Nitrogen Dynamics and Management for Maize Production in Kura Clover Living Mulch

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DEDICATION

For the stewards of the land.

ABSTRACT

Kura clover living mulch (KCLM) systems have been investigated for incorporation into upper-Midwestern row-crop rotations to provide living groundcover during vulnerable spring and fall fallow periods. The extended growing season of the cool season legume crop takes advantage of sunlight energy that is not utilized for photosynthesis in monocrop systems; increasing carbon capture, supplies of root exudates to the soil microbiome, and tightening nutrient cycles through active root growth. These conceptual advantages, as well as observed improvements in water infiltration and reductions of soil erosion and nitrate leaching, may help to mitigate regionally important environmental impacts from agricultural production. Designing KCLM systems for upper-Midwestern row-crop production requires consideration of the current production needs and management strategies, and the full quantification of environmental benefits cannot be determined in the absence of robust nitrogen (N) management guidelines for maize production in KCLM systems. The objectives of this study were to (i) determine spring agronomic management strategies that improve N contributions from the KCLM system, and (ii) determine factors influencing N management guidelines for continuous maize grain and stover production in KCLM. These questions were addressed with two field experiments, both conducted at the Rosemount Research and Outreach Center in Rosemount, MN. To determine the effect of agronomic management techniques on in-season N contributions from the KCLM, soil and gaseous N pools were measured over 12 weeks in 2018 following treatment applications of clover residue removal or return and banded herbicide or rotary zone tillage. Clover residue removal did not influence N pool concentrations, while banded herbicide and rotary zone tillage enriched the soil with inorganic N relative to an unmanaged control, where rotary zone tillage was superior to banded herbicide. This experiment concludes that a producer may harvest clover prior to seeding row-crops without altering N management and rotary zone tillage increases in-season N contributions from the living mulch through greater disturbance and incorporation of above and below-ground N-rich clover biomass pools. To determine factors influencing N requirements for continuous maize production in KCLM, a two-year nitrogen rate trial was conducted in 2017 and 2018 on first-year maize and second-year maize after maize following forage management in a KCLM system. This study determined that first-year maize production after at least one year of forage management is self-sufficient in N, while N contributions for second-year maize production is reliant on the number of years in forage management prior to first-year maize seeding. While spring management of the KCLM enriches the soil with inorganic N, this contribution does not provide the total N requirements for high-yielding maize. Continuous maize production in KCLM depletes labile and biomass N pools that accumulate during forage management and subsequent years of crop production require fertilizer N at similar rates to conventional production systems.

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LIST OF ABBREVIATIONS

KCLM	Kura clover living mulch
N	Nitrogen
EF	Enrichment factor
T	Rotary strip-tillage
K	Banded herbicide application
Rm	Clover residue removed
Rt	Clover residue returned
TIN	Total inorganic N
Rw	Row management
Rs	Residue management
Z	Row/interrow zone
DAP	Days after planting
SOC	Soil organic carbon
SON	Soil organic nitrogen
EONR	Economic optimum nitrogen rate

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CHAPTER 1: KURA CLOVER LIVING MULCH: SPRING MANAGEMENT EFFECT ON NITROGEN

1.1. Synopsis

Kura clover living mulch (KCLM) systems have the potential to provide ecosystem services in intensively managed cropping systems while supplying soil mineral nitrogen (N) to the growing cash crop. Living mulch management relies on strong spring suppression to reduce competition between vigorous kura clover and emerging row crop seedlings, but standard suppression management practices utilize widely different modes of action. The objective of this research was to gain insight into the impact of common KCLM management practices on early season N dynamics. Kura clover was mowed, and residue was either harvested or returned before rows were established via strip tillage or banded herbicide. Soil and gaseous N pools were monitored for 12 weeks post initial application of suppression management treatments. An enrichment factor (EF) approach was utilized to compare N pools under managed treatments relative to an unmanaged clover control. Strip tillage increased soil N by 300%, while banded herbicide row establishment increased soil N by 220% relative to the unmanaged control. Pre-plant clover harvest reduced short term soil NO3–N, but during later time intervals there was no relationship between residue management and soil N. We conclude that, for the dual goals of maintaining clover perenniality while providing greater soil N enrichment, strip tillage is superior to band herbicide for row establishment. Additionally, pre-plant clover harvest may open opportunities for dual harvests in a single growing season, increasing economic return while maintaining in-season N contributions from the living mulch.

1.2. Introduction

Kura clover (*Trifolium ambiguum* M. bieb.) is a rhizatomous persistent perennial legume forage crop native to the mountainous regions of eastern Europe (Bieberstein, 1808). Its dense root and rhizome system allows for frequent defoliation and vegetative repropagation in harsh environments (Peterson et al., 1994a). This extreme persistence, along with its shade, drought, flood, and cold tolerance, low-growing habit, and nitrogen-rich biomass has prompted researchers to investigate its use as a living mulch in cropping systems of the Upper Midwestern U.S. (Speer and Allinson, 1985; Zemenchik et al., 2000).

Kura clover living mulch (KCLM) systems have been recognized for their soil and water conservation benefits, including reduced soil erosion (Siller et al., 2016), increased water infiltration (Baker, unpublished data), and reduced nitrate leaching (Ochsner et al., 2017) and residual N (Ochsner et al., 2010), compared to conventionally managed crop rotations. These environmental benefits, however, are often accompanied by reduced yields of the main cash crop (Zemenchik et al., 2000; Affeldt et al., 2004; Pedersen et al., 2009; Ochsner et al., 2010, 2017; Grabber et al., 2014; Siller et al., 2016). Competition between the living mulch and emerging row crop seedlings is a primary factor for delayed crop development and yield loss (Zemenchik et al., 2000; Sawyer et al., 2010; Grabber et al., 2014). This factor has led to the development of more aggressive suppression management practices during the critical spring establishment period (Zemenchik et al., 2000; Affeldt et al., 2004).

Vigorous spring clover regrowth stores large amounts of organic N in protein-rich biomass early in the growing season. When spring growing conditions allow, clover may be harvested as forage (Pedersen et al., 2009), but most often, residue is returned via suppression management, and low C/N clover tissues are readily mineralized upon senescence and incorporation (Affeldt et al., 2004; Turner et al., 2016). Clover suppression techniques often utilize combinations of pre-plant mowing (Affeldt et al., 2004; Pedersen et al., 2009), broadcast chemical suppression (Sawyer et al., 2010; Grabber et al., 2014; Siller et al., 2016; Ochsner et al., 2017), strip-tillage (Pearson et al., 2014; Turner et al., 2016), and/or chemical banding (Zemenchik et al., 2000; Singer, 2005; Ochsner et al., 2017) before and after the planting date. These management techniques vary in the amount of clover disturbance, the degree of incorporation, and the spatial organization of disturbed residues.

Methods of clover suppression and residue incorporation impact the resulting soil environment, which plays an important role in the spatiotemporal mineral N supply from biomass decomposition (Blackshaw et al., 2010; Liebman et al., 2018). Kura clover living mulch systems could be better utilized if management techniques were designed and chosen based on organic N cycling dynamics. Enhanced understanding of KCLM suppression management techniques may reveal agronomic benefits, such as a reduction in the fertilizer N requirement for the cash crop, while achieving soil and water conservation benefits.

This experiment branches from a two-year N management study for continuous corn in KCLM. Preliminary data from the first year of these experiments suggested increased soil N after herbicide suppression management, leading to our hypothesis that clover disturbance and suppression increases the reactive N supply in KCLM systems (Alexander et al., 2018). The objective of this study was to compare N availability and loss pathways from commonly used KCLM suppression management practices. Understanding nitrogen dynamics and clover recovery after spring clover suppression management could facilitate the design of more stable, resilient, and beneficial companion cropping systems for the Upper Midwestern corn belt.

1.3. Materials and Methods

1.3.1. Study Site and Experimental Design

A field study was conducted from 29 May through 22 August, 2018 to investigate spatiotemporal N dynamics in KCLM systems after spring agronomic management. Plots were located at the University of Minnesota Research and Outreach Center in Rosemount, MN (44.73° N, 93.09° W) on a Waukegan silt loam (fine-silty over sandy or sandy-skeletal, mixed, superactive, mesic Typic Hapludolls). Soils from an adjacent experiment were grid sampled to 0.3 m (n = 64) and contained an average of 20.5 g kg^{-1}

organic carbon and 5.7 pH in KCl (Alexander et al., 2018). Endura kura clover was seeded at 11 kg ha^{-1} in 2006 and used as a living mulch for row crop production from 2008–2009, rhizomes were dug up with a potato digger for vegetative repropagation in 2010 (Baker, 2012), row crop production commenced in 2011–2014, and three hay cuttings and one seed harvest were taken from 2015–2017. In 2015, P and K were applied according to soil test values.

Two main clover management factors were examined: (i) seed-row establishment, with the row prepared either mechanically by rotary strip-tillage (‘T’), or chemically using a banded herbicide or ‘kill’ (‘K’) application, and (ii) residue management, with mowed residue removed from (‘Rm’) or returned to (‘Rt’) the plots, resulting in four residue-row treatment combinations, i.e., T/Rm, T/Rt, K/Rm, and K/Rt (Table 1.1). An additional unmanaged control treatment, which was not mowed and had no row establishment, was also examined. Plots were arranged in a randomized complete block design with three replications of the five treatments and were 4.7 m (6 rows) wide by 7.6 m. In addition, the four plots within each block receiving row and residue management were split by ‘row’ and ‘interrow’ zones after row management treatments were applied (Figure A1). Clover was mowed to 50 mm on 29 May, and on 31 May cut clover residue was raked and removed. Rows were established on 4 June. Kill treatments received N-(phosphonomethyl) glycine (glyphosate) at 9.35 L a.e. ha^{-1} applied with a walk-behind sprayer unit in 0.3-m bands spaced every 0.76 m. Strip-till treatments were tilled using a rotary zone tillage tool (Northwest Tillers, Yakima, WA, USA) which created 0.3-m wide strips on 0.76-m intervals. An additional 2.3 L a.e. ha^{-1} of glyphosate was broadcast on managed plots (all treatments except the control) on 22 June to inhibit clover regrowth (Grabber et al., 2014; Siller et al., 2016; Ochsner et al., 2017).

Row-crops were not seeded into plots after row establishment so that the measured soil N pools could be isolated from soil N uptake by the main cash crop.

1.3.2. Soil N

Soils were collected bi-weekly from 17 May to 5 July and on 17 July, 6 August, and 22 August. Samples were taken at three depths (0–50, 50–150, and 150–300 mm) with a 20-mm i.d. coring device. Sample location was random in the unmanaged control and at the center of a randomly selected pair of row and interrow zones in managed plots. Samples were weighed and homogenized before 38 mL of 2 M KCl solution was added to a 10 g subsample of wet soil and shaken at 120 rpm for 1 h. Soil slurry was filtered through 11 µm filter paper and the extract was analyzed for NO_3–N and NH_4–N concentrations with the Greiss–Ilosvay with cadmium reduction and the sodium salicylate–nitroprusside methods, respectively, modified for flow-through injection analysis (Lachat, Loveland, CO, USA) (Mulvaney, 1996a). Cadmium reduces NO_3–N to NO_2–N, which is detected by the Greiss–Ilosvay method, therefore reported values for NO_3–N are the sum of NO_2–N and NO_3–N. A second subsample of 5 g wet soil was oven dried for at least 24 h and weighed to determine gravimetric water content. Core volume and adjusted dry sample mass were used to calculate bulk density which was used to convert soil N concentrations on a soil mass basis (e.g., mg N g^{-1}) to a per hectare basis (e.g., kg N ha^{-1}) by depth interval.

1.3.3. Soil-Atmosphere Gas Exchange

Nitrous oxide emissions were measured bi-weekly from 24 May–5 July using non-steady-state chambers (Venterea et al., 2016). Chamber bases measuring 0.7 m long × 0.36 m wide × 0.1 m deep were installed by trenching base dimensions with an electric chainsaw and pressing the acrylic base at least 50 mm into the ground. Bases were placed randomly within unmanaged control plots and randomly in managed treatments with the condition that the base spanned one row-width, containing equivalent ratios of row and interrow zones to field scale proportions. Bases in non-tilled treatments were installed 48 h before initial sampling to mitigate high gas flux from disturbance while bases in tilled treatments were installed immediately after tillage and hours before sampling to capture the effect of soil mixing on soil-to-atmosphere gas emissions, as done previously (Reicosky and Lindstrom, 1993; Reicosky and Archer, 2007). Chamber displacement by clover biomass was calculated using fresh clover moisture content determined via oven-drying and density obtained via water displacement so that fresh clover volume could be calculated from in-season dry biomass measurements. The chamber volume used in the flux calculations was adjusted by subtracting the wet clover volume from the total above ground chamber volume (Collier et al., 2016). Biomass that exceeded chamber top height was folded into each top upon chamber placement (Collier et al., 2016). Atmospheric N_2O–N concentration was sampled from the top of the clover canopy from each control plot with a 12 mL polypropylene syringe. These samples represented the initial (time 0) measurement for all chambers within the corresponding block. Insulated and vented chamber tops were then placed and secured onto bases with binder clips. Gas samples were collected from each chamber at 20, 40, and 60 min after chamber top placement and the 12 mL samples were immediately transferred to glass vials sealed with butyl rubber septa. Samples were handled and analyzed according to (Venterea et al., 2016) with a 5890A Gas Chromatography analyzer (Hewlett–Packard, Palo Alto, CA, USA) in conjunction with a 7000 Headspace Autosampler (Teledyne Tekmar, Mason, OH, USA). Gas fluxes were calculated using the restricted quadratic method, where quadratic regression is used unless the second derivative of the resulting quadratic regression function is greater than 0, in which case linear regression is used (Parkin et al., 2012).

Ammonia (NH_3–N) emissions were measured bi-weekly from 24 May to 5 July. Transparent chambers were assembled and modified based on a semi-open chamber design as described previously (Shigaki and Dell, 2015). Twenty milliliters of 0.5 M H_2SO_4, containing 2% *v*/*v* glycerol solution and a 25 × 3 × 200 mm polyurethane foam strip were placed in a 125 mL bottle so that the foam was saturated in the acid solution. The bottle was then placed at the base of each chamber and the foam was suspended vertically with the bottom of the strip placed in the excess acid solution. After 3–4 d, the foam strips were removed from the chambers and placed into their respective bottles for transport to the lab. Fresh strips and solution were then installed into each chamber. Acid glycerol solution was added to the used strip and excess solution to reach the initial 20 mL volume before dilution with 30 mL of H_2O. The total 50 mL solution was shaken for 0.5 h before filtration through 11 µm filter paper and the extract was analyzed for NH_4–N concentration using the sodium salicylate–nitroprusside method modified for flow-through injection analysis (Mulvaney, 1996a) (Lachat, Loveland, CO, USA).

1.3.4. Clover Sampling

Aboveground biomass was collected on 17 May, 31 May, 13 June, 21 June, 28 June, and 26 July. A 0.5 m^2 quadrat was placed in each plot, with the condition that it contained row and interrow zones in proportion to the field scale and was not previously sampled. Clover biomass was cut to 10 mm and collected from within each quadrat. Clover samples were dried at 60 °C for at least 3 days before dry mass was taken. Subsamples were pulverized and analyzed for total C and N concentrations using the Dumas dry combustion method with an elemental analyzer (Bremner and Mulvaney, 1982) (VarioMax, Elementar, Langenselbold, Germany).

1.3.5. Environmental Conditions

Soil moisture and temperature data were collected at the center of each zone in managed plots and randomly within the control. Sensors were installed vertically at the 76-mm depth and measurements were taken at 0.5-hr intervals. Single replicates from each zone in each treatment were monitored from 28 May–10 July. Daily minimum and maximum temperatures from each zone were used to calculate cumulative soil heat units with a minimum threshold value at 10 °C to account for limited microbial activity below this value (Nicolardot et al., 1994; Pietikäinen et al., 2005).

Daily precipitation, minimum air temperature, and maximum air temperature were obtained from the National Weather Service Cooperative Observer Station no. 217107 for the time period beginning on 20 April and ending on 22 August. Daily maximum and minimum air temperatures were averaged to obtain single daily average air temperature values and cumulative precipitation was calculated beginning on 20 April. Daily average air temperature and cumulative precipitation in 2018 were compared graphically to the 1981–2010 historical average.

1.3.6. Data Analysis

Soil NO_3–N, NH_4–N, and the sum of NO_3–N and NH_4–N (total inorganic N; TIN) concentrations in soil from sampled depth intervals were summed across depth intervals.

The summed concentrations were plotted against time and trapezoidally-integrated to represent cumulative soil N availability across the entire analysis period (Burton et al., 2008; Engel et al., 2010; Maharjan and Venterea, 2013; Venterea et al., 2015). Individual N_2O flux measurements were similarly time-integrated to determine cumulative emissions over the sampling period. Cumulative NH_3–N emissions were determined by the summation of individual flux measurements, since these values represented the total cumulative flux between sampling dates.

Values of time-integrated NO_3–N, NH_4–N, TIN, and N_2O–N and NH_3–N emissions were analyzed using an enrichment factor (EF), which was calculated based on Equation 1:

$$EF_C = [C_{treatment,\ b}/C_{control,\ b}]\ 100,$$

where C represents the measured variable and b represents the experimental block. The EF approach was based on calculations commonly used in ^{15}N isotope, contaminant, and mineral-ore analyses, where measured concentrations are compared to baseline or reference isotope, elemental, or mineral concentrations present in earth’s atmosphere, soils, or crust (Mariotti et al., 1981; Brimhall et al., 1988; Loska et al., 2005). For this study, the EF represents the magnitude of the difference between N pools under KCLM management treatments relative to baseline values under unmanaged clover. Categories were defined for enrichment factor values, where, <100% represents depletion, $100 \leq EF_C < 200\%$ represents slight enrichment, $200 \leq EF_C < 400\%$ represents moderate enrichment, and $EF_C \geq 400\%$ represents high enrichment of the N pool variable in the managed treatment relative to the unmanaged clover.

Statistical analysis utilized the MIXED procedure of SAS 9.4 at $p \leq 0.05$ (SAS Institute, Cary, NC, USA). Scatter plots of predicted and residual values were evaluated for homogeneity of variance and normality (Kutner et al., 2004) with the UNIVARIATE procedure of SAS; these requirements were met for all dependent variables. The data were organized into four groups that were subjected to separate statistical analyses, each during different time periods corresponding with management type, as follows:

Short-term soil N response to residue management: This analysis focused on the 6-d period between mowing (29 May) and application of row treatments (4 June), and aimed to evaluate the effects of the management ‘system’ (i.e., Rm, Rt, or control) on time-integrated NO_3–N, NH_4–N and TIN, and the effects of residue management (i.e., Rt or Rm) on the EFs corresponding to these soil N variables.

Zone-differentiated N response to residue and row management: This analysis examined the EF variables NO_3–N, NH_4–N, TIN, and NH_3–N following row establishment (4 June) through the end of the experiment (22 August), and their relationship with residue management, row establishment (i.e., K or T), and zone (i.e., row or interrow).

Zone-weighted N response to residue and row management: This analysis addressed the effect of residue management and row establishment on the EF variables NO_3–N, NH_4–N, TIN, NH_3–N, and N_2O–N over the entire sampling period of the corresponding N variable. For this analysis, N variables observed in the row and interrow zones following row establishment were weighted by relative zone area before time-integration and calculation of the EFs. This analysis also examined clover biomass and biomass-N variables sampled on 28 June as affected by residue management and row establishment, since the majority of clover regrowth was expected to occur prior to row crop canopy closure.

Cumulative N gas emissions: This analysis evaluated the effect of the management system (i.e., K/Rt, K/Rm, T/Rt, T/Rm, or control) on N_2O–N and NH_3–N emissions occurring over the entire study period (24 May–5 July). For this analysis, NH_3–N fluxes observed in the row and interrow zones in the managed plots were combined after weighting by relative zone area. This weighting procedure was not necessary for N_2O–N because measurements were made across both zones with a single chamber.

1.4. Results

1.4.1. Environmental Conditions

Environmental conditions during the study period were near the 1981–2010 climate average (Figure 1.1). The average daily temperature was 19.9 °C in 2018 and

17.6 °C from 1981–2010. Cumulative precipitation was 436 and 457 mm in 2018 and 1981–2010, respectively (Figure 1.1).

1.4.2. Short-Term Soil N Response to Residue Management

Nitrate and TIN were significantly affected by the management system (Table 1.2). Time-integrated soil NO_3–N concentration following mowing and residue management was 105% greater in the residue returned (Rt) system than the unmanaged control while the residue removed (Rm) system was not different from the Rt or control systems (Figure 1.2a). Soil TIN concentrations in the Rt and Rm treatments were 87 and 49% greater than the control, respectively (Figure 1.2b). The EFs for all three soil N variables did not differ between the Rt and Rm systems in the period following mowing and preceding row treatment application. Soil TIN concentrations over this sampling period can be found in the Appendix (Figure B1).

1.4.3. Zone Differentiated N Response to Residue Management and Row Establishment

The row establishment by zone interaction was significant for NO_3–N and TIN EFs following row establishment through the end of the study (Table 1.3). Nitrate and TIN in the row of the tilled (T) treatment were highly enriched compared to the unmanaged control, 675 and 479%, respectively, and were statistically greater than the row of the killed (K) treatment and the K and T interrow zones. The row in the K treatment was moderately enriched in NO_3–N (333%) and was statistically greater than the interrow in the K and T treatments. Nitrate concentrations in the interrow of the K and T treatments were moderately (202%) and slightly enriched (183%), respectively, and were not different from each other. Soil TIN enrichment in the K interrow (188%) was not different from the K row (247%) or the T interrow (160%), but enrichment in the K row was greater than the T interrow (Figure 1.3). Soil TIN concentration over this sampling period can be found in the Appendix (Figure B2).

Zone significantly affected NH_3–N emissions, with greater enrichment in the row (170%) than the interrow (120%), corresponding with cumulative NH_3–N emissions of 0.68 and 0.45 kg ha^{-1}, respectively.

1.4.4. Zone Weighted N Response to Residue and Row Management

Row establishment significantly affected EFs for NO_3–N and TIN following mowing through the end of the experiment (Table 1.4). The EF for NO_3–N was greater in the T treatment (304%) than the K treatment (220%), corresponding with greater enrichment of TIN in the T treatment (229%) than the K treatment (179%). Biomass and biomass-N were not affected by row or residue management. Soil TIN concentration and clover biomass over this sampling period can be found in the Appendix (Figure B3 and B4).

1.4.5. Cumulative N Gas Emissions

Emission of N_2O–N and NH_3–N over the total study period (24 May–5 July) was not affected by management system (Table 1.5).

1.5. Discussion

Soil N concentration responded to mowing and residue management in the 6 d period between mowing and row establishment (Figure B1). The control treatment had less soil NO_3–N than when residue was returned (Rt) and less soil TIN than when residue was returned or removed (Rm). In the context of utilizing kura clover as a living mulch to supply N for a cash crop, an absence of a residue management effect on the measured variables suggests that early surface residue removal does not influence soil N pools. Enrichment of soil NO_3–N and TIN after row establishment was high in the T row, moderate in the K row, and slight in both T and K interrows. Likewise, zone-weighted EFs for soil NO_3–N and TIN were significantly affected by row management, with greater enrichment in the T treatment than the K treatment.

Soil N not taken up by plants can be lost to environmentally damaging gaseous N pools; NH_3–N, NO_x–N, and N_2O–N (Follett and Hatfield, 2001; Cassman et al., 2002; Bertram et al., 2005). Volatilized NH_3–N was slightly enriched in both the row and interrow after row establishment and significantly greater in the row than the interrow. This result may suggest that elevated soil N in the row increased NH_3–N volatilization; however, NH_4–N was not significantly affected by zone, indicating that NH_3–N was volatilized directly from senescing clover residue. Bursts of NH_3–N emissions after chemical or mechanical senescence of legume crops have been reported previously (Dabney and Bouldin, 1985; Harper et al., 1995; Quemada and Cabrera, 1995). Despite enrichment in NH_3–N volatilization in clover under living mulch management, the magnitude of emission was low in comparison to soil mineral N pools and annual NH_3–N emission from local cropland (US EPA, 2017). This may be due to soil acidity (5.7 in the surface layer), since ammonia volatilization is a pH-dependent reaction in which NH_4–N is hydrolyzed when high concentrations of OH^- are present (Kirchmann and Witter, 1989).

In a previous study, N_2O–N emission during the spring management period in a corn-soybean (*Glycine max* L. (Merr.)) rotation was 98% greater in corn and 161% greater in soybean in a KCLM system compared to conventional management (Turner et al., 2016). Findings from the present study further highlight the potential for elevated N_2O–N emissions during living mulch management. Further research is needed to better understand the mechanisms and factors governing partial denitrification and N_2O–N emissions in KCLM systems, including the partitioning of organic and inorganic N sources of N_2O–N, and the feasibility of applying management practices to reduce N_2O emissions, for example, the use of nitrification inhibitors (Duan et al., 2017).

Greater enrichment of soil N with strip tillage row establishment suggests that soil N contribution from the living mulch is most influenced by biomass incorporation, where more intensive row establishment methods increase mineralizable biomass supply, soil-biomass contact, soil temperature, organic matter mineralization rate, nitrification rate, or a combination of these factors (Figure B5) (Licht and Al-kaisi, 2005; Bossche et al., 2009). Despite more intensive management in the tilled treatment, biomass and biomass-

N did not differ among the Rt, Rm, K, or T treatments by mid-summer, indicating that clover regrowth is similar among residue and row management treatments (Figure B4).

These results suggest that zone tillage is preferable to banded herbicide for KCLM cropping systems to supply in-season N to the cash crop. Zone tillage increases soil temperature, soil contact with senescing biomass, and soil N contributions to growing cash crops (Licht and Al-kaisi, 2005). Removal of cut kura clover residues did not reduce soil N pools, supporting a spring forage harvest before seeding the primary cash crop when conditions allow. Clover harvest prior to planting the cash crop would probably be most feasible as haylage rather than bales due to the risk of slow field drying prior to baling, along with greater harvest losses and the increased time commitment of haying during the spring planting season (Pattey et al., 1988; Buckmaster, 1990; Rotz et al., 1993), although, harvest method will be most influenced by the equipment and storage capabilities of the individual producer. The option to utilize kura clover as a forage crop may also offer flexibility within crop rotations when unfavorable field conditions, poor market outlooks, or market opportunities arise near the planting date. These results open new possibilities for optimization of KCLM management practices that might produce multiple harvests in a single growing season, maintain clover perenniality, reduce reliance on fertilizer N, increase system resilience, and improve soil health.

Concepts presented here improve the current understanding of legume-row crop intercropping systems, but further research is necessary to fully quantify N dynamics in KCLM production systems. These findings should be useful for optimization of spring agronomic management practices for KCLM production systems. Future research should address variation in system performance as influenced by kura clover stand age, cropping history, and extreme weather conditions for the development of stable and resilient KCLM cropping systems.

Table 1.1: Chronological clover suppression management by management system.

Treatment	**Mowed**	**Residue**	**Row Management**	**Chemical Suppression**
Date	29 May	31 May	4 June	22 June
Control	No	-	None	No
K/Rm	Yes	Removed	Band-kill	Yes
T/Rm			Zone-till	
K/Rt		Returned	Band-kill	
T/Rt			Zone-till	

Table 1.2. Treatment means and significance of soil N pools post residue management prior to row establishment.

	Time Integration			*** EFs**		
Factor	**NO_3–N**	**NH_4–N**	**TIN**	**NO_3–N**	**NH_4–N**	**TIN**
	----g N m^{-2} * d----			**--------EF, %--------**		
System						
‡ Control	4.6b†	3.1	7.7b			
§ Rt	9.5a	5.0	14.5a	222	161	194
|| Rm	7.3ab	4.3	11.5a	162	135	151
Significance				*p*-value		
	0.038	0.129	0.015	0.249	0.380	0.259

† Within a column means followed by the same letter are not significantly different at $p \leq 0.05$. * Enrichment factor of measured N variables. ‡ Unmanaged clover. § Residue returned after mowing. || Residue removed after mowing.

Table 1.3. Treatment means and significance of N pools following row management.

Fixed Effect	**NO_3–N**	**NH_4–N**	**TIN**	**NH_3–N**
		--------* EF, %--------		
Row management (Rw)				
‡ K	268b†	125	218b	146
§ T	429a	103	320a	144
Residue management (Rs)				
┃ Rt	357	98	270	151
|| Rm	340	130	268	138
Zone (Z)				
Interrow	193b	140	175b	120b
Row	504a	88	363a	170a
Significance		*p*-value		
Rw	0.001	0.535	0.005	0.881
Rs	0.578	0.377	0.954	0.369
Rw × Rs	0.144	0.787	0.218	0.140
Z	<0.001	0.161	<0.001	0.006
Rw × Z	<0.001	0.340	<0.001	0.476
Rs × Z	0.452	0.384	0.378	0.280
Rw × Rs × Z	0.938	0.649	0.749	0.475

† Within a column for a given fixed effect, means followed by the same letter are not significantly different at $p \leq 0.05$. * Enrichment factor of measured N variables. ‡ Banded herbicide row establishment. § Zone tillage row establishment. ┃ Residue returned after mowing. || Residue removed after mowing.

Table 1.4. Treatment means and significance of soil and gaseous N pools over the entire sampling period. Clover biomass and biomass-N are from the 28 June sampling.

Fixed Effect	N_2O–N	NH_3–N	NO_3–N	NH_4–N	TIN	Biomass	Biomass-N
			--------* EF, %--------			Mg ha^{-1}	kg N ha^{-1}
Row management (Rw)							
‡ K	423	144	220b†	116	179b	1.6	47.3
§ T	376	151	304a	110	229a	1.9	45.3
Residue management (Rs)							
│ Rt	349	156	268	105	205	1.9	47.7
‖ Rm	450	139	256	121	203	1.6	45.0
Significance				*p*-value			
Rw	0.594	0.507	0.002	0.765	0.003	0.339	0.789
Rs	0.272	0.149	0.495	0.486	0.850	0.305	0.724
Rw × Rs	0.810	0.102	0.010	0.690	0.082	0.852	0.887

† Within a column for a given fixed effect, means followed by the same letter are not significantly different at $p \leq 0.05$. * Enrichment factor of measured N variables. ‡ Banded herbicide row establishment. § Zone tillage row establishment. │ Residue returned after mowing. ‖ Residue removed after mowing.

Table 1.5. Treatment means and significance of N emission over the entire sampling period.

Fixed effect	**N_2O–N**	**NH_3–N**
	kg ha^{-1}	
System		
† Control	0.65	0.64
‡ K/ ┃Rt	2.12	0.81
K/ || Rm	2.59	0.86
§ T/Rt	1.76	0.97
T/Rm	2.09	0.75
Significance	*p*-value	
System	0.060	0.274

† Unmanaged clover. ‡ Banded herbicide row establishment. § Zone tillage row establishment. ┃ Residue returned after mowing. || Residue removed after mowing.

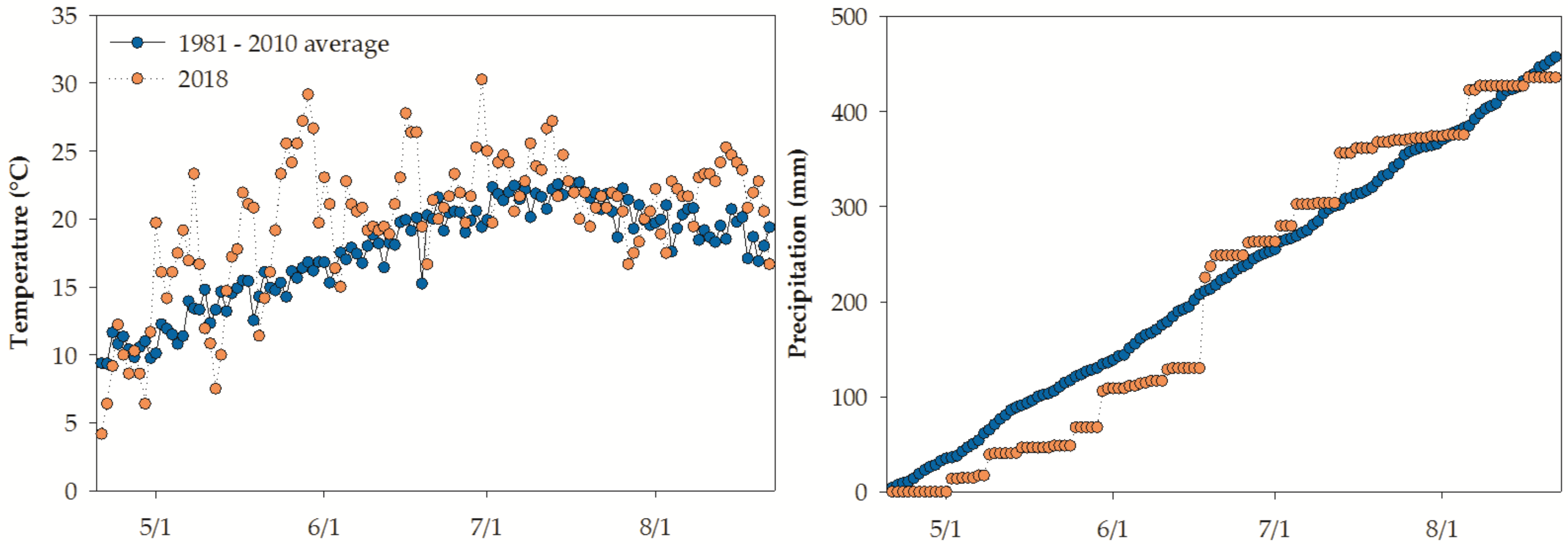

Figure 1.1. Daily average air temperature and cumulative precipitation from 20 April–22 August in 2018 and the 1981–2010 average.

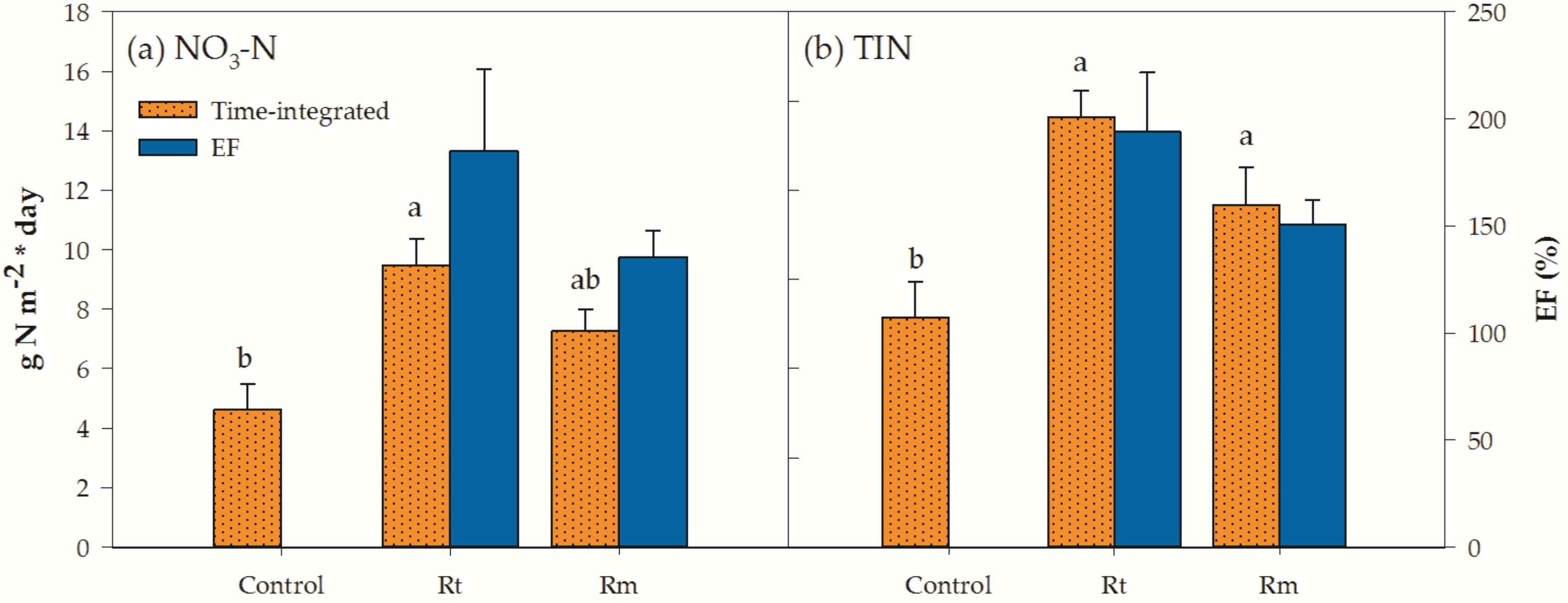

Figure 1.2. Time-integration (left axis) and enrichment factor (EF) (right axis) means and standard error for soil nitrogen variables by management system. (**a**) Soil NO_3–N; (**b**) Soil TIN. Columns with the same letter are not significantly different at $p \leq 0.05$.

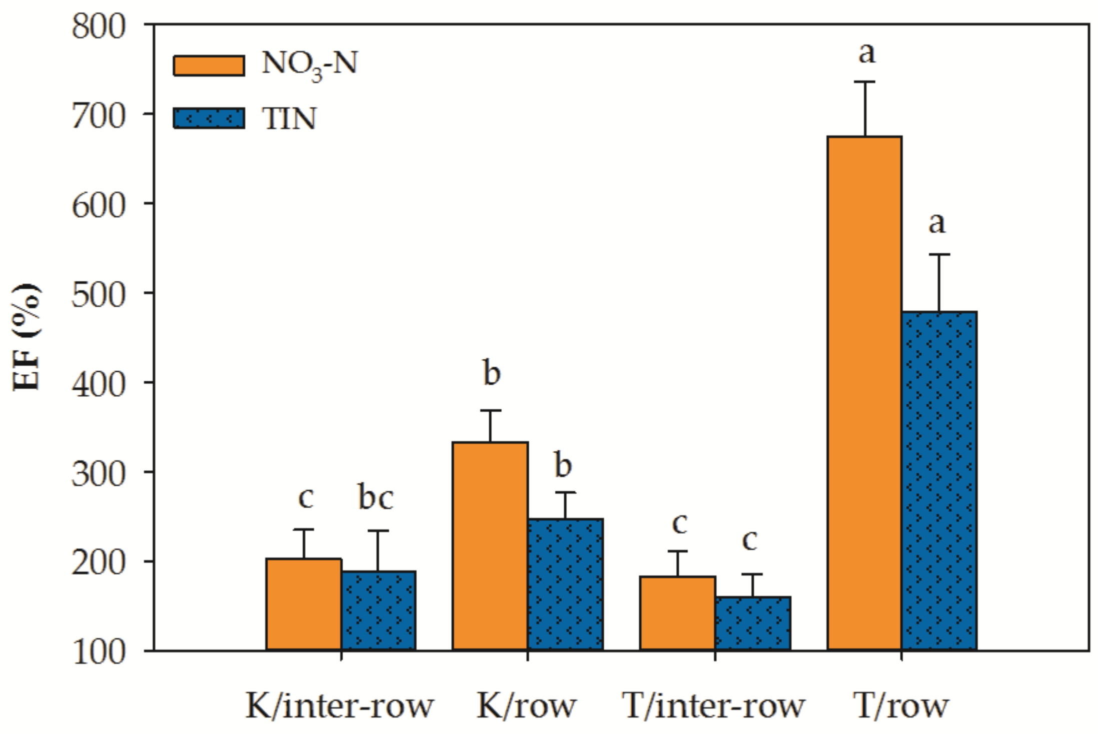

Figure 1.3. Enrichment factor (EF) and standard error for soil NO_3–N and soil inorganic N (TIN) following row establishment as a function of band herbicide (K) or zone till (T) row establishment and zone (row or interrow). Columns with the same letter are not significantly different at $p \leq 0.05$.

CHAPTER 2: KURA CLOVER LIVING MULCH REDUCES FERTILIZER N REQUIREMENTS AND INCREASES PROFITABILITY OF MAIZE

2.1. Synopsis

Kura clover living mulch (KCLM) systems have been investigated for their incorporation into upper Midwestern row crop rotations to provide ecosystem services through continuous living cover. Reductions in soil erosion and nitrate loss to surface and groundwater have been reported, but factors affecting agronomic performance and nutrient management are not well defined. To achieve realized environmental benefits, research must develop agronomic management techniques, determine economic opportunities, and provide management recommendations for row crop production in KCLM systems. Two experiments were conducted in 2017 and 2018 to determine the response to N fertilizer application for maize production in KCLM. The first-year maize experiment was maize following two or three years of forage management, and the second-year maize experiment followed maize after one or two years of forage management. Eight fertilizer N treatments ranging from 0–250 kg N ha^{-1} were applied to each experiment and grain and stover yields were compared to conventionally managed maize hybrid trials that were conducted nearby. First-year maize did not need fertilizer N to maximize yield and profitability in either year, and second-year maize required a fertilizer N rate near local University guidelines for maize following soybean. The net economic return from maize grain and stover in the KCLM averaged over first- and second-year maize experiments and 2017 and 2018 growing seasons was \$138 ha^{-1} greater than the conventional comparison.

2.2. Introduction

Kura clover (*Trifolium ambiguum* M. bieb), a rhizatomous perennial legume forage, is well suited for incorporation into upper Midwestern row-cropping systems as a perennial cover crop or living mulch (Zemenchik et al., 2000). Kura clover’s dense rhizome system holds large stores of metabolite energy that allow for perennial persistence and rapid reestablishment after intensive agronomic management (Affeldt et al., 2004). In the U.S. Midwest., maize (*Zea mays*) and soybean (*Glycine max*) has been successfully grown in a kura clover living mulch (KCLM), and clover forage productivity recovered in the following year (Zemenchik et al., 2000; Pedersen et al., 2009; Grabber et al., 2014). Living cover and active root uptake during the fall and spring months reduce soil erosion and nitrate leaching from maize production by up to three-quarters in the KCLM system compared to conventional management (Ochsner et al., 2010; Siller et al., 2016).

Maize production in KCLM requires stover harvest to prevent smothering of kura clover by crop residues (Zemenchik et al., 2000). Maize silage and stover is an important forage and bedding material in livestock operations and was harvested from 7% of maize acres in the central and eastern U.S. in 2010 (Schmer et al., 2017; USDA, 2018). While harvest of maize residue in conventional production systems can increase soil erosion and negatively affects soil carbon, structure, and fertility (Wilhelm et al., 2007; Johnson et al., 2016), soil physical and chemical properties were unaffected by 5–7 years of continuous maize stover removal under KCLM management in the upper Midwest (Baker, 2019). Improved protection from soil erosion and increased carbon input to soils from living cover maintains soil quality and reduces environmental and economic costs of stover harvest by increasing the sustainable stover removal rate (Pratt et al., 2014). Higher stover removal rates will reduce the land area impacted by stover harvest, thus reducing harvest cost and increasing the sustainability of stover removal in upper Midwestern livestock production systems.

Disparities in the literature exist regarding the agronomic productivity between KCLM and conventional maize systems, where grain and forage yields are either reduced (Pedersen et al., 2009; Ochsner et al., 2010, 2017; Grabber et al., 2014; Siller et al., 2016) or maintained (Zemenchik et al., 2000; Affeldt et al., 2004; Sawyer et al., 2010; Pearson et al., 2014). Previous research comparing maize grain yield in KCLM and conventional management systems are sometimes confounded by nitrogen (N) management, where living mulch treatments were granted legume N credits of 67–146 kg N ha^{-1} (Ochsner et al., 2010, 2017; Siller et al., 2016). Limited understanding of biological N fixation and cycling in KCLM systems has left researchers with little baseline information on N management guidelines, limiting the quantification and understanding of agronomic, environmental, and economic attributes of KCLM systems. Defining more robust N management recommendations for KCLM-row cropping systems requires identification of environmental and agronomic factors that affect in-season N contributions and availability. Recent work to isolate factors affecting in-season N contributions from KCLM identified rotary zone tillage as an important factor in promoting N mineralization from disturbed and incorporated clover residues (Alexander et al., 2019). This aligns with previous studies that identified rotary zone tillage as a promising strategy to reduce living mulch competition with the emerging row crop (Pearson et al., 2014; Dobbratz et al., 2019; Ricks, 2019).

It is necessary to develop N management guidelines based on crop rotations and scenarios that may be utilized by growers to quantify the economic and environmental potentials of KCLM-maize management systems. The potential reduction of fertilizer N requirements for maize through in-season N contributions from KCLM may reduce management costs and improve economic competitiveness with other cropping systems. The objectives of this research are to determine the effect of N fertilizer management on the productivity of maize and kura clover in a KLCM-maize system and assess the economic performance of this system. Greater understanding of N cycling in KCLM systems will facilitate innovation and adoption of perennial-annual intercropping systems that reduce environmental impacts of agriculture while providing economic benefits to crop and livestock growers.

2.3. Materials and Methods

2.3.1. Site Description

Field experiments were conducted in 2017 and 2018 at the University of Minnesota Research and Outreach Center in Rosemont, MN (44.73°N, 93.09°W) on a Waukegan silt loam (Fine-silty over sandy or sandy-skeletal, mixed, superactive, mesic

Typic Hapludolls). Soils at the site contained 20.5 g kg^{-1} organic carbon and were 5.7 pH in the 0–30 cm layer. *Endura* kura clover was established at the site in 2006–2007 and used as a living mulch for maize and soybean from 2008–2009. Rhizomes were dug from the experimental site in 2010 using a potato (*Solanum tuberosum*) harvester for a vegetative repropagation study (Baker, 2012) and kura clover recovered before resuming as a living mulch for maize in 2011 and 2013 and soybean in 2012 and 2014. Phosphorous (P) and potassium (K) fertilizers were applied according to soil test values and University of Minnesota guidelines in 2015 (Kaiser et al., 2011) and clover was harvested for hay once each year in 2015 and 2016.

Daily precipitation and minimum and maximum air temperature from 1 April to 31 October were obtained from National Weather Service Cooperative Observer Station no. 217107. Daily weather observations were averaged over 1981–2010 to represent the climatic normal. Daily minimum and maximum temperature were used to calculate cumulative growing degree days with a base and upper limit of 10 and 30°C, respectively, beginning on 12 May for the climatic average and the planting date for the years of these experiments.

2.3.2. Experimental Design

Two studies, each conducted over two years, investigated fertilizer N management for maize in KCLM. The first-year maize experiment was maize seeded following two and three years of forage management in 2017 and 2018, respectively, and the second-year maize experiment followed maize production after one or two years of forage management in 2017 and 2018, respectively. First-year maize production in preparation for the second-year maize experiment received 150 kg N ha^{-1} as liquid urea-ammonium nitrate banded 10 cm from the center of the row at the six-leaf collar stage of maize phenological development. The second year of each study was placed adjacent to the first; therefore, treatments were not applied to the same plots in both years. The studies investigated fertilizer N rate, where an unfertilized control was compared to plots that received a split application of urea containing urease and nitrification inhibitors (SuperU, Koch Agronomic Services, Wichita, KS, USA) at 40, 80, 120, 180, or 250 kg N

ha^{-1}. SuperU was surface-banded at 40 kg N ha^{-1}onto the center of the tilled row and incorporated with a second pass of the rotary zone tillage tool prior to planting. The remainder of treatment fertilizer N was surface banded 5 cm from the center of the row at the four-leaf collar stage of maize phenological development. Two additional fertilizer N treatments applied urea instead of SuperU, either in a single application of 120 kg N ha^{-1} at planting or as a split application of 40 and 80 kg N ha^{-1} at planting and the four-leaf collar stage of maize phenological development, respectively. Plots were 4.7 m (6 rows) wide by 15.2 m long and the first- and second-year maize experiments were arranged as two 4x2 randomized complete blocks with four replications of the eight fertilizer N treatments.

2.3.3. Agronomic Management

Prior to spring clover management, triple superphosphate, potash, and gypsum fertilizers were broadcast over the entire experimental area in both years based on soil test values and university guidelines (Kaiser et al., 2011). Clover was cut with a flail mower to 5 cm prior to row establishment in 2017 and in the first-year maize experiment in 2018. Rows were established with a rotary zone tillage tool (Northwest Tillers, Yakima WA) that tilled 30-cm-wide rows every 76 cm on 11 May 2017 and 22 May 2018 (Alexander et al., 2019; Dobbratz et al., 2019). Fertilizer N applications at planting were applied with a garden seeder (Earthway, Bristol IN) in the center of the tilled strip before incorporation with a second pass of the rotary zone tillage tool. Maize (Pioneer P0157AMX, Pioneer Dupont, Johnston IA) was planted in 76-cm rows in the center of the tilled strips at 86,000 seeds ha^{-1} with a John Deere 7000 planter (John Deere, Moline IA) on 12 May 2017 and 22 May 2018. An additional clover mowing 15 days after planting (DAP) was necessary in 2017 to reduce clover encroachment into maize rows, but mowing was not needed in the 2018 experiments. Herbicide suppression was used in both years to reduce clover encroachment into maize rows and was achieved with a broadcast application of 2.3 L a.e. ha^{-1} (N-(phosphonomethyl) glycine) (glyphosate) 39 and 31 DAP in 2017 and 2018, respectively (Ochsner et al., 2010; Siller et al., 2016; Alexander et al., 2019).

2.3.4. Crop Sampling and Analysis

Clover biomass was sampled at 160 and 133 DAP maize in 2017 and 2018 by placing a 0.5-m^2 quadrat between the center two maize rows and cutting clover biomass to a 1 cm height (Alexander et al., 2019). Maize was harvested at physiological maturity by hand harvesting ears and cutting stalks to a 15 cm height from 4.6 m in two rows in each plot. Maize stover was weighed in the field before a six-stalk subsample was ground with a biomass chipper, collected, and weighed. Maize ears, stalks, and clover were dried at 60°C for 3 d until reaching a constant mass. Dry stover subsamples were weighed to determine field moisture content to correspond with in-field measurements. Maize ears were shelled before dry grain and cobs were weighed. Subsamples of grain, cob, stover, and kura clover were ground to <0.1 mm and analyzed for organic carbon and N with the Dumas dry combustion method in an elemental analyzer (Elementar, Langenselbold DE) (Bremner and Mulvaney, 1982). The N content of crop components were combined with corresponding dry biomass measurements to determine maize N uptake. Stover and cob yield and N content were summed and are herein referred to as stover yield and N content, respectively.

2.3.5. Residual Soil Nitrogen

Post-harvest soil samples were collected with a 41-mm i.d. hydraulic coring device. Soils were collected from the 0–30 and 30–60 cm soil layers in 2017 and 2018. Samples were homogenized and weighed before a 10-g subsample of wet soil was added to 38 mL of 2M KCl, shaken at 120 rpm for 1 h, and filtered through 11-µm filter paper. The filtered extractant was analyzed for NO_3-N (sum of NO_2-N and NO_3-N) and NH_4-N with the Greiss-Ilosvay with cadmium reduction and the sodium salicylate-nitroprusside methods, respectively, each modified for flow-through injection analysis (Mulvaney, 1996b) (Lachat, Loveland CO). A 5-g subsample of wet soil was dried at 105°C until constant mass to determine gravimetric water content. Core volume and mass were used to determine soil bulk density, and soil N content was the product of soil N concentration and soil bulk density.

2.3.6. Economic Calculations

Partial net economic return was determined with a partial budget analysis of this study's KCLM experiments and conventionally managed maize hybrid trials, where maize hybrids followed soybean and were similar relative maturity as the hybrid used in the KCLM experiments. Hybrid trials were conducted at Rosemount, MN in 2017 and 2018 (Hoverstad et al., 2017, 2018), and trial yields were compared to corresponding KCLM experiment years. Agronomic management costs that differed between conventional and KCLM cropping systems included fertilizer N rate, spring tillage, spring mowing, fall tillage, and baling operations. Baling operations included raking, round baling, bale transportation, bale storage (Edwards, 2014; Plastina, 2018), and nutrient replacement costs associated with stover removal (1.65 kg P Mg^{-1} and 6.65 kg K Mg^{-1} dry stover) (Sawyer and Mallarino, 2014). Management for conventional maize production was assumed as spring field cultivation and fall disk-chiseling, while management for the KCLM system was based on agronomic practices performed on experimental treatments with spring and in-season mowing, rotary zone tillage, and baling operations with the associated nutrient replacement cost (Sawyer and Mallarino, 2014; Plastina, 2018; Dobbratz et al., 2019). All other costs, including land, P and K fertilization excluding stover nutrient removal replacement, seed, planting, pesticide and application of pesticide, harvest costs, and miscellaneous costs were assumed equal across treatments and conventional comparisons (Plastina, 2018). Net return from grain in the variety trials and the KCLM experiments were the product of grain yield at the economic optimum fertilizer N rate (EONR), which was determined from grain yield estimates of the fitted quadratic regression equations where N cost was \$0.86 kg^{-1} and the grain valued at \$133 Mg^{-1} at 155 g kg^{-1} moisture. When there was no grain yield response to fertilizer N, the EONR was set at 0 kg N ha^{-1}. Net return from stover in KCLM experiments was the product of stover yield at the EONR and stover value (\$79.37 Mg^{-1}) at 200 g kg^{-1} moisture (Battaglia et al., 2018). A comparison of system performance was based on parameter estimates of the fitted linear or quadratic regression of the response variables at the EONR when grain yield was affected by fertilizer N and the average response of the unfertilized treatment when grain yield was not affected by fertilizer N.

2.3.7. Data Analysis

Data were analyzed separately for first- and second-year maize experiments. Effects of fertilizer N treatments and year were evaluated using analysis of variance at $P < 0.05$ with the lme4 package of R (Bates et al., 2014). Year and N fertilizer treatment were considered fixed effects and block was considered a random effect. When the main effect of year was significant, means were compared at $P < 0.05$ using pairwise comparisons with the emmeans package of R (Lenth et al., 2018). When the main effect of fertilizer N treatment or the year-by-fertilizer N treatment interaction was significant, means of the three treatments receiving a total of 120 kg N ha^{-1} were compared at $P < 0.05$ using pairwise comparisons with the emmeans package of R. Regression of the parameter response to fertilizer N rate was conducted using the unfertilized treatment and the split SuperU N rate treatments with the lme4 package of R, where quadratic and linear regression functions were evaluated using analysis of variance and the quadratic function was used when $P < 0.05$.

2.4. Results

The 30-year (1981–2010) cumulative precipitation between 1 April and 31 October was 689 mm, and actual precipitation was 798 and 772 mm in 2017 and 2018, respectively (Figure 2.1 a). Accumulated growing degree days were 1141, 1362, and 1435 for the climatic normal, 2017, and 2018, respectively (Figure 2.1 b).

2.4.1. First-year Maize

First-year maize grain yield was not affected by year or fertilizer N treatment (Table 2.1). Grain N yield was 9% greater in 2018 than in 2017. Stover and stover N yield were 25 and 34% greater in 2018 than in 2017, respectively, and late-season clover yield was reduced by 77% in 2018 compared to 2017.

Post-harvest soil NO_3-N and total inorganic N (NO_3-N + NH_4-N, TIN) in the 0–0.6 m layer were affected by the interaction between year and treatment (Table 2.1) (Figure 2.2a, b). Significant differences among 120 kg N ha^{-1} treatments differing in N source and/or timing were found for NO_3-N and TIN. Residual NO_3-N and TIN did not differ between treatments in 2018 but were greater in the 120 kg N ha^{-1} SuperU and pre-plant urea treatments relative to the split-urea treatment in 2017.

2.4.2. Second-year Maize

There was a significant year-by-treatment interaction effect for maize grain, grain N, and stover yields (Table 2.2) (Figure 2.3a, b, 2.4), but N rate and timing treatments were not significantly different within a year. Late-season clover biomass responded negatively to fertilizer N application in 2017 and biomass was reduced in 2018 relative to 2017. Clover biomass was not significantly different between fertilizer N rate treatments in 2018 (Figure 2.5).

Post-harvest soil NO_3-N and TIN were significantly affected by year and treatment, with greater soil N in 2018 than 2017 and increased residual N content with fertilizer N application (Figure 2.6a, b). Nitrate-N and TIN did not differ between source and timing treatments within a year.

2.4.3. Economic Performance

Fertilizer N was needed to maximize economic return from grain production in second-year maize (Table 2.2). The EONR was 0 kg N ha^{-1} for first-year maize in both years, and 177 and 146 kg N ha^{-1} for second-year maize in 2017 and 2018, respectively. At the EONR, crop and soil N variables were consistent, with approximately 13 Mg maize grain ha^{-1} and <20 kg ha^{-1} of post-harvest TIN in the 0–0.6 m layer (Table 2.3).

Partial management cost, the sum of agronomic management costs that corresponded with treatment management and yield at the EONR for the KCLM experiments and the conventional comparison, were greater in the KCLM system than conventionally managed maize (Table 2.4). High spring tillage and stover harvest, handling, and nutrient replacement costs (1.65 kg P Mg^{-1} + 7.65 kg K Mg^{-1}, \$6 Mg^{-1} dry stover) (Sawyer and Mallarino, 2014) increased the partial management cost in the KCLM system by \$101–303 ha^{-1} relative to conventional management. Nitrogen costs were reduced by \$132 ha^{-1} in first-year maize, while the cost of fertilizer N for second-year maize was similar to the conventional comparison.

Net return from maize grain was reduced in the KCLM system compared to conventional management due to reduced yield and increased partial management cost. Net return was greater in 2018 than 2017 due to greater stover yields in first- and second-year maize. Additional revenue from stover harvest increased partial economic net return of the KCLM system averaged between first- and second- year maize to \$-42 ha^{-1} and \$318 ha^{-1} in 2017 and 2018, respectively (Table 2.5).

2.5. Discussion

Results from this study demonstrate that kura clover living mulch may be integrated into current cropping systems to provide economic and environmental benefits. Net economic return at the EONR in the KCLM system was similar or superior to the conventional comparison for first- and second-year maize in both years. Maize grain yield was reduced and management costs were increased in the KCLM system relative to the conventional comparison, but the added value from maize stover maintained or increased economic net return. With KCLM, residual soil TIN was <20 kg N ha^{-1} in the 0–0.6 m soil layer at the EONR in first- and second-year maize in both years. Additionally, KCLM has been shown to protect soils from erosion, decreasing the risk of degradation to soil and water resources during vulnerable fall and spring months when soils under conventional management are fallow (Helmers et al., 2005; Qi et al., 2011).

Maintaining clover vigor in a KCLM system is challenging since spring clover regrowth is affected by variable weather conditions, maize production requires intensive clover suppression management, and shade from maize limits mid- and late-season clover growth, (Grabber et al., 2014). Snowfall (25 cm) at the study site on 16 April 2018 delayed spring clover growth and development relative to 2017 and eliminated the need for mowing prior to row establishment in second-year maize. Following row establishment and maize planting, rapid growing degree day accumulation and maize development shaded the clover canopy before a second mowing was needed to manage competition between clover and maize. Less aggressive in-season suppression management in 2018 reduced mechanical clover disturbance, but late-season clover biomass was reduced by three-quarters relative to 2017 due to heavy shading. Management to mitigate reductions in clover biomass and vigor due to shading is limited, but may be important to reduce clover recovery time in the following spring (Zemenchik et al., 2000; Grabber et al., 2014).

Agronomic management preceding row crop production in KCLM influences N contributions during the maize year. Second-year maize grain yield was affected by the interaction between year and treatment, where grain yield for non-N-fertilized maize in 2018 was 4.8 Mg ha^{-1} greater and increased more gradually with applied N than in 2017. These differences may be attributed to differences in weather during the growing season or grain yield level. However, cumulative precipitation and growing degree day accumulation was similar in both experiment years, and first-year maize did not respond to treatment, year, or the interaction of these factors. Thus, it is most likely that the number of years in forage management prior to treatment application was the main factor affecting maize yield response to fertilizer N in second-year maize experiment, confirming previous findings for maize following alfalfa (*Medicago sativa*) (Yost et al., 2014a, 2015).

Forage legumes increase the soil labile N pool relative to fertilized maize systems (Carpenter-Boggs et al., 2000; Singh et al., 2018). Forage stands ≥3yr old at termination often accumulate enough labile organic N to eliminate the need for fertilizer N in first-year maize (Yost et al., 2015) and in many cases second-year maize (Yost et al., 2014a; b). While labile N accumulates under forage legume production, intensive grazing or harvest of sole kura clover reduces root and shoot biomass productivity over time (Peterson et al., 1994b; a). The intensity of mechanical and chemical suppression of KCLM in the spring disturbs root and shoot tissues (Dobbratz et al., 2019), while maize reduces late-season clover biomass due to competition for light and plant resources (Baributsa et al., 2008). The translocation of metabolites from root biomass during the spring flush of clover growth and limited opportunity for biomass recovery in living mulch management is likely to exacerbate the decline of root biomass, spring vigor, and clover health. Rapid root accumulation after the establishment year has been observed in other forage legumes, where root biomass doubled between the first and second year of establishment (Bolinder et al., 2011). The additional year of forage management preceding treatment application in 2018 relative to 2017 may have allowed for greater recovery of root biomass and accumulation of labile N. The magnitude of these accumulated N pools was large enough to reduce fertilizer N requirements for two years of maize production when the clover was managed as forage for ≥2 yr.

The relationships between clover forage production, maize production, and N contributions from the KCLM-maize cropping system adds complexity to the current understanding of N management in these systems. Early studies found that KCLM systems supply most or all of the N requirements for maize (Zemenchik et al., 2000), suggesting that N contributions from KCLM are supplied in the same year as biological N fixation. Although the response to fertilizer N in second-year maize may have been partially influenced by fall and spring growing conditions and maize development, both of which influence clover growth, early-season clover growth and vigor are closely linked to clover root biomass (Peterson et al., 1994b). The number of years in forage preceding first-year maize is likely an important factor for re-accumulation of root biomass that is translocated to shoots in early spring, linking clover root biomass, spring vigor, and mineralized clover biomass N following suppression management. This study suggests that the N contribution from the living mulch is supplied in-season following row establishment and suppression management, and that mineralized N is sourced from labile and biomass N pools accumulated during forage management. First-year maize following at ≥2yr of forage management does not need fertilizer N and second-year maize requires fertilizer N near University guidelines for maize following soybean. Additional research is needed to confirm these relationships with a greater number of rotation management variables, and to quantify the effect of KCLM management on root biomass pools. Optimization of crop rotation in KCLM systems may balance health of clover and the row crop to realize sustainable N contributions over a greater number of years.

2.6. Conclusions

Maize grown in KCLM was economically similar or superior to that produced conventionally due to additional income from maize stover harvest. Kura clover living mulch reduced the fertilizer N requirement for first-year maize in both years and second-year maize in 2018 relative to the conventional comparison. These benefits promote the use of KCLM systems for continuous maize production; however, further optimization is needed to reduce adoption barriers of KCLM-maize systems. Barriers to adoption of KCLM systems for maize production include slow clover establishment, which may take land out of production for a full growing season, the need for specialized row establishment equipment, suppression management operations that require multiple passes during the spring planting season, and required maize stover harvest that can be challenging during wet fall conditions. These adoption barriers may be offset by agronomic benefits of KCLM systems, including, the potential to reduce P and K application rates by up to one-half through band application with the rotary zone tillage tool relative to broadcasting (Kaiser and Pagliari,; Kaiser and Rosen,). Additionally, increased water infiltration in KCLM systems (Baker, 2019) may distribute precipitation over the landscape more evenly, reducing areas affected by flooding and reducing the time from rainfall until field conditions are suitable for field operations. Potential avenues for system optimization may include research to speed clover establishment, utilization of strip-tillage equipment that is more readily available than the rotary zone tillage tool, suppression techniques to reduce root and rhizome biomass disturbance, and alternative row-crop rotations. Alterations in agronomic management should consider potential impacts on mineralization of accumulated organic N pools and how this may affect the fertilizer N requirement of row crops. Research to address these constraints may improve the competitiveness of KCLM systems with conventional cropping systems, leading to increased adoption and realized environmental and economic benefits of KCLM systems. Kura clover living mulch-row crop systems may be an important strategy for reducing the negative impacts of agricultural production on water quality and soil health through improved water infiltration into soils, reduced residual soil N following row crops, increased protection against soil erosion, and increased gross crop productivity.

Table 2.1: Year and treatment means and statistical significance of dependent variables for first-year maize.

	Grain yield	**Grain N†**	**Stover**	**Stover N**	**Kura clover**	**NH_4-N**	**NO_3-N**	**TIN‡**
Fixed effect	Mg ha^{-1}	kg ha^{-1}	Mg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1} 0–0.6 m		
Year								
2017	12.7	140b*	6.75b	49.6b	616a	4.63b	14.99	19.62
2018	13.0	150a	8.52a	66.5a	140b	7.93a	12.35	20.28
Treatment								
0¶	12.8	134	7.37	59.2	444	6.61	7.96	14.57
40¶	12.7	137	7.41	54.4	330	5.56	9.31	14.87
80¶	13.1	146	7.38	54.7	439	7.98	10.54	18.52
120§	12.6	140	7.70	60.4	407	6.03	15.15	21.18
120‖	12.7	143	7.83	58.7	401	4.75	10.28	15.03
120¶	13.2	158	7.58	58.1	361	5.99	12.31	18.30
180¶	13.0	150	7.95	59.6	357	6.32	15.88	22.21
250¶	12.9	151	7.85	59.2	285	6.97	27.93	34.90
Significance					$P > F$			
Year	0.311	0.020	<0.001	<0.001	<0.001	<0.001	0.203	0.163
Treatment	0.967	0.153	0.753	0.965	0.607	0.697	<0.001	<0.001
Year × treatment	0.959	0.741	0.565	0.954	0.723	0.527	0.002	0.003

† Nitrogen (N). ‡ Total inorganic N (NO_3-N + NH_4-N, TIN). § kg nitrogen (N) ha^{-1} fertilizer N as pre-plant urea. ‖ kg N ha^{-1} fertilizer N as split-applied urea. ¶ kg N ha^{-1} fertilizer N as split-applied SuperU. * Within a column for a given fixed effect, means followed by the same letter are not significantly different at $P < 0.05$.

Table 2.2: Year and treatment means and statistical significance of dependent variables for second-year maize.

	Grain yield	**Grain N†**	**Stover**	**Stover N**	**Kura clover**	**NH$_4$-N**	**NO$_3$-N**	**TIN‡**
Fixed effect	Mg ha^{-1}	kg ha^{-1}	Mg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1} 0–0.6 m		
Year								
2017	11.5	118	6.10	41.9b*	593	4.48b	6.50b	10.98b
2018	12.9	143	8.34	64.2a	125	6.18a	9.02a	15.19a
Treatment								
0¶	9.3	94	6.18	46.6	558	4.37	4.27	8.64
40¶	11.1	113	6.98	56.1	353	6.03	5.40	11.43
80¶	11.6	119	7.18	48.4	327	4.91	5.93	10.85
120§	12.3	132	7.46	54.9	381	4.79	6.44	11.24
120‖	13.2	146	7.29	47.1	253	4.89	7.96	12.85
120¶	13.2	143	7.74	52.9	346	6.17	5.94	12.11
180¶	13.5	146	7.63	56.9	265	5.53	9.22	14.75
250¶	13.3	151	7.30	61.4	389	5.92	16.91	22.83
Significance					*P* > *F*			
Year	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Treatment	<0.001	<0.001	0.014	0.124	0.009	0.944	<0.001	<0.001
Year × treatment	<0.001	0.006	0.032	0.448	0.004	0.982	0.120	0.242

† Nitrogen (N). ‡ Total inorganic N (NO$_3$-N + NH$_4$-N, TIN). § kg nitrogen (N) ha^{-1} fertilizer N as pre-plant urea. ‖ kg N ha^{-1} fertilizer N as split-applied urea. ¶ kg N ha^{-1} fertilizer N as split-applied SuperU. * Within a column for a given fixed effect, means followed by the same letter are not significantly different at $P < 0.05$.

Table 2.3: Level of dependent variables in first- and second-year maize at the economic optimum nitrogen rate (EONR) in 2017 and 2018.

Variable	**Unit**	**2017**		**2018**	
		First-year maize	**Second-year maize**	**First-year maize**	**Second-year maize**
EONR	kg N ha^{-1}	0	177	0	146
Grain yield	Mg ha^{-1}	12.3	13.5	13.2	13.4
Grain N‡	kg ha^{-1}	126	142	141	150
Stover yield	Mg ha^{-1}	6.2	7.0	8.5	8.4
Stover N	kg ha^{-1}	47.7	47.7	70.7	65.6
Clover biomass	kg ha^{-1}	742	457	146	111
Residual TIN§ 0–0.3 m	kg ha^{-1}	8.6	9.1	7.9	5.7
Residual TIN 0.3–0.6 m	kg ha^{-1}	2.4	4.5	10.3	10.3

† Economic optimum nitrogen rate (EONR). ‡ Nitrogen (N). § Total inorganic N (NO_3-N + NH_4-N, TIN).

Table 2.4: Partial management cost (in U.S. $ ha^{-1}) for maize produced conventionally and in KCLM in 2017 and 2018.

Management practice	**2017**			**2018**		
	Conventional	**First-year KCLM**	**Second-year KCLM**	**Conventional**	**First-year KCLM**	**Second-year KCLM**
Spring mowing§		26.93	26.93		26.93	
Spring tillage	13.59†	76.78‡	76.78‡	13.59†	76.78‡	76.78‡
Fertilizer N	131.88	0.00	148.68	131.88	0.00	122.64
Mowing§		26.93	26.93			
Grain handling and storage§	231.10	170.53	186.58	215.88	182.48	185.09
Fall tillage§	48.68			48.68		
Raking§		13.10	13.10		13.10	13.10
Baling§		33.36	33.36		33.36	33.36
Bale handling and storage§		141.16	158.73		192.33	191.08
Stover nutrient removal‖		37.50	42.17		51.09	50.76
Partial management cost	**425.25**	**526.29**	**713.26**	**410.03**	**576.08**	**672.81**

Costs obtained from † Plastina, 2019, ‡ Dobbratz et al., 2019, § Plastina, 2018, ‖ Battaglia et al., 2018. Practices not listed are assumed to be equal between management systems.

Table 2.5: Economic return (in U.S. $ ha^{-1}) for maize produced conventionally and in KCLM in 2017 and 2018.

Year	**2017**			**2018**		
Management	**Conventional**	**First-year KCLM**	**Second-year KCLM**	**Conventional**	**First-year KCLM**	**Second-year KCLM**
Fixed management cost†	1235.47	1235.47	1235.47	1235.47	1235.47	1235.47
Partial management cost‡	425.25	526.29	713.26	410.03	576.08	672.81
Grain value§	2221.10	1638.95	1793.20	2074.80	1753.83	1778.87
Stover value‖	-	619.13	696.19	-	843.57	838.07
Net return	**560.38**	**496.32**	**540.66**	**429.30**	**785.85**	**708.66**
Partial economic net return#	**-**	**-64.06**	**-19.72**	**-**	**356.55**	**279.36**

†Fixed management cost: the sum of land rental, phosphorus and potassium fertilizer not associated with stover removal, fertilizer application, seed, planting, pesticide and application, harvest, labor, and miscellaneous. ‡ Partial management cost (Table 2.4). § Grain value: $133 Mg^{-1} at 155 g kg^{-1} moisture. ‖ Stover value: $79.37 Mg^{-1} at 200 g kg^{-1} moisture (Edwards, 2014). # Partial economic net return is net return of the treatment minus net return of conventional management in the same year.

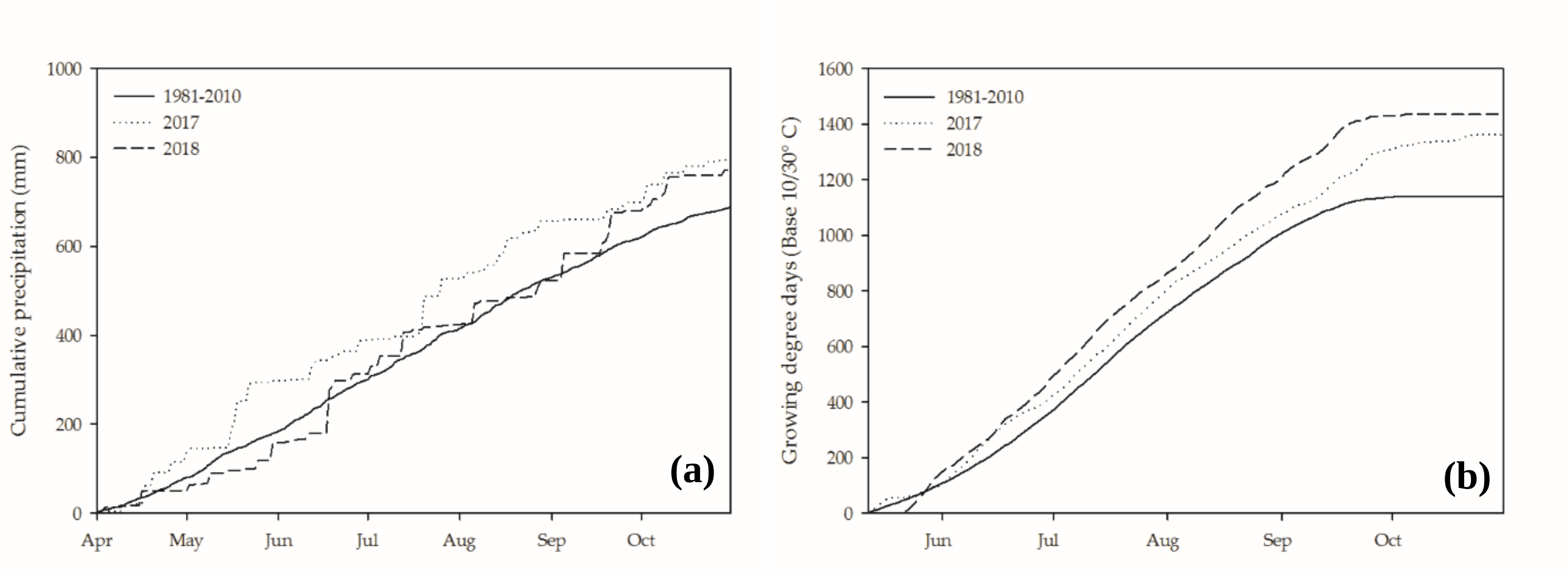

Figure 2.1a, b: (a) Cumulative precipitation between 1 April and 31 October and **(b)** Accumulated growing degree days (base 10°C and upper limit 30°C) following planting for the 1981–2010 historic average and the 2017 and 2018 experiment years.

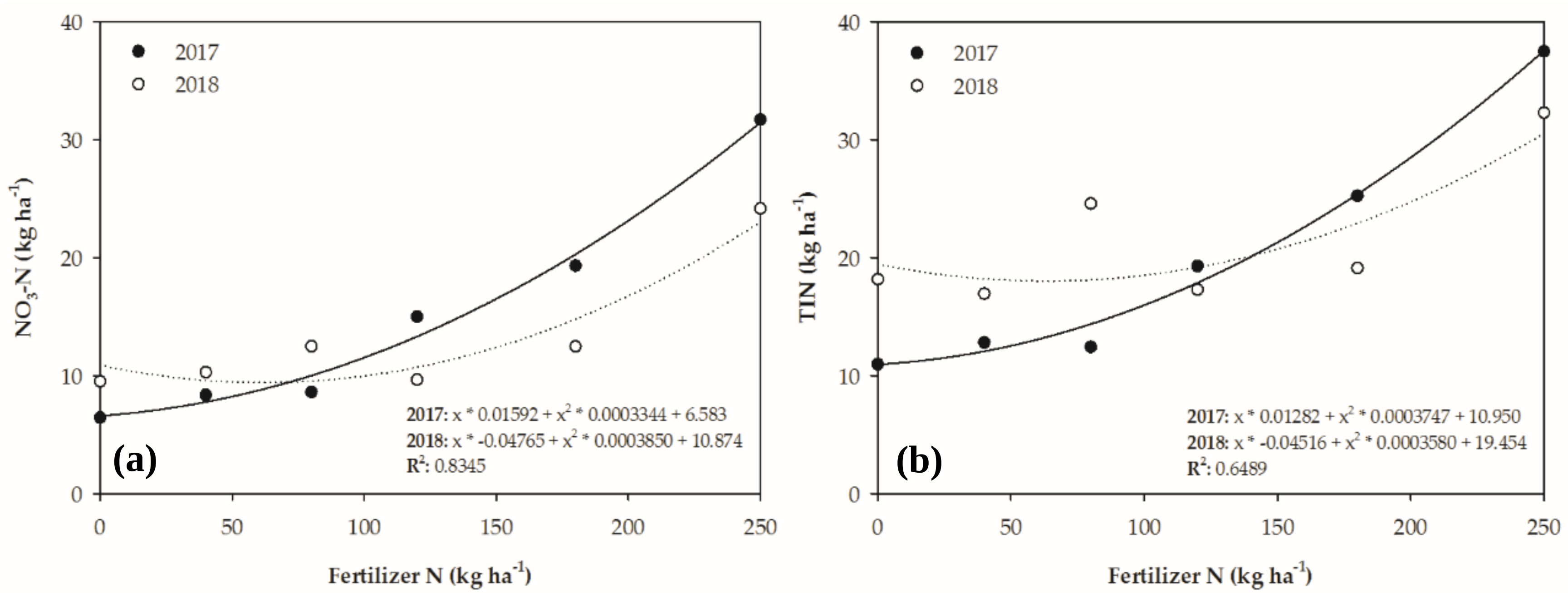

Figure 2.2a, b: Response of post-harvest soil NO3-N **(a)** and total inorganic nitrogen (NO3-N + NH4-N, TIN) **(b)** to fertilizer nitrogen (N) rate for first-year maize in 2017 and 2018.

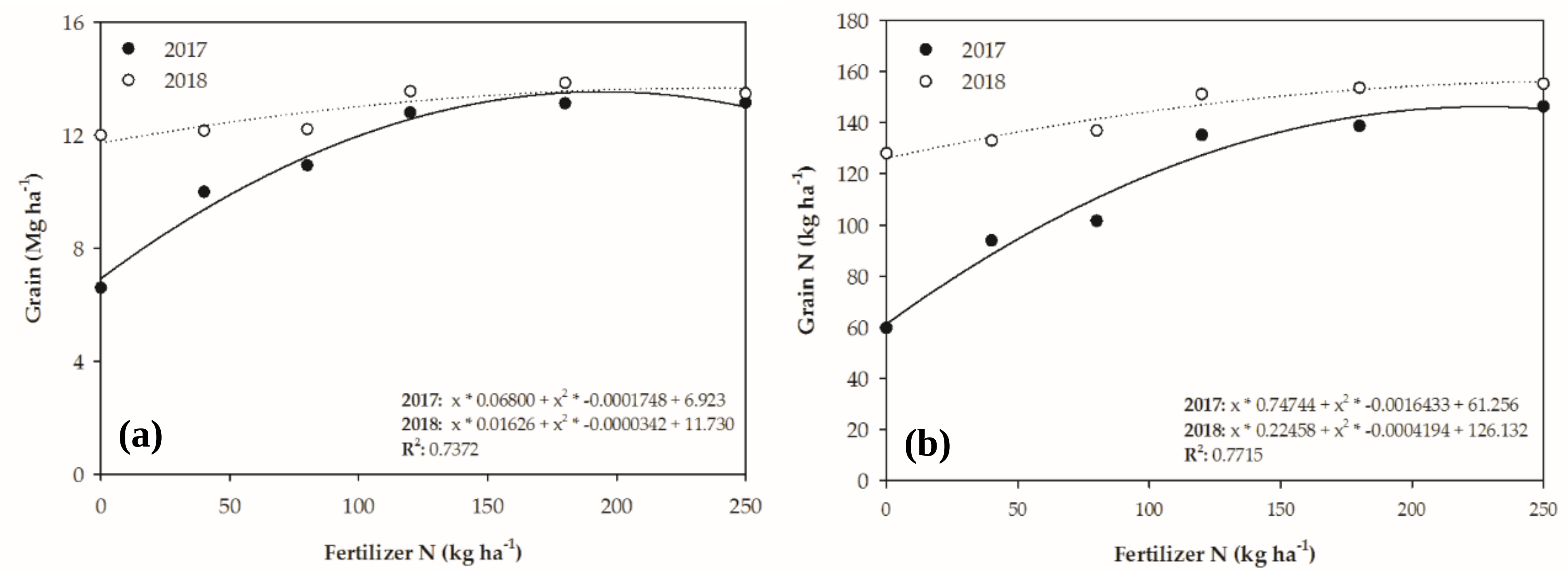

Figure 2.3a, b: Response of grain yield **(a)** and grain N **(b)** to fertilizer nitrogen (N) rate for second-year maize in 2017 and 2018.

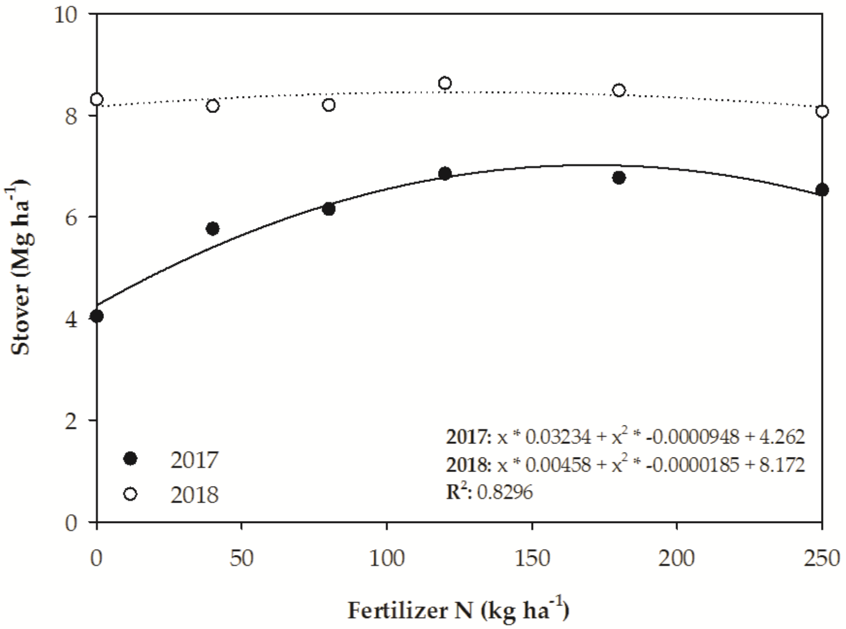

Figure 2.4: Response of stover yield to fertilizer nitrogen (N) rate for second-year maize in 2017 and 2018.

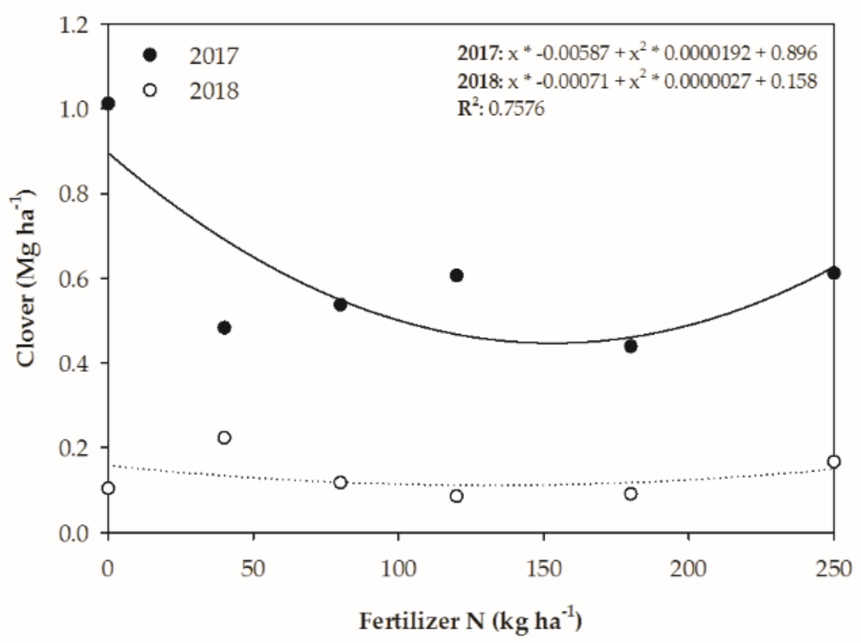

Figure 2.5: Response of late season clover biomass yield to fertilizer nitrogen (N) rate for second-year maize in 2017 and 2018.

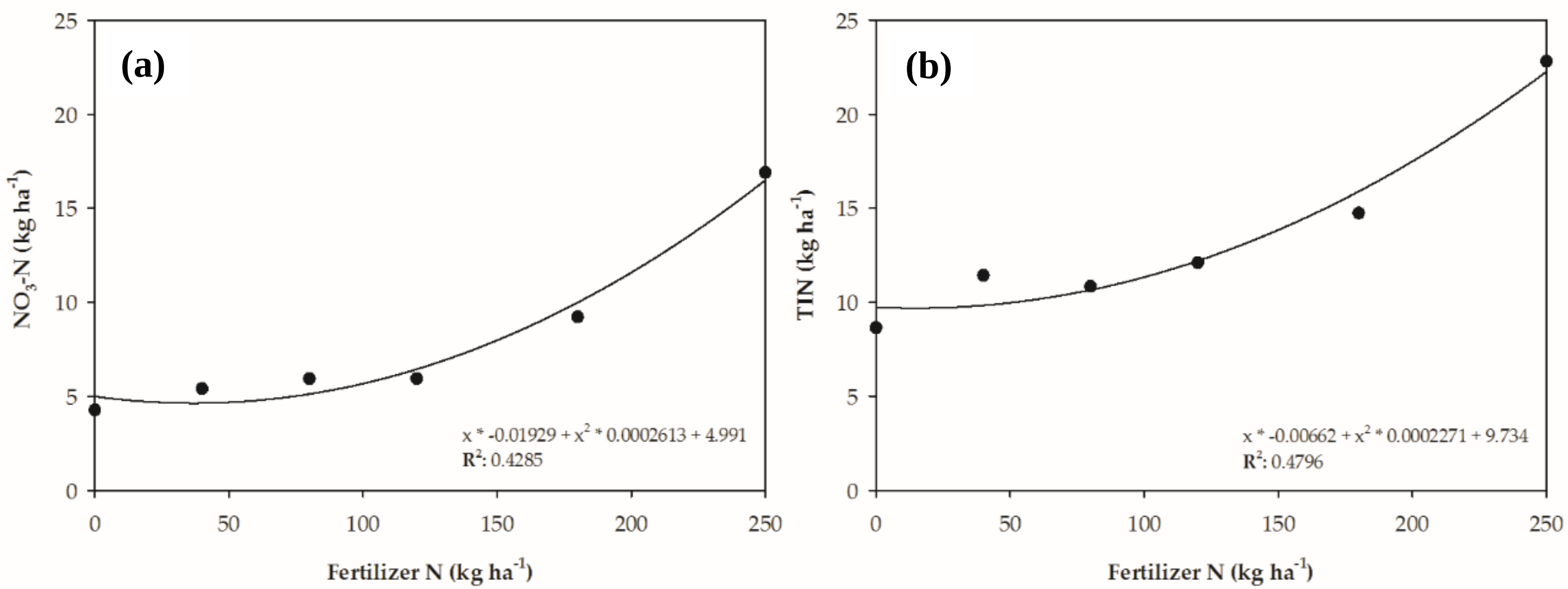

Figure 2.6a, b: Response of post-harvest soil NO3-N **(a)** and total inorganic nitrogen (NO3-N + NH4-N, TIN) **(b)** to fertilizer nitrogen (N) rate for second-year maize, across 2017 and 2018.

CHAPTER 3: CONCLUSIONS

3.1. Agronomic Factors and Optimization Opportunities for Maize (*Zea mays*) Production in Kura Clover Living Mulch

Kura clover living mulch (KCLM) provides nitrogen (N) to row crops through disturbance, incorporation, and mineralization of labile and biomass N pools. Agronomic factors including crop rotation history, management timing, and management technique impacts the magnitude of labile and biomass N pools that supply in-season N to the row crop. Spring clover suppression management and in-season shading during row crop production inhibits the reaccumulation of clover biomass and reduces in-season N contributions to subsequent row crops. Conversely, low intensity management and reduced competition for light and nutrients during forage management replenishes clover biomass and increases the in-season N contribution to subsequent row crops. Mineralization of accumulated labile and biomass N provides the full N requirements for maize production following forage management while the second year of continuous maize production requires fertilizer N at a rate near University guidelines for maize following soybean (*Glycine max* L. (Merr.)). It is likely that factors other than clover management history impact in-season N contributions to the row crop. The identification of these factors may reveal potential for further reductions of fertilizer N in the second year of continuous maize production.

Understanding the timing and magnitude of physiological and management induced reductions of above- and below-ground clover biomass may reveal opportunities for agronomic management optimization to maintain clover vigor under continuous row crop production. Clover nonstructural root carbohydrates are depleted in the early spring to promote rapid shoot development (Peterson et al., 1994b). Seasonal physiological depletions of nonstructural clover root metabolites correspond with KCLM spring suppression management for row crop production. The translocation of root metabolites, suppression of aboveground growth, and subsequent shading under the crop canopy inhibits the recovery of clover biomass, resulting in declining spring vigor and reduced mineralizable N in the following year (Grabber et al., 2014).

Crop rotations and agronomic management may be designed to reduce the competition between the row crop and the living mulch to maintain clover biomass and vigor while reducing practical management constraints. Kura clover may be allowed to grow and recover before spring forage is harvested and short season maize hybrids are established. This management technique would take advantage of the spring clover growth as a forage crop, and since the cool-season clover recovers less vigorously following the first forage cutting, suppression management intensity may be reduced. The short season maize hybrid may be utilized for grain, high moisture grain, or silage and the greater diversity of income with forage and maize production on the same cropland could provide greater economic stability. While crop management techniques may stabilize and improve clover vigor, alternative suppression techniques may reduce practical constraints of KCLM management. Alternative strip tillage tools and high-speed-low-impact residue incorporation with rotary hoes or vertical tillage tools may reduce management time constraints, increase equipment availability, and reduce management costs for producers who adopt KCLM systems. These changes in agronomic management would impact N management recommendations due to changes in the magnitude and disturbance of mineralizable N pools.

3.2. Adoption Opportunities

Kura clover living mulch systems may add economic and environmental resilience to Midwestern commodity production industries. Crop production in KCLM systems increase water infiltration (Baker, 2019), reduce nitrate loss (Ochsner et al., 2010, 2017), reduce soil loss (Siller et al., 2016), and increase gross ecosystem productivity (Wortman et al., 2012) compared to conventional rotations. Projected increased rainfall during planting season and the redistribution of annual precipitation in fewer and larger storm events present growing risks of flooding and rill erosion to upper-Midwestern cropland (Zobel et al., 2018). Increased water infiltration rates may distribute heavy rainfall more evenly over the landscape and increase the utilized storage capacity of poorly drained glacial landscapes. More evenly distributed soil moisture has the potential to reduce yield variability driven by topographical hydrologic variation, improve field scale nutrient use efficiency, and increase agronomic management windows by reducing field areas affected by flooding.

U.S. forage industries are susceptible to risks from unfavorable weather conditions. In the last decade (2009 to 2018), the average price received for alfalfa (*Medicago sativa*) hay in the U.S. ranged from \$125 to 233 Mg^{-1}, corresponding with total U.S. production of 64 and 46 Tg, respectively (USDA, 2018). The range in U.S. alfalfa production and production value can be attributed to poor yields caused by draught or harvest issues associated with frequent rainfall (Putnam et al., 2000; Andresen et al., 2001). Since the nutritional quality of kura clover forage is similar or superior to alfalfa (Seguin et al., 2002), Midwestern U.S. producers with an established KCLM system may be able to respond to production shortfalls in traditional alfalfa producing regions to satisfy demand and stabilize forage markets. This cropping system attribute, accredited to greater flexibility in crop rotations, may be useful when unfavorable market outlooks or challenging local planting conditions reduce the potential profitability of row crop production.

Maize stover production in KCLM systems may be the most economically important quality of the companion cropping system. Added erosion protection from continuous living cover in the KCLM system increases the sustainable maize stover removal rate with reduced risks of nutrient runoff and soil carbon decline (Pratt et al., 2014). Maize stover was retained on 86% of U.S. maize acres in 2010, with the majority of maize residue utilized by grazing (83%) rather than mechanical harvest (17%) (Schmer et al., 2017). Since maize stover utilization by grazing is generally under 25% (Fernandez-Rivera and Klopfenstein, 1988), and upper-Midwestern weather conditions prevent efficient stover grazing, stover utilization in upper-Midwestern KCLM systems will require baling or chopping operations to prevent clover smothering in the following spring.

There are several uses for maize stover that may be better utilized in the coming decades to meet growing global protein demands (Burggraf et al., 2015; Mao et al., 2016). Mechanical harvest of dry maize stover may be difficult when fall harvest conditions prevent stover drying to below 0.2 $g\ g^{-1}$ moisture (Shinners et al., 2007). This potential hurdle may be an opportunity for producers feeding ruminant animals, who may ensile maize stover for maize stalklage production (Johnson et al., 1999). Maize stalklage has been investigated for its replacement value of traditional whole plant maize silage (Berger et al., 1979; Meteer, 2014; Conway, 2019). Ruminant rations that utilize alkaline treated maize stalklage can reduce grains and feeding costs with no negative impacts to feed efficiency or daily gains (Shreck et al., 2014). Maize stalklage as a source of ruminant nutrition fits well into KCLM management for mixed crop-livestock systems by adding value to an underutilized resource while producing readily marketable grain.

3.3. Research Needs

Further research should be conducted on KCLM systems to optimize agronomic management for maintenance of clover vigor and mineralizable N pools. The response of above and below ground biomass to agronomic management, row crop production, and forage management may reveal greater insights into the labile and biomass N sources that provide in-season N contributions to the row crop. Quantification of clover biomass dynamics along with agronomic and environmental information may reveal management intensity thresholds that reduce cropping system productivity due to slowed clover recovery. This information would provide insight into the biological limit of KCLM systems to provide realized agronomic benefits while maintaining sustainable clover growth. These insights would facilitate the design of more reliable crop rotation and nutrient management recommendations.

Nitrous oxide (N_2O) emissions are a potential negative attribute of KCLM cropping systems. Although data included in Chapter 1 did not find that KCLM management increased N_2O emissions relative to unmanaged clover, the measured N_2O flux during the six week spring management period was alarmingly high (>2 kg N ha^{-1}). Turner et al., 2016 found that KCLM management increased seasonal N_2O emissions relative to a conventional maize-soybean rotation and recent work highlights the potential for increased yield scaled N_2O emissions from crop residues in low-input agricultural systems (Pugesgaard et al., 2017). Factors affecting N_2O emissions in KCLM systems must be identified so that management options for mitigation may be investigated.

Practical constraints to KCLM management must be identified to facilitate the design of innovative management strategies that reduce current adoption barriers. Agronomic optimization must consider the number of field passes, time constraints, equipment availability, and nutrient management recommendations. Spring tillage is currently a substantial practical barrier to KCLM management. The rotary zone tillage tool used in this research has large per-row power requirements and is not commonly available in the U.S. Midwest. Alternative strip tillage tools that require less power per-row may be needed to manage larger fields, but reduced clover disturbance and soil mixing may reduce clover suppression and N contributions to the row crop. A hypothesized technique to increase clover suppression while utilizing less aggressive tillage management is subsurface band application of anhydrous ammonia (NH_3) during row establishment. Application of NH_3 fertilizers increase the soil pH and promotes nitrification and accumulation of nitrite in the application band (Myers and Thien, 1988; Tierling and Kuhlmann, 2018), resulting in an inhospitable environment for plant growth (Oke, 1966). Chemical clover suppression with NH_3 fertilizers may improve the effectiveness of conventional strip tillage tools that offer the possibility to apply phosphorus, potassium, and sulfur fertilizers in a subsurface band. Integrating emerging strip tillage technologies into KCLM management could achieve row establishment, nutrient management, and chemical clover suppression in a single field operation.

Kura clover living mulch systems have great potential for integration into current Midwestern cropping systems. Its use for conservation, market stability, and increased management intensity provide new opportunities for Midwestern crop and livestock producers. Agronomic management for KCLM-maize cropping systems must be further developed and long-term economic analysis must be quantified to provide timely information to producers who may benefit from KCLM management systems.

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