to sequester atmospheric CO_2 is receiving renewed interest and investment (Oldfield et al., 2022). Several methods to achieve this goal are being

promoted, including reduced tillage, integration of cover crops, extended crop rotations, and microbial inoculants (Blanco-Canqui, 2021; Srivastava and Singh, 2022). Increased relative primary productivity and annual biomass production in a KCLM relative to conventional maize suggests there is potential to sequester atmospheric CO_2, however, CO_2 is not the only greenhouse gas relevant to agriculture. Crop production is the leading source of N_2O emissions in the US, and 1 kg of N_2O emissions are equivalent to ~298 kg CO_2 (Rasmussen, 1992). As soil C is a difficult parameter to measure in a short timeframe in the rich mollisols of the maize belt region, focusing effort toward quantifying N_2O emissions in these systems will help to determine whether KCLM can be used as a tool to mitigate climate change.

Information regarding nitrous oxide (N_2O) emissions from KCLM systems is sparse. Currently, only two studies have measured N_2O emissions from KCLM systems, and each observed alarmingly high emissions in unfertilized and N rate treatments below typical grower rates (Turner et al., 2016a; Alexander et al., 2019b). One aspect that these previous studies did not address is that N_2O may originate from different sources and be emitted at different magnitudes in the distinct row and interrow zones of the KCLM. It is likely that the row and interrow zone function differentially based on differences in mechanical disturbance and fertilizer N inputs, therefore, measurements from both the row and interrow zones could provide further insight and context into N_2O emissions from these systems.

This study aims to quantify the spatiotemporal distributions of biomass inputs and N species in a KCLM system to identify the magnitude of in-season N contributions from the living mulch and the potential fertilizer N replacement value for maize production. Additionally, we aim to quantify N_2O emissions from the row and interrow zones and correlate distributions of biomass N inputs with environmental data to identify drivers of N_2O emissions from this mixed cropping system. During the years of this study, we observed two distinctively different growing seasons that represent normal precipitation and the extreme case of crop failure due to drought. Characterizing these parameters under extreme weather conditions is important and may be more relevant in the future

given increasing likelihood of extreme weather events due to the changing climate (Ortiz-Bobea et al., 2018).

3.3. Materials and Methods

3.3.1. Site description and experimental design

An experiment was conducted during the 2020 and 2021 growing seasons to investigate the effect of fertilizer N application rate on clover biomass inputs, soil inorganic N pools, and N_2O emissions within a KCLM system for maize production. The site was located at the University of Minnesota's Rosemount Research and Outreach Center on a Waukegan silt loam (fine-silty over sandy or sandy-skeletal, mixed, superactive, mesic Typic Hapludolls). Kura clover was seeded at the site in the spring of 2018 and the stand was managed as a forage crop during the 2018 and 2019 growing seasons. The site was prepared by mowing clover to a 50 mm height on 11 May 2020 and 10 May 2021 before rows were established with a shank-type strip tillage tool on the following day (Orthman Manufacturing, Lexington, NE, USA). Fertilizer N rate treatments were applied as SuperU, urea fertilizer with nitrification and urease inhibitors (Koch Agronomic Services, Wichita, KS, USA), banded within the tilled strip at a 50 mm depth with an EarthWay garden seeder (EarthWay, Bristol, IN, USA) on the same day as strip tillage. Maize hybrid Dekalb 47-54RIB was seeded at 79,000 seeds ha^{-1} on 14 May 2020 and 13 May 2021, and glyphosate was broadcast onto the plot area at 1.4 kg a.e. ha^{-1} on 30 May 2020 and 25 May 2021.

3.3.2. Litter decomposition

Litter bags were prepared by cutting 60 x 120 mm segments of 500-µm nylon mesh material that was folded in half on the long edge before two of the edges were sealed with a soldering iron (Lupwayi et al., 2007). The empty bags were weighed before ~5 g of dry clover biomass of known C, N, and ash content was added, and the final edge was sealed. Bags were placed onto a reciprocal shaker for 2 h at 240 rpm to sieve and remove residue particles that passed through the 500 µm (0.5 mm) mesh. Finally, the

prepared litter bags were weighed to determine the initial dry clover residue mass (Equation 1).

(1) $$Ash\ free\ residue_{initial} = (Total_{initial} - Bag_{initial}) * (1 - Ash_{initial})$$

Soil was excavated adjacent to the row zone to 200 mm with a 70 mm i.d. auger. Litter bags were placed at the 200 mm depth before half of the excavated soil was replaced and packed and a second litter bag was placed at the 100 mm depth. The remaining excavated soil was replaced and packed before the final litter bag was placed adjacent to the excavated hole on the soil surface. One set of litter bags from the 0, 100, and 200 mm depths were collected after 1, 2, 3, 4, 6, and 8 wk after installation. Bags were dried at 60° C until they reached a constant mass before they were weighed, cut open, and the partially decayed clover residue was removed. Empty bags were weighed, and the mass was subtracted from the total bag mass to determine the amount of clover residue remaining after deployment. Root fragments that had penetrated the bags during deployment were removed from the decayed clover residue and their mass was subtracted from the remaining residue mass before samples were pulverized and homogenized with a mortar and pestle. Pulverized litter was subsampled, oven dried at 105 °C for 2 h, weighed, and combusted in a muffle furnace at 900° C for 4 h before the remaining material was weighed to determine the amount of ash introduced from the mineral portion of the clover residue and soil contamination during deployment (Andrén et al., 1992; Mulvaney et al., 2010) (Equation 2).

(2) $$Ash\ free\ residue_{final} = \left(Total_{final} - Bag_{final} - Roots\right) * (1 - Ash_{final})$$

The remaining litter samples were analyzed for total C and N using a combustion analyzer to provide insight into the decomposition dynamics of clover residue (vario MAX cube, Elementar, Langenselbold, DE) (Stevenson, 1996). Preliminary analyses determined that the C:N ratio of the initial ash free clover residue was ~9, and that this ratio did not change over the 8 wk deployment period. We therefore determined the clover decomposition rate based on litter mass as it is correlated with total sample C

(R^2=0.84) and N mass (R^2=0.79). Final litter mass was related to the initial residue mass to determine litter loss and remaining litter after deployment (Equation 3; 4).

(3) $$Remaining\ litter = \frac{Ash\ free\ residue_{final}}{Ash\ free\ residue_{initial}}$$

(4) $$Litter\ loss = 1 - Remaining\ litter$$

Remaining litter was analyzed by quadratic regression against days after deployment. The coefficients of the resulting regression equations were extracted to determine the amount of remaining litter on each day after deployment (Figure 3.1; Equation 5),

(5) $$Remaining\ litter_z = az^2 + bz + c$$

where a, b, and c are model coefficients for litter bags corresponding with surface or buried litter bags in 2020 or 2021 and z = days after bag deployment (deposition).

3.3.3. Clover inputs: spring management

Clover was sampled by manually cutting aboveground biomass from a 0.5 m^2 quadrat that spanned one row width. Clover biomass samples were dried at 60° C until they reached a constant mass before samples were weighed and adjusted to dry kg ha^{-1} basis. The interrow zone spanned 0.56 m of the 0.76 m row spacing and the row zone occupied the remaining 0.2 m. Aboveground clover biomass was collected before and after the clover was mowed in the spring to quantify total surface biomass additions from the mowing portion of spring management. Total surface biomass additions were multiplied by the relative areas of the row and interrow zones to determine surface biomass inputs to the respective management zones.

Clover residue burial and distribution in the row zone was determined with a bead tracer analysis (Allmaras et al., 1996). Five-hundred plastic beads were placed on the soil surface in the area that is intercepted by the strip tillage tool. The beads were tilled under the same conditions as the plot area, and after tillage, beads were recovered from the

surface, 0-50, 50-100, and 100-150 mm depths, in 50 mm increments on each side of the tilled strip; no beads were recovered from the 100-150 mm depth. Bead tracers were recovered from four replications and the average proportion of buried beads was used to determine the relative amount of clover buried by strip tillage. The remaining surface clover biomass after mowing and the mowed clover residues in the row zone were summed and multiplied by the proportion of buried bead tracers to determine buried clover residue inputs in the row zone. The remaining surface clover biomass after mowing was multiplied by the proportion of surface bead tracers to determine surface clover residue inputs in the row zone.

3.3.4. Clover inputs: shade suppression

After treatment application, clover biomass was collected weekly to define the temporal distribution of clover biomass in the KCLM system. Standing clover biomass was estimated for each plot with polynomial regression of clover biomass by date, where the degree of the polynomial was determined by minimizing the AIC (Figure 3.2). Daily clover N deposition was determined using the polynomial clover biomass estimates (Figure 3.2) according to equation 6.

(6) $$Clover\ N\ deposition_x = -0.049(y_{x-1} - y_x), if\ y_{x-1} - y_x \geq 0$$

, where x = DOY, y = clover biomass (kg ha^{-1}), and clover biomass contains on average 4.9% N (unpublished data).

3.3.5. Biomass nitrogen mineralization

Nitrogen mineralization from daily clover N inputs was estimated using the litter decay estimate for z days after deposition (Equation 5) and the estimated clover N deposition from spring management and shade suppression (Equation 6). Because clover biomass decay estimates are limited to the 8 wk after litter bag installation, mineralization estimates are limited to 56 d after deposition (Equation 7).

(7) $$Mineralization\ of\ daily\ N\ deposit_x = Clover\ N\ deposition_x * \sum (Remaining\ litter_{z-1} - Remaining\ litter_z), \{z = 1{,}56\}$$

, where *x*=DOY, and *z*=days after deposition.

Daily N mineralization was calculated as the sum of mineralization of daily N deposits (Equation 7) on that day and the preceding 56 d because of the limit of litter decay estimates (Equation 8; Figure 3.3).

(8) $$Daily\ N\ mineralization_x = \sum Mineralization\ of\ daily\ N\ deposit_i, i = \{x - 56, x\}$$

, where *x*=DOY.

Remaining organic N was estimated with clover N deposition (Equation 6) and daily N mineralization (Equation 8) estimates according to equation 9.

(9) $$Remaining\ organic\ N_x = \sum Clover\ N\ deposition_x - \sum_1^x Daily\ N\ mineralization_x$$

, where *x*=DOY.

Total N mineralization was calculated as the sum of daily N mineralization (Equation 8,10)

(10) $$Total\ N\ mineralization = \sum Daily\ N\ mineralization_x, i = \{1, n\}$$

, where *x*=DOY.

3.3.6. Soil nitrogen

After treatment application, soil was sampled weekly in the center of the maize row and the center of the interrow zone from the 0-0.30 m depth with a 20 mm i.d. corer. Samples were weighed before 8-10 g was subsampled and added to 38 mL of 2M KCl solution and shaken for 1 h at 120 RPM (Mulvaney, 1996). The extractant was filtered with 1 μm filter paper and analyzed for total NO_2-N + NO_3-N (NO_3-N) and NH_4-N concentrations with the Griess-Ilosvay with Cd reduction and sodium salicylate-nitroprusside method, modified for flowthrough injection analysis, respectively (Lachat, Loveland, CO, USA) (Mulvaney, 1996). A second 5 g subsample was collected, weighed, and dried at 105° C for 24 h. Oven dry soil was weighed and related to field moist soil to determine the mass water content which could be related to the whole sample mass to determine soil bulk density. Soil N concentrations were adjusted to the dry soil basis using the soil moisture content and to the kg ha^{-1} basis using bulk density measurements.

3.3.7. Nitrous oxide emissions

Nitrous oxide emissions were measured from the plot area twice-weekly using semi-static chamber methods (Parkin and Venterea, 2010). Because of the distinct spatial and temporal distributions of N between row and interrow zones in the KCLM system, each were measured separately. Chamber bases with a 0.29 x 0.50 m or a 0.15 x 0.30 m footprint were inserted at least 50 mm into the ground in the center of the interrow and 30 mm to one side of the maize row, respectively. On each sampling date, three 12-mL gas samples were collected near the soil surface with a syringe at one location within the plot area. Samples were immediately transferred to glass vials sealed with a butyl rubber septum and used as the T0 gas measurement. The interrow zone contained clover that grew and died back throughout the sampling period. Biomass that exceeded chamber top height was folded into each top upon chamber placement and the displaced chamber volume was estimated using clover biomass measurements and constant values for clover moisture content and biomass density (Alexander et al., 2019b; Lim et al., 2021). Insulated and vented chamber tops were placed onto chamber bases in 60 s intervals and secured using binder clips. After installation, 12-mL samples were collected from each chamber after 30, 60, and 90 minutes. Gas samples were analyzed for N_2O and CO_2

concentration with a 5890A Gas Chromatography analyzer (Hewlett–Packard, Palo Alto, CA, USA) in conjunction with a 7000 Headspace Autosampler (Teledyne Tekmar, Mason, OH, USA). Measured gas peak areas were adjusted by subtracting the peak area of vials that contained ambient concentrations of gasses during vial preparation and adjusting the sampled volume by the air temperature during sampling relative to the ideal gas constant (Parkin and Venterea, 2010). Gas fluxes were calculated using the restricted quadratic method, where the first derivative of the quadratic regression between N_2O concentration and time is used unless the second derivative of the resulting quadratic function is greater than 0, in which case a linear function is used (Parkin et al., 2012).

3.3.8. Analysis

Dependent variables were tested for normality and homogeneity of variance by visual inspection of QQ plots and residual vs fitted plots, respectively (Kutner et al., 2004). When data violated these assumptions, appropriate data transformations were made before dependent variables were retested. Grain yield, stover yield, and crop N uptake data were fit using mixed effects models in the *lme4* package in R (Bates et al., 2015; Team, 2021), with fertilizer N rate and year as fixed effects and block as the random effect (Bickel et al., 2007). Cumulative N_2O emissions were analyzed in the same manner, but with fertilizer N rate, zone, and year, as fixed effects, and block as the random effect. Models were analyzed using type III ANOVA with Kenward-Roger estimated denominator degrees of freedom using base R. When $P \leq 0.05$, treatment means were separated using the Tukey multiple comparison test with the *emmeans* (Lenth et al., 2018) and *multcomp* (Fox and Weisberg, 2019) packages in R, and results were presented using compact letter display (Piepho, 2018).

Clover biomass and soil N variables were evaluated using mixed models with repeated measures. Mixed effects models were fit using fertilizer N rate and year as fixed categorical effects, DOY as a continuous effect, and fertilizer N rate nested within block as the random slope term. DOY was tested as a linear, quadratic, cubic, and quartic function, and the degree of the polynomial was determined by minimizing the AIC of the respective models. Significant main effects and interactions were evaluated with

polynomial regression models, which give insight into the effect of treatments and years based on the mean and shape of the resulting linear relationships (Tremblay et al., 1998).

Daily N_2O emission data were analyzed between zones and years using multiple linear regression. Predictor variables included daily mineralized N, remaining organic N, the 3 d cumulative precipitation prior to sampling, air temperature, NO_3-N concentration, and NH_4-N concentration. Daily mineralized N, remaining organic N, and standing clover biomass were generated from the clover biomass regression and biomass decomposition analysis. Air temperature and cumulative precipitation leading up to gas measurements were obtained from the US-Ro6 weather station of the AmeriFlux network (Novick et al., 2018). Soil NH_4-N and NO_3-N concentrations for the specified dates were generated using linear interpolation of soil N concentrations before and after the sampling date. Predictor variables were tested for normality using visual inspection of QQ plots, and data that violated assumptions of normality were transformed and re-tested. Data were then centered and normalized and fit to the multiple regression models for each combination of zone and year (Marquardt, 1980). Models were parameterized using backward regression, where the predictor variable with the highest *P*-value was removed from the model in succession until one or two highly significant predictors remained (Ruengvirayudh and Brooks, 2016). Models that were generated from the backward elimination protocol were compared using ANOVA, and the model that minimized the AIC was chosen with no significance threshold of the predictor variables.

3.4. Results and Discussion

3.4.1. Weather conditions

Weather conditions varied between the 2020 and 2021 growing seasons (Figure 3.4). On Oct 1, accumulated growing degree days during the 2020 and 2021 growing seasons were 2.6 and 19.0% greater than the 1991 – 2010 average. From late April until the end of Sep, the site received 4.3% more rainfall and 38.1% less rainfall than average in 2020 and 2021, respectively. The hot and dry conditions experienced during the 2021 growing season resulted in severely stunted crops and complete clover dieback in Aug. The wide range of conditions observed during the study years heavily impact the results,

where, in 2020, the results represent N cycling dynamics that could be expected in a normal year, while in 2021, the results represent N variables under the extreme case of crop failure due to drought conditions.

3.4.2. Clover biomass and mineralization

Clover biomass followed different temporal distributions in 2020 and 2021 and were significantly affected by the interaction between DOY and year (Table 3.1; Figure 3.5). Clover biomass followed its usual habit in the beginning of the growing season and accumulated biomass at ~120 – 160 kg ha^{-1} d^{-1} in both years. Clover biomass peaked on 12 Jun in 2020 and 4 Jun in 2021 before declining under shade and severe drought conditions, respectively. In 2021, there was no living aboveground clover biomass between 24 Jul and 16 Aug. Rainfall on 24 Aug promoted clover regrowth that reached its early season peak near the end of the growing season.

Clover residue mineralization added 82 and 102 kg N ha^{-1} back into the KCLM system during the 2020 and 2021 growing seasons, respectively (Figure 3.6). The amount of unmineralized clover residue at the end of the 2021 growing season was 126% greater than in 2020 due to relatively slower residue decomposition. Clover regrowth at the end of the 2021 growing season increased the amount of total N in the organic and mineralized fractions, which ended the season at ~300 kg N ha^{-1} compared to ~160 kg N ha^{-1} at the end of the 2020 growing season. Mineralized N from clover residue enters the soil and behaves as any other N input and can be recycled by crop growth or lost through various N cycling pathways (Groffman et al., 1987). This analysis does not assume that mineralized N accumulates in the soil profile or contributes exclusively to the maize crop, instead, it provides a closer look at the magnitude of N pools and inputs from KCLM management.

3.4.3. Soil nitrogen

Soil N in the interrow zone was affected by interactions between DOY and year (Table 3.1; Figure 3.7). In 2020, soil N concentrations remained relatively steady in the interrow zone despite large biomass N inputs and few maize roots (Chapter 2). This

finding confirms previous research that did not find differences in soil N concentration when mowed clover residues were returned or removed and suggests that mineralized N is recycled by the living mulch in a typical growing season (Alexander et al., 2019b). In 2021, soil NH_4-N and NO_3-N concentrations increased throughout the growing season. The accumulation of NO_3-N was particularly strong, at 0.17 kg N ha^{-1} d^{-1}, and concentrations in the interrow zone ended the season at ~50 kg N ha^{-1}, which is 50% of the estimated mineralized N. This result is unsurprising and can be attributed to the reduced uptake of soil N from severely stunted clover and maize crops.

In the row zone, soil NH_4-N and NO_3-N was affected by the interaction between fertilizer N, DOY, and Year (Table 3.1; Figure 3.8). Soil NH_4 concentrations behaved as expected after application of urea-based fertilizers (Han et al., 2004). Soil NH_4-N concentrations followed similar trends between years, but concentrations were elevated in 2021 relative to 2020. Initial soil NH_4-N concentrations scaled with fertilizer N rate treatments before levels decreased quadratically and remained at a low level after DOY 220. In 2020, soil NO_3-N concentrations peaked shortly after fertilizer application before declining to a low level at DOY 220 (Figure 3.8b). Interestingly, the unfertilized control followed a similar temporal pattern as the fertilized treatments, indicating an increase in plant available N from mineralized clover biomass that was disturbed and incorporated with strip tillage. Soil NO_3-N was elevated at all times in 2021 relative to the 2020 growing season. These data show the accumulation of NO_3-N from clover dieback and mineralization while maize plants were stunted by severe drought conditions. Rainfall in late Aug re-started clover and maize growth and soil NO_3-N was rapidly taken up by the crops.

3.4.4. Crop yield

In 2020, grain and stover yields in the 180 kg N ha^{-1} treatment reached 13.2 and 7.0 Mg ha^{-1}, respectively (Table 3.2; Figure 3.9). This result is in conflict with previous N rate research in KCLM systems that concluded that maize production does not require fertilizer N to maximize grain yield in a year after the clover is used for forage production (Alexander et al., 2019a).

The results outlined in chapter 2 added that kura clover root biomass declines over the growing season as a response to tillage. In that study, root biomass loss represented 23% of the initial concentration and amounted to 3.04 Mg ha^{-1}. These biomass pools are a potentially large source of mineralizable N that likely contributed to reduced N requirements for maize after forage production in Alexander et al., (2019a). Chapter 2 also provides insight into why, despite a large amount of aboveground clover biomass N (82 kg ha^{-1}) deposited in the interrow zone due to shading, maize required N near a typical grower rate due to the spatial separation of maize roots in the row zone and biomass N inputs in the interrow zone.

A broad view of the relationships between the distribution of maize and clover roots, the magnitude of aboveground clover biomass inputs, and the response to fertilizer N in the contexts of this study suggests that KCLM systems cannot reliably replace the need for fertilizer N for maize production under the simplified rule of crop rotation defined by Alexander et al., (2019a). These observations warrant the need for further research into the multiyear cycles of clover root biomass under KCLM management.

3.4.5. Nitrous oxide emissions

Cumulative N_2O-N emissions from the KCLM system responded to the interaction between zone and year (Table 3.3; Figure 3.10). In 2020, 85% (2.90 kg N_2O-N ha^{-1}) of the total cumulative N_2O-N emissions (3.41 kg N_2O-N ha^{-1}) were from the interrow zone, while in 2021, 49% (0.85 kg N_2O-N ha^{-1}) of the total N_2O-N emissions (1.75 kg N_2O-N ha^{-1}) were from the row zone. In comparison to conventional maize and soybean systems in the upper Midwest, which typically emit ~0.6-2.5 kg N_2O-N ha^{-1}, these cumulative emissions from the KCLM system were relatively high (1.75-3.41 kg N_2O-N ha^{-1}) (Venterea and Coulter, 2015; Fernández et al., 2016; Venterea et al., 2016). Increased relative emissions from the row zone can be easily explained in 2021 due to high levels of mineral N, but high N_2O emissions from the interrow zone in 2020 is less intuitive.

The interrow zone did not receive fertilizer N and soil N concentrations in that zone were not affected by fertilizer N rate. In 2020, there was high variability in cumulative non-area weighted N_2O-N emissions in the interrow zone (mean=3.93;

SD=2.57) relative to the row (mean=1.96; SD=0.58). This high relative emission and variability in a management zone that did not receive inorganic N may be an indication of hotspots, where large amounts of N_2O are emitted from microenvironments that promote rapid denitrification of a rich N source (Parkin, 1987). Turner et al., (2016b) defined hotspots, intermediate, and coldspots in a large field of conventionally managed maize using geostatistical approaches. In that study, hotspots and coldspots were defined as sites that emitted 4.6 and 0.9 kg N ha^{-1}, respectively, during the 42 d measurement period. Under a maize-soy rotation at that particular site, the study concluded that hotspots are semi-permanent features that exist due to differences in topography and hydrology. However, the hotspots observed in this study are distinctively different that those defined by Turner et al., (2016b).

The inception of the hotspot hypothesis was provided by Parkin (1987). In that study, a soil core and its components were incubated under aerobic conditions to determine the source of N_2O emissions within an agricultural soil. The core was split into smaller fractions of the total core before it was revealed that 85% of total emissions originated from a single partially decayed leaf. Later work described how hotspots of microbial activity can result in anaerobic microenvironments in an aerobic soil (Schlüter et al., 2019). With readily mineralizable clover residue inputs exceeding 40 kg ha^{-1} d^{-1} and the humid microenvironment under the dense clover canopy, the KCLM system has the essential ingredients to generate microbial hotspots of denitrification. Under this hypothesis, hotspots in a KCLM may be less contained to specific microtopography than those observed by Turner et al., (2016), and instead are defined by the cropping system.

Another possible source of N_2O emissions is from N inputs that exceed crop requirements (Thomson et al., 2012). Turner et al., 2016 presented cumulative emissions from maize production under KCLM and conventional management and found that baseline emissions in the KCLM were relatively increased in the latter half of the growing season. These high baseline emissions may be attributed to large biomass N inputs and low N uptake in the interrow zone when clover is suppressed by shade and maize roots are not present, however, soil N data in 2020 did not indicate accumulation of N in that zone.

The multiple regression analysis explained 15 – 22% of the variability in daily N_2O emissions (Table 3.4). The analysis indicates that, depending on the zone and environmental conditions during the year, daily mineralized N, standing clover biomass, precipitation, and soil NO_3 concentrations are key drivers of N_2O emissions in KCLM systems, while remaining organic N can suppress emissions under certain conditions. Precipitation and NO_3-N concentrations are well known drivers of N_2O emission in many fertilized agroecosystems (Dobbie et al., 1999; Giles et al., 2012). Precipitation was a significant positively correlated predictor of N_2O-N emissions for both zones in each year, but NO_3-N concentration could not be used to predict emissions from the interrow in 2021.

The interrow zone contributed to 85% of the total N_2O-N emissions in 2020 (Figure 3.10), and was influenced by precipitation, soil NO_3-N concentration, and standing clover biomass (Table 3.4). The high emissions and their high variability coupled with a significant response to standing clover biomass support the hypothesis that hotspots are a potential contributor of N_2O emissions in the KCLM system. The clover canopy under a maize crop is dense and humid and the low C:N residues (~9) are readily mineralizable. A water film from precipitation or morning dew could result in an anaerobic microenvironment and promote denitrification processes on partially decaying residues (Kravchenko et al., 2017). In 2021, emissions in the interrow zone were influenced by standing clover biomass and precipitation, but cumulative emissions were 3.4 times lower than in 2021. The limited precipitation in 2021 reduced the rate of residue mineralization and likely reduced the rate of denitrification and the occurrence of hotspots in that zone. The lack of NO_3-N as a driver of N_2O emissions makes sense due to the steady increase in NO_3-N concentrations in that zone over the growing season. This increase was caused by halted clover growth and decay of surface clover residue deposits, but little soil moisture availability likely decreased the occurrence of denitrification processes (Bakken, 1988).

In the row zone, the multiple regression analysis indicated slightly different drivers between growing seasons (Table 3.4). In 2020, precipitation, NO_3-N concentration, daily mineralized N, and NH_4-N concentration contributed to emissions, while remaining

organic N reduced emissions. Because the predictor variables are centered, we can compare the magnitude of their coefficients. Precipitation N had the largest positive coefficient of all significant predictor variables. Daily mineralized N was also a large contributor to emissions, however, the magnitude of the coefficient for remaining organic N is 11% greater than the coefficient for daily mineralized N. Because the row zone receives biomass inputs only during spring management, these predictors are correlated and may offset a portion of their effects. In 2021, N_2O emissions in the row zone were influenced by precipitation and standing clover biomass. The correlation of standing clover biomass with increased emissions may be explained by the presence of clover biomass when moisture was not severely limited, and the lack of NO_3-N availability as a driver of emissions is unsurprising, as the conditions for denitrification were not met for most of the year.

3.5. Conclusions

Kura clover deposits a large amount of biomass N from spring management and shade suppression. These N inputs, however, are most likely recycled by the clover under normal weather conditions. Only a fraction of N inputs from KCLM management are deposited where maize roots are present, and organic N inputs from root biomass is likely a larger contributor to in-season N from the KCLM. Therefore, fertilizer N requirements for maize may depend on kura stand health, which is likely a function of management intensity in previous years. High emissions and high variability of N_2O in the interrow suggests the presence of hotspots as a major contributor to emissions in KCLM systems. Standing clover biomass as a positively correlated contributor to daily N_2O emissions in the interrow supports this finding. Under this hypothesis, there may not be management options to mitigate N_2O emissions from KCLM systems. Future research should investigate the effect of crop rotations on KCLM biomass pools and N requirements for maize as well as the sources of N_2O from KCLM systems.

Table 3.1: Treatment means and standard error of clover biomass and soil N concentrations across fertilizer rate treatments and day of year (DOY) during the 2020 and 2021 growing seasons.

	Row & Interrow Clover biomass	**Interrow**		**Row**	
		NH_4-N	**NO_3-N**	**NH_4-N**	**NO_3-N**
	kg ha^{-1}	kg ha^{-1}		kg ha^{-1}	
Fertilizer N					
0	1507 (82.8) †	5.34 (1.21)	11.90 (1.56)	12.7 (3.95)	7.57 (1.21)
90	1292 (82.8)	5.40 (1.21)	9.86 (1.56)	10.1 (3.95)	8.67 (1.21)
180	1292 (82.8)	6.68 (1.20)	10.80 (1.55)	18.0 (3.91)	9.70 (1.20)
DOY					
x‖	5024	59.56	-0.63	9.29	-20.94
x^2	-36.29	39.23	-6.16	-4.73	3.59
x^3	0.11	11.89	69.78		29.00
x^4	$-1.30 * 10^{-4}$		-35.78		-29.12
Year					
2020	1506 (65.2)	4.79 (0.85)	6.33 (1.01)	5.36 (2.47)	4.97 (0.78)
2021	1204 (69.1)	7.83 (1.19)	19.82 (1.43)	28.19 (3.47)	15.95 (1.10)
Significance			P > F		
N	<0.01	0.89	0.85	0.46	0.28
Year	<0.0001	<0.0001	<0.0001	0.05	<0.0001
DOY	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
N:Year	<0.01	0.95	0.69	0.11	0.25
N:DOY	0.40	0.51	0.58	<0.01	0.03
DOY:Year	<0.0001	<0.0001	<0.0001	<0.001	<0.0001
N:DOY:Year	0.89	0.91	0.58	0.01	0.01

† Standard error. ‖ Coefficients of simple or polynomial linear regression.

Table 3.2: Treatment means and standard error of grain yield, stover yield, and N uptake of maize crops across fertilizer N rate treatments during the 2020 and 2021 growing seasons.

	Grain yield	**Stover yield**	**N uptake**
	Mg ha^{-1}		kg ha^{-1}
Fertilizer			
0	4.31 (0.32) †	3.47 (0.22)	77.8 (6.25)
90	6.28 (0.32)	4.42 (0.22)	98.6 (6.25)
180	7.44 (0.32)	4.64 (0.22)	123.9 (6.25)
Year			
2020	10.22 (0.27)	6.11 (0.18)	151.5 (5.20)
2021	1.81 (0.27)	2.25 (0.18)	48.6 (5.20)
Significance			
N	<0.0001	<0.01	<0.001
Year	<0.0001	<0.0001	<0.0001
N:Year	<0.0001	<0.0001	<0.0001

† Standard error.

Table 3.3: Treatment means and standard error of cumulative N_2O-N emissions across fertilizer N rate treatments and row zones during the 2020 and 2021 growing seasons.

	N_2O-N
	kg ha^{-1}
Fertilizer	
0	2.65 (0.36) †
90	2.26 (0.36)
180	2.94 (0.36)
Zone	
Row	2.69 (0.30)
Interrow	2.54 (0.30)
Year	
2020	2.94 (0.30)
2021	2.29 (0.30)
Significance	
N	<0.0001
Zone	<0.0001
Year	0.04
N:Zone	0.01
N:Year	0.60
Zone:Year	<0.0001
N:Zone:Year	0.01

† Standard error

Table 3.4: Multiple regression analysis of daily N_2O emissions from the KCLM system.

	2020				**2021**			
	Row		**Interrow**		**Row**		**Interrow**	
	Estimate	*P* > *t*	Estimate	*P* > *t*	Estimate	*P* > *t*	Estimate	*P* > *F*
Precipitation	7.72 * 10^{-3}	<0.0001	2.50 * 10^{-2}	<0.0001	2.35 * 10^{-2}	<0.0001	7.03 * 10^{-3}	<0.0001
NO_3	4.09 * 10^{-3}	<0.01	1.26 * 10^{-2}	<0.01	4.76 * 10^{-3}	0.11		
Standing clover			7.42 * 10^{-3}	0.05	6.55 * 10^{-3}	0.02	6.59 * 10^{-3}	<0.0001
Daily mineralized N	6.53 * 10^{-3}	0.03			5.02 * 10^{-3}	0.10		
Remaining organic N	-7.26 * 10^{-3}	0.01						
NH_4	1.97 * 10^{-3}	0.12						
Model		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**
Adjusted R^2	**0.1924**		**0.1491**		**0.2234**		**0.1855**	

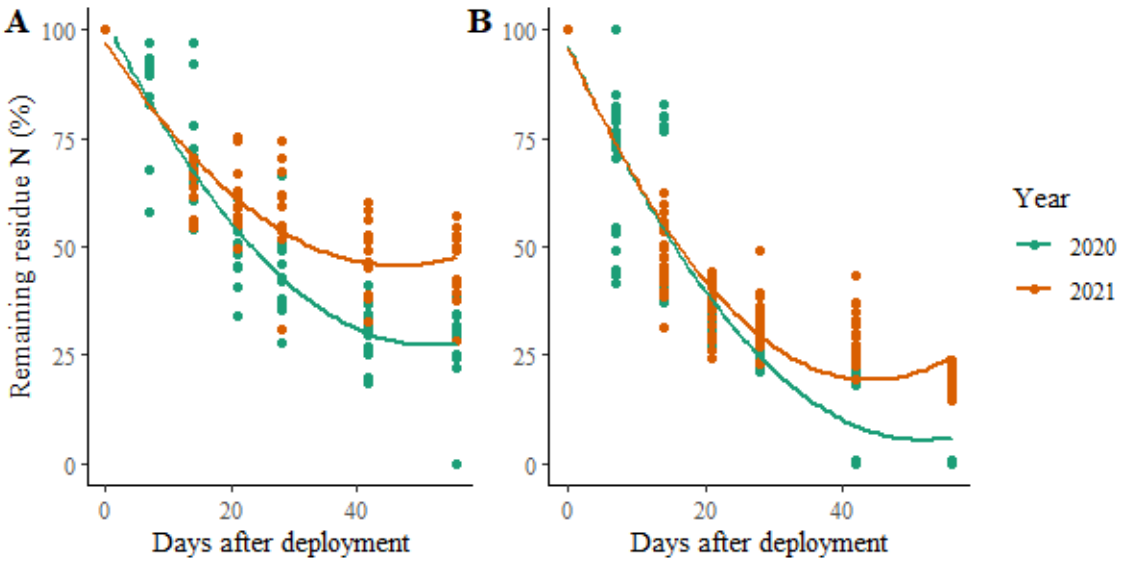

Figure 3.1: Remaining nitrogen in litter bags placed **a)** on the soil surface and **b)** at the 0.10 and 0.20 m depths across all N rate treatments.

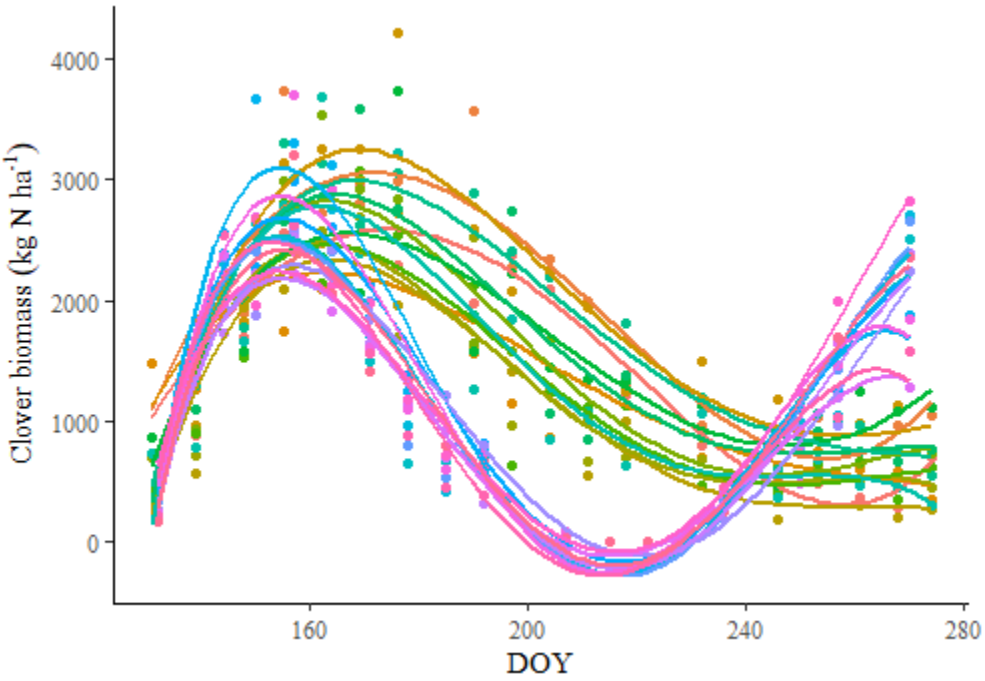

Figure 3.2: Regression of clover biomass data by day of year (DOY) for each plot in each year.

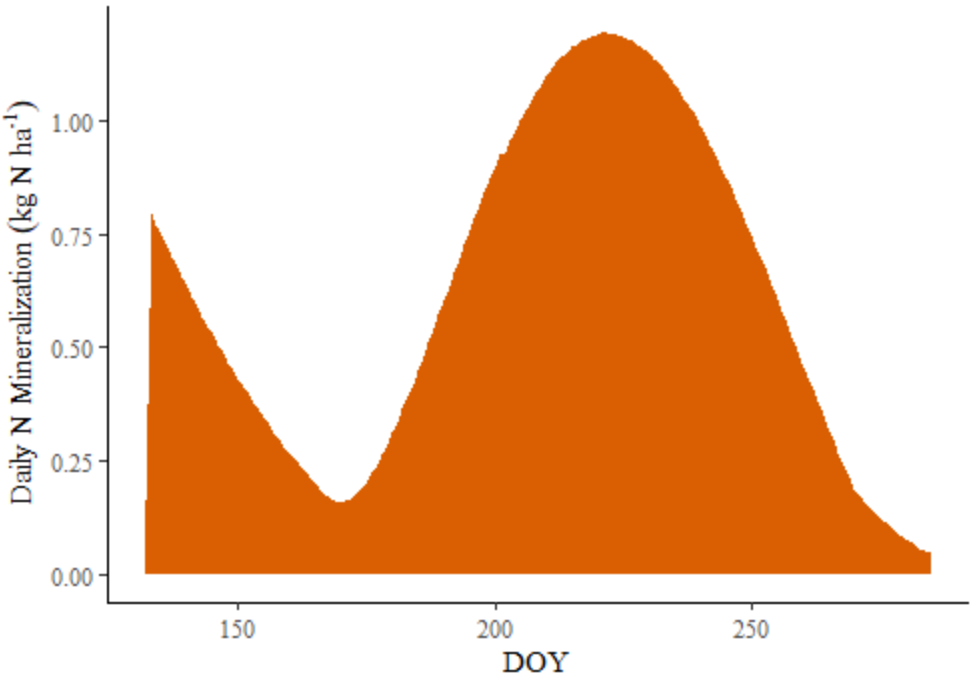

Figure 3.3: Daily nitrogen (N) mineralization.

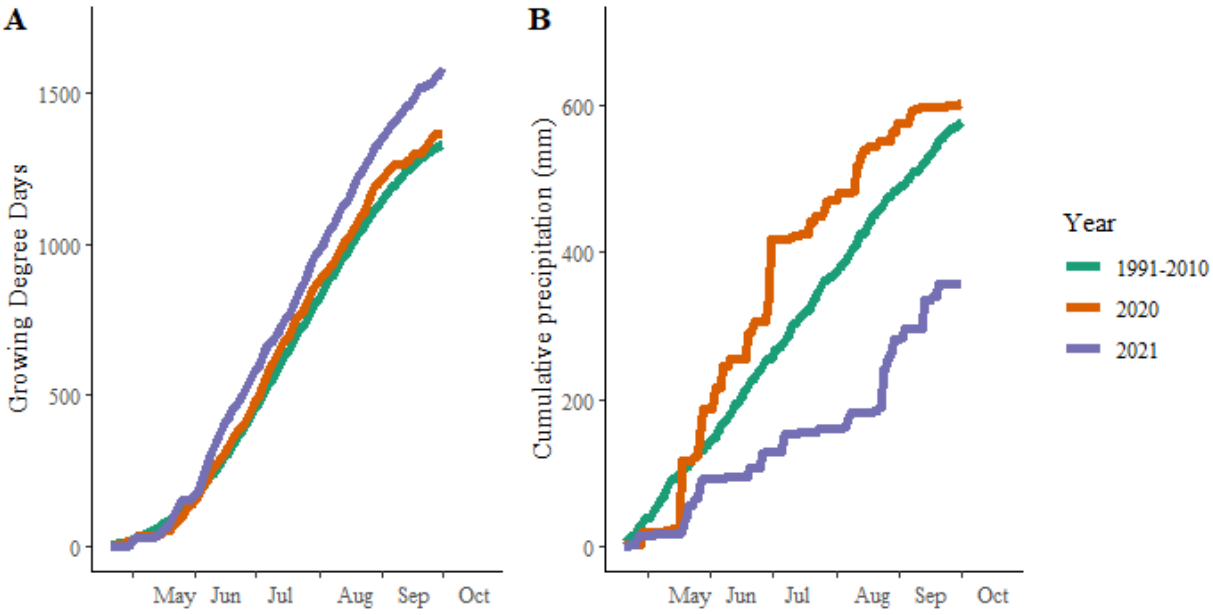

Figure 3.4: a) Growing degree days and **b)** cumulative precipitation at the Rosemount Research and Outreach Center from 4/20 – 10/1 during the 1991 – 2010 average and the 2020 and 2021 growing seasons.

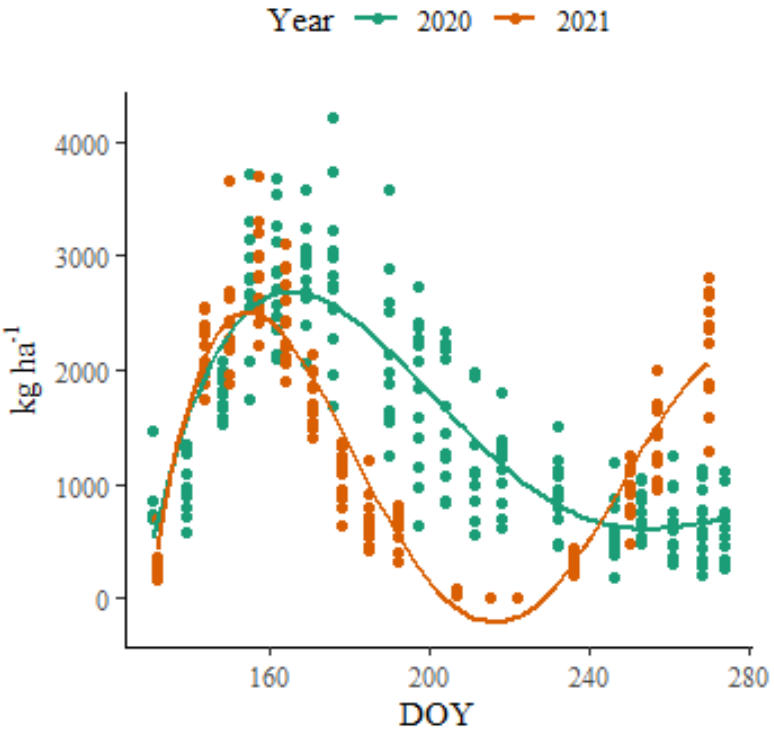

Figure 3.5: Temporal distribution of clover biomass during the 2020 and 2021 growing seasons.

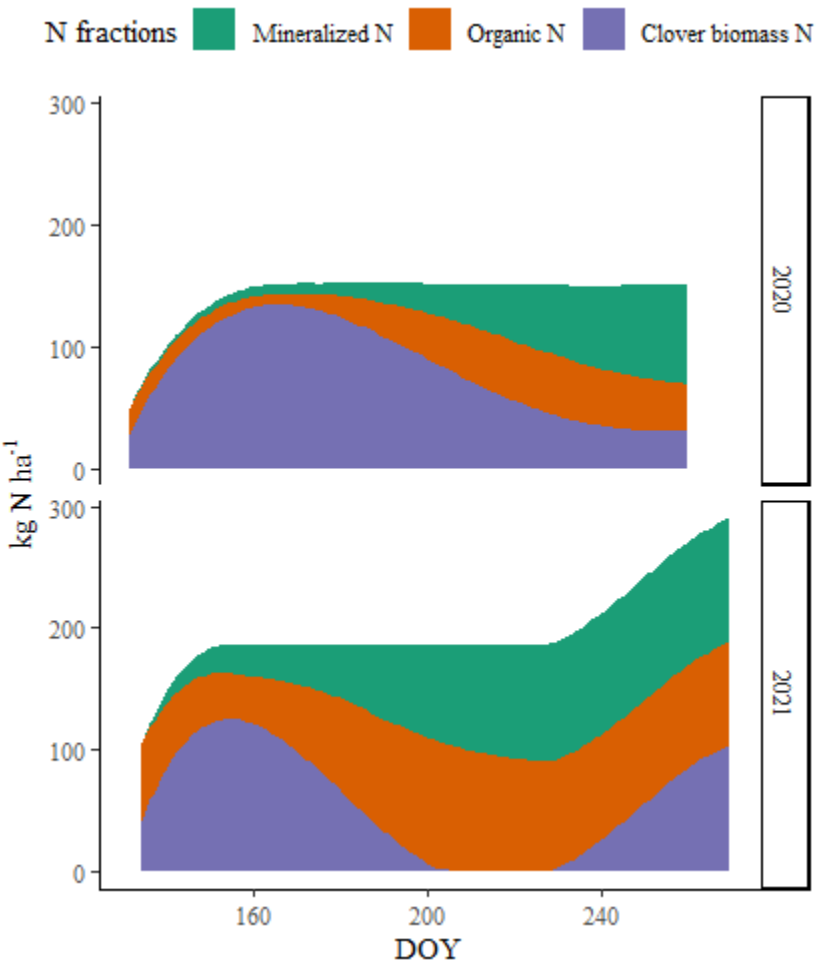

Figure 3.6: Stacked area chart of biological and mineralized N fractions in a KCLM-maize system during the 2020 and 2021 growing seasons.

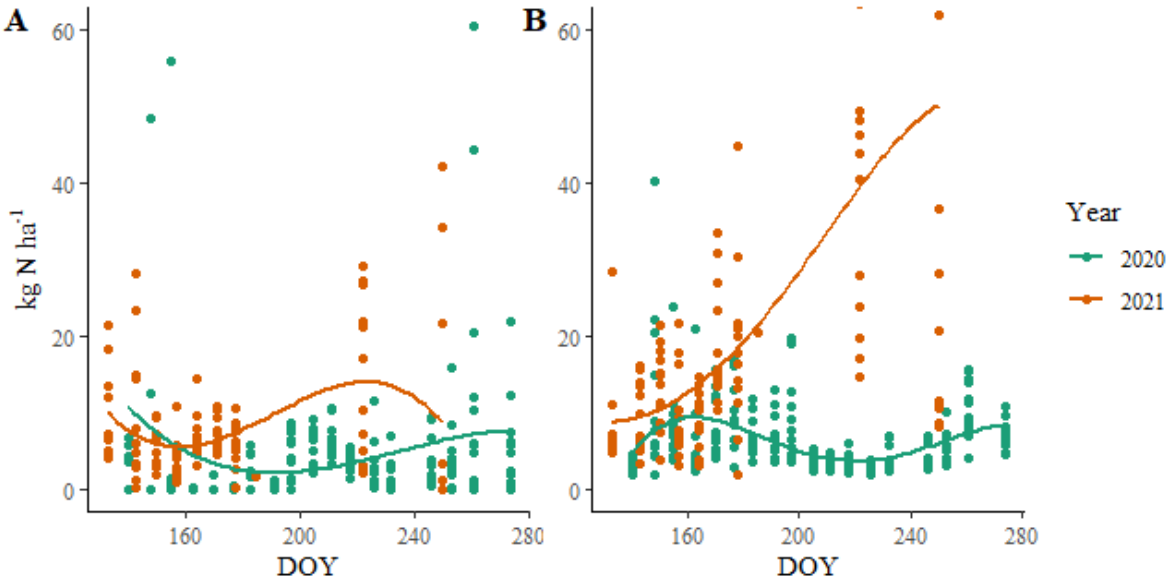

Figure 3.7: Concentrations of soil **a)** NH_4-N and **b)** NO_3-N in the interrow zone during the 2020 and 2021 growing season.

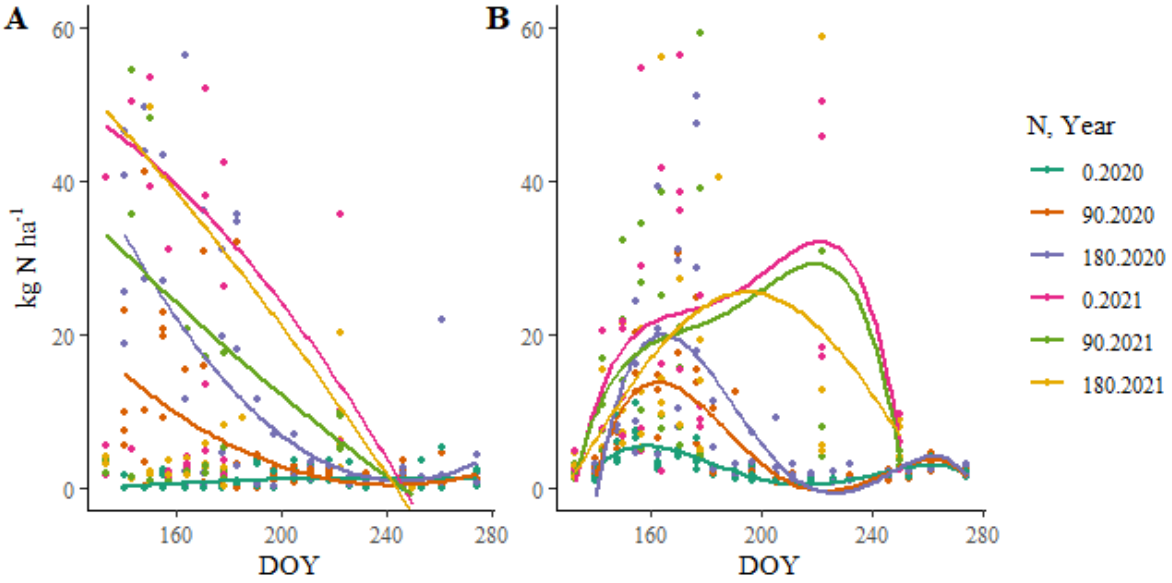

Figure 3.8: Concentrations of soil **a)** NH_4-N and **b)** NO_3-N in the row zone during the 2020 and 2021 growing season.

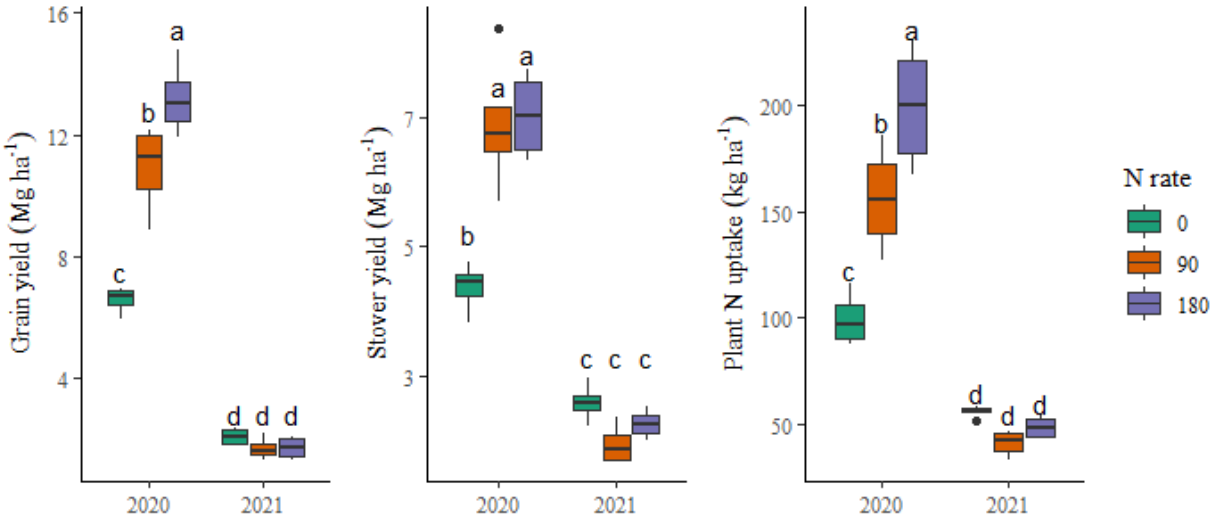

Figure 3.9: Grain yield, stover yield, and crop N uptake by fertilizer N rate treatment and year. Means labelled by the same letter are not significantly different at $p \leq 0.05$.

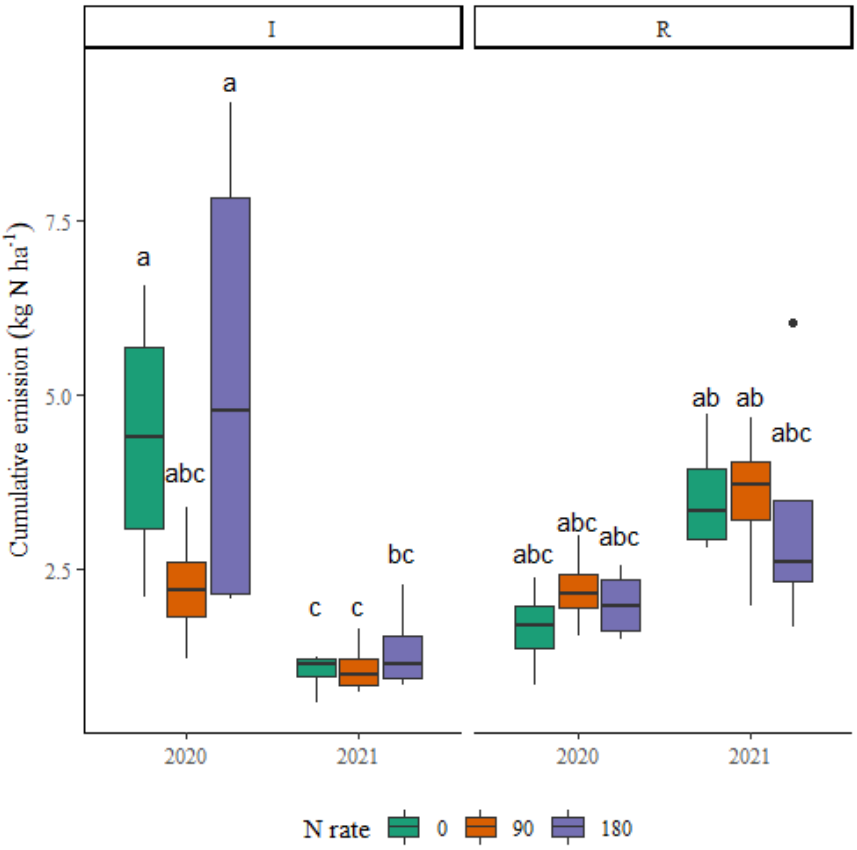

Figure 3.10: Area equivalent cumulative N_2O-N emissions by fertilizer N rate treatments in the row and interrow zones during the 2020 and 2021 growing seasons. Means labelled by the same letter are not significantly different at $p \leq 0.05$.

CHAPTER 4: RESEARCH STATUS AND FUTURE OPPORTUNITIES

4.1. Where does a Kura Clover Living Mulch Fit?

Kura clover living mulch adds perennial living cover to existing row-crop production systems. This acts to protect soils from wind and rill erosion in the spring when row crops are not well established (Pratt et al., 2014; Siller et al., 2016). The robust root system and vigorous spring growth increases water infiltration and primary productivity while reducing runoff and nitrate leaching relative to conventionally managed maize (Ochsner et al., 2010; Baker et al., 2021). These benefits respond to some of the main environmental impacts from maize production and promote its widespread use, however, there are practical considerations that limit its adoptability.

Stover must be removed at the end of the growing season to mitigate smothering in the spring which could compromise the health of the clover stand. This makes the need for maize stover one of the most important consideration for a producer that integrates a KCLM into their farming operation. Maize stover can be grazed, baled, or harvested with the whole plant as silage. In 2010, stover was partially or fully harvested from 2% of maize acres, while maize silage was harvested from 6.4% of the planted maize crop (Schmer et al., 2017; USDA, 2018). Cellulosic ethanol was a promising industry that hoped to utilize a large portion of US maize stover production, however, this industry suffered due to highly competitive energy markets and new technologies that displaced the need for expanded biofuel production (Lynd et al., 2017). Without this major consumer of maize stover, the need for KCLM is limited to livestock production inputs and niche markets.

Producers who have a need for these feedstocks are often large consumers with a need each year. If their access to cropland isn't large enough to maintain a diverse rotation on these fields, they risk soil physical degradation through the repeated removal of residue inputs (Johnson et al., 2016). In this scenario, the KCLM can be used for continuous maize production to protect against soil loss and to maintain soil physical and chemical properties (Pratt et al., 2014; Baker et al., 2021). This solution may provide practical and economic benefits to producers by mitigating risks associated with the long-

term concentration of feed production near feed storage infrastructure (Roth and Undersander, 1995).

An alternative use for KCLM may be within sensitive areas, such as near waterways or on highly erodible land. This strategy has been promoted by the prairie strips program, which encourages the planting of perennial vegetation on vulnerable landscape positions (Tyndall et al., 2013). Research has presented evidence that this strategy can provide disproportional conservation benefits relative to the size of displaced crop production (Schulte et al., 2017). Utilizing a KCLM in this way has not been investigated, but the environmental benefits of the two approaches are similar and may yield similar results.

4.2. Advice for Managing a Kura Clover Living Mulch for Maize

If the needs for a KCLM are met, a producer must make a few choices to optimally manage the system in their operation. The literature describes a few main management considerations, such as row establishment, suppression management, and fertilizer management, but there is not a comprehensive guide that distills this information for potential users. My contributions to this area are related to row establishment, fertilizer N requirements for maize, and interactions between these considerations and stand history. I will present evidence from my findings and the existing literature to provide guidance on the broader subject of successful management of a KCLM for maize production.

The method of establishing a clover free row zone is an important consideration for a producer integrating a KCLM into their operation. Equipment availability is a main determining factor for which method to use. The rotary zone tillage tool that is utilized in my work and other projects in Minnesota is an effective option for establishing a clover free zone and a suitable seedbed, however, the high horsepower requirements, limited working widths, slow working speeds, and low availability of equipment are prohibitive to most producers. Producers who have access to strip tillage equipment with integrated fertilizer application technology may choose these methods to manage a KCLM. However, this option can be costly for those who do not already own this equipment. A

better option for beginners and no-tillers is to utilize banded herbicides and banded liquid N fertilizer. Affeldt et al., 2004 described the banded herbicide technique as a 0.25 m band of 0.56 kg a.e. ha^{-1} of dicamba and 0.05 kg a.e. ha^{-1} of clopyralid (Stinger) and noted that this method could be paired with herbicide resistant maize varieties and other applications of broadcast herbicide suppression before and after planting. Banded herbicides can be applied at planting with a sprayer nozzle in-line with the planter row unit. This method ensures that maize is seeded directly in the center of the row zone and reduces the need for high accuracy guidance systems. While this method does not incorporate clover biomass into the row zone, it is effective in maintaining a clover free zone for maize seedlings at a low cost.

Depending on the spring growing conditions, kura clover growth may be overcompetitive with maize seedlings. This issue is most related to soil temperature and available carbohydrate reserves that promote early season growth. Maize seedlings require soil temperature of 10° C for emergence. Clover growth likely requires similar soil temperatures, but the perennial clover already has a well-established root system to speed spring growth. Federal crop insurance programs allow maize producers in Minnesota to plant maize between 11 April and 25 May for full coverage of planted fields. Planting in the first half of this period is attractive to many conventional producers as the risks associated with slow emergence can be outweighed by the benefits of earlier maize development and the risks associated with planting in poor field conditions (Lauer et al., 1999). In the living mulch system, however, the risk of slow emergence is more critical, as it reduces options to manage competition. The literature has not yet addressed the area of planting date within a KCLM. Across 11 different studies and 17 site years, the average maize planting date was 13 May, with the earliest date on 30 April and the latest date on 10 Jun. The studies which planted maize at earlier dates tended to manage competition with 2 or 3 applications of herbicides, while studies that relied on tillage and mowing for row establishment and suppression were generally planted at later dates. It should be noted, however, that most of the banded herbicide studies were conducted at lower latitudes in Wisconsin, which likely influenced maize planting date more than the method of row establishment. It is difficult to say what approach is best for maize yields

and clover stand health without further research that compares planting date and living mulch management treatments. However, from our experiences and observations managing these systems, it appears that planting in the latter half of the covered crop insurance window is a reasonable tradeoff between managing competition and maximizing accumulated growing degree units for maize.

Fertilizer placement studies have not been conducted for KCLM cropping systems. Given what we know about maize root distribution in the mixed cropping system, it is likely that banded applications are best for nutrient use efficiency. My studies have broadcasted P, K, and S fertilizers before planting and banded N fertilizers during row establishment on the tilled or killed row. While this strategy aligns with the cost and logistics of applying mineral fertilizers and mitigates risks associated with early application of volatile N fertilizers, we recognize the limitations of this within our research. The source of N fertilizers must be considered with the row establishment method, where rows established with strip tillage can utilize anhydrous ammonia, dry, or liquid N fertilizers, and rows established with banded herbicides may benefit from liquid sources applied with a y-drop applicator to mitigate N volatilization in the no-till system (Liu et al., 2019).

Manure application in KCLM research is understudied. The logistics of manure management in integrated crop-livestock systems is always a large hurdle, and even more so when nutrients must be applied in a band. Beef production systems most often produce dry or composted manures, but modern confinement beef operations produce manure in a liquid form. When dry manure is the predominant product produced from a farming operation, there are not practical options for band application. When liquid manures are available, however, they will likely benefit from injection with strip tillage. This approach, in the contexts of large livestock operations, would benefit from fall application to increase the application window and reduce time constraints in the spring. Strip tillage of KCLM in the fall, however, has not been investigated. This approach could be paired with a lighter form of row establishment in the spring, such as banded herbicides or strip freshening to prepare a suitable seedbed and create a clover free zone.

Finally, maize stover must be removed at crop harvest. Depending on the farming operation, this can be accomplished in three main ways. The most economical approach for beef cattle producers is to graze the cornstalks after grain harvest. This method is widely used in the western corn belt where they recommend that cattle graze ~50% of the leaf, husk, and tassel fractions, in order to return residue inputs that maintain the productivity of maize in the following year (Nunes et al., 2021). In the living mulch system, we may want to increase the intensity of grazing to reduce the amount of remaining stover residue to improve clover health in the following spring. However, the remaining stover should be monitored closely so that cattle don't resort to feeding off of stalk and cob fractions, as these fractions are less nutritious and can lead to weight loss and reduced carcass quality. The second option for maize grain producers is to remove stover by baling. Alexander et al 2019a found that this method of stover removal can cost between \$225 – 290 ha^{-1}, but the value of the stover offset these costs and increased the profitability of maize in KCLM relative to conventional grain production. Finally, whole plant maize can be removed and harvested as maize silage. This use is perhaps the ideal scenario for the KCLM system as it introduces no additional costs to the harvesting of the crop and the early relative harvest date allows for clover regrowth in the fall.

4.3. Research Opportunities

There are several research opportunities related to this cropping system. The largest remaining barrier to the adoption of KCLM is the initial stand establishment. Kura stands are costly to establish both due to the high cost of seed and their slow development in the seeding year. Kura clovers slow establishment is attributed to its large investment of photosynthetic energy into belowground root and rhizome structures (Speer and Allinson, 1985). Slow clover growth results in weed pressure which requires mowing or herbicides that increase management cost (Seguin et al., 1999). Additionally, forage yields during the establishment year cannot compete economically with conventional forage or row cropping systems. A few studies investigated the use of nurse crops to increase forage yields in the seeding year, but each noted that interspecies competition reduced clover growth relative to sole seeding (Seguin et al., 1999; Cuomo et al., 2001).

Later work noted that kura establishment can be improved with compatible microbial inoculant and N fertilization (Seguin et al., 2001; Laberge et al., 2004), but still, work must be done to expedite clover establishment to protect growers from high opportunity costs. Currently, work is being conducted to increase seedling vigor and seed yields through improved genetics. Additionally, research is investigating the effect of seeding kura on 0.76 m rows to concentrate kura development in the interrow zone. These genetic and management opportunities must be further tested and developed to solve this fundamental issue.

Once kura stands are established, we encounter different challenges associated with managing the mixed cropping system. While several aspects of KCLM management have been investigated, there are remaining management considerations that should be addressed. The first of which is maize planting date. This subject has not been studied within KCLM systems and is typically dealt with by using deductive reasoning using contexts around spring weather conditions and clover stand condition. This type of management decision can be made by experienced producers, but prescriptive approaches will improve the success of early adopters. This type of research can be described as adaptive management, where the goals are to stabilize the performance of a system under changing conditions and uncertainty (Bybee-Finley and Ryan, 2018). This approach to define the timing of spring management in a KCLM may factor in date, latitude, stand history, accumulated growing degree days, and standing clover biomass.

The intensity of KCLM management likely influences clover health, and the long-term impacts of management on clover root biomass, clover vigor, and environmental benefits are unknown. Many studies recognize the influence of stand history on the performance of row crops in the KCLM but controlling for this variable requires planning years ahead of a potential experiment. These questions will require long-term research to investigate the effect of row crop or forage management on the health and vigor of the living mulch in subsequent years. The identification of optimal crop rotations as it relates to crop productivity and environmental benefits with the recognition of different needs for producers could help to maximize the potential benefits of KCLM and strengthen management guidelines based on stand history.

Finally, further research into the sources of N_2O emissions from KCLM cropping systems is essential to identify and develop mitigation strategies. Nitrous oxide emissions are notoriously difficult to mitigate in fertilized agroecosystems due to their correlation with soil properties and environmental conditions (Thomson et al., 2012; Venterea et al., 2012). While the KCLM may provide N benefits to maize under certain conditions, our data suggests that consistent yields likely require fertilizer N near conventional rates and a large portion of biomass N inputs are not utilized by the row crop. The hypothesis that hotspots are a major contributor to N_2O production in these systems also complicates mitigation efforts, but further testing is required to confirm this source of emissions from KCLM cropping systems.

BIBLIOGRAPHY

Affeldt, R.P., K.A. Albrecht, C.M. Boerboom, and E.J. Bures. 2004. Integrating Herbicide-Resistant Corn Technology in a Kura Clover Living Mulch System. Agron. J. 96(1): 247–251. doi: 10.2134/agronj2004.0247.

Aho, K., D. Derryberry, and T. Peterson. 2014. Model selection for ecologists : the worldviews of AIC and BIC. Ecology 95(3): 631–636. http://www.esajournals.org/doi/full/10.1890/13-1452.1.

Albrecht, K.A., and A. Sabalzagaray. 2006. Maize silage production in a kura clover living mulch. Grassl. Sci. Eur. 11: 59–61.

Alexander, J.R., J.M. Baker, R.T. Venterea, and A. Coulter. 2019a. Kura Clover Living Mulch Reduces Fertilizer N Requirements and Increases Profitability of Maize. Agronomy 9(432). doi: 10.3390/agronomy9080432.

Alexander, J.R., R.T. Venterea, J.M. Baker, and J.A. Coulter. 2019b. Kura clover living mulch: Spring management effects on nitrogen. Agronomy 9(2). doi: 10.3390/agronomy9020069.

Allmaras, R.R., S.M. Copeland, P.J. Copeland, and M. Oussible. 1996. Spatial relations between oat residue and ceramic spheres when incorporated sequentially by tillage. Soil Sci. Soc. Am. J. 60(4): 1209–1216. doi: 10.2136/sssaj1996.03615995006000040036x.

Amos, B., and D.T. Walters. 2006. Maize Root Biomass and Net Rhizodeposited Carbon. Soil Sci. Soc. Am. J. 70(5): 1489–1503. doi: 10.2136/sssaj2005.0216.

Andrén, O., E. steen, and K. Rajkai. 1992. Modelling the effects of moisture on barley straw and root decomposition in the field. Soil Biol. Biochem. 24(8): 727–736. doi: 10.1016/0038-0717(92)90246-T.

De Baets, S., J. Poesen, J. Meersmans, and L. Serlet. 2011. Cover crops and their erosion-reducing effects during concentrated flow erosion. Catena 85(3): 237–244. doi: 10.1016/j.catena.2011.01.009.

Baker, J.M., K.A. Albrecht, G.E. Feyereisen, and J.D. Gamble. 2021. A perennial living mulch substantially increases infiltration in row crop systems. J. Soil Water Conserv.: 80. doi: 10.2489/jswc.2022.00080.

Bakken, L.R. 1988. Denitrification under different cultivated plants: effects of soil moisture tension, nitrate concentration, and photosynthetic activity. Biol. Fertil. Soils 6(4): 271–278. doi: 10.1007/BF00261011.

Bates, D., M. Mächler, B.M. Bolker, and S.C. Walker. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67(1). doi: 10.18637/jss.v067.i01.

Bates, D., M. Maechler, B. Bolker, S. Walker, R.H.B. Christensen, et al. 2014. Package "lme4." Compr. R Arch. Netw.

Bickel, P., P. Diggle, S. Fienberg, U. Gather, I. Olkin, et al. 2007. Linear and Generalized Linear Mixed Models and Their Applications.

Bittsánszky, A., K. Pilinszky, G. Gyulai, and T. Komives. 2015. Overcoming ammonium toxicity. Plant Sci. 231: 184–190. doi: 10.1016/j.plantsci.2014.12.005.

Blanco-Canqui, H. 2021. No-till technology has limited potential to store carbon: How can we enhance such potential? Agric. Ecosyst. Environ. 313(October 2020): 107352. doi: 10.1016/j.agee.2021.107352.

Blevins, R.L., M.S. Smith, G.W. Thomas, and W.W. Frye. 1983. Influence of conservation tillage on soil properties. J. Soil Water Conserv. 38(3): 301 LP – 305. http://www.jswconline.org/content/38/3/301.abstract.

Blue, W.G., and C.F. Eno. 1954. Distribution and Retention of Anhydrous Ammonia in Sandy Soils. Soil Sci. Soc. Am. J. 18(4): 420. doi: 10.2136/sssaj1954.03615995001800040017x.

Boulton, A.J., and A. Williford. 2018. Analyzing skewed continuous outcomes with many zeros: A tutorial for social work and youth prevention science researchers. J. Soc. Social Work Res. 9(4): 721–740. doi: 10.1086/701235.

Bybee-Finley, K.A., and M.R. Ryan. 2018. Advancing intercropping research and practices in industrialized agricultural landscapes. Agric. 8(6). doi: 10.3390/agriculture8060080.

Clay, D.E., S.A. Clay, Z. Liu, and S.S. Harper. 1995. Leaching of dissolved organic carbon in soil following anhydrous ammonia application. Biol. Fertil. Soils 19(1): 10–14. doi: 10.1007/BF00336339.

Colliver, G.W., and L.F. Welch. 1970. Toxicity of Preplant Anhydrous Ammonia to Germination and Early Growth of Corn: I. Field Studies 1 . Agron. J. 62(3): 341–346. doi: 10.2134/agronj1970.00021962006200030009x.

Congreves, K.A., O. Otchere, D. Ferland, S. Farzadfar, S. Williams, et al. 2021. Nitrogen Use Efficiency Definitions of Today and Tomorrow. Front. Plant Sci. 12(June): 1–10. doi: 10.3389/fpls.2021.637108.

Cuomo, G.J., D.G. Johnson, and W.A. Head Jr. 2001. Interseeding Kura Clover and Birdsfoot Trefoil into Existing Cool-Season Grass Pastures. 93(April): 176–180.

Darby, H., and J. Lauer. 2013. Critical Stages in the Life of a Corn Plant. Plant Physiol.: 15–20. doi: 10.2489/jswc.69.3.67A.

Dobbie, K.E., I.P. McTaggart, and K.A. Smith. 1999. Nitrous oxide emissions from intensive agricultural systems: Variations between crops and seasons, key driving variables, and mean emission factors. J. Geophys. Res. Atmos. 104(D21): 26891–26899. doi: 10.1029/1999JD900378.

Dobbratz, M., J.M. Baker, J. Grossman, M.S. Wells, and P. Ginakes. 2019. Rotary zone tillage improves corn establishment in a kura clover living mulch. Soil Tillage Res. 189(October 2018): 229–235. doi: 10.1016/j.still.2019.02.007.

Eleki, K., R.M. Cruse, and K.A. Albrecht. 2005. Root segregation of C3 and C4 species using carbon isotope composition. Crop Sci. 45(3): 879–882. doi: 10.2135/cropsci2004.0170.

Fernández, F.G., R.T. Venterea, and K.P. Fabrizzi. 2016. Corn Nitrogen Management Influences Nitrous Oxide Emissions in Drained and Undrained Soils. J. Environ. Qual. 45(6): 1847–1855. doi: 10.2134/jeq2016.06.0237.

Fox, J., and S. Weisberg. 2019. An R Companion to Applied Regression. Third. Sage, Thousand Oaks, CA.

Ghaffarzadeh, M., F. García Préchac, and R.M. Cruse. 1997. Tillage effect on soil water content and corn yield in a strip intercropping system. Agron. J. 89(6): 893–899. doi: 10.2134/agronj1997.00021962008900060008x.

Giles, M., N. Morley, E.M. Baggs, and T.J. Daniell. 2012. Soil nitrate reducing processes - Drivers, mechanisms for spatial variation, and significance for nitrous oxide

production. Front. Microbiol. 3(DEC): 1–16. doi: 10.3389/fmicb.2012.00407.
Graça, M.A.S., F. BäRlocher, and M.O. Gessner. 2005. Methods to study litter decomposition: A practical guide.
Groffman, P.M., P.F. Hendrix, and D.A. Crossley. 1987. Nitrogen Dynamics in Conventional and No-Tillage Agroecosystems with Inorganic Fertilizer or Legume Nitrogen Inputs. 332: 315–332.
H. Seran, T., and I. Brintha. 2010. Review on Maize Based Intercropping. J. Agron. 9(3): 135–145. doi: 10.3923/ja.2010.135.145.
Han, K.-H., W.-J. Choi, G.-H. Han, S.-I. Yun, S.-H. Yoo, et al. 2004. Urea-nitrogen transformation and compost-nitrogen mineralization in three different soils as affected by the interaction between both nitrogen inputs. : 193–199. doi: 10.1007/s00374-003-0704-4.
Hatfield, J.L., T.J. Sauer, and J.H. Prueger. 2001. Managing soils to achieve greater water use efficiency: A review. Agron. J. 93(2): 271–280. doi: 10.2134/agronj2001.932271x.
Hunter, A.S., and W.A. Rosenau. 1966. The Effects of Urea, Biuret, and Ammonia on Germination and Early Growth of Corn (Zea mays L.). Soil Sci. Soc. Am. J. 30(1): 77–81. doi: 10.2136/sssaj1966.03615995003000010028x.
Irmak, S., M.S. Kukal, A.T. Mohammed, and K. Djaman. 2019. Disk-till vs. no-till maize evapotranspiration, microclimate, grain yield, production functions and water productivity. Agric. Water Manag. 216(November 2018): 177–195. doi: 10.1016/j.agwat.2019.02.006.
Jia, X., M. Shao, X. Wei, and Y. Wang. 2013. Hillslope scale temporal stability of soil water storage in diverse soil layers. J. Hydrol. 498: 254–264. doi: 10.1016/j.jhydrol.2013.05.042.
Johnson, J.M.F., J.S. Strock, J.E. Tallaksen, and M. Reese. 2016. Corn stover harvest changes soil hydrology and soil aggregation. Soil Tillage Res. 161: 106–115. doi: 10.1016/j.still.2016.04.004.
Kovacs, G.E., V. Scoyoc, and T.J. Vyn. 2011. Corn Plant Uniformity Following Shallow NH3 Placement in Precision- Guided , Pre-Plant Nitrogen Applications. North Central Extension-Industry Soil Fertility Conference. p. 181–187
Kravchenko, A.N., E.R. Toosi, A.K. Guber, N.E. Ostrom, J. Yu, et al. 2017. Hotspots of soil N 2 O emission enhanced through water absorption by plant residue. Nat. Geosci. 10(7): 496–500. doi: 10.1038/ngeo2963.
Kutner, M.H., C.J. Nachtsheim, and J. Neter. 2004. Applied linear regression models. Fourth. McGraw-Hill, New York.
Laberge, G., F. Mabood, and P. Seguin. 2004. Kura Clover Early Growth Is Comparable to That of White Clover When Not Nitrogen Limited. 4167(June). doi: 10.1081/PLN-200049171.
Langhans, I., P. Goos, and M. Vandebroek. 2005. Identifying effects under a split-plot design structure. J. Chemom. 19(1): 5–15. doi: 10.1002/cem.900.
Lauer, J.G., P.R. Carter, T.M. Wood, G. Diezel, D.W. Wiersma, et al. 1999. Corn hybrid response to planting date in the northern corn belt. Agron. J. 91(5): 834–839. doi: 10.2134/agronj1999.915834x.
Lenth, R., H. Singmann, J. Love, P. Buerkner, and M. Herve. 2018. emmeans: Estimated

Marginal Means, aka Least-Squares Means. R Packag. Version 1.2.3. doi: 10.1080/00031305.1980.10483031>.License.
Li, L., J. Sun, F. Zhang, T. Guo, X. Bao, et al. 2006. Root distribution and interactions between intercropped species. Oecologia 147(2): 280–290. doi: 10.1007/s00442-005-0256-4.
Lim, J.Y., S.R. Cho, G.W. Kim, P.J. Kim, and S.T. Jeong. 2021. Uncertainty of methane emissions coming from the physical volume of plant biomass inside the closed chamber was negligible during cropping period. PLoS One 16(9 September). doi: 10.1371/JOURNAL.PONE.0256796.
Liu, S., X. Wang, X. Yin, H.J. Savoy, A. McClure, et al. 2019. Ammonia Volatilization Loss and Corn Nitrogen Nutrition and Productivity with Efficiency Enhanced UAN and Urea under No-tillage. Sci. Rep. 9(1): 1–12. doi: 10.1038/s41598-019-42912-5.
Lupwayi, N.Z., G.W. Clayton, J.T. O'Donovan, K.N. Harker, T.K. Turkington, et al. 2007. Nitrogen release during decomposition of crop residues under conventional and zero tillage. Soil Tillage Res. 95(1–2): 231–239. doi: 10.1016/j.still.2007.01.007.
Lynd, L.R., X. Liang, M.J. Biddy, A. Allee, H. Cai, et al. 2017. Cellulosic ethanol: status and innovation. Curr. Opin. Biotechnol. 45: 202–211. doi: 10.1016/j.copbio.2017.03.008.
Marquardt, D.W. 1980. You Should Standardize the Predictor Variables in Your Regression Models. J. Am. Stat. Assoc. 75(369): 74–81. doi: 10.1080/01621459.1980.10477428.
Mulvaney, R.L. 1996. Nitrogen – Inorganic Forms. In: Sparks, D.L., editor, Methods of Soil Analysis Part 3: Chemical Methods. 5th ed. American Society of Agronomy, Madison, WI, WI. p. 1123–1184
Mulvaney, M.J., C.W. Wood, K.S. Balkcom, D.A. Shannon, and J.M. Kemble. 2010. Carbon and nitrogen mineralization and persistence of organic residues under conservation and conventional tillage. Agron. J. 102(5): 1425–1433. doi: 10.2134/agronj2010.0129.
National Drought Mitigation Center, U.S. Department of Agriculture, and National Oceanic and Atmospheric Administration. 2021. U.S. Drought Monitor.
Nicoullaud, B., D. King, and F. Tardieu. 1994. Vertical distribution of maize roots in relation to permanent soil characteristics. Plant Soil 159(2): 245–254. doi: 10.1007/BF00009287.
Novick, K.A., J.A. Biederman, A.R. Desai, M.E. Litvak, D.J.P. Moore, et al. 2018. The AmeriFlux network: A coalition of the willing. Agric. For. Meteorol. 249(May 2017): 444–456. doi: 10.1016/j.agrformet.2017.10.009.
Nunes, M.R., M. De, M.D. McDaniel, J.L. Kovar, S. Birrell, et al. 2021. Science-based maize stover removal can be sustainable. Agron. J. 113(4): 3178–3192. doi: 10.1002/agj2.20724.
Ochsner, T.E., K.A. Albrecht, T.W. Schumacher, J.M. Baker, and R.J. Berkevich. 2010. Water balance and nitrate leaching under corn in kura clover living mulch. Agron. J. 102(4): 1169–1178. doi: 10.2134/agronj2009.0523.
Ochsner, T.E., T.W. Schumacher, R.T. Venterea, G.W. Feyereisen, and J.M. Baker. 2017. Soil Water Dynamics and Nitrate Leaching Under Corn–Soybean Rotation,

Continuous Corn, and Kura Clover. Vadose Zo. J. 0(0): 0. doi: 10.2136/vzj2017.01.0028.
Oldfield, B.E.E., A.J. Eagle, R.L. Rubin, J. Rudek, and D.R. Gordon. 2022. Crediting agricultural soil carbon sequestration. Science (80-.). 375(6586): 1222–1225.
Ortiz-Bobea, A., E. Knippenberg, and R.G. Chambers. 2018. Growing climatic sensitivity of U.S. Agriculture linked to technological change and regional specialization. Sci. Adv. 4(12). doi: 10.1126/sciadv.aat4343.
Parkin, T.B. 1987. Soil Microsites as a Source of Denitrification Variability. Soil Sci. Soc. Am. J. 51(5): 1194–1199. doi: 10.2136/sssaj1987.03615995005100050019x.
Parkin, T.B., and R.T. Venterea. 2010. Chamber-Based Trace Gas Flux Measurements. In: Follett, R.F., editor, Sampling Protocols. p. 3–39
Parkin, T.B., R.T. Venterea, and S.K. Hargreaves. 2012. Calculating the Detection Limits of Chamber-based Soil Greenhouse Gas Flux Measurements. J. Environ. Qual. 41(3): 705. doi: 10.2134/jeq2011.0394.
Piepho, H.P. 2018. Letters in mean comparisons: What they do and don't mean. Agron. J. 110(2): 431–434. doi: 10.2134/agronj2017.10.0580.
Polasky, A.D., J.L. Evans, J.D. Fuentes, and H.L. Hamilton. 2021. Statistical climate model downscaling for impact projections in the Midwest United States. Int. J. Climatol. (February): 1–18. doi: 10.1002/joc.7406.
Pratt, M.R., W.E. Tyner, D.J. Muth, and E.J. Kladivko. 2014. Synergies between cover crops and corn stover removal. Agric. Syst. 130: 67–76. doi: 10.1016/j.agsy.2014.06.008.
Rasmussen, R.A. 1992. The Global Sources of Nitrous Oxide. J. Geophys. Res. 97(D13): 651–660.
Roth, G., and D. Undersander. 1995. Harvesting and Storage. Corn silage production, management, and feeding. ASA, CSSA, and SSSA, Madison, WI. p. 12–31
Ruengvirayudh, P., and G. Brooks. 2016. Comparing Stepwise Regression Models to the Best-Subsets Models, or, the Art of Stepwise. Art Stepwise Regres. Gen. Linear Model J. 42(1): 1.
Sawyer, J.E., P. Pedersen, D.W. Barker, D.A. Ruiz Diaz, and K. Albrecht. 2010. Intercropping corn and kura clover: Response to nitrogen fertilization. Agron. J. 102(2): 568–574. doi: 10.2134/agronj2009.0392.
Schlüter, S., J. Zawallich, H.J. Vogel, and P. Dörsch. 2019. Physical constraints for respiration in microbial hotspots in soil and their importance for denitrification. Biogeosciences 16(18): 3665–3675. doi: 10.5194/bg-16-3665-2019.
Schmer, M.R., R.M. Brown, V.L. Jin, R.B. Mitchell, and D.D. Redfearn. 2017. Corn Residue Use by Livestock in the United States. Agric. Environ. Lett. 2(1): 0. doi: 10.2134/ael2016.10.0043.
Schulte, L.A., J. Niemi, M.J. Helmers, M. Liebman, J.G. Arbuckle, et al. 2017. Prairie strips improve biodiversity and the delivery of multiple ecosystem services from corn–soybean croplands. Proc. Natl. Acad. Sci. U. S. A. 114(42): 11247–11252. doi: 10.1073/pnas.1620229114.
Seguin, P., C.C. Sheaffer, N.J. Ehlke, and R.L. Becker. 1999. Kura clover establishment methods. J. Prod. Agric. 12(3): 483–487. doi: 10.2134/jpa1999.0483.
Seguin, P., C.C. Sheaffer, N.J. Ehlke, M.P. Russelle, and P.H. Graham. 2001. Nitrogen

fertilization and rhizobial inoculation effects on kura clover growth. Agron. J. 93(6): 1262–1268. doi: 10.2134/agronj2001.1262.
Siller, A.R.S., K.A. Albrecht, and W.E. Jokela. 2016. Soil erosion and nutrient runoff in corn silage production with Kura clover living mulch and winter rye. Agron. J. 108(3): 989–999. doi: 10.2134/agronj2015.0488.
Smiley, R.W. 1970. Anhydrous Ammonia as a Soil Fungicide Against Fusarium and Fungicidal Activity in the Ammonia Retention Zone. Phytopathology 60(8): 1227. doi: 10.1094/phyto-60-1227.
Smucker, A., S. McBurney, and A. Srivastava. 1982. Quantitative Separation of Roots from Compacted Soil Profiles by the Hydropneumatic Elutriation System. Agron. J. 74: 500–503.
Speer, G. S. and Allinson, D.W. 1985. Kura Clover (Trifolium ambiguum): Legume for Forage and Soil Conservation. Econ. Bot. 39(2): 165–176.
Speer, G.S., and D.W. Allinson. 1985. Kura Clover (Trifolium ambiguum): Legume for Forage and Soil Conservation. Econ. Bot. 39(2): 165–176.
Srivastava, P., and N. Singh. 2022. Effects of microbial inoculants on soil carbon stock, enzymatic activity, and above ground and belowground biomass in marginal lands of Northern India. L. Degrad. Dev. 33(2): 308–323. doi: 10.1002/ldr.4153.
Stevenson, F.J. 1996. Nitrogen - Organic Forms. In: Sparks, D.L., editor, Methods of Soil Analysis Part 3: Chemical Methods. 5th ed. American Society of Agronomy, Madison, WI. p. 1085–1089
Taylor, N.L., and R.R. Smith. 1997. Kura Clover (Trifolium ambiguum M.B.) Breeding, Culture, and Utilization. Adv. Agron. 63(C): 153–178. doi: 10.1016/S0065-2113(08)60243-6.
Team, R.C. 2020. R: A language and environment for statistical computing.
Team, R.C. 2021. R: A Language and Environment for Statistical Computing. https://www.r-project.org/.
Thomson, A.J., G. Giannopoulos, J. Pretty, E.M. Baggs, and D.J. Richardson. 2012. Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. Philos. Trans. R. Soc. B Biol. Sci. 367(1593): 1157–1168. doi: 10.1098/rstb.2011.0415.
Tremblay, G., P. Legendre, J.F. Doyon, R. Verdon, and R. Schetagne. 1998. The use of polynomial regression analysis with indicator variables for interpretation of mercury in fish data. Biogeochemistry 40(2–3): 189–201. doi: 10.1023/a:1005997430906.
Turner, P.A., J.M. Baker, T.J. Griffis, and R.T. Venterea. 2016a. Impact of Kura Clover Living Mulch on Nitrous Oxide Emissions in a Corn–Soybean System. J. Environ. Qual. 45(5): 1782. doi: 10.2134/jeq2016.01.0036.
Turner, P.A., T.J. Griffis, D.J. Mulla, J.M. Baker, and R.T. Venterea. 2016b. A geostatistical approach to identify and mitigate agricultural nitrous oxide emission hotspots. Sci. Total Environ. 572: 442–449. doi: 10.1016/j.scitotenv.2016.08.094.
Tyndall, J.C., L.A. Schulte, M. Liebman, and M. Helmers. 2013. Field-level financial assessment of contour prairie strips for enhancement of environmental quality. Environ. Manage. 52(3): 736–747. doi: 10.1007/s00267-013-0106-9.
USDA. 2018. National Agricultural Statistics Service.
Venterea, R.T., and J.A. Coulter. 2015. Split application of urea does not decrease and

may increase nitrous oxide emissions in rainfed corn. Agron. J. 107(1): 337–348. doi: 10.2134/agronj14.0411.
Venterea, R.T., J.A. Coulter, and M.S. Dolan. 2016. Evaluation of Intensive “4R” Strategies for Decreasing Nitrous Oxide Emissions and Nitrogen Surplus in Rainfed Corn. J. Environ. Qual. 45(4): 1186. doi: 10.2134/jeq2016.01.0024.
Venterea, R.T., A.D. Halvorson, N. Kitchen, M.A. Liebig, M.A. Cavigelli, et al. 2012. Challenges and opportunities for mitigating nitrous oxide emissions from fertilized cropping systems. Front. Ecol. Environ. 10(10): 562–570. doi: 10.1890/120062.
Zemenchik, R.A., K.A. Albrecht, C.M. Boerboom, and J.G. Lauer. 2000. Corn production with kura clover as a living mulch. Agron. J. 92(4): 698–705. doi: 10.2134/agronj2000.924698x.
Ziyomo, C., K.A. Albrecht, J.M. Baker, and R. Bernardo. 2013. Corn performance under managed drought stress and in a Kura clover living mulch intercropping system. Agron. J. 105(3): 579–586. doi: 10.2134/agronj2012.0427.